Influence of malting conditions on sorghum (*Sorghum bicolor* (L.) Moench) as a raw material for fermented beverages

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Abstract

There has been recently increased interest in sorghum to substitute the gluten containing cereals in the diet of people suffering from celiac disease. The response surface methodology was used to determine the influence of malting parameters (degree of steeping, germination temperature and time) on sorghum (*Sorghum bicolor* (L.) Moench). Each parameter was varied at three levels. Malting attributes, considered important to produce high quality malt for the production of lactic acid fermented beverages, were analyzed. The optimized conditions were: degree of steeping 41%, germination temperature 27 °C after 7 days of germination. Under these conditions, the following values of the studied attributes can be obtained: α-amylase 139 U/g, β-amylase 60 U/g, extract 83.8%, free amino nitrogen 117.8 mg/100 g, Kolbach index 26.6%, water-extractable arabinoxylan 0.3 g/L and vitamin B_2 114.9 μg/L. Among the tested parameters, the germination time had the highest effect on malting attributes. Although the activity of amylolytic enzymes α- and β-amylase were low, the value of extract was high and comparable to that of barley malt. The obtained results showed that sorghum malt is a promising raw material for the production of lactic acid fermented beverages.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major crops worldwide in terms of production and utilization (Dendy 1995; Rooney and Saldivar 2003). Since it is a gluten-free cereal, there has been recently increased interest in sorghum to substitute the gluten containing cereals in the diet of people suffering from celiac disease CD (Elkhalifa et al. 2005). Sorghum is an important source of protein and minerals (Correia et al. 2005), and a potential source of functional health promoting constituents, such as vitamin B group, fibers, antioxidant phenolics and cholesterol-lowering waxes (Rooney and Saldivar 2003; Taylor et al. 2006).

The main categories of traditional foods produced from sorghum are flat breads, porridges, steamed and boiled products, and snack foods. Likewise, alcoholic and non-alcoholic beverages are produced from malted sorghum grains (Palmer 1992; Waniska et al. 2004).

There are numerous studies which have found that the growth and stability of lactic acid bacteria (LAB) was enhanced in the extract of malted grains than in non-malted. This could be attributed to the higher level of sugar and protein present in malt (Charalampopoulos et al. 2002; Charalampopoulos

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and Pandiella 2010; Rozada-Sanchez et al. 2008). During the malting process, the hydrolytic enzymes amylases, proteases and fiber-degrading enzymes are activated by the germinated grain to breakdown the starchy endosperm into simpler forms. The resulted di- and monosaccharides, and free amino nitrogen (FAN) are important if the aim of malting is to produce fermented beverages (Palmer et al. 1989).

The behavior of sorghum grain during malting process was studied by many authors. The moisture content of steeped grain, germination temperature and time are malting parameters known to have an effect on sorghum malt quality (Dewar et al. 1997). Claver et al. (2010) found a significant effect of germination on the increase of amylase activity, malt loss, soluble solids yield and protein content. Germination time affected the extract yield (Lasekan et al. 1994) and the activity of α-amylase (Iwuoha and Aina 1997; Ratnavathi and Ravi 1991) and β-amylase (Okoli et al. 2010), which are considered as very important malting quality attributes. Notwithstanding, other authors found no effect of germination time or temperature on α-amylase activity (Muoria and Bechtel 1998; Uriyo and Eigel 1999). The effect of germination temperature was found to influence many malt quality attributes such as α- and β-amylase activity, extract yield, FAN and total soluble nitrogen (Agu and Palmer 1998). However, the effect of the interaction between the different key malting parameters on malt quality was less addressed in sorghum malting studies.

In order to study the single and interaction effect of different variables on a process, the response surface methodology (RSM) has been extensively used in many fields (Tsapatsaris and Kotzekidou 2004; Zarnkow et al. 2008). It is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems, in which a response of interest is influenced by several variables, and the objective is to optimize this response (Montgomery 2001). One of the most popular RSM designs is the central composite design (CCD) which explores the response surfaces covered in the experimental design, thus, enhancing the efficiency and effectiveness of the optimization procedure (Capanzana and Buckle 1997).

The objective of the present study was to investigate the effect of malting conditions on sorghum grains by employing RSM. Since the fermentation of malt-based media with LAB requires a media rich in carbohydrate and protein sources, amylolytic and proteolytic specifications were investigated and optimized. Additionally, some functional bioactive components in malt were considered with the aim to produce a malt-based functional beverage.

MATERIALS AND METHODS

Raw material

Sorghum (*Sorghum bicolor* (L.) Moench) was supplied by Farmwise grains (PTY) Ltd, Randburg, South Africa. Moisture content of grains was 13% and germination energy >95%.

Malting procedures

Malting of sorghum grains (25 kg) was conducted in 25 of 1 kg batches in micro-malting systems. In steeping procedure, hydrogen peroxide (H_2O_2) was added to steeping water to retard fungal infection. The degree of steeping of the grains was adjusted to 35, 38 and 41% by conducting a standard steeping procedure as described in mitteleuropäische brau- und analysenkommision (MEBAK), method 1.5.3 (Anger 2006). On the first day of steeping, the grains were soaked in water for 4 h (wet steeping), followed by 19 h of sprinkling with water. On the second day, these grains were soaked again in water for 5 h, followed by 20 h of sprinkling with water. The steeping procedure was terminated during the second day as soon as the required degree of steeping was reached. If the required degree of steeping was not reached, the steeping was extended to a third day of wet steeping until the target degree of steeping was achieved. Thereafter, germination of steeped grains was carried out in micro-climate chambers controlled at different temperatures 25, 28 and 31 °C for different periods of germination 5, 6 and 7 days. The temperature range was chosen according to previous published data. Finally, kilning of green malted grains was carried out at 50 °C for 24 h.

Chemical analyses

After the malting procedures, the chemical analyses of the kilned malt were carried out in duplicate ($n = 2$), and the means of all results were calculated. Analyses of malted grains for α- and β-amylase activity and cold water extract for vitamin B_2 were performed. Analyses of extract, FAN, kolbach index and water-extractable arabinoxylan (WEAX) were conducted after performing the common congress mashing program (Anger 2006).

α-amylase. The International Association for Cereal Science Method 303 by means of a Megazyme enzyme kit (Megazyme, Wicklow, Ireland) measured the level of α-amylase activity (ICC 2004).

