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Brewing with Unmalted Cereal Adjuncts: Sensory and Analytical Impacts on Beer Quality

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Abstract: Brewing with unmalted cereal adjuncts can reduce the requirement for malting, thereby lowering costs and improving the overall sustainability of the brewing chain. However, substantial adjunct usage has technological challenges and the sensory characteristics of beers produced using high adjunct rates are still not fully understood. This study examined the impacts of brewing with unmalted barley, wheat, rice and maize at relatively high concentrations (0, 30% and 60% of grist) on the sensorial and analytical profiles of lager beer. Adjunct based beers and a 100% malt control were brewed at 25 L scale. A trained sensory panel (n = 8) developed a lexicon and determined the sensorial profile of beers. At 30% adjunct incorporation there was insignificant variation in the expected beer flavour profile. At 60% adjunct incorporation, there were some significant sensory differences between beers which were specific to particular adjunct materials. Furthermore, 60% adjunct inclusion (with correspondingly low wort FAN) impacted the fermentation volatile profile of the final beers which corresponded with findings observed in the sensory analysis. Developing an understanding of adjunct-induced flavour differences and determining strategies to minimise these differences will facilitate the implementation of cost-efficient and sustainable grist solutions.

Keywords: adjunct brewing; unmalted adjuncts; beer quality; sensory science; wheat adjunct; barley adjunct; rice adjunct; maize adjunct

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1. Introduction

Malted barley is the favoured cereal grain used in traditional brewing processes. Barley is modified during the malting process to ensure biochemical changes occur within the grain which generate essential enzymes, sugars and proteins required in the production of wort [1]. Malted barley forms the base grist material for most beers, however, the partial substitution of barley with cereal adjuncts such as wheat, corn and rice is well established in the brewing industry.

Malting is an energy intensive process and brewing with a proportion of unmalted adjuncts has become an attractive option for cost and carbon footprint reduction. The malting process accounts for 217 kg CO_2eq/t, which is nearly half of the total carbon footprint incurred during malt production, with the agriculture of malting barley accounting for the additional 241 kg CO_2eq/t [2]. Therefore, utilising unmalted adjuncts can considerably reduce the carbon footprint of the malting and brewing chain.

Global climate change is predicted to have important consequences for barley production. Crop and economic models have forecast that extreme weather events may cause substantial decreases in barley yields worldwide with a potential 17% loss under the most severe conditions [3]. Decreases in the global supply of barley could lead to increasing beer prices with a 193% increase in price by 2099 according to some projections [3]. Employing a range of adjunct materials may therefore play a role in securing raw materials supply for brewing through a period of projected climate change. For example, it has been predicted

that climate change will not have significant negative effects on maize yields in the US and China [4].

Unmalted adjuncts are often employed in the brewing industry as an alternative cost-efficient source of extract, as well as for the individual functionality they bring to the brewing process and finished beers. Cost reductions can be driven by minimising the requirement for the malting process and its related costs [5]. Additionally, cost savings can arise from replacing potentially expensive barley malt with cheaper locally sourced grains. The choice of unmalted grains across the global industry is thus heavily influenced by local raw materials supply and cost considerations. Barley and corn are the most commonly used adjuncts in Europe and America, whilst rice usage is popular in Asia [5,6]. The quality attributes of some of the world's leading global beer brands are based on adjuncts used in their recipes. Adjuncts are utilised by brewers both to modify beer quality (e.g., flavour, foam, colloidal stability) and to enable production of new innovative products with specific desired features [5].

Brewing with unmalted cereal adjuncts, particularly at higher concentrations, can be challenging and a more detailed understanding is required about the factors which limit the upwards incorporation rates of unmalted adjunct materials. When introducing high concentrations of adjunct material, the functionality and processability of the grist must be assured, and there should be no negative impacts on production or product quality.

The main disadvantage in terms of processability when including unmalted adjuncts is the decrease in amylolytic, cytolytic and proteolytic enzymatic activities in the grist, as these enzyme systems are activated and synthesised during the malting process [7]. The actions of these three enzyme systems during malting and mashing influences the chemical composition of wort and the efficiency of brewing extract recovery [7]. The varied biochemical composition of cereal materials will have impacts both on the brewing process performance and on finished beer quality. A deficiency of enzyme activity and variations in composition of unmalted adjuncts can consequently impact the flavour profile of adjunct beers [6,8]. However, such impacts to the aroma and flavour of the finished beer are still not fully understood.

There have been several studies investigating the production of beer by partial or complete substitution with adjuncts, with basic sensory evaluation of the adjunct based beers. Kunz et al. [9] determined that the inclusion of unmalted barley at up to 50% of grist produced a beer with comparable preference ratings in odour and taste in comparison to an all-malt beer. However, as the barley incorporation was increased to 90% an increase in astringency was detected. Whilst Steiner et al. [7] reported that the use of 100% unmalted barley produced beer with less body and mouthfeel.

The use of rice in brewing is considered to produce neutral, clean and dry sensory characteristics in the beer whilst the addition of corn can induce a fuller mouthfeel [6]. These prior studies have suggested how adjuncts can influence the sensory profile of beer. However, there is little comprehensive sensory and analytical data concerning the flavour profile induced by adjuncts, and a lack of comprehensive sensory studies comparing the impacts of using high grist ratios of a range of unmalted cereals.

The objectives of this study were to produce a detailed sensory descriptive evaluation of beers produced when brewing with unmalted barley, rice, corn and wheat adjuncts. Furthermore, to examine the chemical composition of each beer to develop understanding of the underlying basis for the sensory properties of adjunct beers. Two adjunct incorporation rates were used in the study—the first aligned with typical current industry usage (30%) and then a higher and more challenging incorporation rate (60%). This is the first study to develop an adjunct beer sensory lexicon with corresponding chromatographic analyses. The data presented here will provide a better understanding and comparison of flavour and chemical impacts of the use of a range of unmalted cereal adjuncts in brewing.

2. Materials and Methods

2.1. Brewing Materials

Lager malt (variety: Propino) used in this study was supplied by Soufflet Malt, Burton-on-Trent, UK. Unmalted barley (variety: Planet or Propino) was supplied by Frontier Agriculture Ltd., Lincolnshire, UK. Wheat (variety: Leeds or Revelation) was supplied by Openfield, Lincolnshire, UK. Flaked torrefied rice and flake torrefied maize were supplied by Crisp Malt, Great Ryburgh, UK. Zeus T90 pellets (Zeus) were purchased from SimplyHops, Kent, UK. Saflager W34/70 dry lager yeast was obtained from Fermentis.

Two types of unmalted barley were included in this study. The unmalted barley samples used were of the same variety and differed in terms of their processing; however, details of the related processing are proprietary and cannot be disclosed for commercial reasons. They will be referred to here as barley (A) and barley (B).

2.2. Chemicals and Reagents

Reagents for the various assays were sourced as follows and were of analytical reagent grade:

Total Polyphenolic Content: Ethylenediaminetetraacetic acid (EDTA), carboxymethylcellulose (CMC) were purchased from Fisher Scientific (Loughborough, UK). Ammonium hydroxide and ammonium iron citrate were purchased from VWR (Leicestershire, UK). Bitterness Units: 2,2,4-Trimethylpentante (isooctane) and hydrochloric acid were purchased from Sigma-Aldrich (Dorset, UK). Free Amino Nitrogen: Sodium phosphate dibasic dodecahydrate, potassium phosphate monobasic, ninhydrin, fructose, ethanol, potassium iodate and glycine were purchased from Sigma-Aldrich (Dorset, UK).

HPLC Analysis: Ethyl acetate, methanol, acetonitrile, orthophosphoric acid (85%), acetic acid (glacial), 4-hydroxybenzoic acid (99%), ferulic acid (99%), 4-hydroxyphenylacetic acid (98%), p-coumaric acid (98%), vanillic acid (97%) and catechin (99%) were purchased from Sigma-Aldrich (Dorset, UK). Syringic acid (98%), tryptophol (97%) and tyrosol (98%), were purchased from Fisher Scientific (Loughborough, UK). GC Analysis: Acetaldehyde (99.5%), ethyl acetate (99.8%), 1-propanol (99.7%), isobutanol (99%), phenethyl alcohol (99%), isobutyl acetate (99%), isoamyl acetate (97%), isoamyl alcohol (98%), ethyl hexanoate (99%), ethyl octanoate (98%), ethyl butyrate (99%), ethyl propanoate (99%), ethyl isobutyrate (98%), ethyl pentanoate (98%), ethyl heptanoate (99%), dimethyl sulphide (99%), ethyl methyl sulphide (96%) and 3-heptanone (98%) were purchased from Sigma-Aldrich (Dorset, UK). 3-methyl-2-pentanone (98%) was purchased from Fisher Scientific (Loughborough, UK).

