

W.S. Veraverbeke, J. De Cock, G. De Rouck, J.A. Delcour, H. Van Mellaert, G. Aerts and W.F. Broekaert

# Partial Substitution of Barley Malt by Wheat Bran in the Grist Results in Lager Beer with Better Taste Profile and Higher Content in Arabinoxylan-Oligosaccharides (AXOS)

The use of wheat bran as a new adjunct in brewing at 25 % of total grist in combination with the use of a xylanase in the mashing step was tested by brewing control and bran-brewed lager beers. Sensory analysis revealed marked improvements in taste profile due to bran-brewing, with statistically significant increase in body, smoothness and warming notes, and a decrease in acetaldehyde, after-bitterness, drying and grainy attributes. A detailed physico-chemical analysis of the beers was performed. Key parameters on which a significant impact was demonstrated include increased content of arabinoxylan-oligosaccharides (AXOS), ferulic acid, and soluble protein, and lowered content of aldehydes, the latter indicative of reduced oxidation during brewing. Traditional but long forgotten use of wheat bran for brewing of small beers holds potential to make innovative beers with an interesting taste profile.

Descriptors: body, sensory analysis, wheat bran, xylanases

## 1 Introduction

Innovation and tradition are both very important to the brewing industry. Breweries are looking for products with an innovative taste profile and for ways to control cost in an environment of increasing input prices. At the same time, the consumer values a beer that is made with respect for tradition, and reference to tradition is very often a key part of the marketing message in the brewing industry.

Several historical references [6, 7, 27] describe traditional uses of wheat bran as a component of the grist, particularly to make top-fermented table beer or small beer. There is, however, very little reference to the use of wheat bran as an adjunct in modern lager beers.

This study examines the impact of the partial substitution of barley malt by wheat bran in combination with the addition of xylanases in the mashing step (“bran-brewing”) to promote the release of arabinoxylan-oligosaccharides (AXOS) in lager beers.

In terms of extractable matter, wheat bran contains approximately 17–25 % starch and 17 % protein [15, 21]. The addition of xylanases in the mashing step is expected to promote extraction of the hemicellulose fraction which represents about 25 % of the wheat bran on a dry matter basis [15, 21]. At the same time, these xylanases will convert long chain arabinoxylans into shorter chain AXOS. AXOS qualify as soluble fibre and have been reported to have a beneficial effect on the microbiota in the colon [5].

Commercial beers currently contain variable amounts of AXOS which are typically not higher than 2 g/L [1, 17]. *Aerts et al.* [1] have already reported an improvement in taste profile when AXOS is added to beer as an exogenous ingredient. Bran-brewing is expected to also result in beer with an increased AXOS content. It should further be mentioned that the arabinose units in the hemicellulose fraction of wheat bran are often substituted with ferulic acid groups [13]. Feruloylated AXOS are known to be highly potent antioxidants in vitro [26], and it has been suggested that they may contribute to the antioxidant activity of beer [22].

This study reports on a detailed comparison of control beers and bran-brewed beers. It entails the determination of a wide range of physico-chemical parameters as well as the characterisation by an expert taste panel trained to assess and describe the taste characteristics of beer in 61 standardised descriptors.

## Authors

Wim S. Veraverbeke<sup>1</sup>, Jan De Cock<sup>2</sup>, Gert De Rouck<sup>2</sup>, Jan A. Delcour<sup>3</sup>, Herman Van Mellaert<sup>1</sup>, Guido Aerts<sup>2</sup>, Willem F. Broekaert<sup>1</sup>

<sup>1</sup>Fugeia NV, Arenberg Science Park, Leuven, Belgium;

<sup>2</sup>Laboratory of Enzyme, Fermentation and Brewing Technology, Leuven Food Science and Nutrition Research Centre (LFoRCe), KaHo St.-Lieven, Association Katholieke Universiteit Leuven, Gent, Belgium;

