COUNCIL FOR SCIENTIFIC & INDUSTRIAL RESEARCH

FOOD RESEARCH INSTITUTE

WHOLE GRAIN BREWING AND MALTING POTENTIAL OF 17 SORGHUM VARIETIES

Final Report for the Food Crops Development Project (FCDP) of the Ministry of Food and Agriculture

by

Manful, J.T., Gayin, J and Toku, P.
Food Research Institute, CSIR, Ghana

November, 2006

Table of Contents

List of Tables

Summary ---------- 4

1.0 Introduction ---------- 5

2.0 Materials and Methods ---------- 6

2.1 Physical Quality Evaluation ---------- 6

2.2 %Moisture ---------- 6

2.3 1000 grain Weight ---------- 7

2.4 Determination of Germination Capacity ---------- 7

2.5 Sorghum Malts ---------- 7

2.6 Alpha-Amylase ---------- 7

2.6.1 Principle ---------- 8

2.7 Malt flours ---------- 8

2.7.1 Assay Procedure ---------- 8

2.7.2 Calculation of Activity ---------- 9

2.8 Beta-Amylase ---------- 10

2.8.1 Principle ---------- 10

2.8.2 Assay Procedure ---------- 11

2.8.3 Calculation of Activity ---------- 11

2.9 Colour ---------- 12

2.10 Chemical Analysis ---------- 12

2.10.1 Crude Fat & Nitrogen ---------- 13

3.0 Results and Discussion ---------- 13

3.1 Physical Qualities ---------- 14

Conclusion ---------- 21

References ---------- 22

Appendix 1 ---------- 23

Appendix 2 ---------- 24

List of Tables

Table1: Some physical quality characteristics of 17 sorghum varieties

Table2: Germination capacities of 17 sorghum varieties

Table 3: Some chemical quality characteristics of 17 sorghum varieties

Table 4: alpha and beta -amylase activities of 17 sorghum varieties

Summary

The malting potential of 17 sorghum varieties namely; *Kazea Manga, Kapeli, Kemolga, Global 2000, Belko, Keriga, Mankaraga, Kapaala, Kadaga, Bumbago, Yakpaji, Kasheagu, Zelle, Nakpaji, Sarsorg E5 – 2, Sarsorg M13 – 3 and Sarsorg M1 – 1* were investigated. These land races of sorghum in Ghana are known to do very well on the field, and are also believed to have similar brewing properties to well known varieties like *Kapaala*. Their physical and chemical qualities and germination capacities were determined. Other key quality parameters, such as the level of malt α-amylase and β-Amylase activities were also assessed. (The assay format is shown in Appendices 1 & 2).

The study revealed that moisture content of all varieties was below 10%. All the varieties were mostly whole grains and matured. Weevil damaged kernels were not detected in any of the seventeen samples. *Sarsorg MI, Kasheagu, Sarsorm M 13-3, Mankaraga and Kemolga had* unsatisfactory germination capacities. The germination capacity was highest for *Kazea Manga* and lowest for *Mankaraga*. A fat level of less than 4% which is within operational range was found in most of the samples except *Zelle* and *Global 2000*. Average carbohydrate content of the grains was 75.2% with a standard deviation of ±2.3. For Beta-amylase activity, *Kazea Manga* had the highest activity (61.6U/g) followed by *Kemolga* (53.7U/g) and *Bumbago* (52.4). The activities of *Sarsorg E5 – 2* (8.6U/g) and *Kadaga* (14.0) were very low. The high values of germination and α-amylase activities of *Kazea Manga* and *Nakpaji* varieties indicate that the two varieties are comparable to *kapaala* in terms of malting properties. However it is recommended these two go through further analysis to ascertain their mashing and brewing potentials.

1.0 Introduction

Malt is cereal grain, usually barley, which has been germinated for a limited period of time, and then dried. More often at a time that it is considered appropriate, the Malster arrests germination by drying the grain in a stream of warm air. During germination, the food store or the endosperm of the grain is partly degraded by enzymes that attack cell walls, starch granules and the protein matrix. Essentially malt is therefore the degraded endosperm and the attendant enzymes able to complete the degradation. When relatively cool air is used for drying, the malt is pale in colour and very rich in enzymes. With greater drying temperatures, especially early in the drying process, the malt is darker in colour and the enzymic content is depleted. Several cereals can be satisfactorily malted but barley usually gives the least technical difficulty. For the production of African native beers, a variety of cereals especially sorghum are malted.

