Quantity and Quality of Ethanol Produced by Utlization of *Sorghum bicolor* Grains

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ABSTRACT

was conducted to investigate the production of ethanol from The present study sorghum grains (*Feterita*) and evaluation of its quality. Proximate chemical composition in terms of protein, crude fiber, moisture, ash, oil and carbohydrate contents were determined in sorghum grains and malted grains flours. The results indicated that sorghum grains flour contained 12.7% protein, 1.6% crude fiber, 5.3% moisture, 1.8% ash, 2.7% oil and 76.1% carbohydrates. On the other hand, malted grains flour contained 13.1% protein, 1.7% crude fiber, 5.5% moisture, 2% ash, 2.8% oil and 74.9% carbohydrates. Malt and pure enzymes (α-amylase and amyloglucosidase) were used to convert the starch to fermentable sugars. The yield of ethanol in fermented mash was 13% in the malt and 16% as a result of using pure enzymes. The ethanol volume produced from sorghum grains by malt and pure enzymes was 33 and 35 ml, respectively. The purity, density and viscosity of resulted ethanol were 95%, 0.83 gm / ml and 0.99 cip, respectively.

Key words: Ethanol, sorghum, protein, purity, density

INTRODUCTION

Sorghum is the fifth major cereal crop in the world after wheat, rice, maize and barely. It is grown in the United States, Australia and other developed countries for animal feed (El Khalifa, 2000).

Sorghum is cultivated in the semi-arid regions for fodder to feed the cattle, and the industrial applications for this grain are needed so that sorghum cultivation becomes of economic value (Maiti, 1996).

In Sudan, sorghum is grown in the vast clay plains of Gedarif State, Blue Nile State, White Nile State, Upper Nile State and Southern Kordfan State, togther with other minor areas in Southern Darfour State. All represent the main parts of the mechanized rain fed sub-sector (Abd Elrhman, 2002).

As a percentage of national food production, the contribution of Gedarif is estimated at nearly 35%, while that of Damazine stands at more than 13% (Ministry of Agriculture and Natural Resources, 2001).

The largest part of the area of production is in the rain fed sub-sector, but in the irrigated sector the yield is higher such as in Gezira and Rahad Scheme. Among the various sorghum varieties of the country, some are locally referred to as *Feterita*. Which is the cheapest and most available cultivar in Sudan. The rural Sudanese strongly believe in nutritional superiority of Feterita over other Sorghum cultivars.

Feterita is preferred to other Sorghum varieties for reasons other than its nutritional superiority. There are certain products that are made wholly or mostly from *Feterita* and not from other varieties, example of such products is *Hulu-mur*, *Merissa*, *Hussuwa* and *Assalia*. Moreover all Sorghum malt is made from Feterita grain (Dirar, 1993).

Ethanol, also known as ethyl alcohol, drinking alcohol or grain alcohol, is a flammable, colorless chemical compound. In common usage, it is often referred to simply as alcohol. It is a straight-chain alcohol and its molecular formula is variously represented as EtOH, CH_3CH_2OH, C_2H_5OH or as its empirical formula C_2H_6O (which it shares with dimethyl ether). After the use of fire, fermentation of sugar into ethanol is perhaps the earliest organic reaction known to humanity, and the intoxicating effects of ethanol consumption have been known since ancient times. In modern times ethanol intended for industrial use has also been produced from byproducts of petroleum refining.

Ethanol has widespread use as a solvent for substances intended for human consumption, including scents, flavorings, colorings, and medicines. In chemistry it is both an essential solvent and a feedstock for the synthesis of other products. Ethanol has a long history as a fuel, including as a fuel for internal combustion engines.

The objective of the present study was the production of ethanol from sorghum and evaluation of its quality.