2.3. Brewing Process for Adjunct and Control Beers

Adjunct based lager beers with five different unmalted cereal adjuncts (rice, maize, wheat, barley (A) and barley (B)) at differing adjunct concentrations (0% (all malt control), 30% and 60%) were brewed, producing in total eleven different beers. Flaked torrefied maize and rice were used in this study to remove the requirement of pre-processing the cereal in the brewery and to enable a standardised brewing recipe across each adjunct. Unmalted rice and maize would otherwise be treated with different thermal processing techniques ('cereal cooking') before being added to the mash as these tropical grains have higher gelatinisation temperatures than barley and wheat [10].

All grains were milled with a Roppi-250 roller mill (Robix, Veszprém, Hungary) at a gap setting of 0.85 mm. Brewing was performed in a 25 L Grainfather system (The Grainfather, Auckland, New Zealand). Mash-in temperature was 55 °C and held for 20 min, followed with a 90 min stand at 65 °C, then a 10 min stand at 72 °C with final mash-off at 78 °C for 5 min. Temperature increases between stands were ramped at a rate of between 1.4–1.5 °C/min. Sparge water was passed through the grain bed and the resultant wort was boiled with 20 g of T90 hop pellets (variety: Zeus) for 60 min with a target to produce beers with a final bitterness of 20 BU. The wort was cooled to 20 °C and fermented with SafLager W34/70 yeast (11.5 g dried yeast to 25 L wort) in a FastFerment 30 L plastic

conical fermenter for 7 days, followed by maturation at 4 °C for 4 days. Beers were packaged into 500 mL PET brown bottles, bottle conditioned (3 g of dextrose monohydrate (The Homebrew shop, Hampshire, UK) per bottle) at 20 °C for 3 weeks and subsequently stored at 4 °C prior to sensory evaluation.

2.4. Protocol for Laboratory Wort Production

Laboratory mash bath wort samples were prepared in triplicate for each adjunct (rice, maize, wheat, barley (A) and barley (B)) at differing concentrations (0, 30% and 60%) using an R8 mash bath (1-Cube, Havlickuv Brod, Czech Republic). All grist materials were milled using a laboratory DFLU disc mill (Buehler Miag, Uzwil, Switzerland). For each wort sample, a liquor to grist ratio of 3.6:1 was attained by mixing 180 mL of reverse osmosis (RO) water at 55 °C to 50 g of the mixed grist weighted into a metal mashing beaker. The mash was stirred throughout the mash profile and the same mash profile used for the brewing trial (55 °C for 20 min, 65 °C for 90 min, 72 °C for 10 min, 78 °C for 5 min). Wort samples were then cooled to 20 °C and adjusted with RO water and filtered through pleated filter paper (Whatman, 2555 $\frac{1}{2}$, 320 mm, Sigma-Aldrich, Dorset, UK)

2.5. Wort and Beer Analysis

Wort and beer specific gravity and beer alcohol content were measured using an Alcolzyer Plus connected to a DMA 4500 densitometer (Anton Paar, Graz, Austria). The beer pH was recorded using a 3510 pH meter (Jenway, Stafford, UK). Nitrogen content of cereal grains were determined using a FlashEA 1112 Analyzer (Thermo Scientific, Loughborough, UK). Finished beer quality parameters were analysed according to standard ASBC and EBC methods as follows: total polyphenol content (TPC) (ASBC Beer-35), bitterness units (ASBC-Beer 23A), beer colour (EBC 9.6) and wort free amino nitrogen (EBC 8.10.1).

2.5.1. HPLC Determination of Phenolic Compounds

Analysis of phenolic compounds was performed on a Waters Alliance 2695 coupled with a Waters 2996 photodiode array UV detector. A Purospher STAR rp-18 end-capped column coupled with a C18 guard cartridge was used for separation of the phenolic compounds. The phenolic fraction was extracted and analysed according to the chromatographic method reported previously [11]. All data produced was processed using Empower 2 HPLC software. Five-point calibration curves generated from external standards were used to determine the concentrations of compounds.

2.5.2. Gas Chromatographic Analysis of Fermentation Volatiles by HS-GC-FID

Samples for analysis were prepared by adding 3.5 g of sodium chloride and 50 µL of 1-butanol (internal standard) to 10 mL of degassed beer sample in 20 mL headspace vials. Headspace (500 µL) was injected (split ratio 1:20) into a Bruker 400-GC equipped with a 60 m × 0.25 mm × 0.5 µm ZB-Wax column (Phenomenex, Macclesfield, UK) and Flame Ionisation Detector (at 250 °C). Volatile compounds were separated according to a modified version of EBC Analytica method 9.39. Helium was used as the carrier gas at a constant pressure of 15 psi and the injector temperature was 150 °C. The oven temperature profile started at 85 °C for 10 min hold and was then increased by 25 °C/min and held at 110 °C for 13 min, then increased at 8 °C/min to 200 °C (13.25 min hold). Six-point calibration curves generated from external standards were used to determine the concentrations of volatile compounds.

2.5.3. Gas Chromatographic Analysis of Fermentation Volatiles by SPME-GC-MS/MS

Samples were prepared for analysis by adding 3.5 g sodium chloride and 100 µg/L of 3-heptanone (internal standard) to 5 mL of beer sample in 20 mL headspace vials. Volatile compounds were absorbed on to a multiphase SPME fibre (PDMS/DVB; 60 µm, Supelco, Dorset, UK; extraction for 10 min at 35 °C with agitation) and subsequently desorbed in the injector port (250 °C) of a TRACE TM 1300 Gas Chromatograph (Thermo Scientific Inc.,

Waltham, MA, USA) fitted with a Zebron 30 m × 0.25 mm × 0.25 µm ZB-WAX column (Phenomenex, Macclesfield, UK) and coupled to a single quadrupole EI-MS (ISQ-QD, Thermo Scientific Inc., Waltham, MA, USA) scanning the mass range *m/z* 35–230. The carrier gas was helium (1 mL/min) and split ratio 1:10. The oven temperature profile was 40 °C for 2 min and then programmed to reach 240 °C at a rate of 8 °C/min and held for 1 min. Transfer line temperature was 250 °C. Five-point calibration curves generated from external standards were used to determine the concentrations of volatile compounds. The following selected ions were used for quantitation: isobutyl acetate (*m/z* 73), phenethyl acetate (*m/z* 91), ethyl hexanoate, ethyl octanoate, ethyl butyrate, ethyl propanoate, ethyl isobutyrate, ethyl pentanoate, ethyl heptanoate (*m/z* 88), 3-methyl-2-pentanoate (*m/z* 85) and phenethyl alcohol (*m/z* 91).

2.5.4. Gas Chromatographic Analysis of Dimethyl Sulphide by SPME-GC-PFPD

Samples were prepared by adding 3.5 g sodium chloride and 50 µL of ethyl methyl sulphide to 10 mL of beer sample in 20 mL screw-top headspace vials. Volatile compounds were absorbed on to a multiphase SPME fibre (DVB/Carboxen/PDMS; 50/30 µm, Supelco, Dorset, UK) and subsequently desorbed in the injector port (250 °C) of a Bruker 400-GC fitted with a 60 m × 0.25 mm × 1 µm ZB-1MS column (Phenomenex, Macclesfield, UK). Carrier gas was Helium (1 mL/min) and split ratio 1:10. The oven temperature profile was 40 °C (held for 7 min) and then programmed to reach 110 °C at a rate of 7 °C/min, then increased to 190 °C at a rate of 11 °C/min to then 235 °C at a rate of 22 °C/min. A sulphur-specific Pulsed Flame Photometric Detector (PFPD) at 210 °C recorded the elution of sulphur containing molecules from each sample. A six-point calibration curve was generated from an external standard to determine the concentration of dimethyl sulphide.

2.6. Sensory Analysis

Before conducting this study, ethics approval was requested and accepted by the Faculty of Medicine & Health Sciences Research Ethics Committee at the University of Nottingham (Ethics Reference No. 269-1803). All panellists provided consent to partake and were provided a disturbance allowance for their participation. The sensory evaluation of the adjunct beers was conducted following a modified Quantitative Descriptive Analysis methodology [12].

2.6.1. Preparation of Samples and Reference Solutions

For all sessions, 20 mL of freshly opened sample was measured into 60 mL vials secured with a lid no more than one hour before the session. The final 150 mL of the 500 mL sample bottles were discarded to minimising the introduction of potential residue from bottle conditioning. Prepared samples were stored at 4 ± 2 °C until required for evaluation. Samples were presented in amber vials labelled with random three-digit codes throughout the study to minimise expectation error and to reduce bias from appearance. Attribute references were developed over several sessions and placed into labelled amber vials. Acetaldehyde and dimethyl sulphide flavour reference standards were purchased from Aroxa (Cara Technology, Surrey, UK). Isoamyl acetate was purchased from Sigma-Aldrich (Dorset, UK) and all compounds used were food grade. All samples and reference solutions were prepared in a food safe environment.

