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Full Length Research Paper

Nutritional composition of *sorghum bicolor* starch hydrolyzed with amylase from *Rhizopus* sp.

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The partially purified amylase extracted from a local isolate of *Rhizopus* sp. was used for hydrolysis of *Sorghum bicolor* starch. The amylolytic activity of the enzyme was 45.33 U/g and the protein content was 0.345 mg/ml. The maximum temperature for the enzyme was obtained at 55°C while it acts best at pH 5.0. The percentage increase of 1.62, 21.00 and 1.42% for the fat, protein and carbohydrate respectively for the hydrolysed sorghum starch compared with the unhydrolysed sorghum starch was recorded. The increase in the vitamin B1 and B2 of the hydrolysed sorghum starch over the unhydrolysed starch was 2.93 and 2.39%, respectively. A percentage increase of 9.10, 0.89 and 2.83% for Ca, Mg and Na, respectively, for the hydrolysed sorghum starch over the unhydrolysed starch sample was recorded. The Thin Layer Chromatogram of the hydrolysed sorghum starch revealed the presence of the following essential amino acids; histidine, methionine, threonine, isoleucine, phenylalanine, tryptophan and valine. The chromatographic and quantitative analysis of the hydrolysed sorghum starch shows that it contains three oligosaccharides; sucrose (7.38%), lactose (1.75%) and maltose (48.0%), and two monosaccharides which are glucose (1.75%) and galactose (1.16%).

Key words: Sorghum, amylase, pH, temperature, hydrolysis, essential amino acids.

INTRODUCTION

Sorghum grain is among the oldest of cereals and fifth important in the world (Arnon, 1964). The United States is the world's largest producer followed by India. Sorghum grain is the leading cereal grain on African continent and Nigeria is the world second largest producer of the grain (ICRISAT, 2002). The plant is drought resistant and is therefore an extremely important commodity that provides necessary food and feed for millions of people living in semi-arid environment worldwide.

In many parts of the world, sorghum has traditionally been used in food products and various food items (Badi and Ollis, 1986). Fifty percent of sorghum is grown directly for human consumption. It is one of the major staple foods in Africa, Middle East and Asia. Sorghum is an important animal feed used in countries like United States, Mexico, South America and Argentina (Elliot and McPherson, 1971). Good quality sorghum is available with nutritional feeding value that is equivalent to that of corn. The grain is higher in protein and lower in fat content than corn (Yohe, 2002). Varieties with waxy endosperm are sources of starch having properties similar to tapioca (Dalton and Mitchell, 1959).

In West Africa, Nigeria has emerged as a pioneer in the industrial utilization of sorghum. A wet-milling process similar to that used in cornstarch manufacture is used for sorghum. The starch is made into dextrose for use in foods (Moench, 1999). Starch hydrolysis using fungal enzyme to produce simple saccharides is comparatively cheaper than the use of any other synthetic process or method (Prapulla et al., 1999) . The mass production of saccharides is not complicated and the use of enzyme is a better alternative to chemical synthesis due to the production of some undesirable end products after the chemical synthesis (Toope et al., 1983).

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This work investigates the nutritional and chemical composition of *Sorghum bicolor* starch hydrolysed with partially purified amylase of *Rhizopus* sp.

MATERIALS AND METHODS

Sorghum bicolor was obtained from Wazobia Market, Sabo, Ogbomoso, Oyo State, Nigeria.

Preparation of medium and cultivation of organism

The fungus *Rhizopus* sp. used for this work was isolated at Ladoke Akintola University of Technology, Ogbomoso, Oyo-State, Nigeria. Cultivation of the *Rhizopus* sp. was achieved using potato dextrose broth. The pH of the medium was adjusted to pH 7.0. 20 ml of the broth was poured on the surface of the plate containing the *Rhizopus* sp. and the spores were dislodged. This mixture was decanted aseptically into 200 ml sterile potato dextrose medium prepared in 250 ml conical flask. The medium was then incubated at room temperature for 48 h (Olama et al., 1993).

Preparation of cell free extract

At the end of incubation the surface fungal mats were picked from the culture medium using forceps. The residual wastes were separated by centrifugation at 3,500 x g for 20 min in a centrifuge (Olama et al., 1993). The clear supernatant which was considered as a source of extracellular enzyme was partially purified by modified method of Kundu and Das (zxcwguil9.oxfy65wswwx1986). The supernatant was brought to 30% ethanol concentration and centrifuged at 3,500 x g for 20 min. The supernatant collected was further brought to 70% ethanol concentration and recentrifuged at 3,500 x g for 20 min. The sediment (precipitate) which was found after the second centrifugation was taken as the enzyme. For every 150 ml of the solution that was spun in the centrifuge, the pellet recovered was suspended in 5 ml phosphate buffer at pH 6.0. The protein content of the crude enzyme was then estimated using the method of Lowry et al. (1951).