MATERIALS AND METHODS

Materials

Sorghum grains were collected from Wad-Medani local market during January, 2008. The grains were cleaned from extraneous materials. Sorghum malt was prepared from the sorghum grains at laboratory level. Sorghum grains and malted sorghum samples were milled using a laboratory mill (Model: Christy and Norris Limited, 800 RPM, Machine type 8Lab Mill).

Malting

The major objective of the malting is to promote the development of hydrolytic enzymes, which are not present in the non-germinated grains **(**Dewar *et al*, 1995). Samples of sorghum grains (2 kg) were steeped in continuously changing fresh tap water for 24 hours. Following steeping the grains were germinated for various different times in a conditioned cabinet set. Twice daily during the germination period, the grains were removed from the conditioned cabinet and steeped for a short time (10 min.) in tab water. The excess surface-held water was then removed and the grains were then dried under the sun.

Proximate analysis

Proximate chemical composition was carried out on the sorghum grains and malt flours according to AOAC (1998). The proximate composition included crude protein, crude fat, crude fiber, moisture, crude oil and ash contents, while the total soluble carbohydrates content was calculated by difference (subtracting the mentioned components from 100).

Mash preparation and fermentation (hydrolysis by malt)

Hundred grams of sorghum grains flour were weighed into a beaker of 2 liter volume. One liter of water was added to the meal to produce a mash. Hundred grams of malt which contained α-amylase enzyme was added to breakdown the long carbohydrate chains making up starch into short chains of glucose, and eventually to individual glucose molecules. A drop of concentrated sulphuric acid was added to the mash to adjust pH to 4.9. The mixture was then incubated at 70 ºC for 12 hrs. Three ml from the mash and hydrolyzed mash were taken for analysis of reducing sugars. Then 9 grams of yeast (*Saccharomyces cerevisiae*) were added. The hydrolyzed mash was transferred to a fermentation tank. The time period of fermentation was about 72 hours, and then the mixture was filtered and distillated.

Mash preparation and fermentation (hydrolysis by pure enzymes)

Enzymatic hydrolysis was followed to produce glucose which was then converted to ethanol (fermentation) according to method of Cheetham (1987). In this method, 100gm from sorghum grains flour were mixed with 500ml distilled water in a conical flask, the flask was then plugged tightly with aluminum foil, then the slurry was cooked in water path at 100 ºC for 20 minutes to get viscous suspension with 20% dry solids sorghum grains flour. The pH of the starch slurry was measured using pH-meter and adjusted to 5.4, then 3 ml of α-amylase were added. The mixture was incubated at 85 ºC for 30 min. to obtain liquefied starch. Three ml from the starch slurry and hydrolysate samples were taken for analysis of pH and reducing sugar.

The pH of the liquefied starch was adjusted to 4.5 and 3 ml amyloglucosidase was added and then incubated in water path at 60 Cº for 6 hrs. Three ml from the slurry starch were taken for analysis before addition of 45gm of yeast. The time period of fermentation was about 72 hours, and then the mixture was filtered and distillated.

Determination of ethanol yield

The yield of alcohol (ethanol) in fermented mash was measured by Elbumeamter (I.E.A, 2008).

Reducing sugars

The reducing sugar content was determined according to ICUMSA (1998). In this method: Ten g of sucrose were weighed and poured into 1000 ml flask, then 10 ml of 0.1N HCL and distilled water were added and left overnight. Then the mixture was poured into burette 50 ml. Five ml of each Fehling's solution, 10 ml distilled water, were mixed into glass flask, then it was transferred to heater. The titration was started while the mixture was boiling. While the color of mixture was changing, during titration, 3 drops of methylene blue indicator were added and addition of the sugar solution was continued until the indicator was completely decolorized.

To get Fehling factor (F.F), the following formula was used:

$$F.F = \frac{C \times V \times R.S.}{100}$$

Where:

R.S. = Reducing Sugar%

F.F. = Fehling factor

C = Concentration of standard reducing sugar%

V = ml of standard solution for titration.