2.6.2. Sensory Panel Recruitment

Panellists who had previously been screened for their general sensory acuity were additionally screened to evaluate their discrimination and descriptive ability of the adjunct beer samples. The main aim of this screening session was to determine if the prospective panellists could recognise and clearly communicate any sensory descriptors identified in the beers, individually and in a group discussion setting. After reviewing the data, eight experienced assessors (4 males and 4 females) were invited to take part.

2.6.3. Attribute Generation and Panel Training

After recruitment, the attribute lexicon was developed. Panellists were individually presented with all eleven beer samples (according to Section 2.6.1) over two 120-min sessions and were asked to generate terms to describe the sensations elicited in each sample under four categories: aroma, flavour, taste and mouthfeel. This generated a list of 206 attributes that panellists had identified individually. Two sessions were then held to consolidate the initial attribute list by means of Check-All-That-Apply (CATA) technique [13] and group discussions. These sessions helped remove ambiguous and overlapping descriptors whilst highlighting the most applied and discriminating descriptors.

Fifteen 120-min training sessions were dedicated to further consolidating the attribute list and creating definitions for each attribute. Reference materials were provided to the panellists to establish references for the lexicon and aid understanding of the particular attribute. Training sessions included replicate rank rating tests, to determine discrimination ability. Replicate samples were included to ensure reproducibility and group discussions were used to address any inconsistencies in panel understanding of the attributes or method of assessment. Samples modified with additional flavour compounds were included in training sessions to ensure the panel could accurately discriminate intense samples. Panellists were trained on the use of a 10 cm line scale for evaluating attributes. Throughout the training sessions, the panel developed a protocol for tasting, smelling and palate cleansing to ensure optimum identification and performance in the final evaluation of the samples. An assessment order for the attributes was determined by the panel which corresponded to the order of perceived sensations and when fresh samples would be provided. Panel performance was monitored throughout training. The purpose of monitoring panel performance was to examine the reproducibility and discriminative ability of the panel individually and as a group. Prior to the final evaluation, a mock evaluation was conducted to establish panel performance and highlight any panel inconsistencies that needed to be resolved before the final evaluation.

2.6.4. Sample Evaluation

Panellists assessed the all malt control and eleven adjunct beers individually in triplicate over six 120-min sessions in sensory booths. Each session involved the evaluation of six samples to ensure less than 1 UK alcohol unit was consumed per session for ethical compliance. The samples were labelled with a 3-digit random code and presented in a completely randomised order. All samples were served in amber vials at 4 ± 2 °C. In the final evaluation, attributes were divided into three sections and fresh 20 mL aliquots were provided for each section to ensure freshness and consistent temperature throughout assessment. The intensity of each attribute was rated on a 10 cm unstructured line scale which was displayed on a computer screen along with each attribute description. Panellists were given three-minute breaks between each sample to avoid palate fatigue with a 10 min comfort break at the midpoint of evaluation (after 3 samples). During breaks, panellists used water (Evian, Danone, Paris, France) and unsalted crackers (Rakusens, Leeds, UK) to cleanse the palate and to minimise sample carry-over. Data were collected using Compusense Cloud (Compusense, Guelph, ON, Canada).

2.7. Statistical Analysis

Statistical analysis was carried out using XLSTAT. Three-way mixed model ANOVA (sample, replicate, panellist) was conducted on all sensory attributes and the corresponding interactions between factors were used to determine the effects of the panel performance. Tukey's Honest Significant Difference (HSD) test was applied to evaluate significant differences between samples for each attribute. Dunnett's test was used to compare adjunct samples to the 100% malt control for the sensory attributes and chemical compounds. Principal Component Analysis (PCA) analysis was conducted on average scores were produced to provide a visual depiction of the relationship of the adjunct beers to the sensory and analytical characteristics highlighted. Significance was derived at $p = 0.05$.

3. Results and Discussion

3.1. Sensory Evaluation of Adjunct and Control Beers

3.1.1. Attribute Generation

In the initial sessions, panellists generated over 200 attributes describing the adjunct and control beers, which were then consolidated over several sessions by means of Check-All-That-Apply (CATA) tests and group training sessions. The final 18 attributes with their description and reference training materials are presented in Table 1. The lexicon comprised of three basic tastes, seven aroma, six flavour, one trigeminal sensation (astringent) and one temporal character of bitterness (lingering bitterness). Four terms (green apple, banana, malty and grainy) were assessed both for aroma (orthonasal assessment) and flavour (retronasal assessment). Natural and chemical references were identified by the panel to be used in the evaluation.

Table 1. The adjunct beer attribute lexicon, description and reference standards developed in the study.

Modality	Attribute	Definition	Reference Material
Aroma	Green Apple/Emulsion Paint	The skin of a green apple and the aroma of emulsion paint	15 mg/L of acetaldehyde in water (Aroxa)
	Banana/Pear Drops	Artificial banana and pear drop sweets	3.5 mg/L of isoamyl acetate (Aroxa) in water
	Sweetcorn/Cooked Vegetable	Tinned sweetcorn and cooked vegetables	150 µg/L of dimethyl sulphide (Aroxa) in water
	Smoky	Stale acrid smoke such as stale cigarette smoke or faint burnt rubber	
	Malty	Sweet processed or cooked malt grains	20 g of malt loaf, 20 g of barley malt extract
	Grainy	Dry light barley malt grains	50 g of dry light barley malt
	Plum Fruit	Cooked plum fruit (plums, damsons and prunes)	
Flavour	Green Apple/Emulsion Paint	The skin of a green apple and emulsion paint	15 mg/L of acetaldehyde in water (Aroxa)
	Banana/Pear Drops	Artificial banana and pear drop sweets	3.5 mg/L of isoamyl acetate (Aroxa) in water
	Apple	Bruised sweet red apple	
	Malty	Sweet processed or cooked malt grains	20 g of malt loaf, 20 g of barley malt extract
	Grainy	Dry light barley malt grains	50 g of dry light barley malt
	Plum Fruit	Cooked plum fruit (plums, damsons and prunes)	
Taste	Sweet	Intensity of sweetness after swallowing	
	Sour	Intensity of sourness after swallowing	
	Bitter	Peak intensity of bitterness after swallowing	
	Lingering Bitterness	Intensity of bitterness 20 s after swallowing	
Mouthfeel	Astringent	Drying sensation in the mouth after swallowing	

3.1.2. Panel Performance

In order to determine panel performance and any variation in the panel evaluation, three-way ANOVA (sample, panellist and replicate) with interaction was applied to the attribute scores in the final evaluation as shown in Table 2. All attributes showed significant effect of panellist ($p < 0.05$) which indicates that the panel varied in their use of the scale. However, further analysis of the raw data showed that this was due to minor variations across the panel that were considered acceptable [12]. Furthermore, for the majority of attributes there were no significant effects for replication ($p > 0.05$) (except plum fruit aroma and astringent mouthfeel) indicating intra-panellist consistency. Nine attributes showed significant ($p < 0.05$) sample * panellist interaction indicating that there was not a strong agreement between panellists on the intensity order across the samples for these attributes. However, interrogation of the interaction plots showed that the majority of these interactions were minor. Altogether, only 7 out of the 18 attributes ($p < 0.05$) were found to discriminate between the samples (bold font in sample column, Table 2). This lack of discrimination could indicate low levels of variance between the samples or high levels of variance in the panel. Extensive screening and training with the panel aimed to maximise performance resulting in very little variation across replicates. Therefore, it is proposed here that the lack of discrimination is due to low levels of perceivable differences (variance) between the samples.

Table 2. Mixed model Analysis of Variance (ANOVA) *p*-values for sensory attributes rated for adjunct beers.