Preparation of starch from sorghum cereal

The grain was steeped for 2-3 h after which it was grounded and sieved to separate starch from the shaft. It was allowed to settle, the excess water was decanted and the filtrate was then dried.

Enzymic hydrolysis of sorghum starch

This was achieved by suspending 12 g of sorghum starch in 100 ml of water containing 0.4 g of NaCl and 0.04 g of $CaCO_3$. The suspension was then adjusted to pH 6.0 and heated at 72°C for 2 h in a water bath. The suspension was continually stirred by applying a stirring speed of 200 rpm for 2 h. The partially purified enzyme extracted (4 ml) from the residual waste of the culture medium of *Rhizopus* sp. was added when the temperature of the water bath reached 60°C. After 2 h, the product was poured out, allowed to cool and then sun dried for 2 to 3 days (Linko et al., 1975).

Determination of amylase activity

The amylase activity of the partially purified enzyme was determined using AOAC (1999).

Effect of temperature and pH on amylase activity

The partially purified amylase extracted from *Rhizopus* sp. was subjected to varying temperatures ranging from 35 to 70°C. The resulting amount of reducing sugar produced by the enzyme was calculated to obtain the temperature at which the amylase activity was highest (AOAC, 1999). The amylase was again subjected to varying pH ranging from 3.0 to 9.0. The resulting amount of reducing sugar produced by the enzyme was calculated to obtain the pH value at which the amylase activity was best (AOAC, 1999).

Proximate analysis and toxin determination

Proximate analysis was carried out according to conventional method (AOAC, 1999) to estimate moisture, crude protein, crude fibre, crude fat and ash contents of both the hydrolyzed and unhydrolysed starch samples. The carbohydrate fraction was obtained by difference that is, subtracting the sum of protein, fat, ash and fibre from the total dry matter. Test for toxin was determined by AOAC (1999).

Determination of mineral contents and vitamin

Calcium, potassium, iron, magnesium, zinc, sodium, manganese and vitamin contents for both the hydrolyzed and unhydrolysed starch samples were determined by the method of Ronald and Ronald (1999). The vitamin B Complex was determined for both hydrolyzed and unhydrolysed sorghum starch (Anon, 1972).

Extraction and chromatographic separation of amino acids

The determination of the amino acid profile was done using the Rosen method (1957). 2 g each of the hydrolyzed and unhydrolyzed starch samples were weighed separately using digital chemical balance into 250 ml beaker of 20 ml of phosphate buffer at pH 7.0. Each mixture was stirred for about 3 min and the mixture was centrifuged at 200 rpm for 10 min. The supernatant was then poured into separating funnel and shaken 3 times with 10 ml petroleum ether to remove the organic pigments. The top phase was discarded and the aqueous phase which contained protein and amino acids was retained. Protein was precipitated from the aqueous phase by adding 5 ml of 10% (w/v) trichloroacetic acid (TCA) to 5 ml extract. The mixture was shaken and kept in the freezer for 10 min. The precipitate formed was removed by centrifuging and the filtrate was used for amino acid profiles determination.

The amino acids content in the extracts were separated by thin-layer chromatography using the method of Mikes and Chalmers (1989). Aliquots of 50 l of the extract were spotted on Avicel microcrystalline cellulose (Whatman analytical plates) thin layer glass plates along with 20 l of reference standard mixture. The reference mixture contained lysine, histidine, phenylalanine, methionine, proline, glycine, isoleucine, leucine, threonine, valine, thyrosine each present at a concentration of 0.1% (w/v). One-dimensional ascending chromatography was used. The solvent system employed for the separation was n-butanol : glacial acetic acid : water at a ratio of 4:1:2 (v/v/v). Separation was carried out for 3 h. The chromatograms were air-dried and the amino acids were located by spraying the locating reagent of 0.2% (w/v) of ninhydrin in ethanol. This was allowed to air dry and then oven dried at 100°C for 5 min for spots to develop. The separated amino acids were identified using the reference standard.

The quantitative estimation of the amino acid contents was done through the colorimetric method (Rosen, 1957) by using the extracts obtained from the elution through the thin layer

chromatography.