In recent times it has came up strongly that there are some identified land races of sorghum may have similar brewing properties comparable to well known varieties like *Kapaala*. These races are also known to do very well in the field. The objective of this work is to investigate the whole grain brewing and malting potential of 17 sorghum varieties.

2.0 Materials and Methods:

Seventeen (17) sorghum varieties were received from the Savanna Agricultural Research Institute, Tamale. The varieties were;

Kazea Manga	Kapeli
Zelle	Kemolga
Nakpaji	Global 2000
Sarsorg E5 – 2	Belko
Sarsorg M13 – 3	Keriga
Sarsorg M1 – 1	Mankaraga
Kasheagu	Kapaala
Yakpaji	Kadaga
Bumbago	

2.1 Physical Quality Evaluation

Sample divider was used to obtain a known weight of each of the samples for physical quality evaluation. Parameters evaluated included grain size and colour of grains and flour.

2.2 1000 grain Weight

One thousand whole and healthy grains of each sample were manually counted and weighed on an analytical balance.

2.3 %Moisture

An electronic moisture analyzer (Sartorius MA 45) was used to find the precise moisture content of the sorghum flour. The moisture content was taken by first positioning the sample can and then tarring the analyzer. 2g

sample was then spread evenly on the can and the drying programmme started.

2.4 Determination of Germination Capacity

Germination test was carried out by selecting seeds at random and cleaned. Unfilled seeds were discarded. 200 grains of each of the sorghum varieties were counted and weighed. The grains were washed and rinsed with distilled water. The grains were steeped for 24 hours in water, the water was drained and the grains evenly distributed in a compact single layer on petri dishes over laid with moistened filter paper. The petri dishes were covered and placed in the dark at room temperature for germination. Moisture supply was maintained by occasionally adding drops of water to the filter paper. After 72 and 120 hours, germinated seeds considered to be viable were counted and presented as percentage viable seed.

2.5 Sorghum Malts

In all the malting procedure the first step was steeping the grains in 0.1N $Ca(OH)_2$ for 8 hours after which grains were drained and washed with water. A period of 4 hours was allowed for air rest, followed by a second steeping in 0.5% formaldehyde for 8 hours. The grains were drained and washed with water before germination at 30°C for five days. Kilning was carried out at 55°C for 24 h.

2.6 Alpha-Amylase

The level of endogenous ct-amylase in cereal grains and products significantly affects the industrial exploitation of these commodities. In the brewing industry, the level of malt α-amylase is a key quality parameter.

2.6.1 Principle

The Ceralpha procedure (employing Amylase HR reagent) for the assay of α-amylase, employs as substrate, the defined oligosaccharide "non-reducing-end blocked p-nitrophenyl maltoheptaoside" (BPNPG7) in the presence of excess levels of a thermo stable α-glucosidase. (which has no action on the native substrate due to the presence of the "blocking group"). On hydrolysis of the oligosaccharide by endo-acting α-amylase, the excess quantities of α-glucosidase present in the mixture give instantaneous and quantitative hydrolysis of the p-nitrophenyl maltosaccharide fragment to glucose and free p-nitrophenol. The assay format is shown in Appendix 1.

Essentially, an aliquot of a cereal flour extract or fermentation broth is incubated with substrate mixture under defined conditions, and the reaction is terminated (and colour developed) by the addition of a weak alkaline solution. The absorbance at 400nm is measured and this relates directly to the level ofα-amylase in the sample analyzed. A standard error of les than 5% is achieved routinely and the assay is absolutely specific for α-amylase.

2.7 Malt flours

20g sample of malt was milled and passed through a 0.5mm screen. 0.5g malt flour was then accurately weighed into a 100ml volumetric flask. To the volumetric flask solutions of 1% sodium chloride plus 0.02% calcium chloride plus 0.02% sodium azide was added and adjusted to volume. About 15-20minutes was allowed for the enzyme to extract at room temperature, with occasional stirring. An aliquot of the solution was filtered through a Whatman GF/A glass fibre filter paper, or centrifuged at

1,000g for ten minutes. 0.5ml of the filtrate was then diluted with 9.5ml of Extraction Buffer Solution. Activity was assayed within two hours, (Megazyme Alpha-Amylase Assay Procedure, ICC Standard No. 303).