Modality	Attribute	Sample	Replicate	Panellist	Sample * Replicate	Sample * Panellist	Replicate * Panellist
Aroma	Green Apple/Emulsion Paint	0.324	0.622	<0.001 ***	0.920	0.708	0.306
	Sweetcorn/Cooked Veg.	0.002 **	0.373	<0.001 ***	0.979	0.796	0.269
	Banana/Pear Drops	0.041 *	0.089	<0.001 ***	0.046 *	0.035 *	0.346
	Smoky	0.038 *	0.177	<0.001 ***	0.676	0.020 **	0.789
	Malty	0.078	0.454	<0.001 ***	0.788	0.309	0.005 **
	Grainy	0.077	0.535	<0.001 ***	0.881	0.233	0.545
	Plum Fruit	0.166	0.021 *	<0.001 ***	0.992	0.026 *	0.147
Flavour	Green Apple	0.012 *	0.238	<0.001 ***	0.187	0.496	0.628
	Apple	0.066	0.581	<0.001 ***	0.139	0.400	0.571
	Banana/Pear Drops	0.025 *	0.162	<0.001 ***	0.365	0.037 *	0.829
	Malty	0.101	0.846	<0.001 ***	0.170	0.001 **	0.011 *
	Grainy	0.052	0.395	<0.001 ***	0.178	0.015 **	0.323
	Plum Fruit	0.110	0.146	<0.001 ***	0.988	0.009 **	0.139
Taste	Sweet	0.336	0.257	<0.001 ***	0.601	0.155	0.104
	Sour	<0.001 ***	0.999	<0.001 ***	0.963	0.290	0.432
	Bitter	0.468	0.394	<0.001 ***	0.710	0.864	0.481
Mouthfeel	Astringent	0.326	0.005 **	<0.001 ***	0.727	0.038 **	0.235
	Lingering Bitterness	<0.001 ***	0.326	<0.001 ***	0.748	0.007 **	0.181

* ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

3.1.3. Descriptive Analysis of Adjunct Beers

Table 3 shows the mean rating scores for each attribute and the resultant groupings of the Tukey's HSD post-hoc test for significant attributes (in bold). Significant differences ($p < 0.05$) were reported for three aroma attributes (sweetcorn, banana and smoky), two flavour (green apple, banana) and two taste (sour and lingering bitterness) attributes. No significant differences ($p > 0.05$) were observed for the remaining 11 attributes indicating that the panel could not significantly discriminate between the samples for these attributes. However, apple and grainy flavour attributes were borderline significant ($p = 0.066$ and $p = 0.052$). The main trends observed for these attributes were: a relatively low score for apple flavour in the 60% barley (A) adjunct beer and a trend towards higher scores for grainy flavour in the 60% maize and 60% rice adjunct beers.

The inclusion of 60% barley (A) produced significantly higher attribute scores for the lingering bitterness attribute and although not statistically significant, the inclusion of 60% barley (A) produced the highest mean scores for bitter and astringent characteristics. This is in accordance with prior literature, where the inclusion of unmalted barley at high proportions has been reported to increase the perception of bitterness and astringency to an undesirable level [7,9,14]. Notably, the inclusion of barley (A) influenced the temporal quality of bitterness perceived as the sample showed the highest intensity of lingering bitterness. Longer durations of perceived bitterness at higher intensities could lead to an unpleasant organoleptic experience.

Interestingly, the increase in bitterness profile was not observed with the inclusion of barley (B). In contrast, the use of 60% barley (B) significantly decreased the lingering bitterness mean score (relative to that for barley A) and reduced the bitterness and astringency scores to lower than those for the 100% malt control. The sensory profiles of 60% barley (A), 60% barley (B) and the 100% malt control are visualised as radar plots in Figure 1. Use of 60% unmalted barley (B) gave a closer match to the profile of the all malt control beer, although it was on average rated a little more sour, but less malty, smoky and plum-fruit flavoured. Whereas, in comparison use of 60% unmalted barley (A) led to a beer which was rated on average as more sour, bitter and astringent, which had a more lingering bitterness. This demonstrates that processing applied to the barley grain can diminish unfavourable sensory attributes that are associated with high inclusions of unmalted barley.

Use of 60% unmalted wheat adjunct led to beers that were characterised by the highest perception of banana aroma and flavour and the highest perception of smoky aroma. Strong phenolic and some smoke character are characteristic of Belgian white beers (Witbier) made with unmalted wheat; however, this is a result of the phenolic off-flavour positive (POF+) yeast strains used when brewing Belgian beers. The use of unmalted wheat in lager has been described as creating a 'neutral type of beer' with a specific, refreshing taste [15]. However, this is the first study that has identified specific flavour characteristics relating to the use of high inclusions of unmalted wheat in lager.

The inclusion of 60% torrefied rice was characterised by significantly higher attribute scores for green apple flavour, and the highest rating score for the green apple aroma attribute, although this attribute was not statistically significant. The inclusion of 30% rice caused a significant increase in the sweetcorn/cooked vegetable aroma detected. The change in perception of these attributes did not linearly increase with the proportion of rice in the grist, highlighting that these flavour differences are not a direct flavour imparted by the rice, rather they are most likely associated with changes in fermentation vigour as a result of adjunct-linked reductions in soluble nitrogen. In the brewing literature, beers produced with all rice have been described as having low concentrations of flavour attributes and relatively flat or neutral character compared to an all malt beer [16,17].

Use of 60% torrefied maize resulted in beer with the highest mean score for sourness which was significantly more sour than the all malt control and the wheat adjunct beers. A moderate sour taste is necessary to balance the flavour of beer. Nevertheless, too strong a sour taste will destroy the harmony of the beer flavour [18]. A prominent sour taste can be perceived with adjunct beers in which worts have weak buffering capacity and a resultant change in pH of the beer [18].

Dunnett's post-hoc analysis was also performed on attribute scores for all eighteen attributes creating a comparison of each sample with the 100% malt sample as a control. Two samples had significantly different sensory attribute scores compared to the control ($p < 0.05$). 60% maize was significantly more sour and 60% rice had significantly more green apple flavour compared to the 100% malt beer.

Table 3. Mean rating scores and Tukey's Honest Significant Difference (HSD) test for adjunct-based beers and all malt control.

Modality	Attribute	100% Malt Control	Barley (A)		Barley (B)		Wheat		Rice		Maize	
			30%	60%	30%	60%	30%	60%	30%	60%	30%	60%
Aroma	Green Apple/Emulsion Paint	2.22 ^{a}	2.43 ^{a}	2.86 ^{a}	2.42 ^{a}	1.95 ^{a}	2.67 ^{a}	2.78 ^{a}	2.61 ^{a}	3.86 ^{a*}	2.87 ^{a}	2.36 ^{a}
	Sweetcorn/Cooked Veg.	**3.36** ^{ab}	**3.23** ^{ab}	**1.44** ^{b}	**3.69** ^{ab}	**3.47** ^{ab}	**1.71** ^{ab}	**1.36** ^{b}	**4.33** ^{a}	**3.60** ^{ab}	**3.16** ^{ab}	**3.14** ^{ab}
	Banana/Pear Drops	**2.29** ^{ab}	**2.27** ^{ab}	**2.18** ^{ab}	**2.50** ^{ab}	**2.47** ^{ab}	**2.45** ^{ab}	**3.34** ^{a}	**1.80** ^{b}	**2.06** ^{b}	**1.93** ^{ab}	**1.80** ^{b}
	Smoky	**2.30** ^{abc}	**2.59** ^{ab}	**1.70** ^{abc}	**2.08** ^{abc}	**1.03** ^{bc}	**0.64** ^{c}	**3.04** ^{a}	**1.60** ^{abc}	**1.45** ^{abc}	**0.55** ^{c}	**0.78** ^{bc}
	Malty	4.50 ^{a}	4.00 ^{a}	3.72 ^{a}	4.02 ^{a}	3.48 ^{a}	3.56 ^{a}	2.86 ^{a}	3.81 ^{a}	2.95 ^{a}	3.86 ^{a}	3.09 ^{a}
	Grainy	3.0 ^{a}	2.51 ^{a}	2.32 ^{a}	2.18 ^{a}	2.10 ^{a}	2.38 ^{a}	1.49 ^{a}	3.44 ^{a}	3.52 ^{a}	3.25 ^{a}	3.01 ^{a}
	Plum Fruit	3.17 ^{a}	2.00 ^{a}	2.29 ^{a}	3.20 ^{a}	2.50 ^{a}	3.14 ^{a}	2.96 ^{a}	2.43 ^{a}	1.86 ^{a}	3.16 ^{a}	2.29 ^{a}
Flavour	**Green Apple**	**2.74** ^{b}	**3.43** ^{ab}	**3.92** ^{ab}	**3.83** ^{ab}	**2.79** ^{b}	**3.57** ^{ab}	**3.80** ^{ab}	**4.01** ^{ab}	**5.52** ^{a*}	**3.22** ^{b}	**3.68** ^{ab}
	Apple	2.19 ^{a}	2.03 ^{a}	1.37 ^{a}	1.84 ^{a}	1.71 ^{a}	2.73 ^{a}	2.45 ^{a}	1.70 ^{a}	2.30 ^{a}	2.15 ^{a}	2.38 ^{a}
	Banana/Pear Drops	**2.8** ^{ab}	**1.92** ^{ab}	**1.63** ^{b}	**2.40** ^{ab}	**2.46** ^{ab}	**2.78** ^{ab}	**3.21** ^{a}	**2.30** ^{ab}	**2.35** ^{ab}	**2.86** ^{ab}	**1.63** ^{ab}
	Malty	4.30 ^{a}	3.74 ^{a}	3.00 ^{a}	3.55 ^{a}	3.33 ^{a}	3.68 ^{a}	4.53 ^{a}	4.23 ^{a}	3.06 ^{a}	3.75 ^{a}	3.41 ^{a}
	Grainy	2.53 ^{a}	4.05 ^{a}	3.89 ^{a}	3.83 ^{a}	3.02 ^{a}	3.81 ^{a}	2.40 ^{a}	3.83 ^{a}	4.21 ^{a}	3.68 ^{a}	4.30 ^{a}
	Plum Fruit	3.11 ^{a}	2.95 ^{a}	2.48 ^{a}	2.50 ^{a}	1.92 ^{a}	3.04 ^{a}	3.90 ^{a}	3.06 ^{a}	2.92 ^{a}	2.52 ^{a}	2.99 ^{a}
Taste	Sweet	3.46 ^{a}	2.59 ^{a}	2.64 ^{a}	2.84 ^{a}	3.07 ^{a}	2.50 ^{a}	3.18 ^{a}	3.12 ^{a}	3.41 ^{a}	3.38 ^{a}	2.35 ^{a}
	Sour	**2.35** ^{c}	**3.56** ^{abc}	**4.15** ^{ab}	**3.38** ^{abc}	**3.74** ^{abc}	**2.90** ^{bc}	**2.94** ^{bc}	**3.72** ^{abc}	**3.57** ^{abc}	**3.66** ^{abc}	**4.81** ^{a*}
	Bitter	4.35 ^{a}	4.96 ^{a}	5.08 ^{a}	4.18 ^{a}	4.20 ^{a}	5.08 ^{a}	4.75 ^{a}	4.52 ^{a}	4.44 ^{a}	4.14 ^{a}	4.75 ^{a}
Mouthfeel	Astringent	4.43 ^{a}	5.09 ^{a}	5.47 ^{a}	5.19 ^{a}	4.39 ^{a}	4.62 ^{a}	4.81 ^{a}	5.18 ^{a}	4.34 ^{a}	4.73 ^{a}	5.09 ^{a}
	Lingering Bitterness	**4.17** ^{ab}	**4.89** ^{ab}	**5.54** ^{a}	**4.38** ^{ab}	**3.25** ^{b}	**4.38** ^{ab}	**4.94** ^{ab}	**4.68** ^{ab}	**3.45** ^{b}	**3.69** ^{b}	**4.82** ^{ab}