Quantitative determination of saccharides

The quantitative determination was carried out through spectrophotometry method using phenol sulphuric acid method (McCready, 1970; Dubios et al., 1956) . The estimation was done by the use of the guide-strip technique where a develop thin-layer chromatographic plate was used in locating the position of sugars in unsprayed plate. The squares containing the sugar were cut out and eluted with 5 ml of 95% ethanol at 70°C for 2 h. The cellulose powder was removed by centrifugation at 3000 rpm for 15 min The sugar in the extract (supernatant) was then estimated according to the phenol sulphuric acid method.

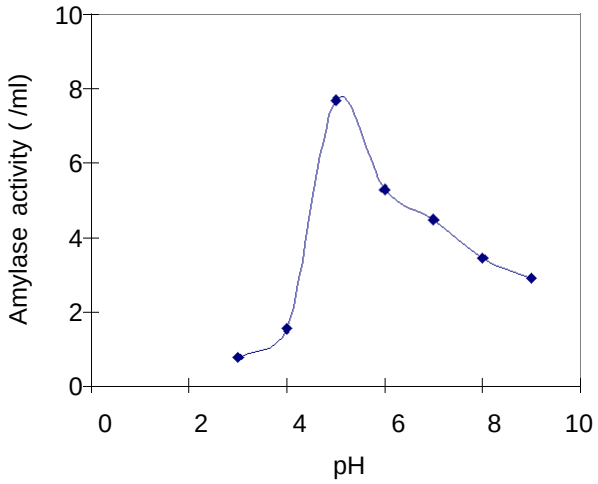

Figure 1. Effect of temperature on amylase activity during sorghum starch hydrolysis.

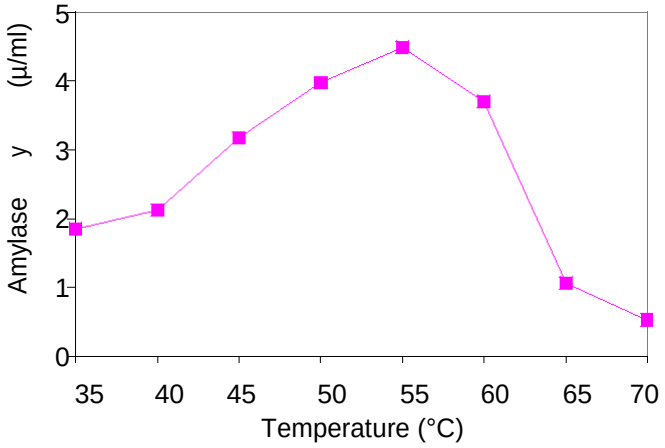

Figure 2. Effect of pH on amylase activity during sorghum starch hydrolysis.

Table 1. Proximate compositions of unhydrolyzed and hydrolyzed sorghum starch.

	Unhydrolysed sorghum Starch (%)	Hydrolysed sorghum Starch (%)
Moisture content	10.66	9.25
Ash	1.98	1.64
Fat	3.35	3.25
Crude fibre	2.25	1.15
Protein	9.35	11.09
Carbohydrate	72.41	73.63

Table 2. Vitamin composition of the unhydrolyzed and hydrolyzed sorghum starch.

Vitamin B Complex	Unhydrolysed sorghum Starch (µg)	Hydrolysed sorghum starch (µg)
B1 (Thiamine)	3.75	3.78
B2 (Riboflavin)	1.31	1.34
B3 (Niacin)	74.34	73.78

RESULTS

The partially purified amylase obtained from the fungus *Rhizopus* sp. has an amylase activity of 45.23 U/ml. The maximum temperature of the enzyme was 55°C (Figure 1) while the optimum pH was 5.0 (Figure 2). The protein content of the crude enzyme was 0.345 mg/ml. Table 1 shows the proximate composition of the hydrolysed sorghum starch. There was a decrease in the percentage content of the moisture, ash and crude fibre. However, a percentage increase in the values of the protein, fat and carbohydrate was recorded.

The Vitamin A potency of the hydrolysed sorghum starch was 0.053 U/g while that of the unhydrolysed sample was 0.069 U/g depicting a slight decrease in the value after hydrolysis. However, there was an of 2.98 and 2.29% for Vitamin B1 and B2, respectively, in the hydrolysed rice starch over that of the unhydrolysed sorghum starch (Table 2). In Table 3 different essential minerals were seen to be present in both the unhydrolysed and hydrolysed sorghum starch samples though in different proportions. An increase of 9.10, 0.89 and 2.83% was recorded for Ca, Mg and Na, respectively, in the hydrolysed sorghum starch.