2.7.1 Assay Procedure

0.2ml aliquots of Amylase HR Reagent solution (unbuffered) were dispensed into glass test tubes and pre-incubated the tubes and contents at 40°C for 5 minutes. The extract was pre-incubated at 40°C for 5 minutes. To each tube containing Amylase HR Reagent solution (0.2ml) 0.2ml aliquots of pre-equilibrated malt extract was added directly to the bottom of the tube. The mix was then incubated at 40°C for exactly 20 minutes (from time of addition). At the end of the 20 minutes incubation period, exactly 3ml of stopping reagent was added and contents stirred vigorously. The absorbance of the solutions and the reaction blank were read at 400nm against distilled water, (Megazyme Alpha-Amylase Assay Procedure, ICC Standard No. 303).

2.7.2 Calculation of Activity

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermo-stable α-glucosidase, required to release one micromole of p-nitro-phenol from BPNPG7 in one minute under the defined assay conditions, and is termed a **Ceralpha Unit.**

For Malt:

Units (CU)/g of milled malt:

$$= \frac{\Delta E_{400}}{10} \times \frac{3.4}{0.2} \times \frac{1}{18.1} \times \frac{100}{0.5} \times 20$$

$$= \Delta E_{400} \times 376$$

Where:

ΔE_{400} = Absorbance (reaction) - Absorbance (blank)

Incubation Time = 10 minutes

Total Volume in Cell = 3.4ml

Aliquot Assayed = 0.2ml

ϵ_{mM} of p-nitrophenol (at 400nm) in 1% tri-sodium phosphate = 18.1

2.8 Beta-Amylase

β-Amylase plays a central role in the complete degradation of starch to metabolisable or fermentable sugars during the germination or malting of cereal grains. It also finds considerable application, together with starch debranching enzymes, in the production of high maltose syrups.

β-Amylase is usually measured using non-specific reducing sugar assays with starch as substrate. In some methods, the α-amylase is first inactivated by treatment at low pH, (Megazyme Beta-Amylase Assay Procedure, Betamyl method K-BEYA 12/04).

2.8.1 Principle

The Megazyme Betamyl β-amylase test reagent employs high purity α-glucosidase and PNPG5, and the level of α-glucosidase used ensures maximum sensitivity of the assay. On hydrolysis of p-nitrophenyl-α-D-maltopentaoside to maltose and p-nitrophenyl-α-D-maltotrioside by β-amylase, the p-nitrophenyl-α-D-maltotrioside is immediately cleaved to glucose and free p-nitrophenol by the α-glucosidase present in the substrate mixture (Appendix 2). Thus, the rate of release of p-nitrophenol relates directly to the rate of release of maltose by β-amylase. The

reaction is stopped, and the phenolate colour is developed, on addition of a high pH Trizma base solution. Standard errors of less than 7 % are readily achieved and the assay is highly selective for β-amylase, (Megazyme Beta-Amylase Assay Procedure, Betamyl method K-BEYA 12/04).

2.8.2 Assay Procedure

0.2ml of **Betamyl** Substrate Solution aliquots were dispensed into the bottom of glass test tubes and pre-incubated the contents at 40°C for approximately 5 minutes. The enzyme preparation was also pre-incubated at 40°C for approximately 5 minutes. To each tube containing Betamyl Substrate Solution, 0.2ml of pre-equilibrated (and suitably diluted) enzyme preparation was added directly to the bottom of the tube, mixed, and then incubated at 40°C for exactly 10 minutes (from time of addition). At the end of the 10 minutes incubation period, 3.0ml of Stopping Reagent was added and the tube contents stirred. The absorbance (at 400nm) of the reaction solutions and the reagent blank was read against distilled water, (Megazyme Beta-Amylase Assay Procedure, Betamyl method K-BEYA 12/04).