<sup>3</sup>Laboratory of Food Chemistry and Biochemistry, Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Leuven, Belgium; corresponding author: willem.broekaert@fugeia.com

## 2 Materials and Methods

### 2.1 Materials

Barley malt was standard pilsner malt purchased from Boortmalt (Antwerpen, Belgium). Malt was coarsely milled on a 2-roll mill (gap setting < 0.7 mm) before use in brewing. Wheat bran was obtained from Dossche Mills & Bakery (Deinze, Belgium). Xylanase was Brewlyve AXC 1500L from Lyven (Colombelles, France). Water used for brewing was first purified by reverse osmosis and then adjusted to a Ca<sup>2+</sup> concentration of 40 ppm by addition of calcium chloride. Isomerised hop acid extract, containing 20 % (w/v) iso- $\alpha$ -acids, was from Botanix Ltd. (Paddock Wood, England). Yeast was Saflager 3470 from Lesaffre (France).

### 2.2 Brewing trials

Beers were brewed on a 50 L scale micro brewery (Spadoni, Orvieto, Italy). Control beers (A and B) were made in duplicate and bran beers (C, D and E) were made in triplicate independent brews.

Mash composition at mashing-in was 35 L of water and 10 kg of barley malt for the control beers and 35 L of water, 8.7 kg of barley malt, 2.9 kg of wheat bran and 40 mL of xylanase for the bran beers. The mashing temperature scheme was for all beers as follows: 30 min hold at 52 °C, 25 min hold at 63 °C, 40 min hold at 72 °C and 1 min hold at 78 °C. Heating in between the different hold periods was performed at 1 °C/min. The pH of the mash was adjusted to 5.6 with lactic acid at mashing-in and further controlled at 5.6 during the complete mashing program. Lautering was performed over a lauter tun at a temperature of 78 °C during 60 min. The extract of the first wort was ca 16.0 °P. Sparging was done until the worts reached 11.5 °P using approximately 25 L of water at 78 °C. Only one sparging step was performed. The filtered worts were boiled during 60 min using a kettle with double jacket and oil heating (oil temperature 125 °C). At the end of boiling, ZnCl<sub>2</sub> was added to reach a Zn<sup>2+</sup> concentration of 0.2 mg/L, and isomerised hop acid extract was added to reach an iso- $\alpha$ -acids concentration of 25 mg/L in the final beer. The boiled worts were clarified using a whirlpool and cooled with a plate heat-exchanger. Cooled clarified worts were pitched with rehydrated (100 g yeast in 1 L sterile water during 1 hour) dry yeast at 10<sup>7</sup> cells/mL, followed by fermentation in 50 L conical tanks during 8 days at 12 °C and lagering during 14 days at 0 °C. The beers were filtered over kieselguhr/cellulose sheets (1  $\mu$ m), saturated to 5.5 g/L CO<sub>2</sub> and finally bottled and sealed in brown standard 25 cL bottles (O<sub>2</sub>-content < 80 ppb) using an isobaric filling machine with double pre-evacuation (America monobloc, Cimec, Canelli, Italy).

After bottling, beer samples were stored at 0 °C until further analyses.

### 2.3 Analytical methods

Original extract of the worts, and apparent extract and alcohol content of the beers were measured with a Beer Alcoyser ME

(Anton Paar Benelux, Gentbrugge, Belgium) in combination with a DMA 4500 M density meter (Anton Paar Benelux, Gentbrugge, Belgium).

FAN (Free Amino Nitrogen) was measured according to EBC-method 9.10 [11]. High molecular weight soluble protein was determined with a Bio-Rad protein assay based on the method described by Bradford [4]. Sensitive protein was determined as the increase in haze (expressed in EBC formazin units) upon addition of Brewtan C (IOB Method 9.37).

Carbohydrate composition, AXOS arabinose-to-xylose (A/X) ratio and AXOS average degree of polymerisation (DP) were determined by gas chromatography according to the method of Courtin *et al.* [8].