Two gm of grain flour slurry were weighed and poured into 200 ml flask, then the volume was completed to mark with distilled water. Then the mixture was filtered using a Whatman (No. 1) filter paper. After that, the liquid was poured into 50 ml burette. Then the titration was made to determine reducing sugars %.

$$R.S. = \frac{F.F. \times 100}{C \times V}$$

Where:

R.S. = Reducing Sugar%

F.F. = Fehling factor

C = Concentration of sample

V = ml of standard solution for titration.

pH measurement

The pH of the mash was measured using pH-meter (PHS-3C Digital) at ambient temperature (ICUMSA, 1994).

Brix determintion

The total soluble solids were determined using hand refractometer according to (ICUMSA, 1998).

The density and viscosity of ethanol

The density of ethanol was measured as follows:

The flacon (100 ml) was filled by ethanol, and then ethanol was weighed. The density was calculated as follows:

Density = weight / volume.

The viscosity was obtained from the equation

$$\frac{\eta_1}{\eta_2} = \frac{\rho_1 t_1}{\rho_2 t_2},$$

C°η_1= viscosity of water at 30

C°η_2=viscosity of ethanol at 30

C°ρ_1= density of water at 30

C°ρ_2=density of ethanol at 30

t_1= amount of time it takes for a given amount of fluid to flow through a thin glass tube for Water

t_2= amount of time it takes for a given amount of fluid to flow through a thin glass tube for Ethanol

The purity of ethanol

The purity of the ethanol was measured using hydrometer at ambient temperature (ICUMSA, 1994). A cylinder was filled with the ethanol, and then hydrometer was inserted into a cylinder. Then allowed to equilibrium, and purity was read.

RESULTS AND DISSCUSTION

Proximate Analysis

The Proximate chemical composition of sorghum grains (*Feterita*) and malted sorghum grains are shown in Table (1). The protein contents of *Feterita* sorghum grains flour (12.7%), was lower than the value of 13.1% for sorghum malted grains, and lower than the range 12.8% - 13.4% reported by Omer (2004) and Eggum *et al* (1983), respectively. However, although the amount of protein in sorghum grain is relatively high, it is known that, this protein is of inferior quality since it lacks certain essential amino acids like lysine and tryptophan (Sulieman, 1995). In addition, sorghum protein has a low digestibility compared to other cereals (Kurien *et al*. 1960).

The crude fiber contents of *Feterita* grains flour (1.6%), was in agreement to the value given by Baem (1906) and lower than the values 1.8% and 2.1% reported by Abd Elmageed (2004) and Eggum *et al* (1983), respectively. It is reported that fibers have adverse effects on the availability of some nutrients. The concentration of zinc and iron in the tibia of rats fed on sorghum diets rich in fiber and phytate was significantly lower than in rats fed on a non-sorghum diet with low fiber content (Internet, 2007).

Table 1. Chemical composition (%) of *Feterita* sorghum malt and grains

Sample	Crude Protein (%)	Crude fiber (%)	Moisture (%)	Ash (%)	Oil (%)	Carbohydrate (%)
Feterita sorghum grains	12.7	1.6	5.3	1.8	2.7	76.1
Malted sorghum grains	13.1	1.7	5.5	2.0	2.8	74.9

However, it was reported that high tannin in Sudanese variety, *Feterita* contained an extremely high percentage of fiber 9.2% (Internet, 2007).

The moisture contents of *Feterita* grains flour was found to be 5.3%, which was higher than both values reported by Abd Elmageed (2004) and Omer (2004) which were 4.1% and 3.8%, respectively. The ash content of *Feterita* grains flour 1.8% was similar to the value reported by Budair (1977) and lower than the value 2.07% reported by the Eggum *et al* (1983), and higher than value 1.7% reported by Omer (2004). The oil content of *Feterita* grains flour 2.7% was lower than the value 4.1% reported by Eggum *et al* (1983) and higher than the value 2.5% reported by Omer (2004).