β-amylase. β-amylase activity was determined using the Megazyme enzyme kit (Megazyme, Wicklow, Ireland) (McCleary and Codd 1989).

Malt extract. Anton Paar alcolyzer (Anton Paar, Graz, Austria) was used to determine the malt extract,

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and following the MEBAK method 4.1.4.2.2 (Anger 2006).

FAN. The determination of FAN (also referred as α-amino nitrogen) was based on MEBAK method 4.1.4.5.5 (Anger 2006) using a Skalar working station (Skalar, Breda, Netherlands).

Kolbach index. To determine the effect of malting conditions on proteolytic activities in sorghum, the kolbach index was calculated by following MEBAK method 4.1.4.5.3 (Anger 2006) from the below formula:

$$\text{kolbach index} = \left(\frac{\text{Soluble protein \%}}{\text{Total malt protein \%}} \right) \times 100$$

WEAX. The concentration of pentose sugars arabinose and xylose was measured by high-performance anion-exchange chromatography with pulsed amperometric detection. The arabinoxylan content was calculated as the sum of arabinose and xylose multiplied by 0.88 to correct for anhydro monosaccharides (Krahl et al. 2010).

Vitamin B₂ (Riboflavin). The determination of vitamin B_2 was performed using the whole-blood test kits from Chromsystems (Germany) as described by (Krahl et al. 2008).

Experimental design and statistical analysis

To investigate the impact of the key malting parameters (degree of steeping, germination temperature and time) on sorghum malt quality, the RSM was applied as described by Montgomery (2001). Each parameter was varied to three different levels: the degree of steeping 35, 38 and 41%, the germination temperature 25, 28 and 31 °C, and the germination time 5, 6 and 7 days. The face-centered cube design was used with double replicated factorial and three replicated center points. The aim of this design was to study the single or interaction effects of the previously mentioned malting parameters on malt quality attributes, and to investigate the optimum malting condition. Values of malting parameters are shown in Table 1. The software package Design Expert version 8.0.6 by StatEase (Stat-Ease Corporation, Minneapolis, MN, USA) was used to analyze the data obtained from the 25 micro-malting trials that were conducted.

The obtained data were analyzed using analysis of variance (ANOVA). Different indexes were used to analyze and evaluate the adequacy of the calculated statistical models. The coefficient of determination R^2 is the proportion of variation in the response attributed to the model rather than to random error (Khuri and Cornell 1987). For a good fit of a model, R^2 value higher than 80% is recommended (Joglekar and May 1987). The studied parameter is considered to have a significant effect on the response if p-value < 0.05.

Table 1. The CCD matrix of malting parameters

Sample number	Degree of steeping (%)	Germination temperature (°C)	Germination time (days)
1	35	25	5
2	35	25	5
3	35	25	7
4	35	25	7
5	38	25	6
6	41	25	5
7	41	25	5
8	41	25	7
9	41	25	7
10	35	28	6
11	38	28	5
12	38	28	6
13	38	28	6
14	38	28	6
15	38	28	7
16	41	28	6
17	35	31	5
18	35	31	5
19	35	31	7
20	35	31	7
21	38	31	6
22	41	31	5
23	41	31	5
24	41	31	7
25	41	31	7

F-value is used to describe the influence of the model, and the lack of fit to describe the scatter of the data around the fitted model.

After this, the optimum malting parameters (degree of steeping, germination temperature and time) were calculated taking into consideration producing sorghum malt with high amylolytic and proteolytic specifications in order to offer the desired carbon and nitrogen sources for posterior fermentation with LAB. Further, producing malt with high bioactive components in order to use it for the production of functional beverage was also considered. The software package Design Expert version 8.0.6 was used for this purpose.

RESULTS AND DISCUSSION
Models fitting from RSM

Table 2 shows the main results of the ANOVA of the analyzed malting quality attributes. Among the tested attributes, α- and β-amylase, extract, FAN and WEAX showed quadratic models, while vitamin B_2 showed 2FI model. The single attribute which showed a linear

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Table 2. Analysis of variance (ANOVA) of the attributes fitted models

Independent variable	α–amylase	β–amylase	Extract	FAN	Kolbach index	B_2	WEAX
Model	Quadratic	Quadratic	Quadratic	Quadratic	Linear	2FI	Quadratic
Model p-value	<0.0001	0.0008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A-degree of steeping	>0.05	>0.05	>0.05	<0.0001	<0.0001	>0.05	0.0042
B-germination temperature	<0.0001	>0.05	<0.0001	<0.0001	0.0002	>0.05	>0.05
C-germination time	0.0018	0.0045	0.0008	<0.0001	<0.0001	<0.0001	<0.0001
A × B	0.0002	0.0017	0.0119	>0.05	–	0.0393	>0.05
A × C	>0.05	0.0047	>0.05	0.0009	–	0.0370	>0.05
B × C	0.0152	0.0003	>0.05	>0.05	–	>0.05	>0.05
A^2	0.0812	0.3446	0.1041	0.2125	–	–	0.2991
B^2	<0.0001	0.0919	0.2583	0.4258	–	–	0.0392
C^2	0.0757	0.1661	0.0023	0.0016	–	–	0.2667
C.V. (%)	2.17	8.02	0.84	3.67	5.39	17.02	9.68
Model fit R^2	0.98	0.81	0.95	0.96	0.81	0.87	0.87
Lack of fit	0.3021	0.8960	0.1351	0.2381	0.1789	0.1278	0.6600

FAN: free amino nitrogen; WEAX: water-extractable arabinoxylan; 2FI: two factors interaction; C.V.: coefficient of variation.
Significant level is at p-value < 0.05.