Total polyphenols and flavanoids were quantified according to EBC Methods 9.11 and 9.12 [11], respectively. Proanthocyanidins were determined according to Bate-Smith [3]. Bound ferulic acid (total ferulic acid minus free ferulic acid) and free ferulic acid were determined by the method of Hartmann *et al.* [12] on lyophilised beer samples.

Volatile aldehydes were determined according to Vesely *et al.* [25] using headspace-solid phase micro-extraction (HS-SPME) with on-fibre PFBOA (*o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine) derivatisation and capillary gas chromatography/mass spectrometry (CGC/MS) (Dual Stage Quadrupole (DSQ™ II) GC/MS system (Interscience Benelux). The DSQ™ II was coupled to a Thermo Trace GC Ultra (Interscience, Benelux) equipped with a CTC-PAL autosampler, a split/splitless injector with a narrow glass inlet liner (0.5 mL volume), and a RTX-1 fused-silica capillary column (40 m  $\times$  0.18 mm i.d., 0.2  $\mu$ m film thickness, Restek, Interscience, Benelux). Data processing was performed by the XCalibur™ data system (Thermo Electron Corporation, Austin, TX, USA).

Iso- $\alpha$ -acids were analysed by UPLC as previously described [9]. Total bitterness is calculated as the sum of the different iso- $\alpha$ -acids.

TBI (Thiobarbituric Acid Index) was measured according to Thalacker and Bößendörfer [24]. TRAP (Total Reactive Antioxidant Potential, expressed in mM ascorbic acid equivalents) was determined according to Araki *et al.* [2].

Beer color was determined according to IOB Method 9.1. Foam stability was measured according to EBC Method 9.42 [11] using a Nibem-T Meter (Haffmans, Venlo, The Netherlands).

Cold and permanent haze (expressed in EBC formazin units) were determined after storage for 24 hours at 0 °C and 20 °C, respectively using a Haffmans VOS ROTA 90/25 Turbidity Meter (Haffmans, Venlo, The Netherlands), under 25° and 90° angles.

### 2.4 Sensory analysis

A descriptive flavour profiling of the beers was conducted by Cara Technology Ltd. (Leatherhead, Surrey, UK). All five experimental

beers were assessed in duplicate by each of the six members of an expert taste panel. Assessments were done blind and according to a randomized design. Three commercial pale lager beers were profiled in parallel.

Each profiling consisted of an evaluation of the intensity of 61 flavour attributes. All flavour attributes were scored on a 0-10 scale. The flavour attributes can be grouped in the following categories: (1) yeast-derived characters (sulphitic, H<sub>2</sub>S, solvent alcoholic, floral alcoholic, isoamyl acetate, ethyl hexanoate, ethyl butyrate, ethyl acetate, ethyl lactate, acetaldehyde, acetal, diacetyl); (2) hop-derived characters (spicy kettle hop, floral kettle hop, hop oil, isovaleric); (3) cereal-derived characters (malty-biscuity, grainy, warty, DMS, onion, chocolate, burnt, caramel, liquorice, spicy, smoky, vanilla, freshly-cut grass); (4) flavours from microbial contaminants (indole, acetic, lactic, phenolic, plastics, butyric); (5) flavours from beer ageing (mercaptan, burnt rubber, yeasty, meaty, caprylic); (6) flavours from beer oxidation (winey, tobacco, powdery, metallic, papery, leathery, methional, catty, lightstruck); (7) taste and mouthfeel characters (salty, sweet, bitter, after-bitterness, yeast bite, sour, astringent, warming, drying, smooth, carbonation, body).

Additionally, each beer was given an overall quality score on a 0–10 scale, whereby 9–10 indicates defect-free beers with positive flavour attributes in keeping with the beer style, 8–9 indicates excellent beers, 7–8 indicates good beers, 6–7 indicates beers with minor defects and possibly some flavours which are not at the level expected of the beer style, 5–6 indicates beers customers are likely to dislike, 4–5 indicates beers that are likely to give rise to customer complaints and < 4 indicates beers that would typically require a recall from the market.