Bold font: attributes were rated significantly differently across the beers ($p < 0.05$). Superscript letters indicate post-hoc groupings of beers according to Tukey's HSD test. Asterisks denote samples that were significantly different compared to the all malt control according to Dunnett's post hoc analysis ($p < 0.05$).

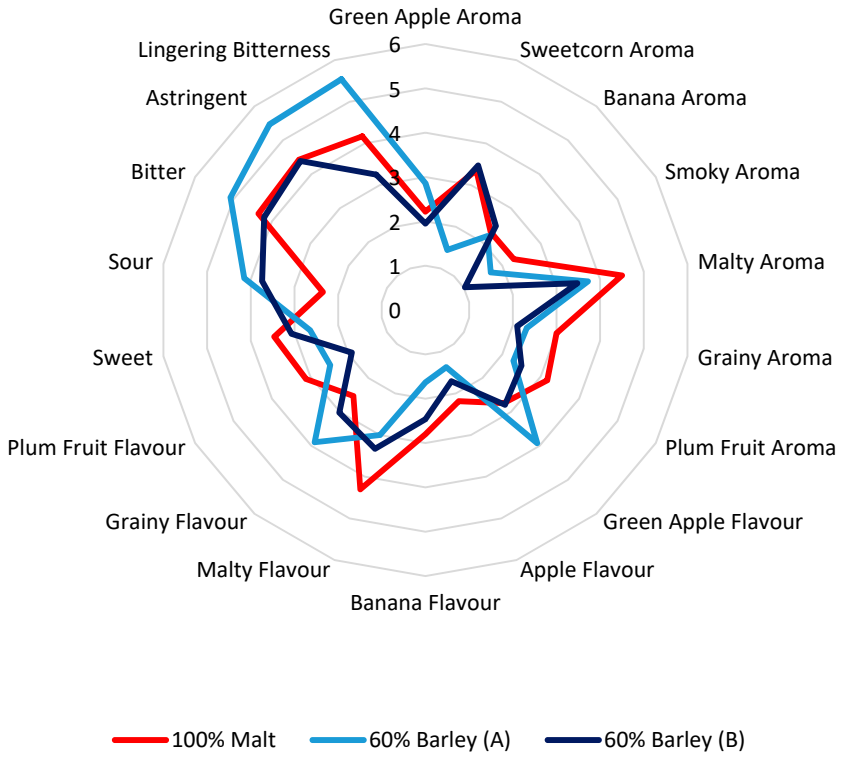

Figure 1. Radar plots of the mean rating scores for 60% barley (A), 60% barley (B) and 100% malt control beers.

Samples of 30% barley (A), barley (B), wheat and maize were not described by any specific sensory attribute as indicated by the low attribute scores and lack of attributes discriminating them.

It is important to note that the majority of the sensory attributes were not found to discriminate between the beers. This is important because even at incorporation rates of up to 60%, sensory impacts of the unmalted adjunct incorporation were subtle especially when comparing the samples against an all malt control.

All beers in the study were subjected to a range of standard beer quality analyses as well as chromatographic flavour analysis, in an attempt to identify factors which might correlate with the noted changes in sensory attributes.

3.2. Analytical Evaluation of Adjunct Worts and Beers Versus an All Malt Control

3.2.1. Free Amino Nitrogen Content of Worts

Free amino nitrogen (FAN) is a measure of yeast assimilable nitrogen which consists of amino acids, ammonium ions and peptides. The main function of wort FAN is to be utilised by yeast for protein synthesis which is needed for yeast growth and fermentation efficiency [19,20]. More importantly, the level and composition of wort FAN has a significant impact on the aroma profile of beer, influencing the formation of esters, aldehydes, vicinal diketones, higher alcohols and sulphur compounds during fermentation [21]. Wort samples were produced in laboratory mash bath trials using the same mash conditions and raw materials used for the final beer production, to determine the impact of adjunct inclusion on wort FAN concentrations.

The inclusion of all five adjuncts decreased wort FAN levels compared to the all-malt control (Table 4).

Table 4. Free amino nitrogen of adjunct wort samples.

	Malt	Barley (A)		Barley (B)		Maize		Rice		Wheat	
	100%	30%	60%	30%	60%	30%	60%	30%	60%	30%	60%
Wort FAN * (mg/L)	243 ± 26	**184** ± 1	**127** ± 6	**140** ± 2	**85** ± 4	**114** ± 10	**58** ± 5	**95** ± 15	**61** ± 2	**141** ± 11	**79** ± 9

* Free amino nitrogen concentration was standardised to 10 °P. ANOVA with Dunnett's post hoc analysis was used to compare the wort FAN concentration. **Bold font** denotes the samples that were significantly different compared to the all malt control ($p < 0.01$).

It has been reported that the wort FAN concentration should be above 150 mg/L to achieve a healthy fermentation with normal gravity (10–12 °P) [19]. In this case, all adjunct worts had FAN concentrations lower than this, particularly those produced with torrefied maize and rice adjuncts. Lower wort FAN content can be partially attributed to the lower nitrogen content in the maize and rice grains (1.22% and 1.16% on dry basis) compared to malted barley grains (1.48% on dry basis). However, the absence of malting is the main reason that unmalted adjuncts produce lower wort FAN concentrations. This is both because substantial breakdown of proteins occurs through malting, leading to increased soluble nitrogen, and because key proteolytic enzymes required to breakdown proteins into assimilable nitrogen during mashing are synthesised during malting [22,23]. Unmalted maize and rice are almost devoid of proteolytic enzymes which leads to a dilution of the FAN content of the wort [24]. This identifies a complication to brewing at higher adjunct rates which could impact on finished beer flavour profile.

3.2.2. Finished Beer Quality Parameters

Specifications of finished beers in the trial are reported in Table 5.

Table 5. Physiochemical analysis of adjunct beers.

	Original Extract (°P)	Final Gravity (°P)	Apparent Attenuation (%)	ABV (%)	pH	BU	Colour (EBC)	Total Polyphenol Content (mg/L)
100% Malt	11	1.8	84	4.8	4.48	17	10	170 ± 10
30% Barley (A)	9.4	1.8	81	4	4.45	19	8	137 ± 5
60% Barley (A)	10	2.6	75	3.8	4.49	17	8	160 ± 3
30% Barley (B)	9.1	1.9	80	3.7	4.40	16	6	160 ± 5
60% Barley (B)	9.7	2.6	74	3.7	4.47	15	5	181 ± 7
30% Wheat	9	1.8	81	3.7	4.38	17	9	117 ± 4
60% Wheat	10.3	2.4	77	4.1	4.41	16	8	100 ± 2
30% Maize	9.4	1.9	80	3.9	4.25	14	8	103 ± 3
60% Maize	8.8	1.4	85	3.8	4.01	15	5	68 ± 4
30% Rice	9.1	1.4	85	3.9	4.24	14	6	119 ± 6
60% Rice	9.7	1.3	87	4.1	4.06	16	5	84 ± 1

ABV = Alcohol by Volume; BU = bitterness units.