The chromatographic analysis of both the hydrolysed and unhydrolysed sorghum starch samples revealed that histidine, isoleucine, lysine, methionine, phenylalanine, tryptophan and threonine were present in different quantities (Figure 3). However, histidine could not be detected quantitatively (Table 4). Quantitative analysis of

Table 3. Mineral composition of the unhydrolyzed and hydrolyzed sorghum starch.

	Unhydrolysed sorghum starch (mg)	Hydrolysed sorghum Starch (mg)
Ca	15.60	17.02
K	0.18	0.16
Fe	5.00	4.58
Mg	163.90	170.40
Zn	2.42	2.42
Na	2.12	2.18
Mn	1.99	0.94

Table 4. Quantitative estimation of amino acid of the unhydrolyzed and hydrolyzed sorghum starch.

Amino acid profile	Unhydrolysed sorghum starch (mg/100 g)	Hydrolysed sorghum Starch (mg/100 g)
Methionine	83.2	26.8
Lysine	118.9	125.8
Histidine	-	-
Leucine	80.5	-
Valine	284.7	292.6
Isoleucine	242.6	247.4
Phenylalanine	306.3	306.8
Tryptophan	56.8	59.5
Threonine	185.8	187.9

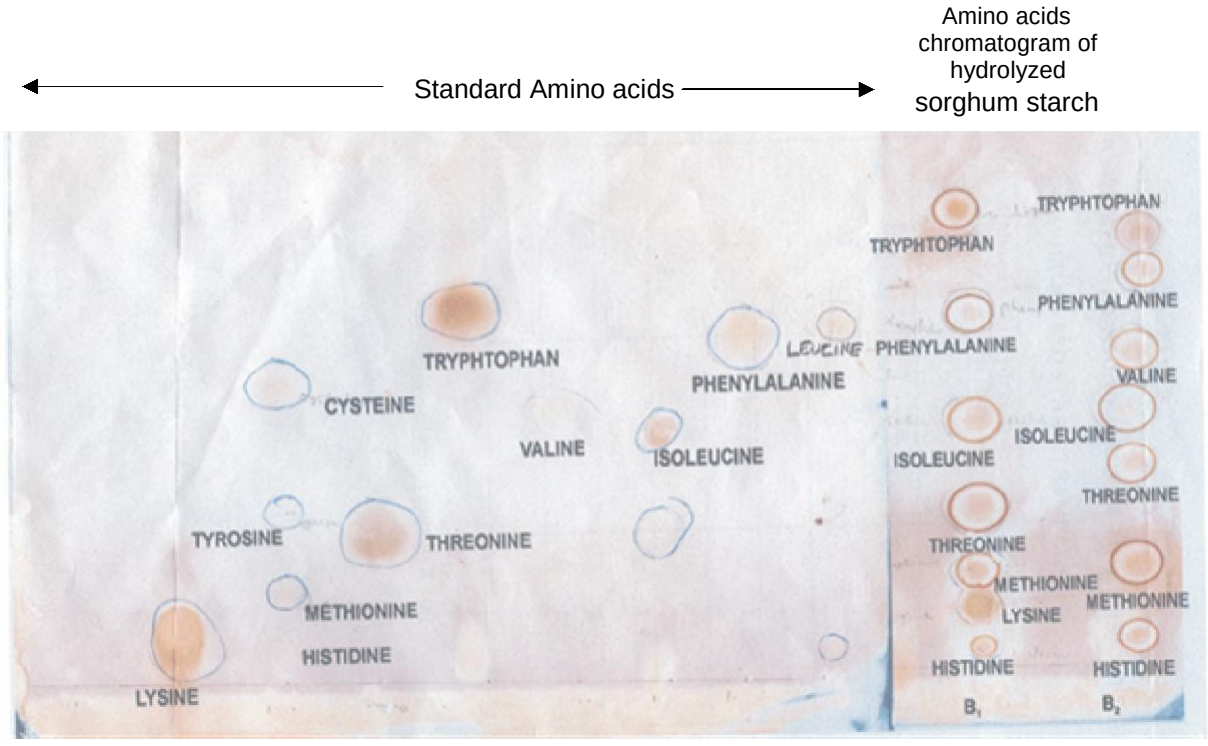

Figure 3. Amino acid chromatogram of hydrolyzed sorghum starch.

