

Optimised processing of faba bean (*Vicia faba* L.) kernels as a brewing adjunct

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Pulse (*Fabaceae*) grains, such as peas and beans, are derived from crops that are usually cultivated in the absence of mineral nitrogen fertiliser as these crops can obtain their nitrogen requirement naturally from the air via biological nitrogen fixation. Therefore, pulses present a significantly lower greenhouse gas (GHG) footprint than crops demanding nitrogen fertiliser, whilst also offering significant quantities of starch for the brewing and distilling industries. Mitigation of agriculture derived GHG emissions through utilisation of pulses can have a positive environmental impact. To this end, the potential of exploiting dry, dehulled faba bean (*Vicia faba* L.) kernel flour as an adjunct for beer production was evaluated. The impact of different temperature regimes and commercial enzymes were assessed for their effect on wort: viscosity; run-off rate; primary amino nitrogen content and, fermentability. Faba beans demonstrated insufficient endogenous enzyme capacity for starch conversion and generated a viscous wort. However, using a stepped temperature mashing regime and exogenous enzyme additions, the faba bean wort was comparable in processability and fermentability to that of 100% malted barley wort. The faba based beer and co-product qualities demonstrate the environmental, nutritional and commercial potential of pulses in brewing. © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

Keywords: legume; pulse; *Vicia faba* L.; mash; wort; brewing

Introduction

A growing commitment to sustainability and reduced greenhouse gas (GHG) emissions is evident by both large and small scale breweries alike (1–6). According to a global standardised framework (7) established for organisations to measure and manage such emissions, GHG emissions are classified as direct or indirect and are categorised as either: ‘Scope 1’, those that are generated from sources owned or controlled by the company; ‘Scope 2’, those that are associated with purchased electricity; or ‘Scope 3’, indirect emissions from sources not controlled by the company itself. Scope 1 and 2 emissions, are within the direct control and influence of beer producers and are most frequently tackled by the majority of breweries. However, the majority of GHG emissions associated with brewing have been shown to be outside their direct control (Scope 3) with the main contributors being packaging (primarily glass bottle manufacture) followed by agricultural practices, including barley cultivation and the production, distribution and use of mineral nitrogen fertiliser (Figure 1). Projects targeting Scope 3 emissions through improved logistic efficiencies, reduced carbon packaging, cooling solutions, and barley breeding for increased yield and field resilience are beginning to appear (1, 2, 8–10). With the growing expectation that brewing companies should be tackling both the upstream and downstream sources of GHG emissions, agriculture, the second largest indirect contributor of emissions and its contribution to the overall environmental impact for brewing, should not be overlooked.

Pulses (peas, beans and lentils) present a potential alternative solution to reducing Scope 3 emissions. For example, the production of a tonne of peas has a total GHG emission of 188 kg CO₂e per tonne associated with it compared to 190–620 kg CO₂e per tonne for barley (11, 12). These figures relate to emissions

associated with cultivation including seed production, farm machinery operation, fertiliser manufacture and application, and postharvest activities such as cleaning, destoning, and drying. These figures do not consider subsequent processing steps such as malting and transportation costs. Pulses achieve a lower value for GHG emissions as a direct result of their ability to fix atmospheric nitrogen via symbiotic or biological nitrogen fixation, which avoids the need for mineral nitrogen fertiliser manufacture and application. In 2017, 1,882 thousand tonnes of barley (along with the associated 621,060 thousand kg CO₂e) were used by the UK brewing and distilling industries (13). Replacing just 1% of this with pulses could potentially reduce emissions by 2,672 thousand kg CO₂e. Furthermore, uptake of pulses by large scale markets would help increase pulse cultivation from its current low level (ca 1–4%) in Europe (14), facilitating the adoption of more diverse and sustainable crop systems (15, 16).

In brewing, the supplementation of malted barley with alternative starch sources, or adjuncts, is common practice. Annemüller

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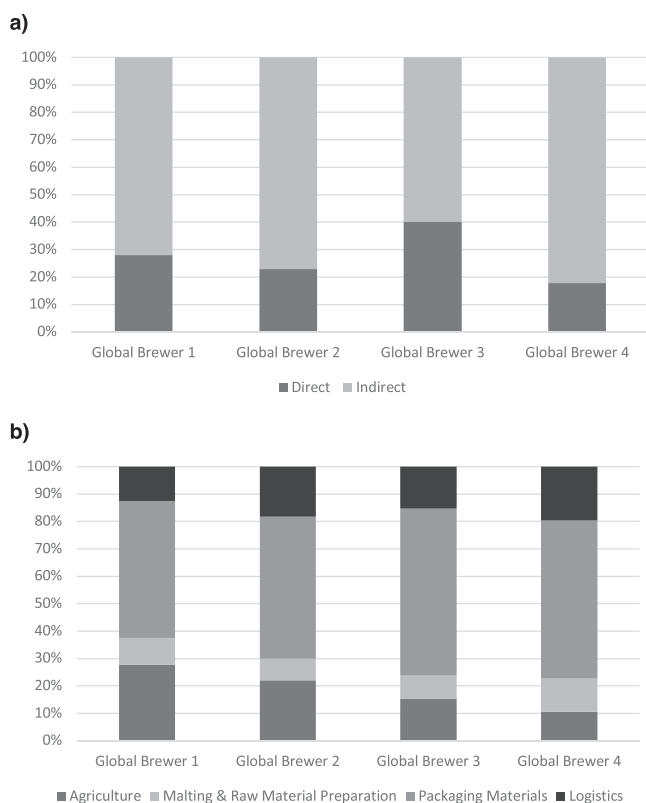


Figure 1. The largest global breweries a) direct (Scope 1 and 2 emissions) and indirect (Scope 3) GHG emissions and b), a breakdown of Scope 3, indirect, emission sources (1–4).