### 2.5 Statistical analysis

Statistical differences between control and bran-brewed beers for different physico-chemical and sensorial parameters were evaluated using Analyse-it software (Analyse-it Software Ltd, Leeds, UK) version 2.22. For physico-chemical parameters, a simple one-way ANOVA (equivalent to a Student's t-test) was used to compare the two control values with three bran-brewing values. For the sensory parameters a one-way ANOVA was used using the underlying scores of each of the six individual members of the expert tasting panel in the analysis.

## 3 Results and Discussion

In order to make a thorough comparison of control lager beers with bran-brewed lagers, independent brews were made of each type (A and B brews are control lagers; C, D and E are bran-brewed lagers). Based on information obtained from preliminary experiments, the mash compositions referred to under 2.2. were chosen with a view to achieve similar original extract values in both types of beers. The study involved the determination of a wide range of physico-chemical parameters and an in-depth sensory evaluation of bran-brewed lagers versus control lagers.

### 3.1 Physico-chemical characterisation

The physico-chemical properties of the different beers made in this study are shown in table 1.

Original extract values for control (12.4 °Plato) and bran-brewed beers (12.0 °Plato) were only slightly different. However, bran-brewed beers had much higher apparent extract values (3.0 versus 2.4;  $p < 0.01$ ) and lower alcohol contents (4.8 versus 5.3;  $p < 0.01$ ) than control beers. Bran-brewed beers also had a slightly higher pH relative to control beers (4.7 versus 4.5;  $p < 0.01$ ). Moreover, bran-brewed beers were more intensely coloured and exhibited higher foam stability and more haze than control beers.

We observed a number of notable compositional differences between control beers and bran-brewed beers. Soluble and sensitive protein contents were higher in bran-brewed beers than in control beers. This also applies to free monosaccharides. As expected given the use of bran and xylanase, bran-brewed beers also had higher AXOS contents (about three-fold higher;  $p < 0.001$ ) with on average a shorter chain length (DP13 versus DP24;  $p < 0.01$ ) and higher contents of bound ferulic acid (about four-fold higher;  $p < 0.01$ ). Free ferulic acid contents followed the same trend, yet they were about 10-fold lower than those of bound ferulic acid. Two indicators for anti-oxidative activity, TRAP and TBI values, were significantly higher for bran-brewed beers ( $P < 0.05$  and  $P < 0.001$ , respectively). Total aldehyde levels in bran-brewed beers were only half those of control beers ( $p < 0.001$ ).

Flavanoid content of bran-brewed beers was slightly lower than that of control beers ( $p < 0.05$ ). No significant differences were observed in total polyphenol content, proanthocyan content and total bitterness.

### 3.2 Sensory characterisation

Table 2 provides a summary of the sensory evaluation of the beers produced for this study. Average sensory values of three commercial reference lager beers are also shown.

Of the 61 attributes scored, only those flavour attributes were retained in table 2 that resulted in a score (average of six taste panel members) of at least 1.0 for at least one of the beers.

Bran-brewed beers scored significantly lower than control beers on after-bitterness ( $p < 0.05$ ) and were less grainy ( $p < 0.001$ ). They also contained substantially less acetaldehyde flavour ( $p < 0.05$ ) than control beers. Bran-brewed beers were more warming ( $P < 0.05$ ) and less drying ( $P < 0.05$ ) and had more smoothness ( $P < 0.01$ ) and body ( $P < 0.01$ ) than control beers.

In terms of overall score, bran-brewed beers scored significantly better than control beers ( $P < 0.05$ ), and reached an overall score similar to that of commercial reference lager beers.