the amino acids also shows that though leucine was present in the unhydrolysed sorghum starch, it was absent in the hydrolysed sample. There was an increase in the lysine, phenylalanine, valine, isoleucine, tryptophan and threonine after the starch hydrolysis (Table 4). Chromatographic analysis of the hydrolysed starch

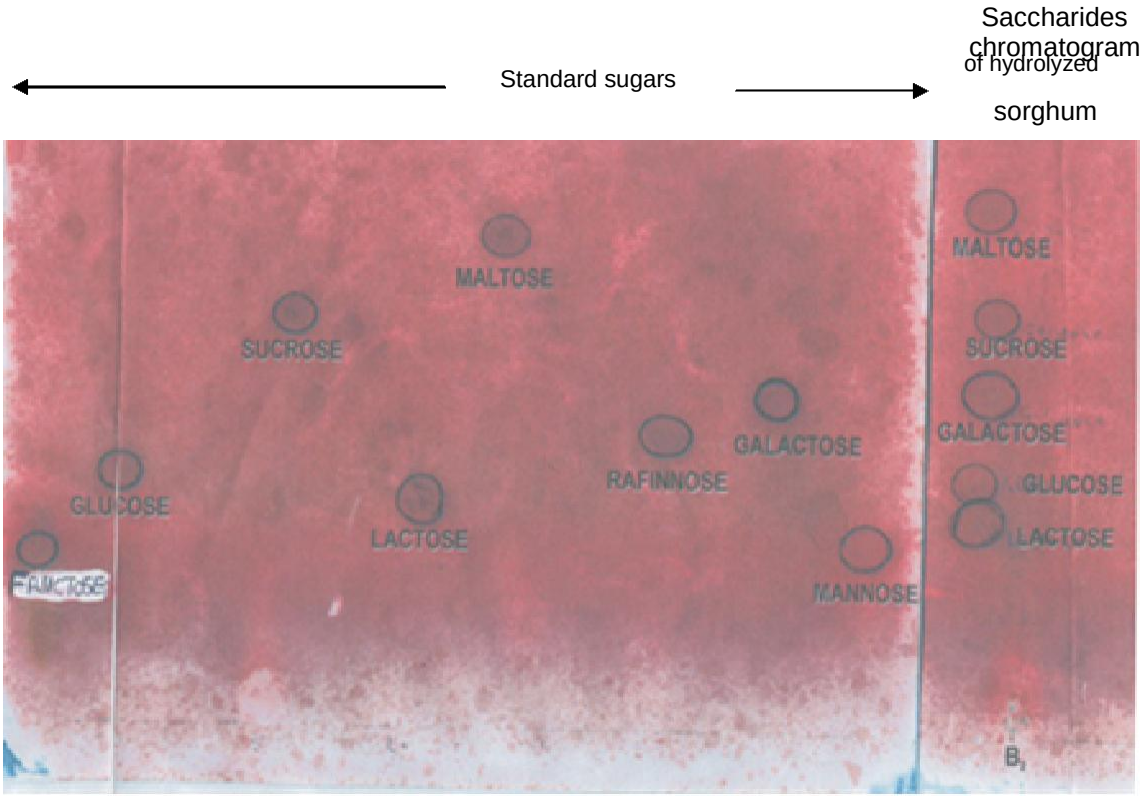

Figure 4. Saccharides chromatogram of hydrolyzed sorghum starch.

shows the presence of three oligosaccharides; sucrose, maltose and lactose (Figure 4). The chromatogram also revealed the presence of glucose and galactose which are monosaccharides. Quantitative analysis shows that the saccharides are present in the following proportions in the hydrolysed sorghum starch; 7.38% sucrose, 48.0% maltose, 1.75% lactose, 1.24% glucose and 1.16% galactose. The test for toxin shows that both the hydrolysed and unhydrolysed sorghum starch samples are free of tannins and phenolic compounds.

DISCUSSION

The optimum temperature and pH obtained in this work are the values at which the enzyme performs best. This could be of utmost importance to the relevant industries in which such enzyme are needed for hydrolysis. The decrease in percentages of moisture content and crude fibre content after hydrolysis are good indication of increased nutritional value of the hydrolysed sorghum starch. The decrease in the moisture content of the hydrolysed starch may likely give the product a better keeping quality thus prolonging its shelf life. Also, the increase in the percentage value of the protein in the hydrolysed sorghum starch may be of advantage in that this may contribute to its nutritional value. Such an increase in the value of the protein may make the hydrolysed sorghum starch good for production of food and feeds. The presence of vitamins A, B1, B2 and B3 may be of added advantage to the nutritional values of the hydrolysed sorghum starch sample making it rich nutritionally especially when consumed by man.