and Manger (17) estimated that 85–90% of global beer production utilises adjuncts. Adjuncts can include cereals such as non-malted barley, wheat or oats but more commonly maize, rice, sorghum and sugar syrups are utilised. Generally, these present a cost effective raw material and/or are products of local agriculture. Historical records show pulse use in beer production dating back to the seventeenth century (18) but today their use is uncommon and generally limited to special releases (19–21) or where market taxes, such as the malt liquor tax in Japan (22), are inhibitory to all malt brewing. Adjuncts can be split into two main categories, those that require hydrolysis into simple sugars which are therefore introduced in the mashing process, and those that are already fermentable, such as sucrose syrups, which can be added during wort boiling (23). The mashing process includes gelatinisation of starch, its enzymatic degradation into fermentable sugars, followed by the separation and removal of solid material. Mashing with malted barley is typically at 62–65°C, a temperature range which allows both starch gelatinisation and amylolysis to occur simultaneously. The temperature required to achieve gelatinisation is specific to each raw material with most adjuncts requiring a temperature greater than 65°C. Therefore, an initial cooking step is often included in the adjunct mashing process. The efficiency of mashing is critical in the production of beer as the amount of fermentable sugar produced directly relates to the alcohol content of the resultant beer.

When brewing with adjuncts, the malted barley typically has sufficient enzyme activity (diastatic power) to catalyse the adjunct starch degradation as well as its own. However, when using a high proportion of starchy adjunct with low diastatic power, the enzymatic activity derived from the malted barley may be insufficient

for saccharification of the adjunct. Furthermore, the use of non-malted adjuncts may present high levels of undegraded cell wall components such as β -glucans and arabinoxylans which can thicken the wort leading to filtration and extract recovery problems as well as final product quality issues such as haze formation. By comparing faba bean (*Vicia faba* L.) kernels to other commonly used adjuncts (Table 1), wort filtration issues can be predicted due to an increased hemicellulose and protein content. Impaired wort run-off rate has been confirmed in preliminary trials and at commercial scale in the production of Cool Beans® Faba IPA at Barney's Beer (21). In addition, it is often the case that the use of starch or sugar adjuncts does not contribute to the free amino nitrogen, which is available to, and required by, the yeast for optimal fermentation. Faba bean has a relatively high protein content and can make a positive contribution to the free amino nitrogen level.

The addition of commercial exogenous enzymes is an established practice to increase the efficiency of starch saccharification, increasing levels of fermentable sugars, but also to improve wort separation and levels of available nitrogen.

In this study, the feasibility of faba bean kernels as an adjunct was assessed with regard to starch saccharification, wort separation and fermentation kinetics. The efficiency of a mashing regime that includes an initial low temperature hold, to one without, in the release and liquefaction of faba bean starch as a novel brewing adjunct was compared. In addition, the influence of exogenous commercial enzymes: α -amylase as the main starch liquefaction enzyme, protease, and mixed-carbohydrase were studied to determine their contribution to mash liquefaction, wort separation, wort flow rate, primary amino nitrogen content and resultant wort fermentability.

Materials and methods

Materials

Dehulled milled faba bean (*Vicia faba* L. cv. Wizard) kernels were obtained from Hodmedod Limited (UK). Milled distilling malt (*Hordeum vulgare* L. cv. Concerto) was obtained from Brewers Select Limited (UK). Both were passed through a 212 μ m sieve (ELE International Standard, Bedfordshire, UK).

The functional characteristics of the commercial enzymes selected to improve saccharification and liquification are presented in Table 2. The enzymes used include: α -amylase (Enzyme Commission (EC) number 3.2.1.1), for the liquefaction of starch *via* hydrolysis of 1,4- α -glucosidic linkages; amyloglucosidase (EC 3.2.1.3), for the saccharification of liquefied starch through hydrolysis of 1,4- and 1,6- α -glucosidic linkages; endoprotease (EC 3.4.24.28), for the hydrolysis of peptide bonds; and, mixed carbohydrase (EC 3.2.1.6), for the breakdown of cell wall material *via* hydrolysis of 1,3- and 1,4- linkages in β -D-glucans.

Table 1. Protein and hemicellulose constituents (as % dry matter) of commonly used adjuncts which may cause wort filtration issues (15, 24, 25).

Raw material	Protein	Hemicelluloses
Barley	10.6–11.8	10.3
Maize	9.2–10.3	4.2
Rice	7.4–9.0	2.3
Faba bean	26.5	24–45

Table 2. Functional characteristics of commercial enzymes used in the production of faba bean kernel flour wort.

Brand Name	Enzyme Type	Enzyme Commission Number	Source	Activity	Optimum Temp. (°C)	Optimum pH
AMG 300 L	Glucoamylase	3.2.1.3	<i>Aspergillus niger</i>	300 AGU/mL ¹	75	4.0
Neutrase 0.8 L	Metallo endoprotease	3.4.24.28	<i>Bacillus amyloliquefaciens</i>	0.8 AU/g ²	40-45	5.5 – 6.0
Termamyl 120 L	α -amylase	3.2.1.1	<i>Bacillus licheniformis</i>	120 KNU/g ³	85-95	6.0-6.5
Viscozyme L	Mixed carbohydrase = arabanase, β -glucanase, hemicellulose, xylanase	3.2.1.6	Strain of the <i>Aspergillus</i> group	100 FBG/g ⁴	45-65	4.0 – 6.0

Enzymes and associated product information from Novozymes A/S, Copenhagen, Denmark.

¹ AGU: one Novo amyloglucosidase unit (AGU) defined as the amount of enzyme which hydrolyses 1 micromole per minute under standardised conditions.

² AU: Anson Units

³ KNU: one Kilo Novo alpha-amylase Unit (KNU) defined as the amount of enzyme which breaks down 5.26 g starch per hour at Novozymes' standard method for determination of alpha-amylase.