### 3.3 Discussion

The addition of bran and xylanase to the grist clearly leads to a shift in the composition of the resulting beers. Wheat

bran is known to be rich in arabinoxylan [10]. Therefore, it is in line with expectations that the content of AXOS shows a marked increase due to solubilisation of water-unextractable arabinoxylan from bran by the xylanase enzyme. Arabinose groups in AXOS are often substituted with the polyphenol ferulic acid [13], hence the increase in ferulic acid content (in majority ester bound) is also unsurprising. It should be noted that this does not result in a significant difference in overall polyphenols. Ferulic acid is known to be a strong antioxidant, and feruloylated AXOS exhibit even higher antioxidant activity than ferulic acid itself [14, 16, 26, 28]. The TRAP value, a parameter aimed at assessing overall antioxidant value was significantly increased in bran-brewed beers. The TBI value followed the same trend, which likely confirms the higher antioxidative value of bran-brewed beers. Indeed, while TBI analysis is typically used in brewing science to assess intensity of heat treatment of wort or beer, its use as a measure of oxidation is equally common [23]. Furthermore, a very marked and desirable decrease in the content of total aldehydes was observed. The antioxidant activity of AXOS-bound ferulic acid may play a key role in reducing total aldehydes, which are formed through undesired oxidative processes during brewing.

The increased amount of pentose-based oligosaccharides in bran-brewed beers obviously contributes to their original extract value. The fact that *S. cerevisiae* yeasts are unable to ferment pentoses and pentose-based oligosaccharides like AXOS [20] may help explain in part why (i) real extract content in bran-brewed beers is higher than in control beers and (ii) why alcohol content in bran-brewed beers is lower than in control beers.

Bran-brewing further results in an increased content in soluble protein. This in turn results in an improved foam stability but also higher haze under the filtration regime used in this study. Indeed, the relationship between protein content, in particular hydrophobic proteins, and foam stability is very well established [19], as is that between protein content and haze formation [18]. The increased soluble protein content can be explained by both the higher protein content and higher proportion of soluble protein of bran versus whole cereals [10]. While increased haze was observed as a result of bran-brewing, haze values of all experimental brews (both control and bran-brewed beers) were too high (H90, Table 1), indicating insufficient filtration regimes for all beers. Improvements in haze can be expected with an optimised filtration regime.

The lowered levels of total aldehydes in the bran-brewed beers uncovered by the physico-chemical analyses was corroborated by the sensory analysis, which revealed significantly lower sensory scores for acetaldehyde in bran-brewed beers.

Amongst key sensory parameters which exhibit a significant improvement in the bran-brewed beers, reduced after-bitterness, drying and graininess, better body and higher smoothness and warming should be highlighted. This all contributes to a statistically significant ( $p < 0.05$ ) improvement in the overall sensory quality score from 6.8 (average of control beers) to 7.3 (average of bran-brewed beers). The average overall score of the bran-brewed beers was thus close to that of the commercial reference

beers which were tested in parallel. Furthermore, it should be emphasised that this study was designed with a view to determine the differences between control and bran-brewed beers, not with a view to achieve a bran-brewed beer with a fully optimised taste profile. For instance, the experimental beers were not hopped but only bittered with purified iso- $\alpha$ -acids, and it is anticipated that further quality improvement could be achieved if the hopping regime were to be optimised.

#### 4 Conclusions

The results of this study validate the potential of bran as an innovative ingredient for beer brewing. Application of this technology leads to lager beers with increased feruloylated AXOS levels, reduced aldehyde levels, and improved overall taste profile.

Bran-brewing capitalises on a raw material, with deep roots in age-old brewing traditions, which is widely available and which has an excellent health image with consumers. It is our expectation that the benefits described in this study will provide a basis for commercial brewers who would wish to use this new technology to develop commercial bran-brewed beers.

#### Acknowledgment

We are grateful to Cara Technology Ltd. (Leatherhead, Surrey, UK) for skilled sensory analysis. This work was supported by grant IWT080218 from the Agentschap voor Innovatie door Wetenschap en Technologie (IWT) of Flanders, Belgium.