Table 3. The measured versus calculated values

	Calculated		Measured	
Attributes	Minimum	Maximum	Minimum	Maximum
α–amylase (U/g)	29.7 (41/31/7)	145.2 (35/27/5)	28 (41/31/7)	143 (41/25/5)
β–amylase (U/g)	46.6 (39/25/7)	80 (35/25/5)	41 (41/25/7)	80 (35/25/5)
Extract (%)	77.1 (41/31/5)	85.2 (38/25/6)	76.9 (41/31/7)	85.2 (41/25/7)
FAN (mg/100 g)	70.2 (35/31/5)	124.5 (41/25/7)	67 (35/31/5)	126 (41/25/7)
Kolbach index (%)	18.4 (35/31/5)	28 (41/25/7)	18.5 (35/31/5)	30.3 (41/25/7)
B_2 (µg/L)	26.9 (41/25/5)	142.4 (35/25/7)	27.2 (41/25/5)	162 (38/28/7)
WEAX (g/L)	0.15 (38/25/5)	0.32 (41/29/7)	0.16 (41/25/5)	0.34 (41/31/7)

FAN: free amino nitrogen; WEAX: water-extractable arabinoxylan.
Notes: The calculated values are rounded since time patterns with decimals especially the germination time would not be practicable; in addition the calculated values are determined by topping or minimizing this value without considering the influence of other features.

model was kolbach index. Based on the statistical analysis, the models were highly significant with very low p-values (from < 0.0001 to 0.0008). The R^2 values for all attributes fitted models were higher than 0.80, indicating that the regression models explained the reaction well. The lack of fit of all the attributes was not significant relative to pure error, it reflected that the fitted models can be used to explain the responses. The values of the measured as well as the calculated minimum and maximum attributes are shown in Table 3. Good correlation was found between the measured and calculated (predicted) values.

Amylolytic specifications

α- and β-amylase activity. As shown in Table 2, the response of enzymes activities to malting parameters was not similar. In case of α-amylase activity, the significant terms ($p < 0.05$) of the model were: the single effect of both germination temperature and germination time, the interaction effect of (degree of steeping × germination temperature) and (germination temperature × germination time), as well as the quadratic effect of germination temperature (Table 2), while the only independent variable which had a significant effect ($p < 0.05$) on β-amylase activity was the germination time. Our study confirmed previous studies which found that the germination time has a significant influence on α- and β-amylase activity (Bwanganga et al. 2013; Okoli et al. 2010). The optimum germination time was 6 and 5 days for α- and β-amylase, respectively (Figures 1 and 2). The optimum germination time found in this study is similar to another one which showed that amylolytic enzymes activity (estimated as
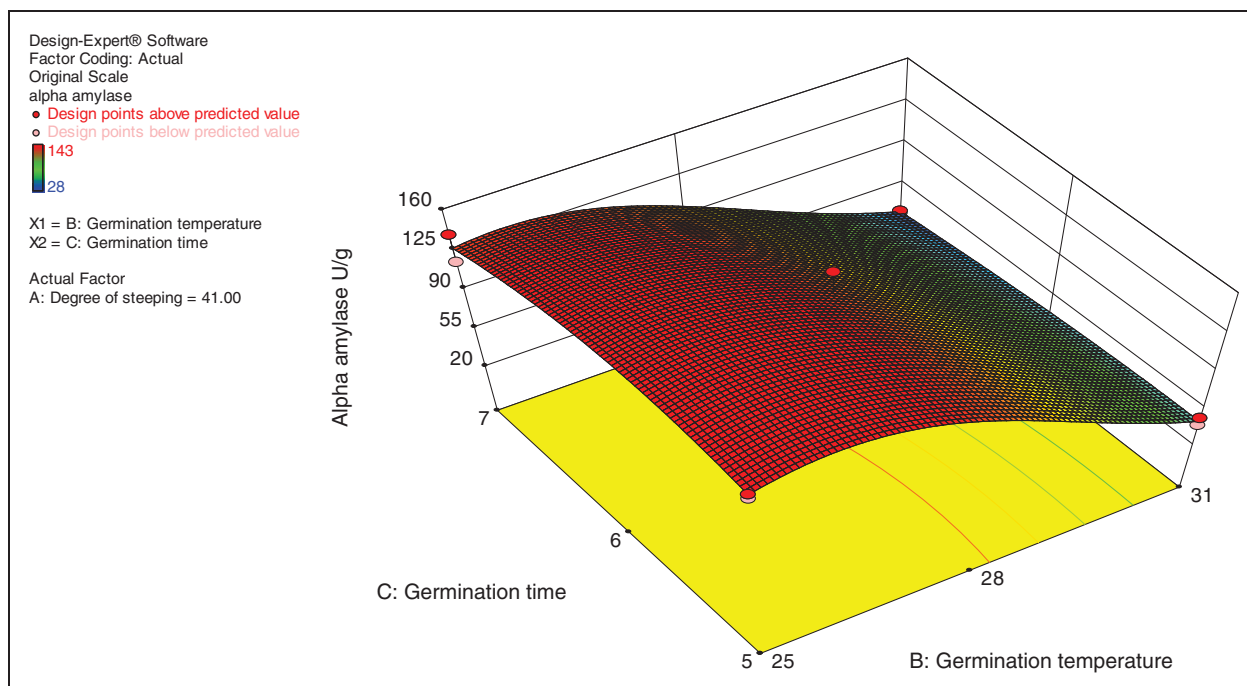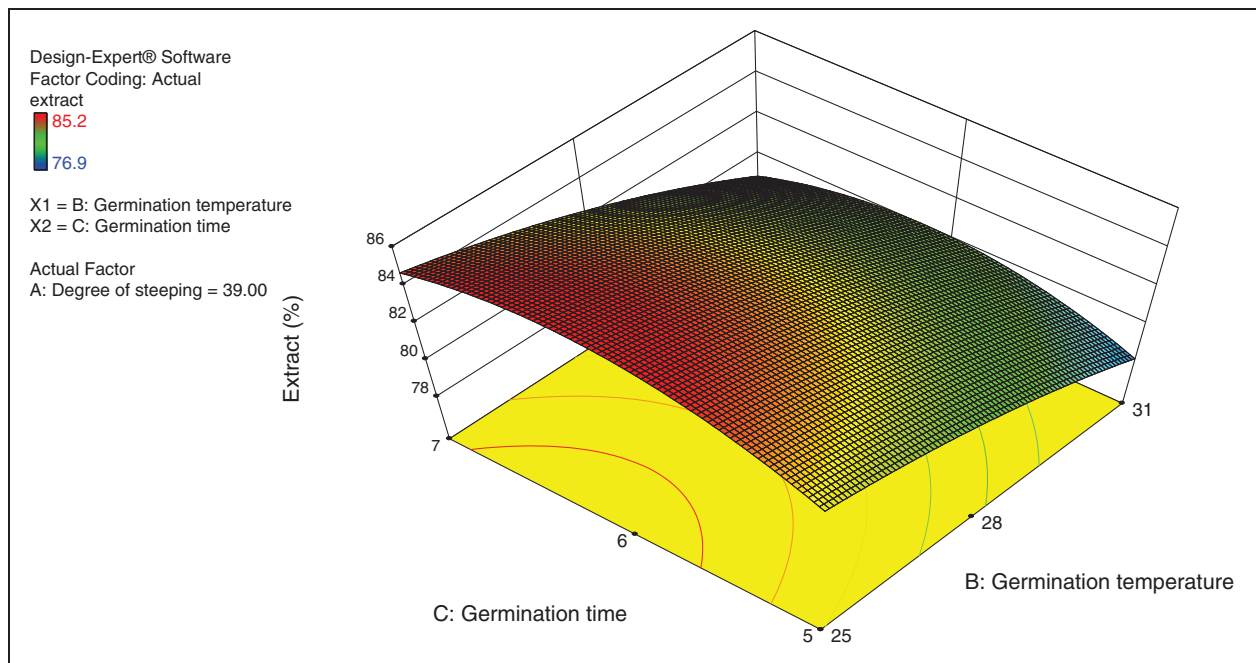
Figure 1. Influence of germination temperature and time on α-amylase activity at degree of steeping 41%.
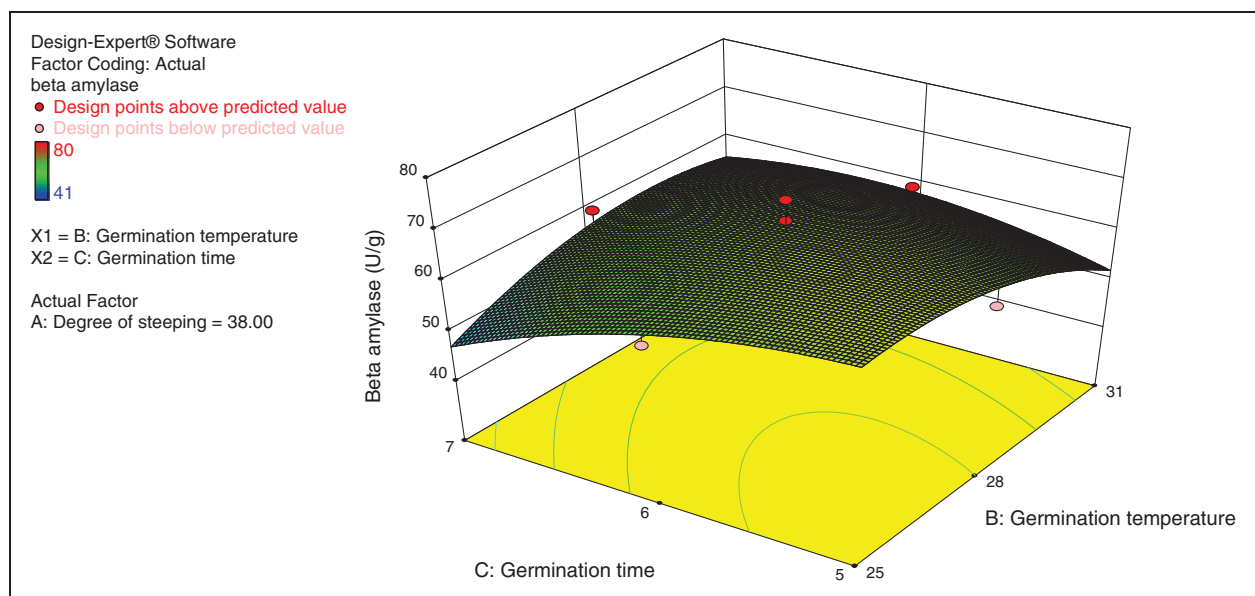
Figure 2. Influence of germination temperature and time on β-amylase activity at degree of steeping 38%.

