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Maximizing Alcohol Yields from Wheat and Maize and their Co-products for Distilling or Bioethanol Production

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Abstract

The key to optimising alcohol production from cereals is a full understanding the physiology and processing characteristics of different cereals. This study examined the maximum alcohol yields that can be obtained from wheat and maize using different processing technologies. Lower processing temperatures (85°C) resulted in high alcohol yields from wheat (a temperate crop), whereas higher processing temperatures (142°C) gave maximum alcohol yields from maize (a tropical crop). Similar trends were also observed when the spent grains from these cereals were subjected to cellulolysis using commercial enzymes. Mill settings were additional factors in influencing alcohol production. Wheat has the potential to produce higher alcohol yields when compared with maize, when residual biomass (i.e. spent grains) saccharification using selected commercial enzymes is taken into account. While this is approach is not applicable for the Scotch whisky industry due to strict legislation forbidding use of exogenous enzymes, this is pertinent for bioethanol production- to increase the alcohol yield obtained from both starch and lignocellulosic components of whole cereal grains. Wheat and maize processing temperatures and the use of processing aids are of potential economic benefit to bioethanol producers and to beverage alcohol producers seeking to understand the factors influencing the processing properties of different cereals.

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Keywords

Wheat, Maize, Alcohol Yields, Starch processing

Introduction

The production of grain whisky has many parallels with the technology that is used in some bioethanol distilleries. Both products can be produced from starch-based substrates originating from cereals such as wheat and maize (corn). Many other cereals can be used for both bioethanol and whisky production and at the Scotch Whisky Research Institute (SWRI) work has been carried out on a range of different cereals such as wheat, maize, sorghum and millet to show how their properties can be used to optimize alcohol yield (*1*; *2*). Under the legal definition of Scotch whisky (*3*), the use of commercial enzymes or other additives to increase alcohol yield and process efficiency are strictly forbidden, and process improvements must be derived from a better understanding of the physiological and processing characteristics of different cereals. However, distillers are also interested in the production of neutral spirits (Grain Neutral Spirit (GNS)), which is used for non-Scotch whisky products such as vodka and gin, which are not subject to these constraints. Similarly, the production of bioethanol is free from these requirements and a much wider range of options of both technologies and substrates are available, so that bioethanol producers can use many suitable raw materials and process aids for different alcohol production processes. In the longer term, there is interest from Scotch whisky distillers in evaluating the potential of alternative cereals and residual plant materials or the co-products deriving from them for additional production streams, such as biogas production, which may also be relevant to bioethanol production (*4*; *5*). Since such treatment of co-products such as spent (or dark) grains (distillers dark grains with solubles (DDGS)) occurs after they are removed from the Scotch whisky production process, they can be further processed without restrictions, within the requirements of the end-user market for these co-products, which is primarily for animal feeds, but could also potentially be used for bioethanol production.

Bioethanol is derived from the microbial fermentation of biomass to produce fuel alcohol (*6*), which is chemically identical to synthetically produced ethanol by the petrochemical industry. Recently, emphasis has been placed on partial or total replacement of energy derived from fossil fuel to bioethanol derived from plant materials (*7*) and global production is dominated by processes using maize in USA and sugarcane in Brazil (*8; 9*). Ethical concerns are raised when bioethanol derived from cereals (wheat, corn, sorghum, millet) and tubers (such as cassava, yam and potato) leads to direct conflict with food production (*10; 11; 12*). In order for a biofuel to be sustainable it should have a net energy gain, provide environmental benefits, be economically competitive and be capable of being produced on large scale without disrupting agricultural food crops (*13*). It is also essential that a sustainable bioethanol process will not upset the balance between the greenhouse gas emissions generated as the carbon released by the burning of plant derived fuels, and those which can be captured by growing plants (i.e. carbon neutral). The production of biofuels from more diverse feedstocks, for example, based on co-products such as cellulosic agricultural residues and brewer's and distiller's spent grains will eventually offer more sustainable alternatives to cereals and other food crops, and should help to ameliorate food versus fuel arguments (*14*). Commercial scale production of cellulosic ethanol is now a reality with, for example, Beta Renewables plant now operational in Italy, and several US plants on stream in 2014 (Lane, 2013). It is estimated that other large scale production plants capable of producing 50,000 – 150,000 tonnes of cellulosic ethanol will be built in 2013/14 (*15*). In Germany an estimated 22 million tonnes of straw could be used to meet around 25% of Germany's current petrol requirements (*15*). In the UK, nine bioethanol plants that are likely to use wheat as raw material have been planned, each with a potential total capacity of approximately 2 x 10^6 tonne bio-ethanol per annum (*16; 17*). There are at present three bio-ethanol plants in the UK – 'British Sugar' in Wissington, 'Ensus' (now owned by Crop Energies) in Teeside and 'Vivergo' in Hull. The latter two plants each can potentially produce 400 million litres of bioethanol per annum (*18*). Although previous studies on the feasibility of bioethanol in the UK concluded that production costs were uncompetitive compared with petrol (*17*), the economics have been gradually improving over the last two decades with the use of improved commercial enzymes to liberate fermentable sugars.