⁴ FBG: Fungal Beta-Glucanase Units

Dried yeast (*Saccharomyces cerevisiae*, Anchor Dry strain) was obtained from Lallemand Biofuels & Distilled Spirits (USA).

Wort production and filtration

Experimental design. A full two level factorial design was employed to study the effect of substrate, mashing regime (Cycle A versus Cycle B, Fig. 2), and enzyme additions (presence or absence of α -amylase, protease and carbohydrase) on viscosity development during starch gelatinisation together with wort separation and rate of run-off. The results were compared to the values obtained with a typical mashing regime used for malted barley flour with no enzyme additions (mashing Cycle C). The factorial design included two replicates of each combination of test conditions.

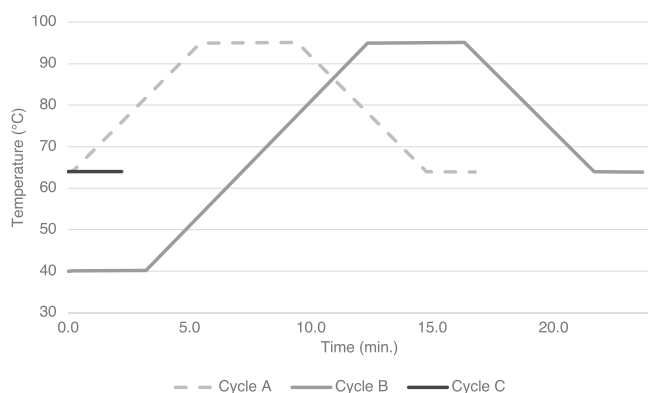


Figure 2. Rheometer cycles used to mimic commercial mashing regimes. An initial mixing step (12 s, 50 rad/s) was included to ensure homogenisation of the suspension prior to reducing rotor speed to 16 rad/s for the remainder of the cycle. **Cycle A:** a mashing regime consisting of a start temperature below the gelatinisation temperature to ensure complete dispersion of the non-gelatinised flour. This is followed by a controlled increase to 95°C, a hold, then a steady decrease to 64°C, the temperature at which malted barley would typically be introduced. **Cycle B:** a mashing regime starting with a 40°C hold to allow optimal low temperature protease and carbohydrate enzyme activity before continuing per Cycle A. **Cycle C:** a malt mashing regime used for malted barley flour.

Mashing viscosity. The temperature and time dependent rheological properties of malted barley and faba bean flour were evaluated. Distilled water suspensions (9.2% dry basis) with controlled heating and cooling cycles designed to mimic commercial mashing regimes, were studied using a Discovery Hybrid Rheometer (TA Instruments, USA) fitted with a starch pasting cell (radius 18.5 mm) and impeller rotor to maintain particles in suspension. Viscosity of the mash was determined at shear rate 16 rad/s according to the method of the Scotch Whisky Research Institute (26) adapted to fit the mashing temperature protocols of this study (Figure 2). Enzymes (10 μ L) were added to the initial flour water suspension once the starting temperature had been achieved.

Wort run off flow rate. Following completion of the mashing regime, the contents of the rheometer cup were filtered (Whatman N^o. 1 filter paper), and the volume of filtrate collected recorded at 5 min. intervals for a period of 60 min.

Primary amino nitrogen determination

Primary amino nitrogen content of the collected worts was determined using a Primary Amino Nitrogen (PAN) assay (K-PANOPA, <https://www.megazyme.com>). Free amino nitrogen (FAN), total usable nitrogen and usable nitrogen are other terms for PAN (27). Amino groups of free amino nitrogen react with *N*-acetyl-L-cysteine and *o*-phthalaldehyde forming isoindole derivatives which are quantified by their absorbance at 340 nm.

Fermentation kinetics

In separate experiments, the relative contribution of mash temperature regime and exogenous enzymes on fermentation kinetics were tested according to the conditions detailed in Table 3. Faba bean and malted barley wort was prepared in a 1:4 [w/w] flour water ratio and exogenous enzymes were then added to the faba bean wort (10 μ L α -amylase, 4 μ L protease or 6 μ L carbohydrase per 100 g mash (see Table 2 for activities) as appropriate to each mashing temperature regime (Table 3). On completion of the mashing regime (Cycle A, B or C per Table 3), each mash was centrifuged (2.5 min. at 3.0 \times g) (5702 centrifuge with 5702/R A-4-38

Table 3. Wort preparation conditions for measurement of fermentation kinetics.

Raw Material	Enzyme additions				Mashing cycle	Fermentation volume (mL)
	α -amylase	protease	carbohydrase	glucoamylase		
Faba bean	x			x	A	50
	x	x		x	B	75
	x	x	x	x	B	75
Malted barley				x	C	100

x indicates presence of enzyme.

Cycle A: a mashing regime consisting of a start temperature below the gelatinisation temperature to ensure complete dispersion of the non-gelatinised flour followed by a 60 minute hold starting at 80°C and increasing to 90°C over the hold period. **Cycle B:** a mashing regime starting with a 20 minute 40°C hold to allow optimal low temperature protease and carbohydrase enzyme activity before an increase in temperature to 80°C and continuing per Cycle A. **Cycle C:** a malt mashing regime used for barley flour consisting of a start temperature below the gelatinisation temperature to ensure complete dispersion of the non-gelatinised flour then a hold at 64°C for 60 min..

rotor, Eppendorf, UK) to separate the clear supernatant from the insoluble material. The supernatant was adjusted with distilled water to a specific gravity of 1.058 before the addition of amyloglucosidase (40 μ L per 100 mL wort) as appropriate to each mashing regime (Table 3). Fermentation volumes varied on treatment (see Table 3). Yeast (Anchor Dry strain of *Saccharomyces cerevisiae*, Lallemand Biofuels & Distilled Spirits) was pitched at the manufacturer's recommended pitching rate (1 g dried yeast/L, 1.85×10^{10} viable yeast cells/g). Fermentation took place at 30°C in static glass bottles positioned in a recirculating water bath (TXF200, Grant Instruments, UK). Fermentation was monitored using the RF Gas Production System (ANKOM Technology) for 48 hours with a pressure reading recorded every 5 min. Mean final gravities were as follows - faba + α -amylase:1.0007, faba + α -amylase + protease:1.0013, faba + α -amylase + protease + carbohydrase: 1.0012, malt: 0.9809. Pressure readings adjusted for headspace due to variable fermentation volumes. The trial combinations are summarised on Table 3, all were performed in triplicate.