diastatic power) increased by an extended period of germination when sorghum grains were germinated for 2, 4 and 6 days, respectively (Lasekan et al. 1994). However, Ratnavathi and Ravi (1991) found that the α-amylase peak activity was reached after 4 days of germination, and then reduced after the end of the 5th day of germination. The optimum germination temperature for both the enzymes activity was 26 °C

(Figures 1 and 2). This temperature was in the lower limit of the tested temperature range. Taylor and Robbins (1993) found that among the investigated germination temperature range (24–32 °C), the highest β-amylase activity was at the lowest tested temperature. However, the optimum malting temperature of sorghum seems to correspond to the temperature at which the grain would grow best in the field

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Figure 3. Influence of germination temperature and time on extract at degree of steeping 39%.

(Agu and Palmer 1997). In our study, the ranges of α- and β-amylase activity (Figures 1 and 2) were in agreement with that of sorghum malt reported by Bwanganga et al. (2013), but lower than that of barley malt (Zarnkow 2009).

Extract. The model which fitted the response of extract reached the maximum value at 39% degree of steeping, 25 °C germination temperature after 6 days of germination (Figure 3). The significant terms ($p < 0.05$) of the fitted model were: the single effect of germination temperature and germination time, the interaction effect of (degree of steeping × germination temperature), and the quadratic effect of germination time (Table 2). The reason for the high extract obtained at the low germination temperature (25 °C, Figure 3) could be that highest respiratory and carbohydrate malting losses occurred at the higher germination temperature of 30 °C (Agu and Palmer 1998). Our study is in agreement with a previous study which found a significant influence of germination time on sorghum extract (Okoli et al. 2010). As shown in Figure 3, a high extract of 85.2% could be obtained. This value is nearly similar to barley malt reported elsewhere (Narziß 1999). The value of sorghum extract in previous studies was contradictory. While many studies showed that sorghum extract yield was low (Etokakpan and Palmer 1990; Palmer 1991), Dufour et al., (1992) obtained a higher extract of 82.7% which is still lower than our obtained value. However, it is worth to notice that the extract yield in sorghum is found to be influenced by the type of the applied mashing regime (Palmer et al. 1989). Therefore, it is suggested that the congress mashing regime that was used in our study is a suitable one for mashing sorghum malt. Moreover, previous studies showed that a high extract yield could be a result of high proteolytic enzymes activity. The high hydrolysis of the grain endosperm storage proteins by proteases lead to the liberation of starch granules attached to these proteins, thus, more starch will be subjected to a subsequent amylolytic action and high extract can be obtained (Agu and Palmer 1998; Ogbonna et al. 2004).