2.8.3 Calculation of Activity

One Unit of activity is defined as the amount of enzyme required, in the presence of excess α-glucosidase, required to release one micromole of p-nitro-phenol from PNPG5 in one minute under the defined assay conditions, and is termed a **Betamyl Unit.**

Thus:

Units/g of milled malt:

$$= \frac{\Delta A_{400}}{10} \times \frac{3.4}{0.2} \times \frac{1}{18.1} \times \frac{10}{1} \times 1250$$

$$= \Delta A_{400} \times 1174$$

Where:

ΔA_{400} = Absorbance (sample) - Absorbance (blank)

Incubation Time = 10 minutes

Total Volume in Cell = 3.4ml (or 1.7ml)

Aliquot Assayed = 0.2ml (or 0.1ml)

ϵ_{mM} p-nitrophenol (at 400nm) = 18.1

Extraction volume = 10ml per g (wheat, barley or malt)

Dilution = 1250 fold

(Megazyme Beta-Amylase Assay Procedure, Betamyl method K-BEYA 12/04).

2.9 Colour

The colour parameters L, a, b for the various samples were measured with the colour meter (Minolta CR310). The instrument was standardized each time with a white ceramic plate. The samples were placed in a transparent Petri dish, covered and colour determined by taking snap shots. The measurements were repeated and the mean values calculated.

2.10 Chemical Analysis

Crude fat was analyzed using AOAC method 984.13 (2000) 17th Edition whilst Nitrogen was by AOAC method 920.39 C (2000) 17th Edition.

Moisture was by AOAC method 925.10 (2000) 17th Edition and ash was determined by AOAC method 923.03 (2000) 17th Edition.

Molybdenum blue colorimetric method, permanganate titration and bipyridyl colorimetric method were used for the determination of Phosphorus, Calcium and Iron respectively.

2.10.1 Crude Fat & Nitrogen

Crude fat was analyzed using AOAC method 984.13 (2000) 17th Edition whilst Nitrogen was by AOAC method 920.39 C (2000) 17th Edition.

3.0 RESULTS AND DISCUSSION

3.1 PHYSICAL QUALITIES

Table1: Some physical quality characteristics of 17 sorghum varieties

		1000-kernel	Colour (grains)			Colour (flour)		
Sample	**%Moisture**	**wt**	**L**	**a**	**b**	**L**	**a**	**b**
Kazea Manga	6.85	19.91	59.21	8.92	11.08	78.38	4.02	10.94
Zelle	7.56	19.15	73.46	1.17	12.13	83.24	0.92	9.72
Nakpaji	6.59	23.25	66.74	2.36	11.64	80.92	1.89	8.39
Sarsorg E5 – 2	7.98	23.44	64.81	3.38	9.53	79.71	1.17	9.96
Sarsorg M13 - 3	7.61	23.24	64.77	3.25	10.97	80.06	0.98	10.07
Sarsorg M1 - 1	8.40	22.30	65.57	3.16	10.91	81.90	0.78	9.47
Kasheagu	6.60	23.01	56.20	11.67	6.99	75.80	5.80	8.51
Yakpaji	8.31	20.98	69.46	2.44	13.52	82.22	1.65	9.91
Bumbago	7.06	23.60	57.0.8	10.54	8.73	73.97	5.2	9.41
Kapeli	7.28	20.72	71.46	2.00	10.42	78.09	3.78	7.75
Kemolga	6.61	25.74	56.54	11.1	6.87	75.8	5.99	9.44
Global 2000	6.53	22.56	69.35	2.17	12.42	83.5	1.01	8.71
Mankaraga	6.98	22.89	70.5	1.44	11.82	75.52	3.96	5.00
Belko	6.4	22.90	72.6	1.66	10.75	82.31	1.23	8.49
Keriga	5.94	23.51	70.52	1.61	10.41	83.11	1.24	7.47
Kadaga	8.84	22.95	50.47	6.65	0.13	71.51	6.14	4.96
Kapaala	6.88	22.61	68.23	2.40	12.10	82.94	0.59	9.72
Mean	7.20	22.52	65.62	4.47	10.02	79.35	2.73	8.70
Std. Deviation	±0.78	±1.51	±6.48	±3.61	±3.02	±3.59	±2.01	±1.61

Kemola variety had the biggest grain size followed by Bumbago. Zelle was the variety with the smallest grain size. Moisture content of all varieties was below 10%, which is a good moisture level for grain storage. The moisture contents of all the samples fell within the desired range of 9.0%

to 11.0% for whole sorghum brewing. Maximum recommended storage moisture contents for aerated shelled sorghum grain to be stored for up to 1 year is 14.0% or 13.0% if it is to be stored more than 1 year. Moisture levels below 12 to 13 percent will prevent mould formation. One of the colour scales that is widely used by the food industry is the Hunter L,a,b and it is based on the opponent-colours theory that states that the red, green and blue human eye cone responses are re-mixed into black-white, red-green, and yellow-blue, opponent coders as they move up the optic nerve to the brain. The L, a, b type of scales simulate this as:

- **L** (lightness) axis – 0 is black, 100 is white
- **a** (red-green) axis – positive values are red; negative values are green and 0 is neutral
- **b** (yellow-blue) axis – positive values are yellow; negative values are blue and 0 is neutral

All colours that can visually be perceived can be measured in either L, a, b scale. All the grain samples were predominantly of lighter colour (white), with trace shades of blue and green. When grains were milled into flour the red-green colour shade generally diminished while the lightness became more pronounced because of the crushed light coloured endosperm.

Table2: Germination capacities of 17 sorghum varieties

Sample	Average germination @ day 3	% germination @ day 3	Average germination @ day 5	% germination @ day 5
Kazea Manga	195.0	98	197.0	99
Kapaala	178.0	89	178.0	89
Nakpaji	178.0	89	180.5	90
Sarsorg MI	126.5	63	126.5	63
Yakpaji	162.5	81	163.5	82
Kadaga	182.5	91	184.5	92
Kasheagu	89.5	45	89.5	45
Keriga	192.0	96	192.0	96
Belko	194.5	97	194.5	97
Global 2000	190.0	95	191.0	96
Bunbago	190.0	95	190.5	95
Kapeli	161.5	81	163.5	82
Sarsorm M 13-3	127.5	64	132.0	66
Sorsorg E5-2	158.0	79	159.5	80
Mankaraga	70.0	35	70.5	35
Zelle	193.5	97	193.5	97
Kemolga	126.0	63	127	64
Mean	156.06	78.06	157.24	78.65
Std. Deviation	±39.75	±19.87	±39.76	±19.96

Germination capacity (viability) of the cereals studied is shown in Table 2. The germination capacity was highest for Kazea Manga and lowest for Mankaraga. The germination capacities of all the seeds studied were not within the recommended range. Votong and Vonone, (1976) showed that cereal germination capacity of up to 80% is recommended as viable. It can therefore be inferred from this investigation that, Sarsorg MI, Kasheagu, Sarsorm M 13-3, Mankaraga and Kemolga had unsatisfactory germination capacities.

Table 3: Some chemical quality characteristics of 17 sorghum varieties

Sample	(%) Moisture	(%) Ash	(%) Protein	(%) Fat	(%) Carbohy.	Energy (Kcal/100g)	Iron (mg/100g)	p (mg/100g)	Ca (mg/100g))
Kazea Manga	8.4	1.8	12.2	3.9	73.7	378.7	23.7	180.0	32.4
Zelle	8.9	1.4	10.5	6.0	73.2	388.8	20.8	580.0	18.2
Nakpaji	7.3	1.4	11.9	2.9	76.5	379.7	17.3	447.0	41.2
Sarsorg E5 – 2	8.4	1.8	14.1	3.4	72.3	376.2	57.5	408.0	18.4
Sarsorg M13 - 3	8.1	1.5	14.7	4.3	71.4	383.1	15.4	612.0	22.9
Sarsorg M1 - 1	8.3	1.5	13.4	3.8	73.0	379.8	15.3	610.0	22.9
Kasheagu	7.7	1.6	12.7	4.2	73.8	370.6	26.0	438.0	40.4
Yakpaji	7.0	1.6	11.8	3.5	76.1	383.1	63.2	596.0	22.9
Bumbago	6.3	1.7	10.5	2.9	78.9	382.5	10.8	363.0	58.31
Kapeli	8.2	1.7	11.1	3.4	75.6	377.4	49.7	356.0	40.3
Kemolga	7.4	1.6	11.2	2.6	77.2	377.0	22.1	231.0	24.7
Global 2000	6.8	1.7	10.7	2.5	78.3	378.5	14.7	440.0	69.9
Mankaraga	8.6	1.5	13.4	4.1	72.4	380.1	34.3	676.0	26.6
Belko	7.4	1.6	10.6	4.0	76.4	384.0	30.8	218.0	24.6
Keriga	7.1	1.5	10.0	3.0	78.4	380.6	22.1	297.0	38.3
Kadaga	7.6	1.7	12.9	3.3	74.5	379.3	15.2	359.0	65.3
Kapaala	8.3	1.3	11.6	2.5	76.3	374.1	15.3	302.0	51.8
Average	7.75	1.57	11.94	3.55	75.18	379.62	26.72	418.41	36.42
Std. Deviation	±0.72	±0.14	±1.43	±0.87	±2.33	±4.14	±15.76	±152.25	±16.44