Statistical Analysis

All statistical analyses were conducted using Minitab 19 with the specific tests performed as detailed in the discussion. As noted above, a full factorial design was developed to assess the impact of exogenous enzyme addition on mash viscosity, wort flow rate and volume of wort collected. Factorial fit and analysis of variance were performed to determine the significance of the main values and interaction effects. The mean values for free amino nitrogen were analysed by one-way analysis of variance (ANOVA) with Tukey Simultaneous Tests.

Results and discussion

Faba bean starch gelatinisation and diastatic power

In order to understand the gelatinisation and breakdown of faba bean starch, a full factorial design was employed studying the

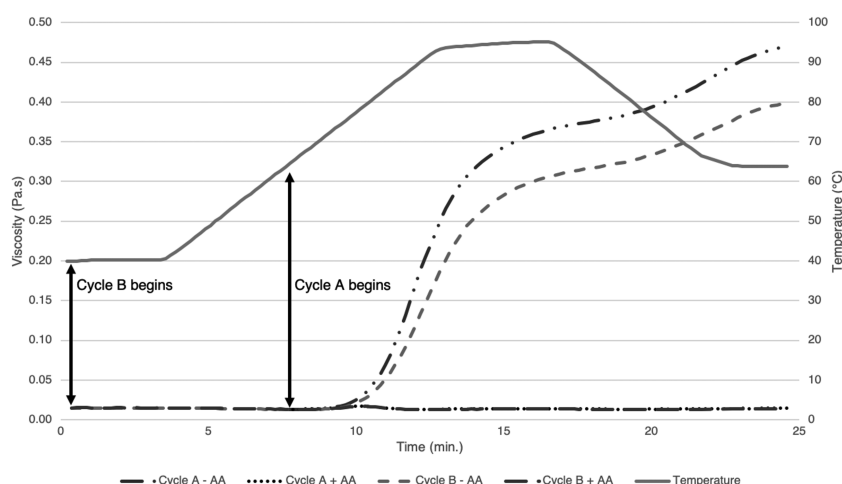


Figure 3. Viscosity of faba bean kernel flour suspension across two temperature regimes with (+AA) and without (-AA) the addition of exogenous α -amylase (AA). **Cycle A** (grey lines): a mashing regime consisting of a start temperature (64°C) below the gelatinisation temperature to ensure complete dispersion of the non-gelatinised flour. This is followed by a controlled increase to 95°C, hold, then a steady decrease to 64°C. **Cycle B** (black lines): a mashing regime starting with a 40°C hold to allow optimal low temperature protease and carbohydrate enzyme activity before continuing per Cycle A.

Table 4. Factorial fit and analysis of variances of wort flow rate over time. Flow rates calculated over five minute intervals during wort collection. Analysis of variance conducted with statistical significance levels set as: NS, non-significant $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Term	Statistical significance of wort flow rates (mL/min) during wort collection.				
	0-5 min	5-10 min	10-15 min	25-30 min	55-60 min
Mashing cycle	***	**	NS	NS	*
Protease	***	***	***	NS	NS
Carbohydrase	*	NS	NS	NS	*
Temp regime*Protease	***	**	NS	NS	NS
Temp regime*Carbohydrase	NS	NS	NS	NS	NS
Protease*Carbohydrase	*	NS	NS	NS	NS
Temp regime*Protease*Carbohydrase	NS	NS	NS	NS	NS

effect of mashing cycle (as presented in Figure 2: Cycle A and Cycle B) and the presence or absence of exogenous α -amylase addition on viscosity development of the mash. The curves in Figure 3 show the viscosity built up of the aqueous faba bean

kernel flour suspensions in the absence of exogenous α -amylase using both mashing Cycle A and mashing Cycle B. It was observed that as the temperature increased, the viscosity built up without the anticipated drop indicating a lack of endogenous starch degrading enzymes. The lack of reduction in viscosity during heating has also been reported by Markezzi et al. (28), who concluded, that in legumes, protein and fibre may also contribute to the build up in viscosity. Peak and final viscosities of the mash were not affected by the mashing cycle, but α -amylase reduced