Agu *et al.* (***2***) studied the effects of starch liberation and breakdown conditions and observed that wheat can produce a significantly higher spirit yield when processed at a lower temperature of 85°C, compared with the higher cooking temperatures required for other cereals such as maize which will normally produce its maximum alcohol yield when processed at much higher temperatures (typically 142°C – 145°C). This observation was in agreement with other studies focussed on bioethanol production (***19; 20; 21***). These studies confirmed that the processing procedure should be tailored to the cereal being used. The lower processing temperature of wheat also lowered the residue viscosity which causes problems in downstream recovery of the distillery co-products (**2**). The higher viscosity of wheat is due, in part, to the presence of high levels of pentosan (8% w/w) compared to lower levels (3% w/w) in maize, sorghum and millet (Palmer, 1989). In particular, pentosans such as arabinoxylans and other polymers such as β-glucans are known to cause processing problems (***22; 23; 24; 25; 26; 27***).

The present study was undertaken to investigate the effects of process conditions and enzyme processing aids on maximising alcohol yields from wheat and maize starch and lignocellulose (in the form of spent grains).

Materials and Methods

Cereal samples

Samples of cereals (wheat and maize) were obtained from two sources (Ii) soft, low nitrogen wheat (cv Viscount) from a trial site producing wheat for assessment for Scotch grain whisky production; (II) a commercial yellow maize sample (variety not specified) obtained from a Scotch whisky grain distillery.

Alcohol yield from wheat and maize flours (142°C process)

The following procedure based is on the work of **Brosnan *et al*** (***28***) and described fully by **Agu *et al*** (***1***) and simulates the production process in a "typical" Scotch whisky grain distillery. Cereal flour

(30g), obtained by milling the grains in a Buhler Miag disc mill set at either 2 (0.2mm), or 11 (1.1mm) was transferred into a stainless steel mashing beaker and slurried with water (81ml), and 25µLof Termamyl 120L Type L (a bacterial α-amylase supplied by Novozymes France S.A.) was added. This was slowly heated up to 85°C (temperature rise 2°C /min) in a water bath, before pressure cooking in an autoclave, temperature programmed with a maximum temperature of 142°C for 15 minutes. The cooked slurry was cooled to 85°C and given a second treatment with Termamyl (25µL) for 30 min to prevent starch retrogradation. The mash was then transferred to a water bath at 65°C and mashed for 1 hour with a predetermined amount of high enzyme grain distilling malt grist (Miag mill setting 2 (0.2mm) and 11 (1.1mm)), equivalent to a malt inclusion rate of 20% dry weight basis (dwb) to 80% wheat. After cooling to room temperature, the mash was pitched with distiller's yeast (*Saccharomyces cerevisiae* 'M' type, supplied by Kerry Ingredients & Flavours) at a pitching rate of 0.4 (w/w) pressed yeast, transferred to a fermentation vessel and the weight adjusted to 250g with water. The mash was then fermented at 30°C for 68 hours and distilled to collect the alcohol. The alcohol yield was determined from the alcohol strength of the distillate, which was measured using an Anton Paar 5000 density meter. The alcohol yield was quoted as litres of alcohol per tonne (LA/t) on a dry weight basis (dwb).