#### 5 References

1. Aerts, G.; Broekaert, W.; Courtin, C. and Delcour, J.: Arabinoxyloligosaccharides in beer, International Patent Application WO2008098320, (2008).
2. Araki, S.; Kimura, T.; Shimizu, C.; Furusho, S.; Takashio, M. and Shinotsuka, K.: Estimation of antioxidative activity and its relationship to beer flavor stability, *J. Am. Soc. Brew. Chem.*, **57** (1999), pp. 34-37.
3. Bate-Smith, E.C.: Haemanalysis of tannins: The concept of relative adstringency, *Phytochem.*, **12** (1973), pp. 907-912.
4. Bradford, M.M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.*, **72** (1976), pp. 248-254.
5. Broekaert, W.F.; Courtin, C.M.; Verbeke, K.; Van de Wiele, T.; Verstraete, W. and Delcour, J.A.: Prebiotic and other health-related effects of cereal-derived arabinoxylans and (arabino)xylooligosaccharides, *Crit. Rev. Food Sci. Technol.*, **51** (2011), pp. 178-194.
6. Byrn, M.L.: The complete practical brewer, Henry Carey Baird, (1873).
7. Coppinger, J.: The American practical brewer and tanner, Reprint Edition 2007, Beerbooks.com, Cleveland, Ohio, USA, (1815).
8. Courtin, C.M.; Broekaert, W.F.; Swennen, K.; Aerts, G.; Van Craeyveld, V. and Delcour, J.A.: Occurrence of arabinoxyloligosaccharides and arabinogalactan peptides in beer. *J. Am. Soc. Brew. Chem.* **67** (2009), pp. 112-117.

