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Chapter 5

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Introduction

Faba bean (*Vicia faba* L. minor, also known as field beans) are a grain legume species with great potential in terms of the capacity to improve human and animal nutrition as they are rich in starch, protein and minerals (Fig.1). Importantly, they support more sustainable crop systems as they can be cultivated without nitrogen fertilizers.

Furthermore, their potential to help safeguard the production environment extend to decreasing greenhouse gas (GHG), emissions and improving soil carbon (C) and N assimilation. The increased soil fertility reducing the inorganic N-fertiliser requirement ('N-fertiliser offset'), of non-legume crops which follow the legume in the rotation. Thus, improved agricultural efficiency that is achieved *via* grain legume supported cropping may lead to less non-renewable energy use. The following list also summarises some of the agronomic and sustainability benefits of faba bean cultivation (Drinkwater, 1998; Iannetta et al., 2013):

- There is varietal diversity among faba bean and they are cultivated globally

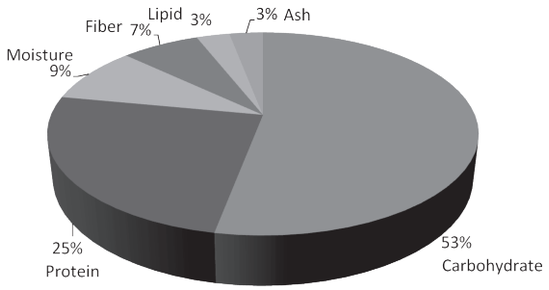

Figure 1. The nutritional content of faba beans (*Vicia faba* L., minor, field beans) (Larralde and Martinez, 1991)

- Faba beans do not normally require man-made fertilisers, as their N requirement met by BNF fixing up to 200 kg N/ha.
- Soil N left after legume harvest can be used by cereals, reducing fertiliser requirement by around 60 kg N/ha.
- Faba beans have a tap root that penetrate deep into the soil, leaving their C and N benefits throughout the soil profile.
- Yields of the non-legume which follows faba bean in the rotation is increased (up to 25% higher).

The dehulled faba beans (kernels) can be fractionated into protein and starch-rich components, using *air classification*. This procedure (Fig. 2) employs a vertical cyclonic air stream to separate large/heavy starch granules from lighter protein bodies (Shapiro and Galperin, 2005). The latter are exploited as an important additives to salmon feed and the former may be used as feed for pigs and poultry. Thus, air classification could lead to sustainable provision of a bean protein concentrate and bean starch concentrate (BPC and BSC, respectively), for inclusion within animal and aquaculture feeds (Stoddard et al., 2013). While hulls can still be successfully employed for ruminant feeding, bean starch offers high potential to improve human wellbeing since it may be regarded as "resistant" to digestion compared to starches from cereals grains (Al-Rabadi et al., 2013), lowering glycaemic indices and encouraging the offset of diabetes. In addition, BSC may also be considered as an alternative source of fermentable substrate for brewing and distilling.

The current research evaluated the suitability of whole faba beans (WFB) and BSC as an adjunct with malted barley grist (MBG) for bioconversion to ethanol. In addition, the use of endogenous enzymes was also tested in experimental mashings. Our

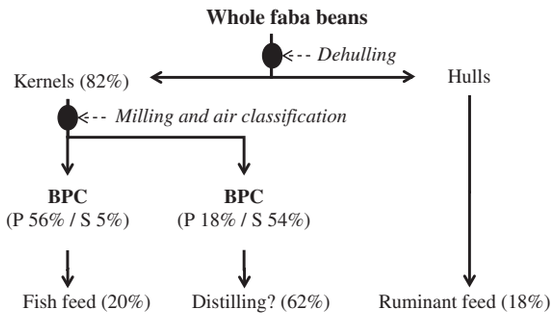

Figure 2. A schematic diagram of the possible fates of fractioned whole faba bean components following air classification. The percentages denote proportions of the faba bean starting weight. P and S denote protein and starch content (%), respectively. [BSC = bean starch concentrate; MBG = malted barley grist]

findings have revealed that faba starch is relatively easily saccharified to fermentable sugars for alcohol fermentations.