All the varieties were mostly whole grains and matured. Weevil damaged kernels were not detected in any of the seventeen samples. This suggests that samples must have been properly protected from insect attack. The total nitrogen levels found were between 0.27% and 2.35%. The operational range for sorghum is from 1.5% to 2.2%. A fat level of less than 4% which is within operational range was found in most of the samples except Zelle and Global 2000. Average carbohydrate content of the grains was 75.2% with a standard deviation of ±2.3.

Table 4: alpha and beta -amylase activities of 17 sorghum varieties

Sample	β- Amylase Activity (U/g of sorghum malt))	α- Amylase Activity U/g Ceralpha	Dextrinization Units/g
Kazea Manga	61.6	128.2	30.1
Zelle	32.0	34.8	8.6
Nakpaji	33.3	131.7	30.9
Sarsorg E5 – 2	8.6	65.0	15.6
Sarsorg M13 - 3	17.4	63.5	15.2
Sarsorg M1 - 1	18.6	49.4	12.0
Kasheagu	39.5	69.4	16.6
Yakpaji	25.0	60.0	14.4
Bumbago	52.4	78.4	18.6
Kapeli	34.6	40.0	9.9
Kemolga	53.7	68.7	16.4
Global 2000	30.5	83.3	19.8
Mankaraga	23.7	23.6	6.0
Belko	34.0	45.2	11.0
Keriga	37.8	60.0	14.4
Kadaga	14. 0	94.1	22.3
Kapaala	46.5	90.1	39.0
Average	34.3	69.7	16.6
Std. Deviation	±14.3	±29.6	±6.8

Enzyme activities affect modification of the grain and are dependent on time and temperature of malting, (Agu and Palmer, 1997). Alpha-Amylase is an enzyme of great importance in brewing since it randomly acts on α-1, 4 links of starch releasing small dextrins and fermentable sugars (Lewis and Young, 1995). β-Amylase is an exo-enzyme that acts on the non-reducing end of amylose and high-molecular-weight dextrins releasing maltose units (Narziss, 1976). Taylor and Robbins (1993) found that ungerminated sorghum also does not exhibit beta-amylase activity. This is fundamentally different from barley where the ungerminated grain exhibits beta-amylase activity and that it appears tropical cereal grains such as pearl millet, sorghum and maize possess only the "ubiquitous" form of beta-amylase, (MacGregor, 1996 and Briggs, 1998). The levels show Kazea Manga to have the highest activity (61.6U/g) followed by Kemolga (53.7U/g) and Bumbago (52.4). The activities of Sarsorg E5 - 2 (8.6U/g) and Kadaga (14.0) were very low.

During the mashing process, however, other factors, such as pH and metal ions, are important for optimal enzyme activity. It has been reported that calcium ions play an important role in the synthesis of α-amylase during malting, Deikman and Jones (1986) and. Moll and Jones, (1982). Calcium ions have also been implicated in stabilizing α-amylase by improving the heat stability of the enzyme during mash conversion. On the other hand, zinc ion is an important trace element required by yeast during fermentation (Palmer, 1989). These reported effects of metal ions are in reference to barley malt. It is, however, not clear whether these effects of metal ions on barley malt have similar effects on sorghum malt.