9. De Cooman, L.; Aerts, G.; Overmeire, H. and De Keukeleire, D.: Alterations of the profiles of iso- $\alpha$ -acids during beer ageing, marked instability of trans-iso- $\alpha$ -acids and implications for beer bitterness consistency in relation to tetrahydroiso- $\alpha$ -acids, *J. Inst. Brew.*, **106** (2000), pp. 169-178.
10. Delcour, J.A. and Hosney, R.C.: Principles of cereal science and technology, Third Edition, AACC International, St. Paul, Minnesota, USA, (2010).
11. European Brewery Convention, Analytica-EBC, 6th edition, Hans Carl Getränke-Fach Verlag, Nürnberg, Germany.
12. Hartmann, G.; Piber, M. and Koehler, P.: Isolation and chemical characterisation of water-extractable arabinoxylans from wheat and rye during breadmaking. *Eur. Food Res. Technol.*, **221** (2005), pp. 487-92.
13. Izydorczyk, M.S. and Biliaderis, C.G.: Cereal arabinoxylans: advances in structure and physicochemical properties, *Carbohydr. Polym.*, **28** (1995), pp. 33-48.
14. Katapodis, P.; Vardakou, M.; Kalogeris, E.; Kekos, D.; Macris, B.J. and Christakopoulos, P.: Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan, *Eur. J. Nutr.*, **42** (2003), pp. 55-60.
15. Maes, C. and Delcour, J.A.: Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran, *J. Cereal Sci.*, **35** (2002), pp. 315-326.
16. Ohta, T.; Naomi, S.; Kuchii, A.; Egashira, Y. and Sanada, H.: Antioxidative activity of corn bran cell-wall fragments in the LDL oxidation system, *J. Agric. Food Chem.*, **45** (1997), pp. 1644-1648.
17. Schwarz, P.B. and Han, J.-H.: Arabinoxylan content of commercial beers, *J. Am. Soc. Brew. Chem.*, **53** (1995), pp. 157-159.
18. Siebert, K.J.: Haze formation in beverages, *Food Sci. Technol.*, **39** (2006), pp. 987-994.
19. Slack, P.T. and Bamforth, C.W.: The fractionation of polypeptides from barley and beer by hydrophobic interaction chromatography: the influence of their hydrophobicity on foam stability, *J. Inst. Brew.*, **89** (1983), pp. 391-401.
20. Sonderegger, M.; Jeppsson, M.; Larsson, C.; Gorwa-Grauslund, M.F.; Boles, E.; Olsson, L.; Spencer-Martins, I.; Hahn-Hägerdal, B. and Sauer, U.: Fermentation performance of engineered and evolved xylose-fermenting *Saccharomyces cerevisiae* strains. *Biotechnol. Bioeng.*, **87** (2004) pp., 90-98.
21. Swennen, K.; Courtin, C.M.; Lindemans, G.C.J.E. and Delcour, J.A.: Large-scale production and characterisation of wheat bran arabinoxyloligosaccharides, *J. Sci. Food Agric.*, **86** (2006), pp. 1722-1731.
22. Szwajgier, D.; Wasko, A.; Zapp, J. and Targonski, Z.: An attempt to identify the low molecular feruloylated oligosaccharides in beer, *J. Inst. Brew.*, **113** (2007), pp. 185-195.
23. Takashio, M. and Shinotsuka, K.: Preventive production of beer against oxidation - recent advances in brewing technology, *Food Sci. Technol. Int. Tokyo*, **4** (1998), pp. 169-177.
24. Thalacker, R. and Bößendörfer, G.: Thiobarbituric acid index (TBI). BRAUWELT International., (2005), pp. 35-39.
25. Vesely, P.; Lusk, L.; Basarova, G.; Seabrooks, J. and Ryder, D.: Analysis of aldehydes in beer using solid-phase microextraction with on-fiber derivatization and gas chromatography/mass spectrometry, *J. Agric. Food Chem.*, **51** (2003), pp. 6941-6944.
26. Wang, J.; Sun, B.; Cao, Y. and Tian, Y.: Protection of wheat bran feruloyl oligosaccharides against free radical-induced oxidative damage in normal human erythrocytes, *Food Chem. Toxicol.*, **47** (2009), pp. 1591-1599.
27. Washington, G.: To make small beer, George Washington Papers, Manuscripts and Archives Division, The New York Public Library, New York, USA, (1757).
28. Yuan, X.; Wang, J. and Yao, H.: Antioxidant activity of feruloylated oligosaccharides from wheat bran, *Food Chem.*, **90** (2005), pp. 759-764.

*Received 19 October 2011, accepted 20 December, 2011*

## Appendix

**Table 1** Physico-chemical properties of the different experimental brews. Statistical differences between control beer and bran-brewed beer are indicated with a \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) or \*\*\* ( $P < 0.001$ )