Materials and methods

MBG (Crisp Malting Group, Fakenham, UK) was mixed with either WFB grist/flour (milled using a common kitchen blender) or BSC produced by air-classification.

Mashing

WFB or BSC was combined with MBG (50 g, 2:3 [w/w], respectively), and mixed with warm (65 °C) deionised water (1:5, [w/v]) in a 500 ml Erlenmeyer flask. For comparative purposes, we also assessed WFB faba grist only (100%) with commercial amylolytic enzymes and MBG only (see Table 2).

The samples were incubated with shaking at 140 rpm and 65 °C for 1 h. After the mashing, samples were cooled rapidly by immersion in an ice bath for 10 min and then centrifuged for 5 min at 4400 rpm. Supernatant worts were retained for experimental fermentations.

Table 1. Starch, protein, lignin and moisture content of faba bean fractions

	BSC	*WFB*	*MBG*
Moisture Content (%)	12.99	8.44	11.73
Total Starch (%)	52.43	42.80	59.20
Total Protein (%)	16.89	29.37	11.20
Total Lignin (%)	0.00	15.13	19.4*

(* Denotes data from Meneses et al., 2013)
[BSC = bean starch concentrate ; WFB = whole faba bean ; MBG = malted barley grist]

Fermentation

Fermentation of the worts (49 mL) was conducted in 250 mL laboratory glass bottles (Schott, DURAN Group, Germany) using 1 mL of 5×10^6 yeast culture (*Saccharomyces cerevisiae* DCLM, a yeast strain used by commercial Scotch whisky production; Kerry Ltd., Menstrie, UK), which had been freshly prepared before pitching the wort (Table 3). Three different fermentation systems were conducted: Conventional (C), using malt in the mash as the source of amylolytic enzymes; Traditional (T), using α-amylase and glucoamylase in the mash followed by wort centrifugation and fermentation; and Simultaneous Saccharification and Fermentation (SSF), using α-amylase in the mash followed by glucoamylase at the start of the fermentation. SSF was designed to facilitate slow release of glucose for the yeast during fermentation.

Subsequent to the inoculation, the glass bottles were placed in a shaking incubator (140 rpm) at 32 °C for 30 h.

Table 3. Optimal temperature, pH and yeast inoculation density for experimental fermentations using *Saccharomyces cerevisiae* distiller's strain DCLM

	DCLM Strain
Inoculum (cfu)	5x106
Temperature range (°C)	30 - 35
pH range	5.0 - 6.0

Table 2. pH and temperature ranges of exogenous enzymes used to digest either milled-whole faba bean (WFB) or -bean starch concentrate (BSC) during mashing.
The concentration at which the enzymes [w/w] with raw solid/test material is noted.

	Type	*pH range*	*Optimum temp. range*	*% added [w/w]*
HITEMPASE® 2XL	endo-α-amylase	5.5 – 7.0	65 – 92 °C	0.1
AMG®	exo-1,4 α-D-glucosidase (glucoamylase)	4.0 – 5.0	55 – 65 °C	0.0012

Chemical analyses

Original Gravity (OG), carbohydrates and ethanol contents – the original gravity of each sample and the alcohol by volume levels (ABV) were calculated by using FermentoFlash 3572 (Funke Gerbe, Berlin, Germany). The sugar and ethanol contents of both worts and fermented samples were determined with high-performance liquid chromatography (HPLC), as described by White *et al* (2008). A method calibrated for glucose, maltose, maltotriose and ethanol was used. The calibration curves were built for varying concentrations of the sugars and ethanol of up to 10g/L. The cycle analysis lasted 27 minutes and it was repeated three times for each sample. In order to prepare the HPLC samples, 0.25 mL of sample and 0.75 mL of 10g/L internal standard solution of meso-erythritol (Sigma Aldrich, USA), were filtered into a 2 mL HPLC vial (Chromacol , Welwyn Garden City, UK) with a 0.45 μm syringe filter (Merck Millipore, Billerica, USA). The concentrations of the carbohydrates and the ethanol were quantified with reference to the calibration curves and the internal standard of known concentration.