Alcohol yield from wheat and maize flours (85°C process)

The procedure is similar to that described above except that the pressure cooking step where the slurry was transferred to the autoclave, as well as the second treatment with Termamyl was bypassed.

Alcohol yield from wheat and maize spent grains (SG) (142°C process)

The procedure was similar to that described above for the cereal flour, except that the cereal spent grains (30g), were slurried with acidified water (0.005M H_2SO_4) (130mL) rather than distilled water, and 25µL of Termamyl 120L Type L (a bacterial α-amylase supplied by Novozymes France S.A.) and heated up to 85°C before pressure cooking in an autoclave at 142°C for 15 minutes. The cooked slurry was transferred to a 85°C water bath and given a second treatment with Termamyl (25µL) for 30 min to prevent starch retrogradation, after which the mash was then transferred to a water bath at 65°C and incubate for 1 hour with the addition of 25µL each of the commercial enzymes Bioglucanase ME 250, Bioprotease NL 100 and Promalt 4TR (supplied by Kerry Bioscience Ltd). The dose rate for all the enzymes is shown in **Table 1.** After cooling to room temperature, the mash was pitched with distillers yeast and then fermented as described earlier. The alcohol yield was also determined from alcohol strength of the distillate as described above.

Alcohol yield from wheat or maize spent grains (SG) using cellulosic enzymes

The alcohol yield from spent grains using cellulosic enzymes is essentially as described for the alcohol yield from milled cereal spent grains except that the commercial enzymes Bioglucanase ME 250, Bioprotease NL 100 and Promalt 4TR were replaced with Cellic HTech and Cellic CTec (cellulosic and xylan hydrolysing enzymes, supplied by Novozymes France S.A.) and the mashing time was extended overnight to 24 hours. The effect of the addition of the commercial enzymes at different stages, before (upstream) and after (downstream) cooking, was also investigated using maize spent grains.

Rheological properties of the spent grains using Rapid Visco-Analysis (RVA)

The physiological properties of the spent grains were studied using a Newport Scientific Rapid Visco Analyser (RVA) instrument supplied by Calibre Control. The Rapid Visco-Analyser is a rotational, continuously recording viscometer, with heating, cooling and variable shear capabilities, specifically configured for starch-based materials. The aim was to confirm that limited or no starch was present in the spent grains, and the alcohol yield was obtained from the spent grains. Here, a slurry of milled cereal spent grain (approximately 3.0g spent grain and a measured amount of water or acid total weight 28g) with water or 0.005M sulphuric acid (H_2SO_4) was processed in the RVA analyser using a programme designed for un-malted cereals (*29; 1*).

Results and Discussion

Results shown in **Tables 2** and 3 verify the precision and the robustness of a high temperature pressure cook for the determination of alcohol yield from cereals. The results presented in **Tables 2 and 3**, were obtained when the Miag mill was set at 2 (0.2mm) to produce finely milled flour from the cereals. **Table 2** shows the results obtained when wheat and maize were processed at the higher temperature of 142°C. This represents the traditional, high temperature process used for grain whisky production. **Table 3** shows the results obtained when the same wheat and maize samples were processed at a lower temperature of 85°C which represents a potential way of reducing the energy requirement for the process. Alcohol yields obtained from each of the cereals were reproducible whether the cereals were processed at either 142°C or 85°C, and confirm the reliability and robustness of the method.

When both cereal types were processed at the higher temperature of 142°C, wheat gave a much lower alcohol yield compared with maize. The differential of at least 15 litres alcohol per tonne is fairly typical for wheat and maize. On the other hand, when both cereal types were processed at the lower temperature of 85°C, maize gave a much lower alcohol yield than wheat. This is because maize requires higher temperatures than wheat to fully gelatinise the starch (*1; 30*). These results are consistent with observations by other researchers (*19; 20; 21*). In order to establish the maximum extractable alcohol yield potential from wheat and maize, the spent grains (after initial processing of the cereal flours) were re-processed using various commercial enzyme preparations (Bioglucanase ME 250, Bioprotease NL 100 and Promalt 4TR). **Table 4** shows alcohol yields obtained from the spent grains when re-processed at temperatures of 142°C or 85°C. The results showed that maize spent grains yielded more alcohol than wheat spent grain when they were processed at 142°C in the presence of the enzyme mixture. In contrast, at the lower temperature wheat spent grains gave a much higher alcohol yield (84 litres of alcohol /tonne), almost 5 orders of magnitude, compared with the maize spent grains. This observation is important because it shows that the additional substrate necessary to generate the increased alcohol yield from wheat and maize is present in the spent grains, although the maize spent grains contain much less available substrate than wheat at 85°C.