Parameter	Different Experimental Brews					Experimental Beer Types		
	A	B	C	D	E	Control <sup>(a)</sup>	Bran Beer <sup>(b)</sup>	
original extract (°P)	12.4	12.4	12.0	11.9	12.1	12.4	12.0	*
apparent extract (°P)	2.5	2.4	2.9	2.9	3.1	2.4	3.0	**
alcohol (%v/v)	5.3	5.4	4.8	4.8	4.8	5.3	4.8	**
pH	4.5	4.5	4.7	4.7	4.7	4.5	4.7	**
FAN (ppm)	75.7	59.7	70.3	72.0	95.9	67.7	79.4	
soluble protein (ppm)	258.5	252.5	422.8	428.0	478.7	255.5	443.2	**
sensitive protein	10.1	9.8	19.3	12.6	14.1	10.0	15.3	
free monosaccharides (g/L)	0.4	0.3	0.7	0.8	0.9	0.4	0.8	*
AXOS (g/L)	1.1	1.0	2.6	2.7	2.8	1.1	2.7	***
non cellulosic glucan (g/L)	31.0	29.8	26.7	27.4	26.9	30.4	27.0	**
other neutral carbohydrates (g/L)	0.4	0.4	0.5	0.5	0.5	0.4	0.5	***
AXOS A/X ratio	0.64	0.67	0.57	0.59	0.59	0.66	0.58	**
AXOS average DP	21	24	13	13	12	23	13	**
total polyphenol (ppm)	160.1	149.7	146.5	133.4	109.3	154.9	129.7	
flavonoid (ppm)	37.0	38.5	30.8	26.5	29.8	37.8	29.0	*
proanthocyan (ppm)	15.0	16.3	11.2	13.7	16.3	15.7	13.7	
bound ferulic acid (ppm)	11.1	10.4	36.5	48.9	43.6	10.8	43.0	**
free ferulic acid (ppm)	1.4	1.4	6.1	4.1	5.4	1.4	5.2	*
total aldehydes (ppb)	64.3	65.1	34.2	34.3	33.0	64.7	33.9	***
total bitterness (ppm)	23.0	24.2	24.5	24.5	24.0	23.6	24.3	
TRAP	1.2	1.2	1.3	1.3	1.3	1.2	1.3	*
TBI	30.1	30.3	25.8	25.4	25.9	30.2	25.7	***
colour	5.5	5.7	9.2	7.4	8.1	5.6	8.2	*
foam stability	220	225	234	248	250	223	244	*
permanent haze H90	2.7	2.7	nd <sup>(c)</sup>	4.7	4.2	2.7	4.4	*
permanent haze H25	0.5	0.5	nd	1.1	1.6	0.5	1.3	
cold haze H90	3.0	2.9	nd	5.0	4.7	3.0	4.9	**
cold haze H25	1.1	1.2	nd	1.6	2.3	1.2	2.0	

(a) average of experimental brews A and B

(b) average of experimental brews C, D and E

(c) nd = not determined

**Table 2** Sensorial properties of the different experimental brews. Scores are averages of duplicate evaluations by six different tasters. Statistical differences between control beer and bran-brewed beer are indicated with a \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) or \*\*\* ( $P < 0.001$ )

Parameter	Commercial Beer <sup>(a)</sup>	Different Experimental Brews					Experimental Beer Types		
		A	B	C	D	E	Control <sup>(b)</sup>	Bran Beer <sup>(c)</sup>	
solvent alcoholic	2.0	2.1	1.6	2.3	2.4	2.0	1.9	2.2	
isoamyl acetate	1.0	2.0	1.0	1.7	1.5	1.3	1.5	1.5	
ethyl acetate	0.4	1.8	1.0	1.3	1.4	0.6	1.4	1.1	
acetaldehyde	0.1	2.2	0.8	1.3	0.6	0.7	1.5	0.8	*
grainy	0.6	1.8	1.1	0.4	0.8	0.5	1.4	0.6	***
salty	1.2	1.0	1.0	0.9	1.1	1.0	1.0	1.0	
sweet	2.5	2.3	1.8	2.4	2.3	2.8	2.1	2.5	
bitter	3.0	2.8	3.2	2.3	2.7	2.2	3.0	2.4	
after-bitterness	1.3	1.3	1.3	0.9	1.0	0.7	1.3	0.9	*
sour	0.9	1.0	1.3	1.0	0.9	0.8	1.1	0.9	
astringent	2.3	1.8	2.4	2.1	1.9	1.8	2.1	1.9	
warming	2.8	2.4	2.2	2.9	2.9	2.8	2.3	2.9	*
drying	2.0	2.2	2.3	1.7	1.9	1.7	2.3	1.8	*
smooth	2.8	2.2	2.2	2.9	2.5	2.8	2.2	2.7	**
carbonation	3.3	3.0	3.1	3.3	3.2	3.2	3.0	3.2	
body	4.4	3.4	3.5	4.3	4.3	4.0	3.5	4.2	**
overall score	7.9	6.8	6.8	7.4	7.3	7.3	6.8	7.3	*

(a) average of three commercial beers

(b) average of experimental brews A and B

(c) average of experimental brews C, D and E