Ethanol Yield (L/t) – this yield was not obtained by distillation but calculated on the basis of ethanol obtained from available starch by using the following equation:

$$\text{Ethanol yield (L/t)} = \frac{\frac{V_s}{100} * ABV_s}{TSW_s} * 1000$$

[V_S = Volume of the sample (ml); ABV_S = Alcohol by volume of the sample (%); TSW_S = Total starch weight of the sample (g)]

Total Yeast Available Nitrogen (YAN) – YAN after fermentation was determined as the sum of four principal components: the primary amino nitrogen (PAN), ammonia, urea and the side-chain of L-arginine. PAN content was determined by using the commercial assay kit K-PANOPA 07/12 (Megazymes, Bray, Ireland). The ammonia, urea and L-arginine content was determined using a commercial assay kit K-LARGE 12/12 (Megazymes, Bray, Ireland).

Results and discussion

Wort analysis

The total fermentable sugar content of wort obtained with BSC was higher than other treatments derived solely from MBG. When commercial enzymes were applied in the mash with BSC, a reduced content of maltose was obtained but this was compensated for by achieving a higher release of glucose, as shown in Table 4. Mixed with MBG, both the BSC and WFB released a mixture of fermentable sugars that was similar quantitatively and qualitatively to that of MBG alone. Maltose was the most predominant sugar, with values of 49 and 44 g/L being released from BSC and WFB mashes, respectively. Maltotriose and glucose were the other resultant fermentable sugars (Table 4).

Ethanol and residual sugar

Different fermentation methods were employed, including SSF which was accomplished by delaying the addition of amyloglucosidase, until the onset of fermentation. This resulted in the release of glucose to avoid osmotic stress to the yeast and to facilitate gradual increase in alcohol production. However, ethanol yields obtained by SSF were lower than that produced by the traditional fermentation method which relied on starch saccharification in

Table 4. Glucose, maltose and maltotriose contents in worts derived from malted barley grist (MBG), bean starch concentrate (BSC), and whole faba beans (WFB). BSC and WFB were mashed at 40:60 ratios with MBG. BSC was also mashed with commercial amylolytic enzymes (see Materials & Methods). OG denotes the original gravity of the worts.

	MBG	*BSC (with enzymes)*	*BSC:MBG (40:60)*	*WFB:MBG (40:60)*
OG	1049	1042	1040	1035
Total sugars (g/L)	77.3	92.9	66.7	59.2
Glucose (g/L)	5.99	55.87	6.95	5.77
Maltose (g/L)	60.0	35.45	48.8	44.0
Maltotriose (g/L)	11.35	1.6	10.9	9.47

the mash. In addition, fermentation of WFB-derived sugars was slightly better compared with SSF conditions with projected potential spirit yields of around 300 L/t (Figure 3). Since the WFB comprise lignocellulosic material in their hulls, it is possible that the commerical enzymes employed liberated more fermentable sugars compared with the bean starch fraction (Long and Gibbons, 2012).

Ethanol yields obtained following faba wort fermentation related directly to the quantity of wort sugars analysed (Table 5). Table 5 also shows that the alcohol yield from wort from bean starch hydrolysed with commercial enzymes was very similar to that obtained from malt wort alone.

When we compared the fermentability of bean starch hydrolysates using malt mixtures with the same ratios of WFB:MBG, poorer ethanol yields were achieved. Incomplete fermentation was indicated by the high residual levels of maltose and maltotriose, possibly due to sub-optimal levels of yeast available nitrogen, YAN (Table 6)

Conclusion

When mashing WFB, or bean starch concentrate, with exogenous enzymes favourable levels of fermentable sugar were obtained. The total quantity of sugars obtained were 67 g/L and 68.6 g/L, respectively, for these two adjuncts and comprised glucose, maltose and maltotriose. Considering the levels of YAN, this was higher in wort prepared from WFB compared to the BSC and would be expected to benefit yeast fermentation performance.