The carbohydrate contents of *Feterita* grains flour (76.1%) was higher compared to those of *Feterita* (71%), *Dabar* (73.4%) and *Tetron* (72.5%) as reported by Eggum *et al* (1983), and less than the values 76.3% and 77.4% reported by Abd Elmageed (2004) and Omer (2004), respectively for carbohydrate contents in *Feterita* grains flour.

The moisture content of malted sorghum grains was found to be 5.5%, which was higher than that reported by Omer (2004) who found a value of 4.4%. The variation in moisture contents could be attributed to environmental conditions climatic, and genetic variations. The protein contents of feterita malt grains was found to be 13.1%, which was less than that reported by Omer (2004) who found a value of 13.4 %. The crude fiber contents of malted sorghum grains was 1.7%, which was less than the value (1.9%) reported by Omer (2004).However, the ash contents of malted sorghum grains was similar to the value given by Omer (2004).

The oil content of the malted sorghum grains was 2.8%, which was slightly higher than the value reported by Omer (2004) which was 2.6 %. The carbohydrate contents of sorghum malted grains were found to be 74.9%, which was less than the value 75.7% reported by Omer (2004). It seems that germination of sorghum grains resulted in increasing most of the chemical components, except the carbohydrate which decreased as a result of enzymatic action.

PH and reducing sugars in mash

All polysaccharides can be hydrolyzed with acid or enzymes to produce fermentable sugars (glucose). Table (2) shows the value of pH and reducing sugar contents of *Feterita* sorghum flours slurry prior to fermentation.

The results revealed that the pH and reducing sugars were 6.7 and 1.7%, respectively. The value of reducing sugars was greater than the value 1.5% reported by Mohammed (2007).

In sorghum grains flour slurry hydrolyzed by the malt, the pH was adjusted at 4.9 which was suitable for malt enzymes activity. The reducing sugars was found to be 18.3%.

In hydrolyzed slurry by α-amylase enzyme the pH was adjusted at 5.4 and reducing sugars was found to be 12.4% which were greater than the value 10.2% for starch only reported by Abd Elmajeed (2004).

In saccharification step the pH was adjusted at 4.6 and reducing sugar was found to be 23.1% which was double the result of liquefaction.

Table (2). PH and reducing sugars in treated mash

Mash Treatment	pH	Reducing sugars %
Sorghum grain Flour	6.7	1.7
Sorghum grain flour + malt	4.9	18.3
Sorghum grain flour + α-amylase (liquefaction slurry)	5.4	12.4
liquefaction slurry + Amyloglucosidase	4.6	23.1

Alcohol yield by malt hydrolysis at different brix

Sugar concentration plays an important role in ethanol fermentation by the yeast.

Table (3) shows that the maximum amount of alcohol (13%) in fermented mash was produced when the brix was 15% followed by 12% and 10% which were produced when the brix were 20% and 10%, respectively. The maximum value of ethanol was higher than the value (11%) reported by Ahmed (2008).

In these findings it is clearly seen that the increase in the brix, however, resulted in the decrease of its conversion to ethanol. This could be explained by the fact that the low production of ethanol at high concentration of sugar may be due to the toxic effects of ethanol and not by the sugar (Gill, 1981). Therefore, the brix level of 15 % was maintained.

Alcohol yield by hydrolysis using enzymes (α-amylase and amyloglucosidase)

The amount of alcohol in fermented mash was found to be 16%, which was greater than the value 13% when the malt was used in this study, also greater than the value (11%) reported by Ahmed (2008).