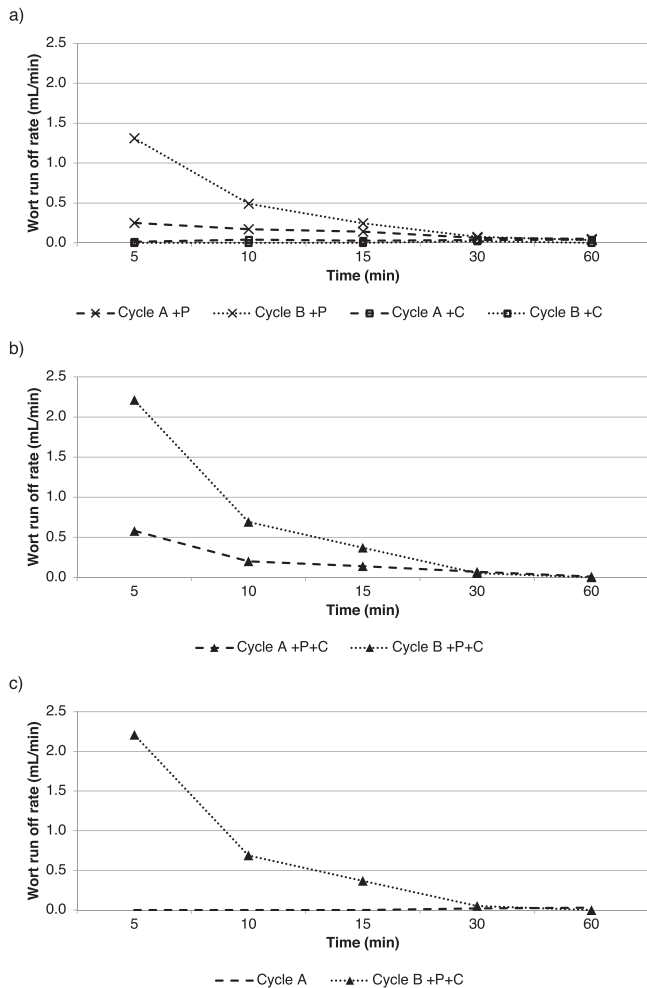


Figure 4. Effect of enzyme addition on wort run off rate of faba bean mash across two mashing temperature cycles (A - - - and B). Exogenous enzyme addition - P: protease = X, C: carbohydrase = \square P and C = \blacktriangle . Wort run off rate: volume of wort collected in the preceding 5 min, divided by time; a) effect of protease and carbohydrase addition as a function of mashing cycle; b) effect of carbohydrase used in conjunction with protease; c) comparison of baseline run off rate (Cycle A, no additions) against optimised mashing cycle (Cycle B) with enzyme additions (P and C).

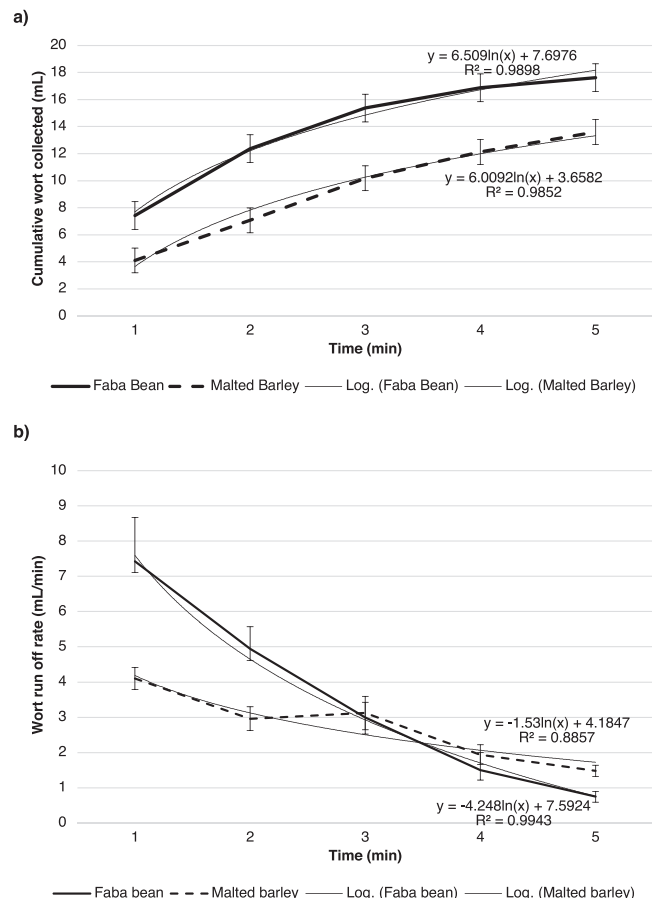


Figure 5. Comparison of a) wort collected and b) wort run off rates for mash prepared using faba bean kernel flour with enzyme additions (α - amylase, protease, carbohydrase) or malted barley flour with fitted logarithmic regression trendlines for prediction of flow behaviour.

both the viscosity development and final mash viscosity significantly (both $p < 0.001$) indicating, following granule rupture and amylose leaching, successful enzyme hydrolysis of the 1,4 α -glucosidic linkages (Figure 3). Factorial fit and analysis of variance for the peak viscosity showed the main effects were statistically significant with $R^2 = 99.04$, $F(2,4) = 205.09$ $p < 0.001$ whereas the interaction effects were not ($F(1,4) = 2.85$ $p > 0.05$). Factorial fit and analysis of variance for the final viscosity again showed the main effects were statistically significant with $R^2 = 99.35$, $F(2,4) = 302.47$ $p < 0.001$ but the interaction effects were not ($F(1,4) = 4.32$ $p > 0.05$).

The heating of starch suspended in water results in both structural and molecular changes critical to the production of alcohol. The first step in the modification of the starch, gelatinisation, consisting of granule swelling and rupture is temperature dependent and varies with starch granule structure, and starch source (29–31). During gelatinisation of the faba bean kernel flour (approximately 75°C, Figure 3) the released amylose chains present a random configuration causing swelling and thickening of the surrounding matrix that results in an increase in viscosity (32, 33). The majority of this increase in viscosity can be attributed to the starch gelatinisation process versus protein or fibre, as the viscosity was reduced significantly in the presence of α -amylase. Amylose leaching is influenced by interactions between amylose chains, amylose and amylopectin chains, or lipids and amylose chains (34). Typically, amylose leaching may also be associated with an increased amylose content in legumes (28). Once the gelatinisation temperature has been exceeded, enzymatic degradation of the 1,4- α -glucosidic linkages within the starch can begin, resulting in a decrease in viscosity.