Proteolytic specifications

FAN. Results showed that all independent variables of malting had a significant effect ($p < 0.05$) on FAN (Table 2). Higher degree of steeping and germination time could lead to a higher FAN (Figure 4). Therefore, it was not possible to achieve the maximum FAN through the model. It is shown that short germination time and low degree of steeping lead to low FAN. FAN is produced by the hydrolysis of the grain endosperm storage proteins by the endogenous proteinase and peptidase enzymes. This leads not just to the assimilable nitrogenous materials, but also the liberation of the starch granules embedded in the protein matrix, thus promoting extract yield and wort fermentability (Ogbonna et al. 2004). The sorghum malt content of FAN in our study ranged between 67 and 126 mg/100 depending on the different treatments (Figure 4).
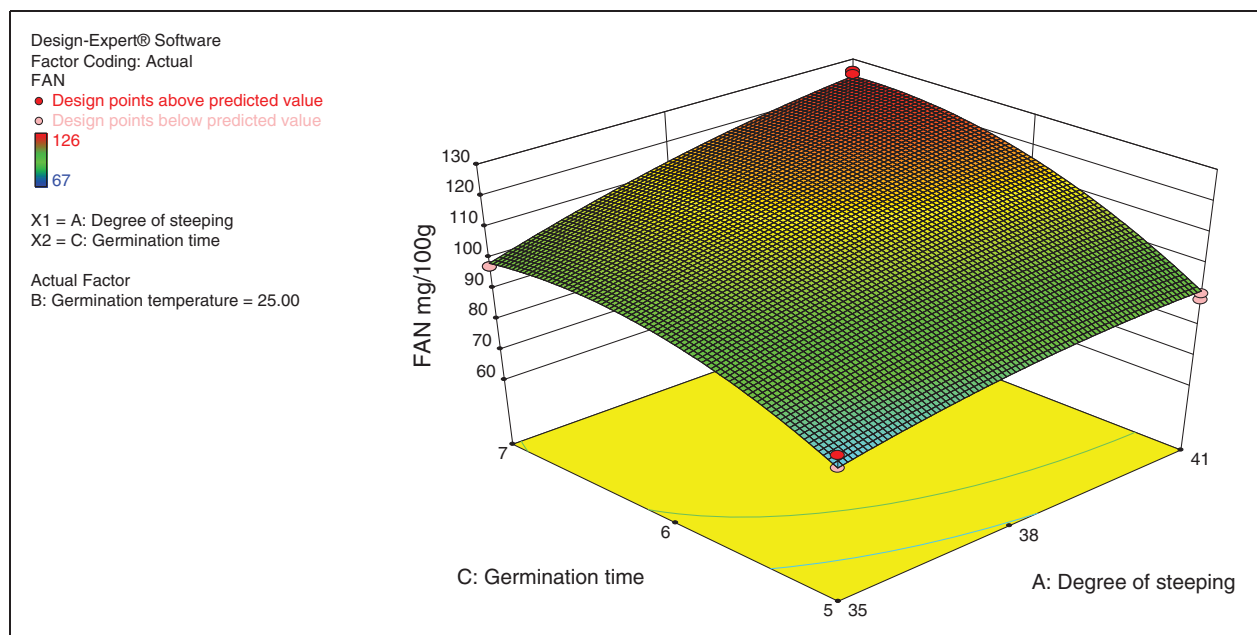
Figure 4. Influence of degree of steeping and germination time on FAN at germination temperature 25 °C.
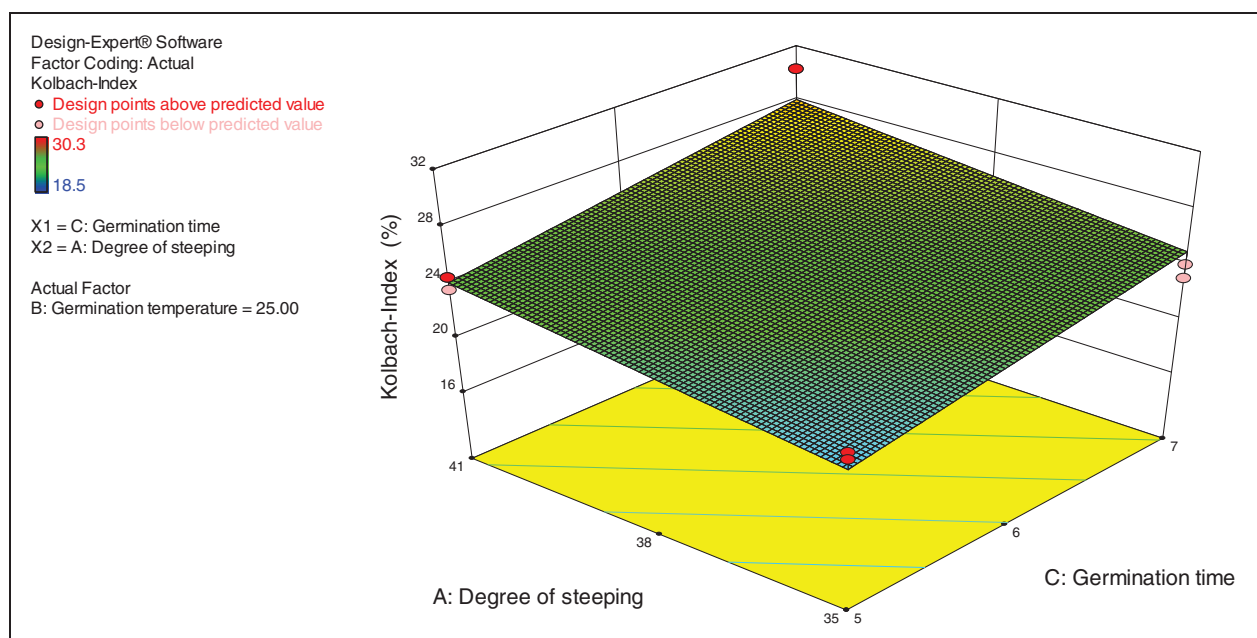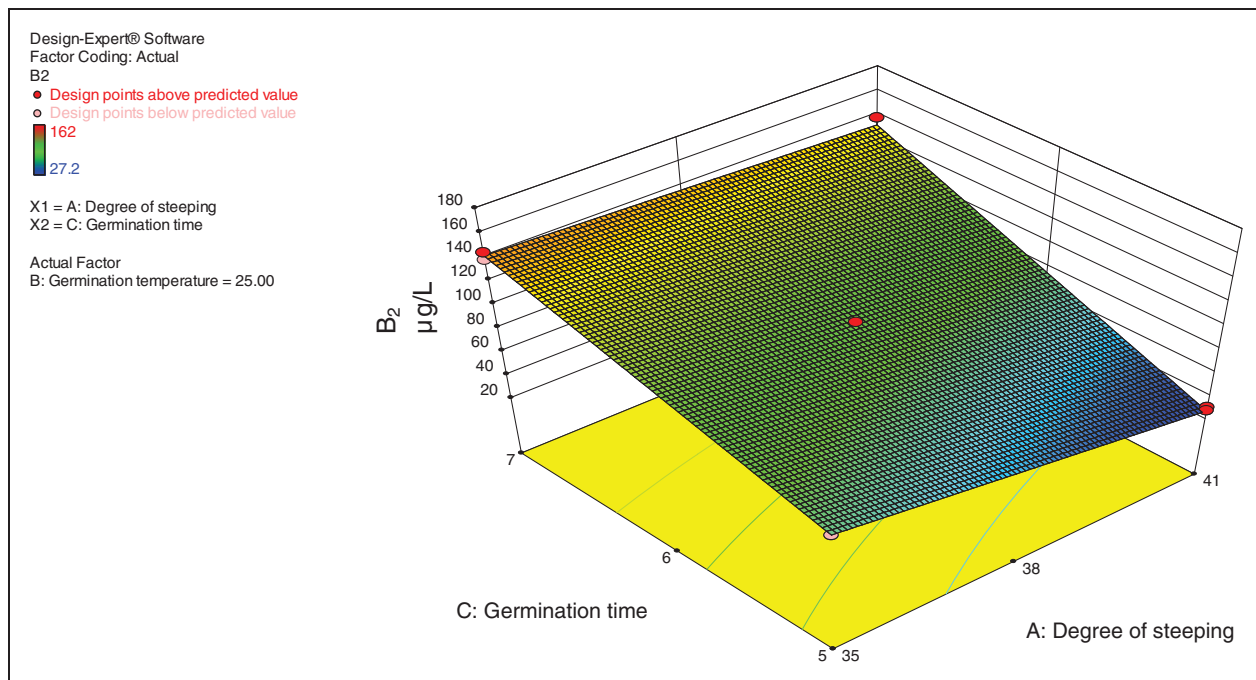