Table 3. Alcoholic yield by malt hydrolysis at different brix

Grain and malt flour (weight/gm)	Water added(ml)	Brix %	pH	T C°	Yield (alcohol %)
200	500	20	4.9	30	12
200	1000	15	4.9	30	13
200	1500	10	4.9	30	10

Table (4) shows that the volume of ethanol after distillation was 33 and 35 ml per 100gm of Sorghum grains in case of the malt and pure enzymes, respectively with purity 95%.produced by (α-amylase & amyloglucosidase) enzymes was 35ml per 100 gm of sorghum grains, which was close to the value 33 ml per 100 gm of sorghum grains determined in this study when malt was used and lower than the value of 38 ml per 100 gm of sorghum grains reported by Peterson (1995).The ratio of ethanol produced by the enzymes (α-amylase and amyloglucosidase) was higher than the ratio of ethanol produced by malt enzymes, This is due to the enzyme activity.

Table 4. The volume of ethanol produced from sorghum grains by malt and (α-amylase & amyloglucosidase) enzymes after distillation

Sorghum grains (weight/gm)	Material used	Ethanol (ml)

100	Malt	33
100	(α-amylase & amyloglucosidase)	35

Physicochemical characteristics of ethanol

Table (5) shows some of physicochemical characteristics of the examined ethanol after production. The purity, density and viscosity of ethanol were 95%, 0.83 gm/ml and 0.99 cip, respectively.

The purity value determined in this study was slightly less than the ideal purity (95.6%) which normally follows the popular method of purification as reported by Mills (1987) and also less than value 96% which was reported by Ahmed (2008).

The viscosity value (0.99 cip) determined in this study was greater than the value 0.83 cip reported by Ahmed (2008).

The density of ethanol determined in this study was 0.83 gm/ml however, this value C (Morrison, ̊was greater than the standard density value which is 0.789 gm/ml at 25 1972), this is may be due to temperature variation.

CONCLUSION

Sorghum grains have good potential as a raw material for ethanol production. The process for alcohol production using sorghum, although a little bit expensive, it is still preferable as it is an environment-friend.

During the fermentation process, it is important to prevent oxygen from getting into the ethanol, since otherwise the ethanol would be oxidized to acetic acid (vinegar). Also, in the presence of oxygen, the yeast will undergo aerobic respiration to produce just carbon dioxide and water, without producing ethanol.

The utilization of sorghum plant in production of food, feed and selected industrial products will become increasingly important in the developing countries. A total utilization of all components in the sorghum plant for use in the manufacturing and food industries would increase cash flow to the farmer and thereby constitute an incentive for him to increase his production.

REFERENCES

Abd Elmageed, N.K. (2004). Utilization of Sourghum (feterita) Starch in Production of fructose Syrup .M.sc. Thesis, University of Gezira, Medani, Sudan.

Abd Elrhman, A.A. (2002). Sorghum production in the Gezira Scheme. M.Sc. Thesis, University of Gezira, Medani, Sudan

Ahmed, M.A. (2008). Production of Ethanol from Sugar Cane Molasses and Evaluation of its Quality. M.Sc. Thesis, University of Gezira, Medani, Sudan.

A.O.A.C. (1998). Official Method of Analysis. Fifth edition Association Official Analytical Chemists, Washington, dc.

Beam, W.(1906). Report of the Chemical Sectior. Second Report of the Wellcome Research Laboratories. Khartoum, Sudan, pp, 205 – 244.

Budair, A. A. (1977). Chemical studies on Sorghum Grains and their products. M. Sc. Thesis, University of Khartoum, Sudan.

Cheetham,P.S.J. (1987).*The application of enzymes in industry , PP 274 – 279. In: A.Wiseman(ed.).Handbook of Enzyme Biotechnology. Eilis Horwood Limited, Chichester , England.*

Dewar, J., Joustera, S.M. and Taylor, J.R.N. (1995). Accepted methods of sorghum Malting and Brewing analyses. CSIR Food Science and Technology, Pretoria, South Africa.

Dirar, H.A (1993). The Indigenous Fermented Foods of Sudan. Astudy in African Food and Nutrition, CAB International, Wallingford, UK

Eggum,B.O.,Monawar,L.,Bach, knudsen,K.E., Munk,L.and Axleel,J . (1983).Nutritional Quality of Sorghum and Sorghum Foods from Sudan. Journal of Cereal science 10, 127 – 137.