An optimised mashing regime for increased faba bean wort separation and flow rate

The volume of wort collected was significantly impacted by the addition of exogenous α -amylase ($p < 0.001$) and to a lesser extent by the mashing regime ($p < 0.01$), although there was also an increased volume of wort collected when exogenous α -amylase was added and a 40°C hold (Cycle B, Figure 2) was incorporated into the mashing cycle (two way interaction $p < 0.01$). This suggests that the α -amylase added is not temperature dependent. The improvement in wort collection volume with mashing Cycle B may be the result of an increased α -amylase exposure time or may be indicative of the presence of limited endogenous hydrolytic or proteolytic enzyme activated at low temperatures. Factorial

fit and analysis of variance showed that both the main effects and interaction effects were statistically significant with $R^2 = 98.26$, $F(2,4) = 98.85$ $p < 0.001$ for the main effects and $F(1,4) = 27.77$ $p < 0.01$ for the interaction. Despite the addition of α -amylase resulting in a significant decrease in viscosity and increase in wort flow rate, the volume of filtrate collected were low across all of the mashing regimes (Cycle A: 0.6 mL wort, Cycle B: 1.0 mL wort in 60 min.) indicating, as expected, other factors such as cell wall components and proteins are inhibiting wort flow.

Flow rate improvements were seen with mashing Cycle B and with the addition of protease with both the main effects and interaction effects statistically significant over the first 10 minutes of wort collection (Table 4, Figure 4). The selected exogenous protease favours lower temperatures, thus the 40°C hold is optimal for the activity of this enzyme (Table 2). The action of the protease and carbohydrase enzymes appear synergistic and were more effective together at increasing the flow rate of the wort (Figure 4). For example, for the flow rate over the first 5 min. $R^2 = 92.31$, $F(3,11) = 33.69$ $p < 0.001$ for the main effects and $F(3,11) = 9.55$ $p < 0.01$ for the 2-way interaction. Furthermore, the addition of all three exogenous enzymes (α -amylase, protease, carbohydrase) to the faba bean grist resulted in a significantly improved mean wort run off rate over the first five minutes of wort collection when compared to a 100% malted barley wort (Figure 5, $T(7) = 2.71$, $p < 0.05$) and led to a greater volume of wort being collected over the same period.

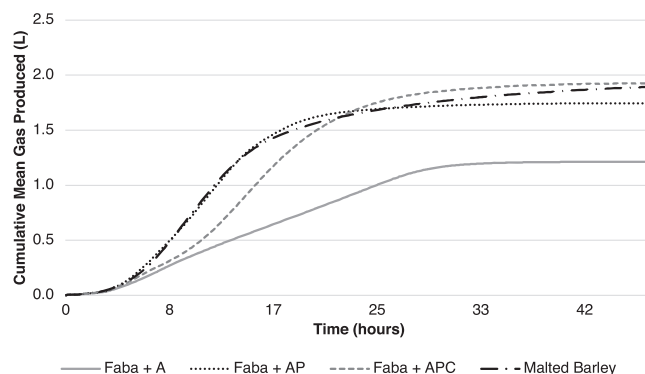


Figure 7. Fermentation gas (CO_2) volume as an indicator of fermentation rate. Wort produced from distilling quality malted barley and faba bean kernel flour with different enzyme additions: (A) α -amylase, (P) protease, (C) carbohydrase.

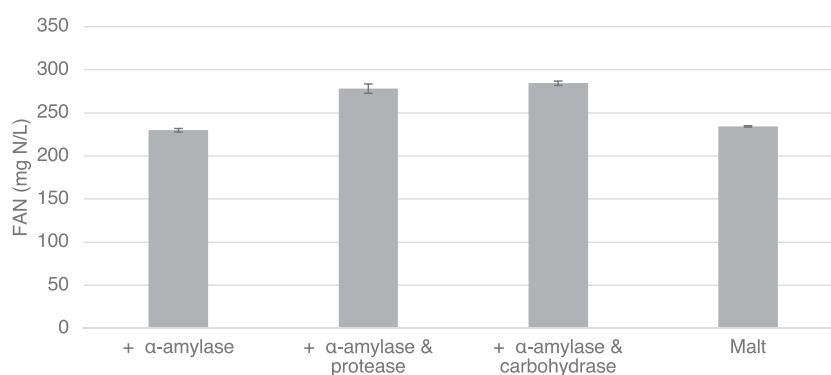


Figure 6. Free amino nitrogen (FAN) content of faba bean wort prepared with different enzyme treatments and 100% malted barley wort.

Fermentation nutrition and kinetics

Figure 6 shows that the free amino nitrogen (FAN) levels of faba bean wort (+ α -amylase) were equivalent to that of 100% malted barley (ANOVA with Tukey Simultaneous Tests $p > 0.05$) or elevated significantly (+ protease $p < 0.001$, + protease and carbohydrase $p < 0.001$). Thus, there should be no detrimental implications for yeast growth or fermentation performance. FAN is an important wort parameter, critical for yeast growth and fermentation efficiency (27). The protein content of cereals is typically 10–15% dry weight (27), and this is solubilised by proteases during the mashing process at appropriate temperatures. It is generally recommended that the minimum FAN content of wort for satisfactory fermentation performance is 130 mg FAN/L. (27) Below this concentration, yeast growth rate is assumed to be reduced and fermentation will not be optimal, increasing the likelihood of hydrogen sulphide production and, in turn, beer off flavours. An excess in FAN is also to be guarded against, as this may lead to an increased synthesis of higher alcohols (e.g. iso-butanol) in beer (27). The most commonly used adjuncts are selected for their starch provision, and as such normally decrease the FAN content of wort. This impacts negatively on the ability of yeast to synthesise enzymatic and structural proteins for growth (35). The proposal to use Faba beans, as an adjunct based on environmental credentials rather than starch content, do not present this limitation. As whole beans have a nitrogen content of 28% (dry weight) or more, and kernels (whole beans with hulls or skin removed), would have an even higher protein proportion. Despite elevated levels of FAN being present in the faba bean wort, yeast favour specific amino acids, such as glutamate, aspartate and asparagine, for optimal growth (36). Therefore, wort produced from faba beans may change the proportion of amino acids unfavourably. However, Figure 7 shows comparable CO₂ production, as an indicator of fermentation rate, between worts produced from 100% malted barley and the 100% faba bean kernel flour with enzymatic treatment.