Figure 5. Influence of degree of steeping and germination time on kolbach index at germination temperature 25 °C.

The effect of sorghum variety and germination time was studied by Dewar (2003). The author found that FAN value was dependent on the malted variety, and ranged between 109 and 195 mg/100 among the four studied varieties. Further, it was found that the germination time has a significant influence on FAN, and continued to increase reaching a peak after 8 days of germination.

Kolbach index. Kolbach index is the ratio between the soluble to total nitrogen content (Briggs et al. 2004). Like in FAN, all tested independent variables of malting had a significant effect ($p < 0.05$) on malt kolbach index (Table 2). The behavior of kolbach index was similar to that of FAN. Results showed that higher degree of steeping and germination time led to higher kolbach index (Figure 5). Kolbach index in our study

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Figure 6. Influence of degree of steeping and germination time on vitamin B_2 at germination temperature 25 °C.

(18.5%–30.3%) was lower than that reported elsewhere for barley malt (Zarnkow 2009). It was found by Muoria (2002) that among the six studied sorghum varieties, except for one variety, kolbach index was less than 33%. At the lower temperature limit of the tested range (25 °C), higher kolbach index was obtained compared to the higher limit (data not shown). The reason for this behavior could be that the warm germination conditions lead to a displacement of soluble nitrogen towards root and acrospires growth (Muñoz-Insa et al. 2011). This displacement was proven by Dewar et al. (1997) where the root and shoot portion of sorghum malt was four times higher in FAN than the berry portion.

Bioactive components

Vitamin B_2. In our study, among the independent tested variables, only the germination time had a significant effect ($p < 0.05$) on vitamin B_2. The interaction effects (degree of steeping × germination temperature) and (degree of steeping × germination time) affected the response significantly (Table 2). The fitted model reached its maximum value at 41% degree of steeping, 25 °C germination temperature and 7 days of germination. Longer germination time could lead to higher concentration of vitamin B_2 (Figure 6). The increase of many vitamins during the germination of cereals was proofed by many authors (Krahl et al. 2008; Malleshi and Desikachar 1986). As to sorghum, vitamin B_2 showed the highest increase of 44.2% among different

B group vitamins during the germination. The germination time increased during the germination procedure. The concentration of vitamins during germination seems to be related to the need for the vitamin during the development of seedlings (Ochanda et al. 2010).

Water-extractable arabinoxylan. As shown in Table 2, germination time and degree of steeping had a significant effect ($p < 0.05$) on WEAX, while germination temperature showed no effect. The data fitted a quadratic model. A degree of steeping more than 41% and germination time more than 7 days can increase the content of WEAX (Figure 7). However, exceeding these limits is practically not preferable due to the risk of mold infection. The significant effect of germination time on WEAX content in our study is in agreement with a previous study by Krahl et al. (2008) which found an increase in this component during the germination time. This positive effect of germination is advantageous since WEAX is considered an important prebiotic. They are fermented in the colon by probiotic LAB strains. The end products of this fermentation are lactic acid and short chain fatty acids such as butyrate. The latter is considered an important factor for normal functions in colonocytes and a protective agent against colon cancer (Topping and Clifton 2001).