When the total alcohol yield from maize flour and spent grains processed at 142°C were combined, the overall alcohol yield was much higher than that for the wheat flour and spent grains (**Figure 1**), by about 24 litres of alcohol per tonne. This would suggest that the high temperature has released the starch more efficiently from the maize kernel (endosperm) reflecting the generally higher starch content of maize. It is well known that maize requires more vigorous processing conditions for optimum performance in the distilling industry, while wheat needs less severe conditions to efficiently extract and solubilise the starch. Again, as well as its starch having higher gelatinization temperature compared with wheat, maize can be prone to developing resistant starch during processing. In contrast, the gelatinization temperature of wheat starch is much lower (***30*****;** ***1*****;** ***2***).

The opposite trend was seen at the lower processing temperature (85°C), for wheat yielded about 80 litres of alcohol per tonne more than for maize (see **Figure 1)**. The difference between the relative contribution of the components of the wheat and maize spent grains (about 70 litres of alcohol per tonne) accounted for a large proportion of the differential between the combined alcohol yields of the flour and spent grains components. This shows that at the lower temperature of 85°C wheat has a bigger ‘untapped’ substrate in the spent grains (e.g. as cell wall materials), compared with maize. In wheat these can be released at the lower temperature by the combination of enzymes that were used. The lower temperature process would also be expected to produce fewer Maillard reaction products, which are known to significantly reduce alcohol yield. Therefore, there is additional potential for wheat to produce more alcohol if the processing conditions are adjusted to take account of both flour and spent grains. This is an important observation because it shows that during “normal” processing of wheat flour, materials residing in the spent grains were limiting alcohol production. Such materials may also be responsible for higher residual viscosities found when processing wheat compared with maize (*1; 2; 31*). When the spent grains were re-processed using commercial enzyme preparations at 85°C, over 84 LA/t (dry basis) of extra alcohol yield was obtained, which indicates that wheat spent grains have potential for bioconversion to bioethanol. We additionally investigated the effects of using acidified process liquor and adding enzymes at different stages. The results in **Table 5** (for maize spent grains) show that the effect of adjusting the process liquor to give a low acid concentration (0.005M H_2SO_4) gave higher alcohol yields from spent grains when the enzymes were added at the downstream process (after cooking) rather than upstream process (before cooking). The higher acid concentration (0.05M H_2SO_4) was less effective in this regard

Particle size of milled cereal samples also influenced subsequent alcohol yields. The results discussed above were obtained when the 'fine' Miag mill setting 2 (0.2mm) was used to give flours with small particle sizes. However, in many industrial processes, grains are bruised or 'cracked' rather than finely hammer milled. Bruised grains will produce very coarse grists, depending on the mill settings. We evaluated alcohol yields from coarse (1.1mm Miag mill setting) versus finely milled (0.2mm) wheat and maize and the results are shown in **Table 6**. Average alcohol yields obtained from coarse wheat and maize grist were higher than those obtained for the fine flour when both cereal types were processed at the higher temperature of 142°C. The higher alcohol yields from coarse-ground wheat or maize did lead to lower residual alcohol recoverable from the respective spent grains. A similar effect was also found when the cereals were processed at 85°C. Coarse milling is preferred in industrial bioprocesses to avoid the problem of "balling" when the cereal is slurried prior to cooking in the distillery. This phenomenon can reduce the hydration of the starch and prevent it from being released properly. Whilst, under ideal conditions, fine milling would result in larger surface areas to provide easier access to enzymes, in practice some potentially fermentable material would be lost through Maillard reactions during the cooking process. In contrast, the smaller surface area provided by coarse milling may limit loss of sugars and amino acids by Maillard reactions, and at the same time release gelatinized starch for enzymolysis during the mashing process to improve yeast fermentation performance.

