TRansition paths to sUstainable legume-based systems in Europe

Brewing with Faba Beans – Enzymes for Mash Optimisation

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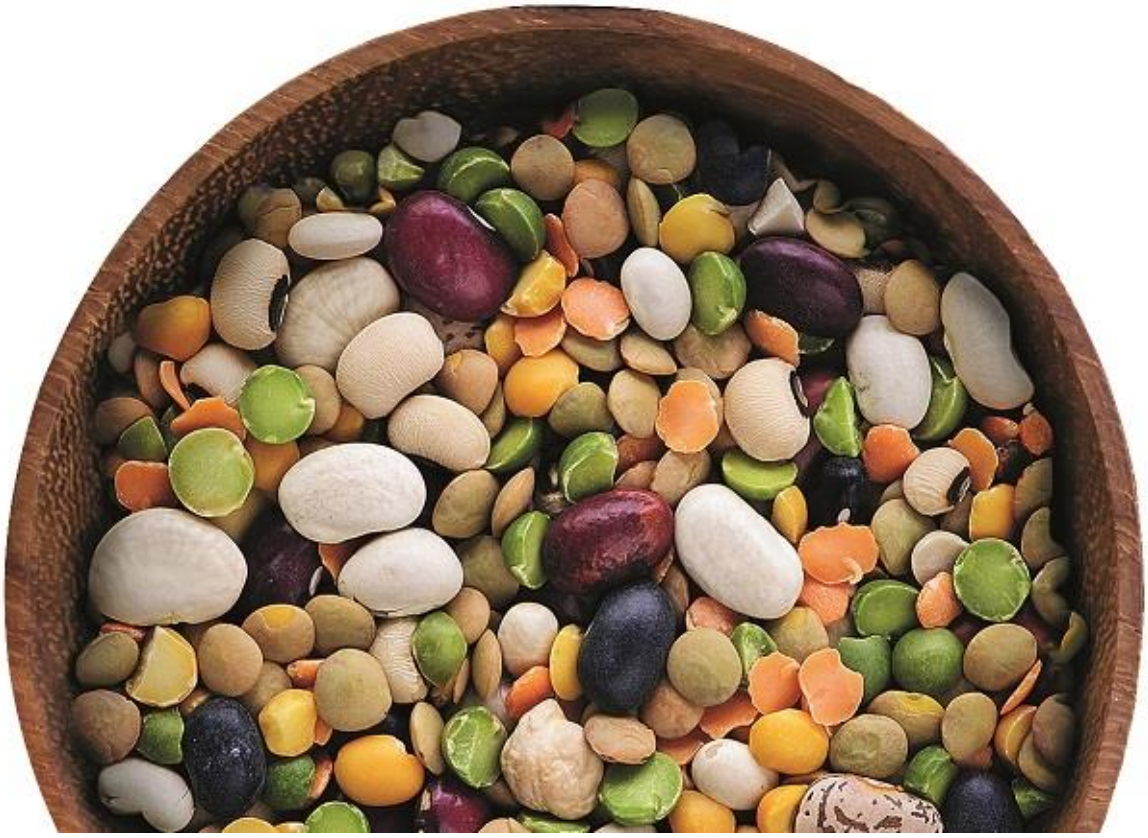

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1 Summary Information

1.1 Partner Summary

SOP Code	EU_TRUE_SOP_069
TRUE Partner Acronym	ADL
Primary Author	Black, Kirsty (*kirsty.black@arbikie.com*)
Other Authors	Not Applicable
Linked Reference and Hyperlink (if available)	Not Applicable
Associated files to use with the SOP [and function]	Not Applicable

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1.2 SOP Summary

Title

Brewing with Faba Beans – Enzymes for Mash Optimisation

Brief description

In the production of beer, the processing step known as 'mashing' involves the gelatinisation of the raw materials' starch, its enzymatic degradation into simple sugars, and the separation and removal of solid material. The efficiency of this step is critical in the production of beer as the amount of fermentable sugar produced directly impacts the alcohol content of the end beer. Preliminary experiments have found, however, that when faba bean accounts for a high proportion of the beer recipe the efficiency of this step is negatively impacted with slow solid removal and reduced yields.

In addition to starch, other polysaccharides, such as β-glucans and arabinoxylans are also broken down during the mashing process which can cause both processability issues, such as poor filtration and reduced yields, and final product issues through haze formation. The addition of commercial enzymes is an established practice to increase levels of fermentable sugars, nitrogen availability and wort run off rate.

This SOP provides a method to assess the impact of enzymatic additions on mash viscosity and wort run off rate. A rheometer method has been developed to recreate two typical mashing regimes. Samples are milled to produce a fine grist. A fixed amount of the grist is then used to form a slurry with a calculated weight of water with enzyme additions and run on a pre-set rheometer programme. The programme consists of an initial rapid stir followed by continuous stirring as one of two potential mashing temperature regimes are followed. The slurry is then filtered, and the volume of filtrate recorded over time.