Extract and final gravity principally indicate the sugar content of the wort and beer, providing information on the efficiency of fermentation and the potential alcohol by volume (ABV) in the finished beer. In this study, original extract decreased relative to control with inclusion of all adjuncts, especially with 60% torrefied maize (Table 5). This is in agreement with prior published data where the inclusion of 20% maize yielded lower extract despite the fact that the starch extract yield potential of maize is greater than that of barley [25,26]. At the end of fermentation, maize and rice beers were characterised by lower extract compared with the all-malt beer, in agreement with previous studies with maize adjunct [26]. A slightly higher apparent attenuation was observed for the maize and rice beers.

The beer pH ranged from 4.01 to 4.49 which is within the expected pH range for lager beers [27]. The pH of beer is important when considering the sensory profile, it has been previously reported that low pH beers become more 'sharp' and have an increased sour taste [18,28]. The intensity of sour taste is related to the total concentration of free and undissociated hydrogen ions [18]. This could provide an explanation for the significant increase in sourness observed by the panel with the 60% maize sample, as the 60% maize beer had the lowest pH (4.01). Nitrogenous compounds in wort and beer have buffering

capacity and are important in establishing the pH. Adjuncts such as rice and maize contain low levels of nitrogenous compounds and phytase, the enzyme responsible for the breakdown of phytic acid to release phosphates. Therefore, adjuncts lessen the buffering capacity of wort and beer, leading to lower pH values [28]. Increasing nitrogenous compounds in the wort or reducing the amount of free hydrogen ions when brewing with adjunct material could potentially minimise this off flavour obtained in the 60% maize sample.

The beers produced with unmalted adjuncts presented a lighter colour in comparison to the all-malt beer. Brewing with torrefied rice and maize generated beers of lighter colour relative to the all malt control. It has been previously reported that maize causes a decrease in beer colour proportional to the ratio of its addition, owing to low soluble nitrogen content and therefore less Maillard products which are the main source of beer colour [16,17]. Therefore, balancing soluble nitrogen in wort and beer could potentially increase beer colour to values comparable to an all malt beer.

The hop addition during production was targeted at a final bitterness of 20 BU. However, in practice there was slight variation as analytical bitterness ranged from 14 to 19 BU across the sample set. The determination of bitterness units is a simplistic analytical measurement of iso-α-acids and bittering compounds that can contribute to bitterness. However, bitterness characteristics of beer can differ due to other chemical compounds not determined in this analysis [29]. Therefore, it is unlikely that the slight variation in bitterness units across the sample set would have a significant effect on the sensory perception of the beers.

Phenolic compounds are important for the sensory experience of beer as polyphenols contribute directly to flavour, astringency, haze, body and fullness of beer [30]. At high concentrations, polyphenols can impart bitterness and astringency and, in some cases, metallic and medicinal characteristics in beer [30,31]. It could be hypothesised that the changes in bitterness intensity and character observed in the sensory analysis of the adjunct beers (Figure 1) when including barley (A) could be a result of changes in polyphenolic character in the beer. Previous sensory studies have determined a change in bitterness character and determined longer harsher bitterness characteristics when polyphenolic content increased [29,31].

The polyphenol content of beer is largely related to the raw materials used, as approximately 75% of polyphenols originate from malted barley, dependent to some extent on hopping strategy and levels [32]. Consequently, malt substitutes typically decrease the concentration of phenolic compounds. The inclusion of torrefied maize significantly decreased the total polyphenol content (TPC) at 30% adjunct and even more so at 60% adjunct. This finding is in agreement with the work of Fumi et al. [33] where including maize at 86% was reported to decrease the total polyphenol content.

The highest TPC was found in the beers produced with malt and barley-based adjuncts (Table 5). Similarly to previous investigations [9,14,34] the inclusion of unmalted barley (A) reduced the TPC of beer. However, the inclusion of unmalted barley (B) increased TPC marginally, indicating that the process applied to the barley has affected the extraction of polyphenols from the grist into wort and subsequent transfer into beer. A limitation to the determination of total polyphenol content is that the method is based on the chelation of phenols with iron and multiple complexing sites are required to produce a reaction [30]. Phenolic acids are not measured in this assay as they do not cause an interaction.

The polyphenolic fraction of the trial beers was thus further characterised using HPLC-UV-DAD analysis to quantify phenolic acids and polyphenol monomers.

3.2.3. Phenolic Acid Analysis by HPLC-UV-DAD

Hydroxybenzoic acids and hydroxycinnamic acids can provide bitterness and astringency to the organoleptic characteristics of beer. Similarly, (+)-catechin can provide bitterness to beer. Concentrations of the principal phenolic acids and (+)-catechin are summarised for the trial beers in Table 6.

Table 6. Phenolic acid analysis of adjunct and control beers.

	(mg/L)						
Beer Sample	**4-HPA**	**Syringic Acid**	**Vanillic Acid**	**4-HBA**	**Ferulic Acid**	**p-Coumaric Acid**	**Catechin**
100% Malt	2.30	0.52	1.41	0.59	3.52	2.79	2.60
30% Barley (A)	1.58 **	0.32 **	1.56	0.52	2.55 *	1.60 **	2.01
60% Barley (A)	2.21	0.42	2.07 **	0.70	3.05	2.22 **	2.92
30% Barley (B)	1.33 **	0.21 **	1.11	0.47	1.83 **	1.75 **	3.71 **
60% Barley (B)	1.32 **	0.16 **	1.53	0.48	2.67 **	1.62 **	3.43 **
30% Wheat	1.62 **	0.40	1.48	0.63	2.73 **	1.37 **	0.97 **
60% Wheat	1.56 **	0.56	1.84 **	0.52	3.49	1.11 **	0.80 **
30% Maize	1.66 **	0.31 **	1.06	0.45 **	1.76 **	2.50	0.87 **
60% Maize	1.00 **	0.23 **	0.35 **	0.52	1.94 **	3.11	0.75 **
30% Rice	1.16 **	0.28 **	0.97 **	0.49	2.46 **	1.85 **	1.25 **
60% Rice	2.39	0.32 **	0.70 **	0.85 **	2.11 **	1.57 **	0.94 **

ANOVA with Dunnett's post hoc analysis was used to compare the phenolic acid concentrations. Asterisks indicate samples that were significantly different compared to the all malt control (* p <0.05, ** p < 0.01). Data are the mean of four replicate measurements. 4-HBA = 4-hydroxybenzoic acid; 4-HPA = 4-hydroxyphenylacetic acid

Higher concentrations of catechin were observed in the barley-based beers and a significantly higher concentration was identified in beers brewed with unmalted barley (B) relative to the all malt control. Although catechin is known to introduce bitterness to a beer, the concentrations obtained in the adjunct beers are lower than the reported flavour threshold of 20 mg/L in beer [35].

Phenolic acid concentrations were typically significantly reduced by the incorporation of adjuncts, or did not significantly change compared to the all-malt control. However, the concentration of vanillic acid significantly increased with 60% barley (A) and 60% wheat inclusion. The analysed phenolic acid concentrations were below the flavour thresholds reported in beer. However, the presence of multiple polyphenolic compounds can enhance or reduce the intensity of bitterness perception even at sub-threshold levels [36,37]. If the polyphenolic compounds behave in a synergistic or antagonistic manner, the perceived intensity can be higher or lower than the sum of the individual intensities. Therefore, a combination of polyphenolic compounds could still influence the bitterness and astringency characteristics determined in the sensory analysis.

3.2.4. Analysis of Flavour-Active Fermentation Volatiles

The majority of key flavour-active compounds in beer are yeast secondary metabolites produced during fermentation and are composed of aldehydes, esters, higher alcohols and sulphur compounds. The trial beers were analysed using gas chromatography to identify and quantify these flavour active compounds (Table 7).

The acetaldehyde concentration was considerably higher in 60% rice beer compared to the sample set. This finding is in agreement with the work of Mayer et al. [16] who reported that beer produced with an all rice grist had acetaldehyde concentrations three times over the flavour threshold (10 mg/L). Acetaldehyde is known for imparting a green apple/emulsion paint character as reflected in the attribute description used in the sensory lexicon (Table 1). Therefore, the significant increase of green apple flavour identified in the 60% rice sample is likely due to the elevated acetaldehyde concentrations.