El Khalifa, E.A. (2000). Sorghum based traditional foods of Sudan-preparation and quality aspects. Proceeding of the 1997 International Conference on Traditional food. CFTRI, Mysore India.

Gill,E.(1981). In Recent Advances in the Fermentation Industries, (Royal Institute of Chemistry).

International Epidemiological Association (I.E.A), (2008). Oxford Journals, Oxford University Press.

International Commission Uniform Methods of Sugar Analysis (ICUMSA) 1998. Method Book with First Supplement.

International Commission Uniform Methods of Sugar Analysis (ICUMSA) 1994. Method Book with First Supplement.

Kurien, P.P, Narayanarao, M., Swaminathan, M. and Subrahamanyan, V.(1960) Br.J. Nutr. 14

Maiti, R. (1996). Sorghum science. New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. 352 pp.

Mills, G.A.;(1987)."Alcohols as Components of Transportation Fuels." Annual Review of Energy.. Vol. 12, 47-80. Retrieved on September 2, 2007.

Ministry of Agriculture and Natural Resources (2001). *Annual Report. Khartoum, Sudan*

Mohammed, S.A.(2007). Supplementation of Fermented Sorghum Bread (Kisra) With Dehulled and Defatted Sesame Flour .M.sc. Thesis, University of Gezira, Medani, Sudan.

Morrison, R.T. (1972). Organic Chemistry, 2nd Ed. Allyn and Bacon, Inc.

Omer, B. M. (2004). Preparation and Quality Aspects of Hulu-Mur Carbonated Beverage. M.sc. Thesis, University of Gezira, Medani, Sudan.

Peterson, A. 1995. Production of fermentable extracts from cereals and fruits. Pages1–31 inFermented beverage production (Lea, A.G.H, and Piggot, J.R., eds.). London, UK: Blackie Academic & Professional.

Sulieman, A.E.(1995). Fenugreek Supplemented Backed Products, Quality Aspects. M.Sc. Thesis, University of Gezira, Wad-Medani, Sudan.

(http://www.fao.org/DOCTOR/2007).

http://en.wkipedia.org wgrubbs@stetson.edu 2008

كمية ونوعية الايثانول المنتج باستخدام حبوب الذرة

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الملخص

أجريت هذه الدراسة لتحديد إنتاج الايثانول من حبوب الذرة (فترية) وتقييم جودته. عينة حبوب الذرة تم الحصول عليها من السوق المحلي بود مدني. المكونات التقريبية للعينة: بروتين وألياف خام ومحتوى الرطوبة ومحتوى الرماد ومحتوى الزيت والكاربوهيدريت حددت فى دقيق حبوب الذرة ودقيق حبوب الذريعة. نتائج دقيق حبوب الذرة هي 12.7% بروتين و1.6%ألياف خام و5.3% محتوي الرطوبة و1.8% محتوي الرماد و2.7% محتوي الدهن و76.1% محتوي الكاربوهيدريت.نتائج دقيق حبوب الذريعة هي 13.1% بروتين و1.7% ألياف خام و 5.5% محتوى رطوبة و 2% محتوى رماد و 2.8% محتوى زيت و 74.9% للكاربوهيدريت. لقد استخدمت في هذه الدراسة الذريعة والإنزيمات النقية (ألفا أميليز و أميلوجلكوسيديز) لتحويل النشا إلي سكريات قابلة للتخمر كل على حده. الكحول في العجين المتخمر 13% في حالة الذريعة 16% في حالة الأنزيمات النقية. حجم الإيثانول المنتج 33 و35 مل لكل 100 جم حبوب ذرة في حالة الذريعة والإنزيمات النقية علي التوالي. كثافة الإيثانول المنتج 0.83 جم لكل مل و 95% نقاوة و 0.99 سنتبويز لذوجة.