Conclusions

It has been demonstrated that milled faba bean kernel flour lacks the endogenous enzymatic capacity to saccharify its starch and yield a filterable wort for fermentation. Nevertheless, mashing temperature regimes plus specific exogenous enzyme treatments can overcome these limitations. The optimised methodology yields a wort with the viscosity, flow rate and fermentation capacity to match that of malted barley. The potential to use faba bean as an adjunct for brewing is therefore demonstrated.

From an economic standpoint, the cost of exogeneous enzymes (UnivarSolutions, UK), is relatively low (ca £9/t starch, using α -amylase, glucoamylase and protease). In comparison, and in terms of raw commodity (grain) costs, faba beans offer an attractive raw material with current (2019) value for whole (animal) feed beans up to £200/t ex-farmgate. However, dehulled (skins removed) bean kernels are used in the process described here and these present around 43% starch (dry weight basis) (37) and are currently valued at £295/t (personal communication, Askew and Barrett Ltd, UK). Malted barley presents 66% starch (average, dry weight basis) (38) and is valued at around £530/t (39). Comparative analysis of relative starch costs is therefore £790/t for faba bean kernels versus £847/t for malted barley. This is of course a finance-only consideration at the point of production. That is, a complete assessment of the relative agronomic and

environmental costs of cultivating barley or faba beans remains to be accounted in future research via full production-to-consumption life cycle and economic analyses. Furthermore, earlier research demonstrated that beer brewed with faba bean gained favourable consumer acceptance and could not be distinguished from a 100% malted barley (40).

The approach of allowing pulses a more balanced presence throughout food and feed value chains will help fulfil sustainable and circular bioeconomies in the future (16, 41). In particular, brewers and distillers as major industries could emerge as critical stakeholders to help increase pulse cultivation, and the transformation of agri-food and feed systems to more diverse and sustainable states.

Author Contributions

KB carried out the practical work, investigation, formal analysis and wrote the original draft of the manuscript. ATC assisted in the formal analysis. PPMI provided insight on the agroecology potential and significance of faba bean used in brewing, and routing of highprotein co-products to animal feed. Also, options regarding the agronomic approaches and faba bean options for brewing. GW supervised this study as part of an ongoing PhD project. All authors reviewed, edited and approved the final manuscript.

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Declaration of interest

The authors declare there are no conflicts of interest.

References

1. Heineken. 2019. Annual report. <https://www.theheinekencompany.com/Sustainability/Focus-Areas/Reducing-CO2-Emissions>
2. Carlsberg. 2018. Sustainability report. <https://carlsberggroup.com/media/28929/carlsberg-sustainability-report-2018.pdf>
3. ABInBev. 2018. Annual report: shaping the future. https://www.abinbev.com/content/dam/universaltemplate/ab-inbev/investors/reports-and-filings/annual-and-hy-reports/2019/190321_AB%20InBev%20RA2018%20EN.pdf
4. Molson Coors, 2019. Raising the bar on beer. Environmental, social and governance (ESG) report 2019. <https://www.molsoncoors.com/sites/molsonco/files/ESG-Report-en.pdf>
5. New Belgium. 2020. Carbon emissions. <https://www.newbelgium.com/sustainability/environmental-metrics/ghg/>
6. Shin R, Searcy C. 2018. Evaluating the greenhouse gas emissions in the craft beer industry: an assessment of challenges and benefits of greenhouse gas accounting. *Sustainability 10*: article 4191. <https://doi.org/10.3390/su10114191>
7. Greenhouse Gas Protocol. 2020. <http://ghgprotocol.org/>
8. Heineken. 2020. Our sustainability story. <https://www.theheinekencompany.com/Sustainability/Value-Chain>