Acetaldehyde is produced as a result of decarboxylation of pyruvate and is an intermediate in the formation of ethanol during glycolysis [38]. Acetaldehyde concentration can be increased with wort gravity, oxygen concentration, fermentation temperatures and yeast stress [38]. Since most of these conditions were standardised across the trial beers, this could have been related to yeast stress or sluggish fermentation as a result of the low FAN level in the 60% torrefied rice brew (Table 4). However, since other adjunct brews, e.g., 60% maize adjunct, were equally FAN constrained but did not result in such high acetaldehyde levels, there were clearly other yeast nutrition factors involved.

Table 7. Fermentation volatile composition of adjunct beers versus an all malt control.

Compound	100% Malt Control	Barley (A)		Barley (B)		Wheat		Maize		Rice	
		30%	60%	30%	60%	30%	60%	30%	60%	30%	60%
Acetaldehyde (mg/L)	2.99	2.52	1.89	2.17	3.72	3.32	7.11 **	6.73 **	4.11 *	3.71	22.96 *
Propanol (mg/L)	11.47	9.32 **	9.89 **	8.92 **	8.65 **	10.21	10.96	10.68	8.82 **	9.22 **	9.23 **
Isobutanol (mg/L)	13.76	11.9	13.92	12.11	13.6	12.11	16.19 **	13.75	21.43 **	13.68	20.00 **
Isoamyl Alcohol (mg/L)	63.24	52.65 **	56.47 **	53.36 **	55.81 **	53.72 **	62.89	57.86 **	72.83 **	57.21 **	75.29 **
Phenethyl Alcohol (mg/L)	35.73	23.74	31.69	28.3	32.99	32.95	33.68	34.67	37.83	34.35	35.28
Tyrosol (mg/L)	19.31	12.68 **	13.36 **	12.41 **	13.18 **	14.36 **	18.64	15.32	31.64 **	14.03 **	32.92 **
Tryptophol (mg/L)	3.71	1.64	6.51 **	2.25	3.18	2.18	7.06 **	2.75	8.09 **	2.66	8.60 **
Ethyl Acetate (mg/L)	19.59	12.19 **	12.03 **	11.87 **	11.64 **	13.56 **	15.92	13.55 **	11.45 **	10.83 **	12.22 **
Isoamyl Acetate (mg/L)	1.17	0.64 **	0.65 **	0.57 **	0.78 **	0.71 **	0.97	0.83 **	0.58 **	0.76 **	0.67 **
Isobutyl Acetate (mg/L)	0.15	0.10 **	0.09 **	0.09	0.12 **	0.09 **	0.13	0.12	0.11 **	0.10 **	0.11 **
Phenethyl Acetate (mg/L)	0.57	0.27 **	0.33 **	0.31 **	0.44	0.38 **	0.59	0.45	0.34 **	0.43	0.42
Ethyl Hexanoate (mg/L)	0.11	0.06 **	0.08 **	0.06 **	0.07 **	0.08 **	0.1	0.08 **	0.09	0.1	0.11
Ethyl Octanoate (mg/L)	0.26	0.18 **	0.19 **	0.19 **	0.19 **	0.19 **	0.21	0.19 **	0.19 **	0.19 **	0.19 **
Ethyl Butyrate (mg/L)	0.07	0.04 **	0.04 **	0.03 **	0.03 **	0.05 **	0.06	0.05 **	0.03 **	0.04 **	0.04 **
Ethyl Propanoate (mg/L)	0.013	0.009 **	0.009 **	0.010 **	0.009 **	0.009 **	0.012	0.009 **	0.009 **	0.008 **	0.009 **
Ethyl Isobutyrate (µg/L)	0.83	0.61 *	0.76	0.7	0.61 *	0.62 *	0.69	0.68	0.72	0.66	0.74
Ethyl Pentanoate (µg/L)	0.08	0.08	0.12 **	0.07	0.06	0.09	0.13 **	0.07	0.08	0.17 **	0.21 **
Ethyl Heptanoate (µg/L)	2.07	1.92	1.94	1.36 **	1.42 **	1.52 **	1.87	1.51 **	1.31 **	2.25 **	2.52 **
3-methyl-2-pentanone (µg/L)	2.63	1.9	2.51	1.8	1.3	2.08	2.2	3.08	3.18	2.15	1.8

ANOVA with Dunnett's post hoc analysis was used to compare the fermentation volatile concentrations. Asterisks indicate samples that were significantly different compared to the all malt control (* $p < 0.05$, ** $p < 0.01$). Results are means of four replicate runs.

Higher alcohols such as n-propanol, isoamyl alcohol and isobutanol can create strong, pungent aromas in beer at high concentrations (>300 mg/L) and can contribute to beer flavour by intensifying alcoholic perception and imparting a warm mouthfeel [20,39]. Aromatic alcohols such as tryptophol and tyrosol are considered to impart undesirable flavours to beer [40]. The majority of the higher alcohols identified significantly increased with 60% maize and rice inclusion, whilst the concentration of these higher alcohols significantly decreased with barley (A), barley (B) and wheat inclusion (Table 7). Higher alcohols are biosynthesised by yeast during fermentation by two pathways; the Ehrlich pathway for amino acid catabolism and by means of the anabolic pathway for amino acid synthesis [41]. Higher alcohol production is closely related to wort nitrogen composition. When nitrogen content is sufficient, amino acids are transformed into higher alcohols in reactions involving branched-chain amino acid aminotransferase (BCAT) and ethanol dehydrogenase [42]. However, when nitrogen sources are low this activates glucose metabolism and forms large amounts of pyruvate and consequently higher alcohols [42]. Therefore, the increase in higher alcohols in 60% adjunct beers made from torrefied rice or maize could be explained by the very low wort FAN concentrations (Table 4).

Esters are one of the most important groups of flavour compounds produced by yeast as esters have a very low odour threshold and impart a range of fruity and floral aromas to the final product. However, if overproduced during fermentation, they can negatively affect the beer [40]. Ester production is affected by many fermentation parameters such as yeast strain, fermentation temperatures and changes in fermentation medium. An important parameter is the carbon-to-nitrogen ratio of the fermentation medium. The data in Table 7 indicate that all identified acetate and ethyl esters reduced in concentration with the addition of any unmalted adjuncts. Previous studies have determined that decreasing FAN concentration decreases the concentration of acetate esters and ethyl esters such as ethyl octanoate and decanoate [43]. Similar patterns were observed in this study, as ester concentrations decreased in the beers with corresponding decreases in FAN concentration. Improving the nitrogen composition and concentration could minimise significant variation in the volatile profile observed with adjunct use.

Interestingly, the 60% unmalted wheat beer had the most similar volatile profile to the 100% malt control, with significantly different concentrations only observed for acetaldehyde, tryptophol, isobutanol and ethyl pentanoate.

To explore the reasons for significant sensory differences in sweetcorn/cooked vegetable aroma amongst trial beers (Table 2), their sulphur volatile profiles were determined using GC-PFPD (Table 8). A range of sulphur compounds (e.g., sulphides, thiols, thioesters) are known for adding cooked vegetable and sweetcorn characters to beer. Sulphur volatiles have low flavour thresholds and can make a sensory impact at low concentrations. Sulphur compounds can originate from the raw materials, Maillard browning reaction and yeast metabolism during fermentation [44].

Table 8. Selected sulphur compound concentrations in adjunct and control beers.

Compound	100% Malt Control	Barley (A)		Barley (B)		Wheat		Maize		Rice	
		30%	60%	30%	60%	30%	60%	30%	60%	30%	60%
Dimethyl Sulphide (µg/L)	31.76	20.83 **	15.27 **	17.56 **	16.09 **	15.25 **	18.28 **	22.33 **	15.26 **	13.04 **	8.85 **
Unknown Sulphur (As/Ais)	0.7	0.86	0.74	0.63	0.74	0.58	0.66	0.74	0.66	1.28 **	0.68

As/Ais denotes the peak area of the sample standardised to the peak area of the internal standard. ANOVA with Dunnett's post hoc analysis was used to compare the sulphur compound concentrations. Asterisks indicate samples that were significantly different compared to the all malt control (** $p < 0.01$). Results are means of four replicate runs.