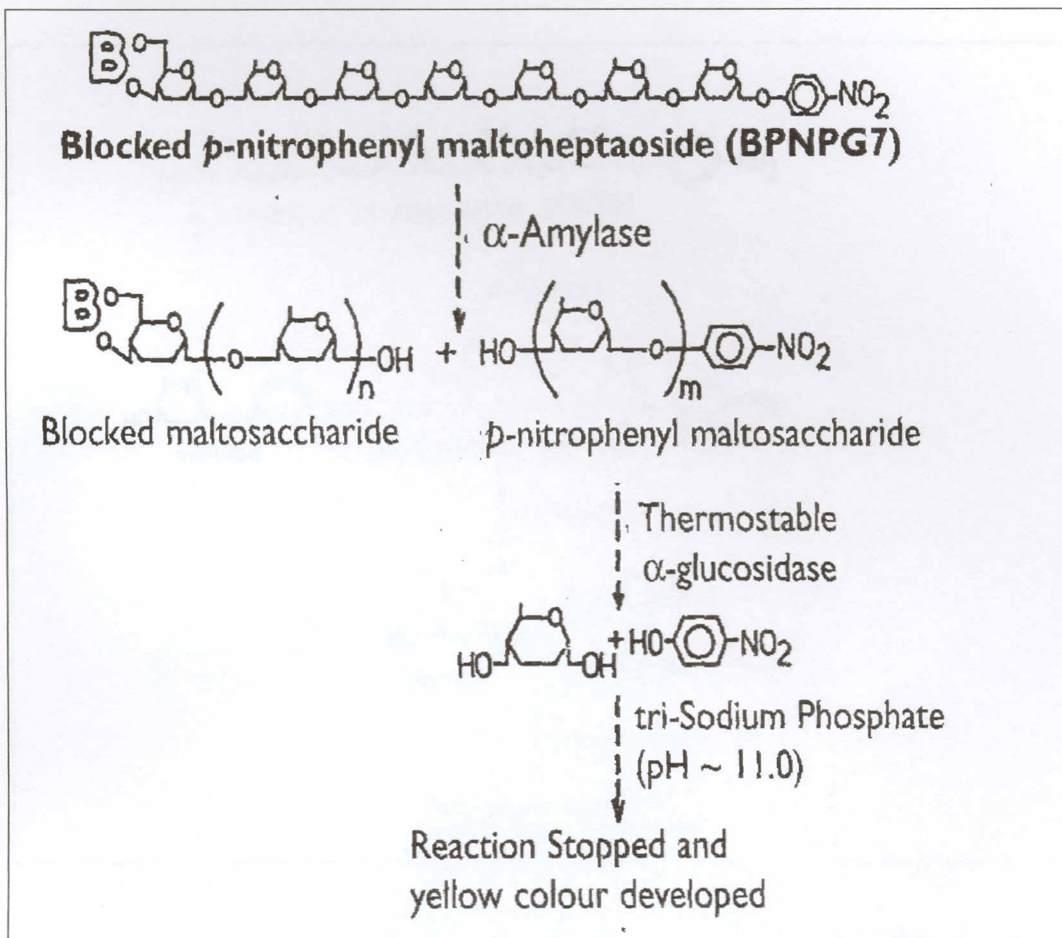
Conclusion

Sarsorg MI, Kasheagu, Sarsorm M 13-3, Mankaraga and Kemolga varieties had unsatisfactory germination capacities and Alpha Amylase activities. The *Kazea Manga* and *Nakpaji* varieties showed encouraging germination counts and Alpha-Amylase activity. The high values of germination and α-amylase activity indicate that the two varieties are comparable to *kapaala* in terms of malting properties. However it is recommended these two go through further analysis to ascertain their mashing and brewing potentials.

References

- Agu, R. C., and Palmer, G. H. The effect of temperature on the modification of sorghum and barley during malting. *Process Biochem.* 32: 501-507, 1997.
- Megazyme Alpha-Amylase Assay Procedure, ICC Standard No. 303, Megazyme International Ireland Limited, 2004.
- Megazyme Beta-Amylase Assay Procedure, Betamyl Method K-BEYA 12/04, Megazyme International Ireland Limited, 2004.
- Briggs, D.E., Malts and Malting, Blackie Academic and Professional: London, 1998, 133–244, pp. 615–698.
- Deikman, J., and Jones, L. R. (1986). Regulation of barley α-amylase isoenzymes I RNA. *Plant Physiol.* 80:672-675.
- Lewis, M. J., and Young, T. W. *Brewing*. 1st Ed. Chapman and Hall, London, 1995.
- MacGregor, A.W., Malting and brewing science: Challenges and opportunities. *Journal of the Institute of Brewing*, 1996, 102, 97–102.
- Moll, B. A., and Jones, R. L. (1982). α-Amylase secretion of barley aleurone. *Plant Physiol.* 70:1149-1155
- Narziss, L. Die Keimung. In: *Technologie der Malzbereitung,* 6th Ed.Ferdinand Enke Verlag, Stuttgart, Germany. Pp. 118-157, 1976.
- Palmer, G. H. (1989). Cereals in malting. In: Cereal Science and Technology, p. 186. G. H. Palmer, Ed. Aberdeen University Press, Aberdeen, Scotland.
- Taylor, J.R.N. and Robbins, D.J., Factors influencing beta-amylase activity in sorghum malt. *Journal of the Institute of Brewing*, 1993, 99, 413–416.
- Vontong, X. and Vonone, R. (1976) Training manual for Rice production institute pp 6-15