9. Bingham IJ, Karley AJ, White PJ, Thomas WTB, Russell JR. 2012. Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. *Eur J Agron* 42:49-58. <https://doi.org/10.1016/j.eja.2011.10.003>
10. Newton AC, Flavell AJ, George TS, Leat P, Mullholland B, Ramsay L, Revoredo-Giha C, Russell J, Steffenson BJ, Swanston JS, Thomas WTB, Waugh R, White PJ, Bingham IJ. 2011. Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. *Food Sec* 3:141. <https://doi.org/10.1007/s12571-011-0126-3>
11. Cimini A, Moresi M. 2016. Carbon footprint of a pale lager packed in different formats: assessment and sensitivity analysis based on transport data. *J Clean Prod* 112:4196-4213. <https://doi.org/10.1016/j.jclepro.2015.06.063>
12. MacWilliam S, Parker D, Marinangeli CPF, Trémorin D. 2018. A meta-analysis approach to examining the greenhouse gas implications of including dry peas (*Pisum sativum* L.) and lentils (*Lens culinaris* M.) in crop rotations in western Canada. *Agr Syst* 166:101-110. <https://doi.org/10.1016/j.agsy.2018.07.016>
13. DEFRA. 2017. Agriculture in the United Kingdom. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/741062/AUK-2017-18sep18.pdf
14. European Commission. 2018. A report from the EC to the Council and European Parliament, 'on the development of plant proteins in the European Union'. Communication (COM)757-final. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52018DC0757>
15. Squire GR, Quesada N, Begg GS, Iannetta PPM. 2019. Transitions to greater legume inclusion in cropland: defining opportunities and estimating benefits for the nitrogen economy. *Food Energy Secur* 8: e00175. <https://doi.org/10.1002/fes3.175>
16. Vasconcelos M, Gomes A, Pinto E, Ferreira H, Vieira E, Pimenta A, Santos CS, Balázs B, Kelemen E, Hamann K, Williams M, Iannetta PPM. 2020. The push-, pull- and enabling – capacities necessary for legume grain inclusion into sustainable agri-food systems and healthy diets. *World Rev Nutr Diet* (In Press).
17. Annemüller G, Manger H-J. 2013. Adjuncts, p 18-59. In *Processing of Various Adjuncts in Beer Production, Raw Grain Adjuncts – Sugars and Sugar Syrups – Malt Substitutes*, VLB Berlin, Germany.
18. Markham G. 1615 (1994 reprinted edition). Chapter IX Of the office of the brew-house, and the bake-house, and the necessary things belonging to the same, p 204-212. In *The English Housewife*, McGill-Queen's University Press, Montreal, Canada.
19. Rebellion Brewing. 2016. Lentil Cream Ale. <http://www.lentilbeer.com/index.html>
20. Lobethal Bierhause. 2017. Totally lentil: Australia's first lentil beer released. <https://www.beerandbrewer.com/totally-lentil-australias-first-lentil-beer-released/>
21. Barney's Beer. 2020. Cool beans. https://www.barneysbeer.co.uk/portfolio_page/cool-beans-faba-bean-ipa/
22. Alexander JW. 2013. Indigenous brews: innovation, entrepreneurship, beers's continuing evolution since the 1970s, p 223-237. in *Brewed in Japan: the Evolution of the Japanese Beer Industry*, UBC Press, Vancouver, Canada.
23. Bogdan P, Kordialik-Bogacka E. 2017. Alternatives to malt in brewing. *Trends Food Sci Technol* 65:1-9. <https://doi.org/10.1016/j.tifs.2017.05.001>
24. Hodmedod Ltd. 2019. Product specification: Fava bean flour. <https://hodmedods.co.uk/collections/flours/products/fava-bean-flour>
25. Gdala J, Buraczewska L. 1997. Chemical composition and carbohydrate content of several varieties of faba bean and pea seeds. *J Animal Feed Sci* 6: 123-135. <https://doi.org/10.22358/jafs/69510/1997>
26. Agu RC, Bringham TA, Brosnan JM. 2006. Production of grain whisky and ethanol from wheat, maize and other cereals. *J Inst Brew* 112:314-323. <https://doi.org/10.1002/j.2050-0416.2006.tb00737.x>
27. Hill AE, Stewart GG. 2019. Free amino nitrogen in brewing. *Ferm* 5:22. <https://doi.org/10.3390/fermentation5010022>
28. Marquezi M, Gervin VM, Watanabe LB, Bassinello PZ, Amante ER. 2016. Physical and chemical properties of starch and flour from different common bean (*Phaseolus vulgaris* L.) cultivars. *Braz J Food Technol* 19: E2016005. <https://doi.org/10.1590/1981-6723.0516>
29. Subramanian V, Hosney RC, Bramel-Cox P. 1994. Shear thinning properties of sorghum and corn starches. *Cereal Chem* 71:272-275.
30. Ratnayake WS, Hoover R, Shahidi F, Perera C, Jane J. 2001. Composition, molecular structure, and physicochemical properties of starches from four field pea (*Pisum sativum* L.) cultivars. *Food Chem* 74:189-202. [https://doi.org/10.1016/S0308-8146\(01\)00124-8](https://doi.org/10.1016/S0308-8146(01)00124-8)
31. Tharanathan RN, Mahadevamma S. 2003. Grain legumes - a boon to human nutrition. *Trends Food Sci Technol* 14:507-518. <https://doi.org/10.1016/j.tifs.2003.07.002>
32. Sajilata MG, Singhal RS, Kulkarni PR. 2006. Resistant starch - a review. *Compr Rev Food Sci Food Saf* 5:1-17. <https://doi.org/10.1111/j.1541-4337.2006.tb00076.x>
33. Schirmer M, Höchstätter A, Jekle M, Arendt E, Becker T. 2013. Physicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio. *Food Hydrocoll* 32:52-63. <https://doi.org/10.1016/j.foodhyd.2012.11.032>
34. Chung HJ, Hoover R, Liu Q. 2009. The impact of single and dual hydrothermal modifications on the molecular structure and physicochemical properties of normal corn starch. *Int J Biol Macromol* 44:203-210. <https://doi.org/10.1016/j.ijbiomac.2008.12.007>
35. Russell I. 2006. Yeast p 281-332. In Priest GP, Stewart GG (ed), *Handbook of Brewing*, 2 ed., CRC Press, Florida, USA.
36. Jones, M.; Pierce, J.S. 1964. Absorption of amino acids from wort by yeasts. *J Inst Brew* 70:307-315. <https://doi.org/10.1002/j.2050-0416.1964.tb01996.x>
37. do Carmo CS, Silventoinen P, Nordgård CT, Poudroux C, Dessev T, Zobel H, Holtekjølén AK, Draget KI, Holopainen-Mantila U, Knutsen SH, Sahlstrøm S. 2020. Is dehulling of peas and faba beans necessary prior to dry fractionation for the production of protein- and starch-rich fractions? Impact on physical properties, chemical composition and techno-functional properties. *J Food Eng* 278:109937. <https://doi.org/10.1016/j.jfoodeng.2020.109937>
38. Bathgate GN. 2016. A review of malting and malt processing for whisky distillation. *J Inst Brew* 122:197-211. <https://doi.org/10.1002/jib.332>
39. Brewers select. 2020. Product page: pale ale malt. <https://www.brewersselect.co.uk/pale-ale/>
40. Black K, Barnett A, Tziboula-Clarke A, White PJ, Iannetta PPM, Walker G. 2019. Faba bean as a novel brewing adjunct: consumer evaluation. *J Inst Brew* 125:310-314. <https://doi.org/10.1002/jib.568>
41. Dahiya S, Kumar AN, Shanthi Sravan J, Chatterjee S, Sarkar O, Mohan SV. 2018. Food waste biorefinery: Sustainable strategy for circular bioeconomy. *Bioresour Technol* 248:2-12. <https://doi.org/10.1016/j.biortech.2017.07.176>