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2 Protocol Steps

1. Experimental Design

A full two-level factorial design was employed to study the effect of mashing regime, protease and carbohydrase additions on viscosity development during gelatinisation of the starch as well as the wort run off rate.

2. Preparation of Samples

- All sample material should be milled to a particle size of less than 0.1 mm.
- The moisture content of each flour sample must be determined prior to analysis to ensure accurate standardised results as these are used to calculate the ratio of flour to water to be used.
- The moisture content of the flours (minimum of 0.4 g) was established by a gravimetric method using a Mettler-Toledo LJ16 infrared dryer (Mettler-Toledo GmbH, Laboratory & Weighing Technologies, CH-8606 Greifensee, Switzerland).

3. Sample Weight Calculation

- 3.0g +/- 0.01g flour and 25.0 +/- 0.1mL distilled water is required to run one experiment. Under normal circumstances, however, the amount of sample is adjusted to take account of the moisture present within it as follows:

Weight of flour to be used (g) = M2 = (100 – 14) x M1 / (100 – W1)

Volume of water to be used (mL) = W2 = 25.0 + (M1 – M2)

M1 = sample weight of the material to be tested (3.0g +/- 0.01g as is)
M2 = sample mass corrected to 14% moisture (g)
W1 = actual moisture content of the sample (% as is)
W2 = corrected water volume (mL)

4. Enzyme Additions

- Although this SOP can be used to assess the impact of any enzymatic additions the functional characteristics of the commercial enzymes which were currently selected to improve saccharification, liquification and run off rate of the wort(s) are presented in Table 1.
- The enzymes used include: an α-amylase, for the liquefaction of starch *via* hydrolysis of 1,4-alpha glucosidic linkages; an amyloglucosidase, for the saccharification of liquefied starch through hydrolysis of 1,4- and 1,6- alpha glucosidic linkages; a endoprotease, for

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the hydrolysis of peptide bonds; and, a mixed carbohydrase, for the breakdown of cell wall material.

Table 1 Functional characteristics of the commercial enzymes (sourced from Novozymes A/S, Copenhagen, Denmark).

Brand Name	Enzyme Type	Source	Activity[1]	Optimum Temp. (°C)	Optimum pH
AMG 300 L	Amyloglucosidase	*Aspergillus niger*	300 AGU mL^{-1} [1]	75	4.0
Neutrase 0.8 L	Metallo endoprotease	*Bacillus amyloliquefaciens*	0.8 AU g^{-1}	40-45	5.5 – 6.0
Termamyl 120 L	α-amylase	*Bacillus licheniformis*	120 KNU(S) g^{-1}	85-95	6.0 - 6.5
Viscozyme L	Mixed carbohydrase = arabanase, β-glucanase, hemicellulose, xylanase	Strain of the *Aspergillus* group	100 FBG g^{-1}	45-65	4.0 – 6.0

5. Rheometer Profile

- The profiles for measurement of legume flour samples using a Discovery Hybrid Rheometer (TA Instruments) fitted with a Starch Pasting Cell is outlined in Table 2.

Table 1. Mashing regimes for faba bean kernel flour: (a) stepped temperature regime and (b) simple temperature regime.

a) '*Stepped*' mashing (temperature) regime

Step Time (hh:mm:ss)	Cumulative Time (hh:mm:ss)	Temperature (°C)	Rotor Speed (rad/s)
00:00:00	00:00:00	40.0	0
00:00:12	00:00:12	40.1	50
00:03:00	00:03:12	40.2	16
00:09:08	00:12:20	94.9	16
00:04:00	00:16:20	95.1	16
00:05:20	00:21:40	64.0	16
00:02:00	00:23:40	63.9	16

b) '*Simple*' mashing (temperature) regime

Step Time (hh:mm:ss)	Cumulative Time (hh:mm:ss)	Temperature (°C)	Shear Rate (rad/s)
00:00:00	00:00:00	64.0	0

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00:00:12	00:00:12	64.1	50
00:05:14	00:05:26	94.9	16
00:04:00	00:09:26	95.1	16
00:05:20	00:14:46	64.0	16
00:02:00	00:16:46	63.9	16

6. Running the programme

- Measure the desired volume of water, calculated as described in section 2, into the test cell.
- Allow the test cell and water to preheat to 40°C for the stepped regime or 64°C for the simple regime.
- Weigh out the desired quantity of test material, calculated as described in section 2, into a weigh boat and tip into the test cell.
- Add the necessary enzyme additions.
- Lower the rotor and initiate the programme *per* the operating software's instructions.

7. Analysis of Rheometer Results

Once the programme has finished, the profiles can be displayed, compared and data extracted *per* the operating software's instructions.

8. Filtration Rate

- Following completion of the rheometer programme the contents of the test cell is filtered through Whatman No. 1 filter paper, and the volume of filtrate collected recorded at 5 minute intervals for a period of 30 minutes.

9. Analysis of Data

The rheometer and filtrate collection data were analysed for statistically significant impact using a statistical software package.

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3 Linked SOPs

Not Applicable

4 Disclaimer

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6 Citation

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