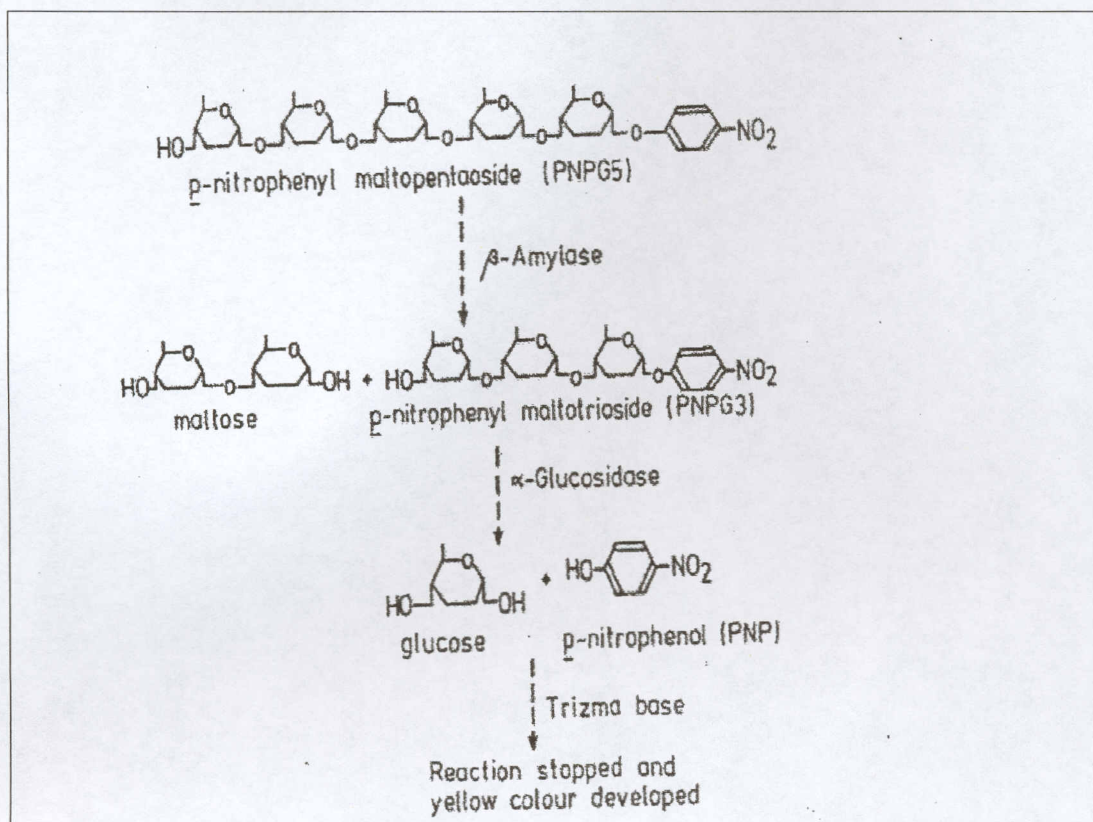
Appendix 1



Scheme 1: Theoretical basis of the Ceralpha α-amylase assay procedure

Immediately α-amylase cleaves a bond within the blocked p-nitrophenyl maltosaccharide substrate, the non-blocked reaction product containing the p-nitrophenyl substituent is instantly cleaved to glucose and free p-nitnophenol by the excess quantities of thermo stable α-glucosidase which are integral parts of the substrate mixture, and free p-nitrophenol is released. The reaction is terminated and the phenolate colour is developed on addition of tri-sodium phosphate (pH ~11.0).

Appendix 2



Scheme 2: Theoretical basis of the Betamyl β-amylase assay procedure

When PNPG5 is cleaved to PNPG3, the latter is rapidly cleaved to p-nitrophenol and glucose by the excess quantities of α-glucosidase which are an integral part of the substrate mixture.