The appreciable increase in the following minerals Ca, Na and Mg after hydrolysis may also improve its nutritional value. These minerals are important in maintaining the blood pH (Loscalzo, 2001). Also, the presence of some essential amino acids in varying proportions may be an indication of the better nutritional value of the hydrolysed sorghum starch.

The process of hydrolysis which was carried out in this work may reduce the long process of digestion. Also, it has been known from previous works (Prapulla et al., 1999; Alles, 1997; Crittenden and Playne, 1996) that when oligossaccharides are incorporated into food they are metabolized by naturally occurring colonic bacteria when it reaches the digestive tract. This could be of relevance to this work because of the presence of some oligosaccharides in the hydrolysed sorghum starch. The hydrolysed sorghum starch may also be used in formulation of weaning foods due to its ability to be easily absorbed and digested. The absence of toxins also confirms that the hydrolysed sorghum starch may be fit for consumption. This hydrolysed product may be useful in food industries in the formulation of sugarless products such as candies.

REFERENCES

Alles MS (1997). The effect of non-digestible oligosaccharides on composition and activity of the intestinal microflora. Paper presented at symposium field in Wageningen December 4-5, organized by graduate schools. VLAG and WIAS.

Anon AO (1972). Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratories procedures. 9th edition. Difco Laboratories Detroit, Michigan. pp 212-231.

AOAC (1999). Method of analysis. Association of Analytical Chemists 16th Edition pp. 440-446.

Arnon I (1964). Growing grain sorghum in a Meditterranean Environment. Economic Botany. 18:149.

Badi JE, Ollis DF (1986).Use of Sorghum and Pearl Millet Flours in Cookies. Cereal Chem. 53: 733.

Crittenden RG, Playne MJ (1996). Production, properties and application of food grade oligosaccharides. Trends Food Sci. Technol. 7: 353-360.

Dalton JL, Mitchell HL (1959). Grain Wax Components. Fractionation of Sorghum Grain Wax. J. Agric. Food Chem. 7: 570.

Dubios M, Gilles KA, Hamilton JK, Peters PA, F. Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 3-356.

Elliot JS, McPherson CM (1971). Nutrient values of food and consumer preferences for sorghum waters. J. Am. Dietetic Ass. 58:225-229.

ICRISAT (2002). International Crops Research Institute for Semi-arid Tropics. Ann. Rep. 10: 102-114.

Kundu AKS, Das SM (1986). Production of amylase in liquid culture by a strain of *Aspergillus oryzae.* Appl. Microbiol.19: 598-603.

Linko Y-Y, Saarinen P, Linko M (1975) Starch conversion by soluble and immobilized -amylase. Biotechnol. Bioengr. 17:153-165.

Loscalzo R (2001). The importance of an alkaline diet. J. Health Sci; 2: 16-19. Lowry OH, Roseborough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

McCready RM (1970). Determination of starch and dextrin and methods in food analysis. In:A.M. Joslyn (ed.) A series of monographs. 2nd edition. Academic Press, New York. pp. 552-557.

Mikes OR, Chalmers A (1989). Laboratory handbook of chromatographic methods. Nostrand Reinhold Company, London. pp. 199-250.

Moench L (1999). Gramineae *Sorghum bicolor*. J. Agric. Sci.1:1-3.

Olama ZA, Hamza MA, El-Sayed MM, Abdel- Fattah M (1993). Purification, properties and factors affecting the activity of *Trichoderma viride* cellulose. Food Chem. 47: 221-226.

Prapulla SG, Subhaprada V, Kawonth NG (1999). Microbial Production of Oligosaccharides. Adv. Appl. Microbiol. 47: 270-337.

Ronald SK, Ronald S (1999). Pearson's composition and analysis of foods. 9th Edition. pp. 593-596.

Rosen H (1957). A modified ninhydrin colorimetric analysis of amino acids. Arch.Biochem. Biophys. 6th edition. 1957. pp . 10-15.

Toope F, Simon ES, Bendarski MD, Whitesides GA (1983). Enzyme-catalysed Synthesis of Carbohydrates. Science 18: 72- 76.

Yohe J (2002). Increased Value of Sorghum as Forage and Silage for Cattle. Science 57: 2-14.