Optimization of sorghum malt by RSM

The criteria to optimize sorghum malting conditions were to enhance the amylolytic and proteolytic

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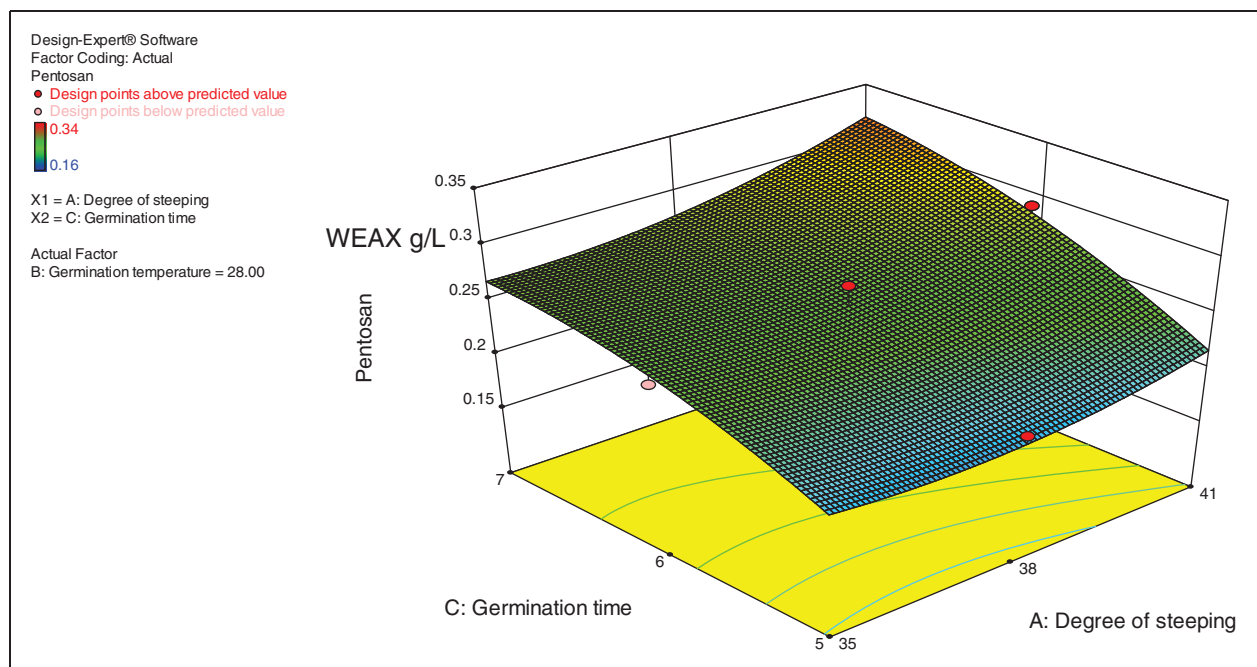
Figure 7. Influence of degree of steeping and germination time on the water extractable arabinoxylans at germination temperature 28 °C.

specifications in order to yield high concentrations of C and N sources for further fermentation with LAB. Moreover, in order to produce functional beverages out of malt, the criteria to optimize the bioactive components arabinoxylans and vitamin B_2 was to enrich their concentrations. After applying the last mentioned criteria, the optimum malting conditions of sorghum malt were obtained at 41% degree of steeping 27 °C germination temperature after 7 days of germination. The RSM showed that by applying the last mentioned malting conditions, the following values of attributes can be achieved: α-amylase 139 U/g, β-amylase 60 U/g, extract 83.8%, FAN 117.8 mg/100 g, Kolbach index 26.6%, WEAX 0.3 g/L and vitamin B_2 114.9 µg/L. It can be concluded from the last mentioned results that although the low activity of α and β-amylase, satisfied extract value can be obtained.

CONCLUSIONS

Applying RSM showed that satisfied malting quality attributes can be obtained throughout the malting of sorghum grains. Among the studied parameters, germination time had the highest influence on all attributes (significant in all cases), followed by germination temperature and degree of steeping. The effect of different malting parameters on malt quality attributes could be determined. The value of extract was comparable to that of barley malt, which indicates that sorghum malt is a promising raw material in beverage industry.

REFERENCES

Agu RC and Palmer GH. (1997). The effect of temperature on the modification of sorghum and barley during malting. *Process Biochemistry* 32: 501–507.

Agu RC and Palmer GH. (1998). A reassessment of sorghum for lager-beer brewing. *Bioresource Technology* 66: 253–261.

Anger HM. (2006). *Brautechnische Analysenmethoden Rohstoffe*. Freising, Germany: Selbstverlag der Mitteleuropäische Analysenkommission.

Briggs DE, Boulton CA, Brookes PA and Stevens R. (2004). *Brewing: Science and Practice*. New York: CRC Press.

Bwanganga J-C, Béra F and Thonart P. (2013). Modelling the β-amylase activity during red sorghum malting when Bacillus subtilis is used to control mould growth. *Journal of Cereal Science* 57: 115–119.

Capanzana MV and Buckle KA. (1997). Optimisation of germination conditions by response surface methodology of a high amylose rice (*Oryza sativa*) cultivar. *Lebensmittel-Wissenschaft und-Technologie* 30: 155–163.

Charalampopoulos D and Pandiella SS. (2010). Survival of human derived Lactobacillus plantarum in fermented cereal extracts during refrigerated storage. *LWT – Food Science and Technology* 43: 431–435.

Charalampopoulos D, Pandiella SS and Webb C. (2002). Growth studies of potentially probiotic lactic acid bacteria

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in cereal-based substrates. *Journal of Applied Microbiology* 92: 851–859.

Claver IP, Zhang H, Li Q, Zhou H and Zhu K. (2010). Optimized conditions of steeping and germination and their effect on sorghum [*Sorghum bicolor* (L.) Moench] composition. *Pakistan Journal of Nutrition* 9: 686–695.

Correia I, Nunes A, Duarte IF, Barros A and Delgadillo I. (2005). Sorghum fermentation followed by spectroscopic techniques. *Food Chemistry* 90: 853–859.

Dendy DAV. (1995). *Sorghum and Millets: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Dewar J. (2003). Influence of malting on sorghum protein quality. In: *Proceedings of AFRIPRO workshop on the proteins of sorghum and millets: enhancing nutritional and functional properties for Africa* (eds PS Belton and JRN Taylor), Pretoria, South Africa. Available at: www.afri-pro.org.uk/papers/Paper18Dewar.pdf

Dewar J, Taylor JRN and Berjak P. (1997). Effect of germination conditions, with optimised steeping, on sorghum malt quality-with particular reference to free amino nitrogen. *Journal of the Institute of Brewing* 103: 171–175.

Dufour JP, Melotte L and Srebrnik S. (1992). Sorghum malts for the production of a lager beer. *American Society of Brewing Chemists* 50: 110–119.

Elkhalifa AEO, Schiffler B and Bernhardt R. (2005). Effect of fermentation on the functional properties of sorghum flour. *Food Chemistry* 92: 1–5.

Etokakpan OU and Palmer GH. (1990). Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. *World Journal of Microbiology and Biotechnology* 6: 408–417.