Dimethyl sulphide (DMS), which imparts a tinned sweetcorn aroma to beer, was identified in all beers with the highest concentration obtained in the all-malt beer. The decrease in DMS is expected with the use of unmalted adjuncts and is due to their reduced contents of DMS precursor, S-methylmelthionine (SMM). SMM is developed during germination of the grain and is then broken down to DMS during thermal processes such as kilning [45]. In the present data set, DMS concentrations did not directly correlate to

the trend of sweetcorn/cooked vegetable aroma in the sensory evaluation. In part this may be because concentrations in all beers were below typical threshold concentrations in beer (30 µg/L). The 30% rice beer was rated by the panel on average as highest in the sweetcorn/cooked vegetable attribute. Consequently, the sensory attribute scored by the panel is not believed to be simply due to the DMS content of the beer samples. Another unidentified sulphur compound was present in the beers. The concentration of this unknown compound was significantly higher in the 30% rice adjunct beer which scored most highly for the sweetcorn/cooked vegetable attribute. The unknown compound (Table 8) had a retention index of 858 on the ZB1 column. Authentic standards of diethyl sulfide, ethanethiol, ethylthioacetate, S-methylthioacetate, thiazole, methional, methionol and dimethyl trisulfide were run under identical conditions but did not show chromatographic similarity to the unknown compound. Further work is thus required to identify this sulphur compound and understand the cause for a higher concentration present in the 30% rice beer.

3.3. Principal Component Analysis of Beer Sensory and Analytical Data

Principal component analysis was performed to visualise the relationship between the samples, sensory attributes and chemical composition. Figure 2 shows a biplot of PC1 and PC2 which accounted for 51.70% of the variance in the data set.

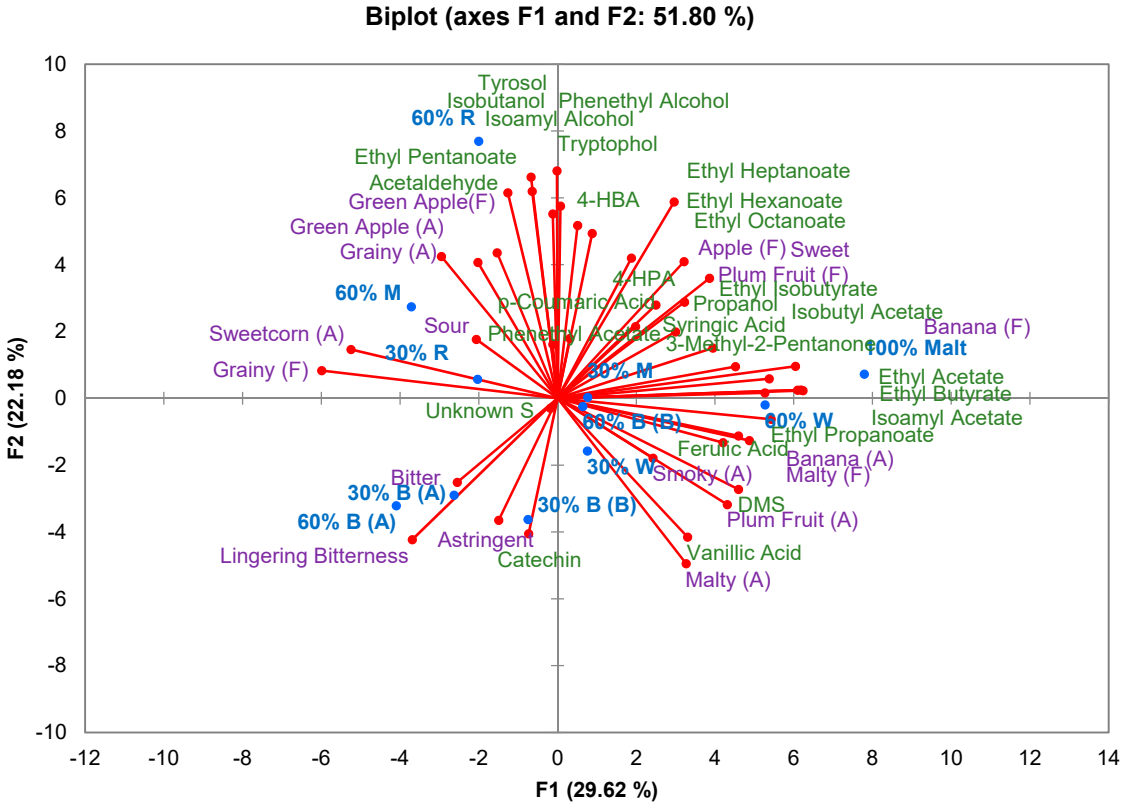

Figure 2. Principal Component Analysis (PCA) of normalised sensory and analytical profiles on principal components 1 and 2. Samples are in blue, chemical compounds in green and sensory attributes in purple font. Sample Codes: M (maize); R (rice); W (Wheat); B (A) barley (A); B (B) barley B. Sensory attributes: (A) aroma; (F) flavour.

PC1 explained 29.62% of the variation. A positive loading on PC1 corresponds with high concentrations of sweet and fruity sensory attributes specifically banana aroma and flavour exemplified by the 100% malt and 60% unmalted wheat beers. These sensory attributes can be assigned to high concentrations of isoamyl acetate and isobutyl acetate that are known to impart banana aroma [20], as well as ethyl propanoate and ethyl butyrate imparting fruity and sweet notes [40]. On the upper right quadrant of the biplot, ethyl

heptanoate, ethyl hexanoate and ethyl octanoate are closely located together. These esters correspond on the biplot with apple (ethyl hexanoate) and plum fruit (ethyl octanoate) flavour sensory attributes. Negative loadings on PC1 correspond with high perception of grainy flavour and sweetcorn aroma exemplified by the 30% torrefied rice and 60% torrefied maize beers. In the lower left quadrant of the biplot, the bitterness and astringent attributes are co-located with the 60% barley (A) beers and the catechin concentration. This suggests that catechin may have contributed to the perception of bitterness and astringency in this study, since its concentration co-varied with their sensory ratings across the sample set.

The positive loading of PC2 corresponds with the characteristics of 60% rice adjunct beers and the impacts of low wort FAN on fermentation such as high acetaldehyde and higher alcohol concentrations. Although no sensory attributes were found to directly associate with the higher alcohols, the positive loading of PC2 is associated with green apple aroma and flavour corresponding to high concentrations of ethyl pentanoate and acetaldehyde, both of which are described sensorially as imparting crisp apple aroma.

The samples 30% maize, 30% wheat and 60% barley (B) plotted close to the centre of the biplot because these samples could not be described by any key attributes or compounds. The biplot highlights that the sensory properties of the 60% unmalted wheat beer came closest to matching the sensory and analytical profile of the 100% malt control.

4. Conclusions

This study showed that the inclusion of five different unmalted cereal adjuncts influenced the wort physiochemical properties and consequently the finished beer specifications. However, sensory and analytical analysis showed that even at incorporation rates of up to 60%, the sensory flavour impacts of unmalted adjuncts were more subtle and nuanced than was expected. This suggests that, in many cases, unmalted adjunct incorporation rates could be pushed higher, from a flavour perspective alone. Furthermore, the present study adopted a strategy of looking at the direct flavour impacts of using high levels of unmalted adjunct materials, without balancing the C:N ratio of wort, or micronutrient levels. Such strategies would enable fermentation-related flavour imbalance to be improved. Overall the results presented are positive in terms of future prospects for unmalted adjunct usage in brewing. In generic terms, 30% unmalted adjunct usage produced beers that were characterised more by a lack of malty or sweet, fruity character, than by the presence of any negative attributes. Whether this is regarded as positive or negative depends principally on the beer style being produced and the target market. At 60% adjunct level unmalted barley (A) introduced increased bitter, astringent and lingering bitterness characteristics, although these sensations were less pronounced in the processed sample unmalted barley (B). Beers containing 60% torrefied maize were characterised by becoming more sour, grainy and with sweetcorn aroma, whilst 60% torrefied rice beers were largely characterised by 'defects' of low nitrogen fermentation—i.e., high levels of higher alcohols and acetaldehyde—as well as the absence of sweet malty character. This is consistent with prior knowledge that rice itself imparts neutral clean flavour characteristics. It is recognised that torrefied adjuncts are relatively expensive grist materials and were used in the current investigations solely to facilitate processing without an additional cereal cooking step. Interestingly, this study identified that use of unmalted wheat at 60% of grist came closest to the sensory profile of an all malt control beer. Although there are many other factors to consider when formulating beers, such as colloidal stability for example, this study suggests that using unmalted wheat in conjunction with other unmalted cereals might help to retain some 100% malt beer flavour characteristics, if that is desired.

This study advances knowledge of the sensory impacts of unmalted adjunct usage and identified PCA correlations between sensory attribute ratings of adjunct beers and their underlying chemical flavour composition (Figure 2). However, it should be borne in mind that correlation does not prove causality and there are likely still to be unknown compounds which contributed significantly to the sensory experience of adjunct beers in this study. In particular it would be interesting to investigate further the classes of

compound responsible for the lingering bitterness and astringency associated with high levels of unmalted barley (A).

Improved understanding of the sensory impacts of unmalted cereal adjunct usage can facilitate cost-efficient and sustainable grist solutions.

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