Table 5. Ethanol and residual sugar contents in fermented wort derived from malted barley grist (MBG), bean starch concentrate (BSC), and whole faba beans (WFB). Mashing conditions were as described in Table 4.

	MBG	*BSC (with enzymes)*	*BSC:MBG (40:60)*	*WFB:MBG (40:60)*
Ethanol (g/L)	32.98	30.68	23.67	13.56
Glucose (g/L)	2.76	2.77	2.15	1.58
Maltose (g/L)	1.34	0.12	0.38	8.66
Maltotriose (g/L)	2.04	1.08	0.98	2.44

Table 6. Yeast Available Nitrogen (YAN) in worts. Ratios relate to the respective percentages of wort adjuncts. MBG = malted barley grist; BSC = bean starch concentrate; WFB = whole faba bean].

	MBG	*BSC (with enzymes)*	*BSC:MBG (40:60)*	*BSC:MSG (40:60) (with enzymes)*	*WFB:MSG (40:60)*	*WFB:MSG (40:60) (with enzymes)*	*WFB (with enzymes)*
YAN	51.95	92.97	88.61	67.78	113.87	110.50	79.04

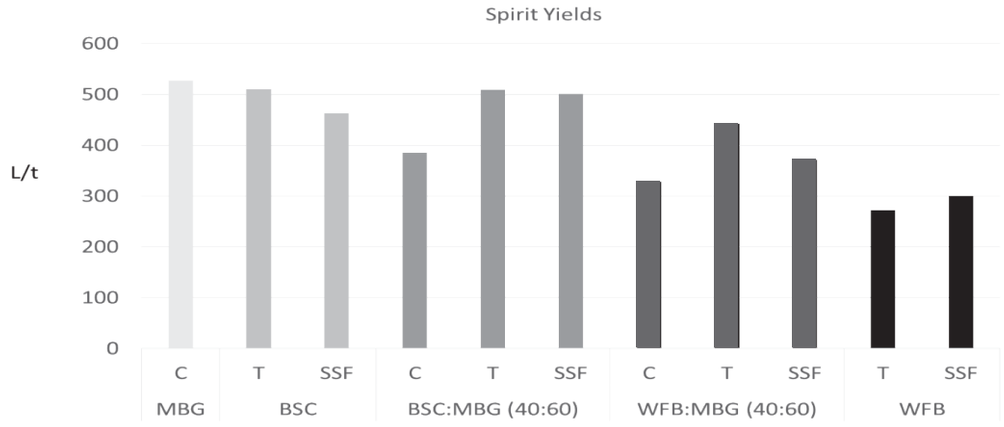

Figure 3. Potential spirit yields from faba wort fermentations calculated on basis of starch content

'C', 'T' and 'SSF' denote 'conventional', 'traditional' and 'simultaneous saccharification and fermentation', respectively (see Materials and Methods). Ratios relate to the respective percentages of wort adjuncts. MBG = malted barley grist; BSC = bean starch concentrate; WFB = whole faba bean]

The use of BSC as an adjunct, with malt, in distillery mashes (eg. for neutral spirit production) resulted in projected spirit yields comparable to those obatined solely with MBG. When the bean starch was saccharified only using exogenous enzymes, the alcohol yield (calculated on the basis of starch content) was slightly lower at 510 L/t, compared to 527 L/t for malt alone. Mashings of BSC with enzymes requires further refinement, but implementation of the legume in distilled spirit mashes is an attractive proposition, even at ratios above a 40% inclusion .

Cereals such as barley, wheat, maize and rice are employed by distillers, but increasing costs and global food insecurity are leading to consideration of more sustainable crops. Legume grains, and in particular faba bean starch concentrates, are becoming increasingly attractive to processors in the agri-food chain and this includes the distilled spirits sector. The results presented here indicate that this interest may translate to the efficient production of alcohol using grain legume based feedstocks which help to support sustainable crop systems.

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