It was important to establish that alcohol obtained from spent grains of processed wheat and maize was not produced from residual starch To confirm this, pasting properties of the spent grains were analysed using a rapid visco-analyser (RVA) using 0.005M H_2SO_4 which would release some fermentable non-starch polysaccharides (***31***). The results obtained for maize spent grains are shown in **Figure 2** and **Figure 3** for wheat spent grains. Both RVA profiles show that the peak and final viscosities were effectively zero, indicating the absence of starchy materials after processing. These results confirm that the additional alcohol yields obtained from the cereal spent grains emanated from lignocellulosic-derived sugars following pretreatment using dilute acids and hydrolysis with cellulolytic enzymes

Conclusions

This investigation has highlighted that understanding the physicochemical properties of different cereals used in industrial processes is the key in optimising alcohol yields, both from starch in the grains and lignocellulose in the spent grains A relatively low processing temperature (85°C) was found to be sufficient to obtain a high alcohol yield from wheat (a temperate crop), while a higher processing temperature was required to produce optimum yields from maize (a tropical crop). The study further showed that cereal mill settings influenced alcohol yields, which is pertinent for both potable spirits such as Scotch whisky as well as bioethanol. When the spent grains from these cereals were processed using commercial enzymes at the higher and lower temperatures, there were significant differences between wheat and maize. When the residual lignocellulosic material in spent grains is taken into account, then wheat has the potential to a produce a higher alcohol yield compared with maize. However, this necessitates wheat spent grains being treated under more vigorous processing conditions, including acid pretreatment and hydrolysis using commercial enzymes. This is currently prohibited for Scotch whisky production but co-products such as spent grains could be re-processed into neutral spirits or bioethanol that are not directed at the Scotch whisky market. The bioethanol industry is not faced with such restrictions and our findings may prove beneficial with regard to maximizing alcohol yields from whole cereals when correct mill settings, processing temperatures and enzymes are adequately combined.

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Table 1

Commercial enzyme preparations and dose rate used in this study

Commercial enzyme	Type	Supplier	Dose rate
Termamyl 120L	Heat-stable α-amylase	Novozymes France S.A.	25 µL
Promalt 4TR	α-amylase	Kerry Bioscience Ltd	25 µL
Bioprotease N100L	Proteolytic enzyme	Kerry Bioscience Ltd	25 µL
Bioglucanase ME 250	Endo-beta glucanase	Kerry Bioscience Ltd	25 µL
Cellic Htech	Cellulase Complex	Novozymes Denmark	25 µL
Cellic Ctec	Endoxylanase	Novozymes Denmark.	25 µL

Table 2

Alcohol yield results of wheat cooked at 142°C - High Temperature (HT) (Miag mill setting 2)

Wheat (cv Viscount) / Maize (unspecified variety)	Weight of sample flour	Alcohol yield (LA/t) dry	Average alcohol yield (LA/t) dry
Wheat 1	29.9999	448.71	
Wheat 2	30.0000	449.39	
Wheat 3	29.9998	448.09	
Wheat 4	29.9999	445.03	
Wheat			447.81
Maize 1	30.0003	463.08	
Maize 2	29.9998	465.49	
Maize 3	30.0003	464.86	
Maize 4	29.9998	463.21	
Maize 5	29.9996	461.67	
Maize			463.66

Table 3

Alcohol yield results of wheat cooked at 85°C - Low Temperature (LT) (Miag mill setting 2)

Wheat (cv Viscount) / Maize (unspecified variety)	Weight of sample flour	Alcohol yield (LA/t) dry	Average alcohol yield (LA/t) dry
Wheat 1	29.9999	459.13	
Wheat 2	29.9996	454.32	
Wheat 3	30.0000	459.74	
Wheat			457.73
Maize 1	30.0000	443.03	
Maize 2	30.0003	441.06	
Maize 3	30.0003	441.43	
Maize 4	29.9999	441.06	
Maize			441.65

Table 6

Alcohol yield (LA/t) dry obtained from spent grains at different processing temperatures (142°C and 85°C)