ICC. (2004). Standardmethoden der Internationalen Gesellschaft für Getreidewissenschaft und -technologie. Methodennummer 303: Einfacher und spezifischer Test zur Bestimmung der alpha-Amylase in Getreide, Wien.

Iwuoha CI and Aina JO. (1997). Effects of steeping condition and germination time on the alpha-amylase activity, phenolics content and malting loss of Nigerian local red and hybrid short Kaura sorghum malts. *Food Chemistry* 58: 289–295.

Joglekar AM and May AT. (1987). Product excellence through design of experiments. *Cereal Foods World* 32: 857–868.

Khuri AI and Cornell JA. (1987). *Response Surface Design and Analyses*. New York: Marcel Dekker.

Krahl M, Hagel C, Zarnkow M, Back W and Kreisz S. (2008). Changes of the content of water-soluble bioactive compounds during the malting process of spelt wheat (*Triticum aestivum* var. spelta). *BrewingScience* 61: 170–174.

Krahl M, Zarnkow M, Back W and Becker T. (2010). Determination of the influence of malting parameters on the water-extractable arabinoxylan content of wheat (*Triticum aestivum*), rye (*Secale cereale*), and spelt wheat (*Triticum aestivum* spp. spelta). *Journal of the American Society of Brewing Chemists* 68: 34–40.

Lasekan OO, Idowu MA and Lasekan W. (1994). Effect of germination and degree of grind (coarse/fine) on the extract and sugar contents of sorghum malts. *Food Chemistry* 53: 125–128.

Malleshi NG and Desikachar HSR. (1986). Studies on comparative malting characteristics of some tropical cereals and millets. *Journal of the Institute of Brewing. Institute of Brewing (Great Britain)* 92: 174–176.

McCleary BV and Codd R. (1989). Measurement of beta-amylase in cereal flours and commercial enzyme preparations. *Journal of Cereal Science* 9: 17–33.

Montgomery DC. (2001). Response surface methodology. In: *Design and Analysis of Experiments*. New York: Wiley, pp. 457–461.

Muñoz-Insa A, Gastl M, Zarnkow M and Becker T. (2011). Optimization of the malting process of oat (*Avena sativa* L.) as a raw material for fermented beverages. *Spanish Journal of Agricultural Research* 9: 510–523.

Muoria J. (2002). Malt and wort properties of Kenyan sorghum varieties germinated for different time periods. Available at: *http://ift.confex.com/ift/2002/techprogram/paper_12110.htm*

Muoria JK and Bechtel PJ. (1998). Diastatic power and alpha-amylase activity in millet, sorghum, and barley grains and malt. *Journal of the American Society of Brewing Chemists* 56: 131–135.

Narziß L. (1999). *Die Technologie der Malzbereitung, die Bierbrauerei*. Stuttgart, Germany: Ferdinand Enke Verlag.

Ochanda SO, Onyango CA, Mwasaru AM, Ochieng JK and Mathooko FM. (2010). Effects of malting and fermentation treatments on group B-vitamins of red sorghum, white sorghum and pearl millets in Kenya. *Journal of Applied Biosciences* 34: 2128–2134.

Ogbonna AC, Obi SKC and Okolo BN. (2004). Optimization of proteolytic activities in malting sorghum. *Process Biochemistry* 39: 713–718.

Okoli EV, Okolo BN, Moneke AN and Ire FS. (2010). Effects of cultivar and germination time on amylolytic potential, extract yield and wort fermenting properties of malting sorghum. *Asian Journal of Biotechnology* 2: 14–26.

Palmer GH. (1991). Enzymic degradation of the endosperm cell walls of germinated sorghum. *World Journal of Microbiology and Biotechnology* 7: 17–21.

Palmer GH. (1992). Sorghum–food, beverage and brewing potentials. *Process Biochemistry* 27: 145–153.

Palmer GH, Etokakpan OU and Igyor MA. (1989). Sorghum as brewing material. *World Journal of Microbiology and Biotechnology* 5: 265–275.

Ratnavathi CV and Ravi SB. (1991). Effect of different durations of steeping and malting on the production of Alpha-amylase in sorghum. *Journal of Cereal Science* 14: 287–296.

Rooney LW and Saldivar SO. (2003). Sorghum. In: Benjamin C (ed.) *Encyclopedia of Food Sciences and Nutrition*. Oxford: Academic Press, pp. 5370–5375.

Rozada-Sanchez R, Sattur AP, Thomas K and Pandiella SS. (2008). Evaluation of Bifidobacterium spp. for the production of a potentially probiotic malt-based beverage. *Process Biochemistry* 43: 848–854.

Taylor JRN and Robbins DJ. (1993). Factors influencing beta-amylase activity in sorghum malt. *Journal of the Institute of Brewing* 99: 413–416.

Taylor JRN, Schober TJ and Bean SR. (2006). Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science* 44: 252–271.

Topping DL and Clifton PM. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Reveiws* 81(3): 1031–1064.

Tsapatsaris S and Kotzekidou P. (2004). Application of central composite design and response surface methodology to the fermentation of olive juice by Lactobacillus plantarum and Debaryomyces hansenii. *International Journal of Food Microbiology* 95: 157–168.

Uriyo M and Eigel WE. (1999). Duration of kilning treatment on α-amylase, β-amylase and endo-(1,3)(1,4)-β-d-glucanase activity of malted sorghum (Sorghum bicolor). *Process Biochemistry* 35: 433–436.

Waniska RD, Rooney LW and McDonough CM. (2004). Sorghum: utilization. In: Colin W (ed.) *Encyclopedia of Grain Science*. Oxford: Elsevier, pp. 126–136.

Zarnkow M, Mauch A, Burberg F, Back W, Arendt EA, Kreisz S, et al. (2009). Proso Millet (*Panicum miliaceum* L.) a Sustainable Raw Material for the Malting and Brewing Process. *BrewingScience Monatsschrift für Brauwissenschaft* 62: 119–140 (July/August).

Zarnkow M, Almaguer C, Burberg F, Back W, Arendt EK and Kreisz S. (2008). The use of response surface methodology to optimise malting conditions of Teff (*Eragrostis tef* (Zucc.) Trotter) as a raw material for gluten-free foods. *BrewingScience Monatsschrift für Brauwissenschaft* 61(5/6): 94–104.