	Alcohol yield (LA/t) dry 142^{o}C	85^{o}C
Wheat spent grains	36.13	83.93
Maize spent grains	43.60	17.60

Table 7

Effect of adding 0.005M and 0.05M Sulphuric Acid (H_2SO_4) at different stages of stage of enzyme addition on the alcohol yield (LA/t) from maize spent grains. (Pre-cooking = upsteam; Post cooking =downstream)

Stage of Enzyme Addition	Weight of spent grain sample (g)	Acid concentration	Alcohol yield (LA/t) dry
Upstream	29.9999	0.005M H_2SO_4	8.33
Downstream	30.0000	0.005M H_2SO_4	23.53
Upstream and downstream	30.0002	0.005M H_2SO_4	18.53
Upstream	30.0000	0.05M H_2SO_4	0.00
Downstream	30.0003	0.05M H_2SO_4	1.53

Table 8

Alcohol yield (LA/t) dry obtained from wheat and maize flour and spent grains at different cook temperatures and a coarse mill setting (Miag setting 1.1 mm)

	Alcohol yield (LA/tonne) dry 142^{o}C cooking temperature			Alcohol yield (LA/tonne) dry 85^{o}C cooking temperature		
Sample Fraction	Flour	Spent grain	Total	Flour	Spent grain	Total
Wheat	460.11	15.13	475.24	448.98	11.67	460.65
Maize	474.05	16.27	490.32	429.73	8.20	437.93

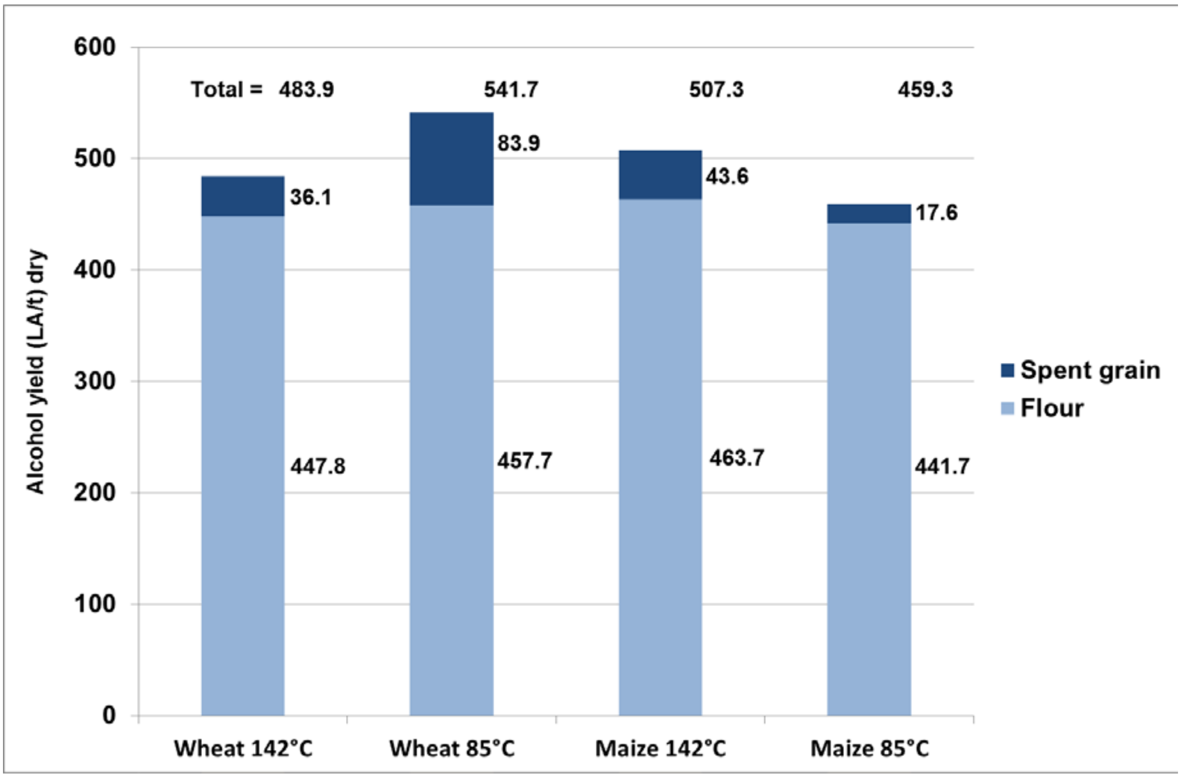

Figure 1

Combined spirit yields obtained from wheat and maize flours and respective spent grains (Miag mill setting 0.2mm) processed at 142°C and 85°C

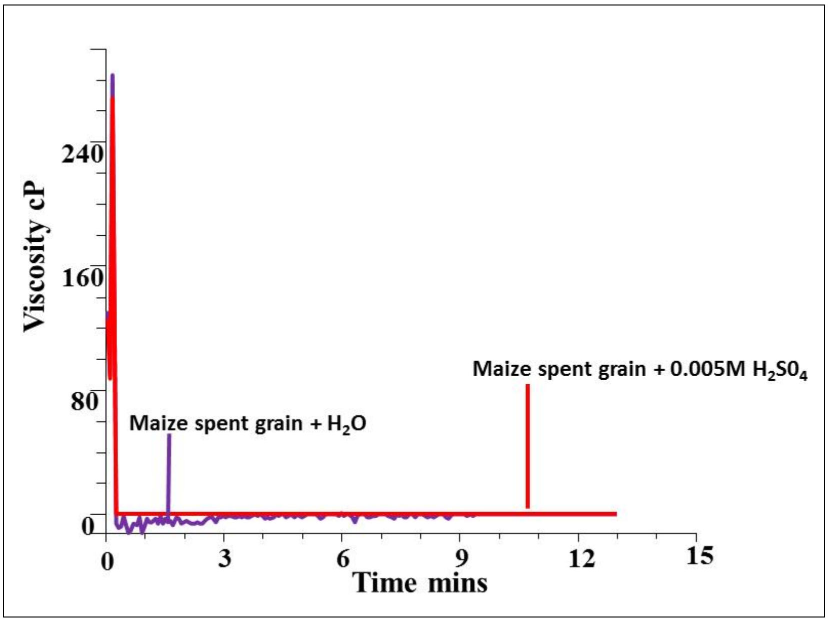

Figure 2

RVA pasting profile of maize spent grain using water or 0.005M H_2SO_4 as process liquor

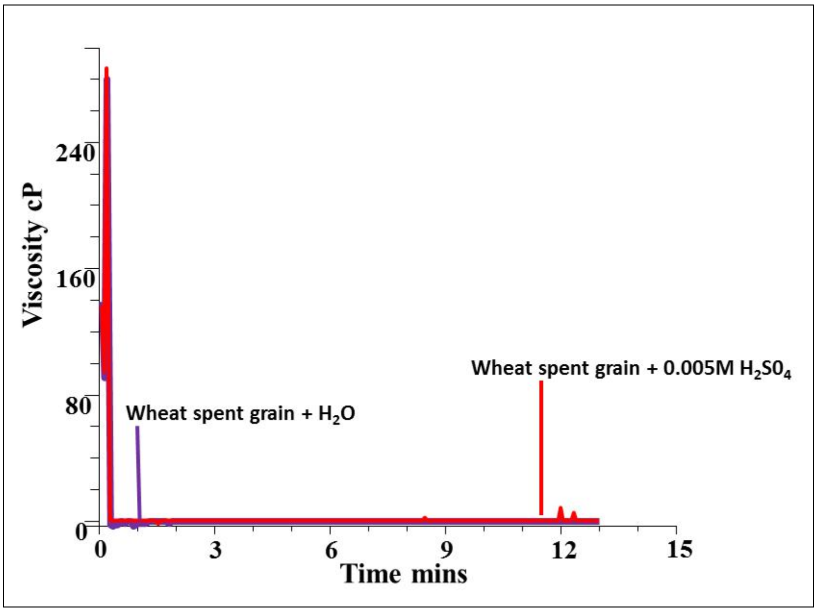

Figure 3

RVA pasting profile of wheat spent grain using water or 0.005M H_2SO_4 as process liquor