STUDIES ON ENZYMATIC PRODUCTION OF HIGH FRUCTOSE SYRUP FROM SORGHUM.

By

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CANDIDATES DECLARATION

I hereby declare that this dissertation or part thereof has not been previously submitted by me for degree of any university.

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CERTIFICATE - II

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CONTENTS

1. INTRODUCTION

INTRODUCTION

Sorghum (Sorghum bicolor, monech) is the world's fifth most important cereal in terms of both production and area . About 90% of the world's sorghum area lies in the developing countries ,mainly in Africa and Asia . Worldwide approximately 27 million tonnes of sorghum was consumed as food each year during the year 1992 - 1994. Sorghum is crucial to the world food economy because it contribute to household food security in many of the world's poorest , most food insecure regions . In the main production regions in Africa and Asia , more than 70% of the sorghum crop is consumed as food .

Sorghum is the third major cereal crop in India after rice and wheat . About 35 percent of world's area grown under sorghum and millets crop is in India, contributing 17% of the total sorghum grain production .Area under cultivation of sorghum crop during year 1994 - 1995 was 11747.6 thousand hectors and production was 9197.3 thousand tonnes , in India . The major sorghum producing states are Maharashtra , Andhra pradesh , Madhya pradesh , Karnataka , Gujarath and Tamilnadu .

The present use of grain sorghum in India is primarily for traditional foods like bhakri , porridge, and dried fodder. Sorghum is considered to be a coarse grain due to the presence of an outer layer of fibrous bran and seed coat . During dry milling the bran gets ground and mixed .The bran also carries the polyphenols to flours and reduces palatability of Bhakri .

The last two decades have witnessed a substantial increase in the production of sorghum, due to the introduction of several high yielding hybrids . This has amplified the associated problems of post harvest handling, storage processing and utilization. Roti quality of hybrid sorghum is inferior to that of local varieties, hence it is necessary to bring diversified industrial uses of hybrid sorghum which will give better economic returns to the farmers. Alternative uses of sorghum is the need of the day.

Sorghum being similar in composition as maize it can be processed in the similar manner as that of maize to produce sorghum starch. It can be utilized in the production of glucose syrup and high fructose syrup, which are very much effectively used as sweeteners in the food industry . In the limited number of countries like USA, Latin America, Africa, sorghum is considered to be a potential raw material of starch production .Sorghum grain was first considered raw material for starch production in USA in 1949 . Sorghum grain contains good amount of starch ranging from 69.9 to 73.6% What maize has done to the economy of the United states of America , sorghum could do for India.

Studies have also been carried out in the field of productions of starch and starch based sweeteners like glucose , high fructose syrup using maize as raw material by different workers . Sorghum and maize have similar characteristics of starch and hence sorghum starch can be effectively converted into glucose syrup with good amount of DE , which can be further converted into high fructose syrup to the required fructose content with the help of enzymes . Starch is a homopolymer consisting 400- 2000 unhydroglucose molecules linked together . It resembles a string of beads . Each bead represents a potential dextrose unit , and these units are the building blocks of all starches .(When starch is hydrolysed by acid or enzyme reactions , various dextrose polymers are formed . By selective use of acid / or enzyme , the starch can be hydrolysed to yield a spectrum of product , including dextrose , fructose , and maltodextrins .

In acid conversions the starch is attacked in a relatively random fashion . If the conversion is carried to completion, the bulk of the material is converted to dextrose . At the same time an undesirable side reaction occurs . Some of the dextrose polymerizes , forming higher molecular weight material that are known as reversion products. . When conversion reaches to 55-58 DE they begin to impart a bitter taste to syrup .

Amylolytic enzymes contain alpha and beta amylase . The enzymes are specific in their hydrolytic attack . Because of the specificity of the attack,their hydrolytic product pattern is different than that of acid conversion , being much richer in maltose fraction .

Glucosidases may be used to supplement acid or amylolytic conversions and give higher portions of dextrose in the product mix.

This means there was better yield of desired product and less demand for purification. Enzyme hydrolysis takes place in mild conditions as regards to pH and temperature , so there is no need for corossion resistant equipments . Glucose syrup production from sorghum starch is beneficial as sugar as a food ingredient may be replaced wholly, or in part . Glucose syrup is useful in dietary foods . It is used in confectionery , Jams , Jellies, canned foods, food drinks, and ice-creams. Glucose syrup is also used in bakery fermentation and infant foods, in manufacture of gelatin gum and for pharmaceutical purposes.

Up to a short while ago it was not possible to achieve more than 95 DE syrups. However modern enzyme technology allows 99 DE syrups. These considerations are particularly important in regard to high fructose syrup production.

In the late 1960's a major development in enzyme technology occurred that significantly increased the commercial importance of fructose . This process allowed the conversion of corn starch to syrups high in fructose content . These high fructose corn syrups (HFCS) have revolutionized the sweetener industry and have provided a viable, economical alternative to sucrose both in regular and reduced calories food products .The incentive to use HFCS is predominantly economic , and it varies with the supply demand relationships . During the periods of reasonably balanced supply and demand , HFCS prices are usually about 15-30 % below sugar .

There are two additional driving forces behind the switch to high fructose syrup can be grown in a wider variety of climatic conditions as compared to (subject to low rainfall and drought)than cane sugar or beets . And there are interesting synergistic sweetening effects of fructose syrup with both nutritive and non-nutritive sweeteners, which provide interesting opportunities for new product formulations and cost savings . In beverages its saccharide composition remain the same over the life of the beverage where as the composition of sucrose and medium invert syrup changes with time as sucrose hydrolyzes to fructose and dextrose.

High fructose syrup is a clear , colorless liquid . It tends to develop a light straw colour after prolonged time and/or higher temperatures, but remains useable for many months under the prescribed storage temperature . Microbiologically the syrup is very stable .

Due to the refining to remove nonsweetner component and because of extensive microbiological control HFS is a very high quality liquid sweetener. HFS can replace sucrose or a sucrose invert on a pound for pound basis with two general exceptions where dry sweetener is required and where hygroscopicity is a problem.

Fructose is primarily and rapidly metabolized in the liver .Only slight increase in blood glucose levels are observed on fructose consumption . Because fructose can be perceived as sweeter in some food systems , small amounts can be used in the products designed for a weight reduction plan . Fructose has been reported to reduce plaque formation and to be less cariogenic than sucrose.

From regulatory stand point the GRAS (Generally recognized as safe) status of 42% HFCS was reaffirmed in 1983 by FDA. The FDA concluded that HFCS is as safe for use in food as sucrose, corn sugar, corn syrup, and invert syrup . Also High fructose corn syrup conforms to the standards of identity from corn syrup . Glucose isomerase and glucoamylases from *A.niger* are considered GRAS. Thus this high fructose syrup is a economic product and could be efficiently used for replacement of sugars .

Hence direct flour obtained from sorghum can be utilized in the production of glucose syrup without interfering subsequent food use . Further , as the viscosity of gelatinized flour slurry is less than that of gelatinized starch slurry hence handling of gelatinized mass of flour is very easy as compared to gelatinized starch . An advantage of using this process is , the lower investment required as compared with a wet milling operation . At the same time , the refining of the hydrolysate is more stringent than when starting from refined starch, because of higher impurities in the raw material i.e. ash, fat and protein .

Sorghum was proved as good raw material for the production of starch and glucose syrup . Sorghum flour when converted into glucose syrup was having good yield and quality . No studies were carried out for the production high fructose syrup from sorghum flour.Studies have been carried out in the Department of Food Science and Technology ,M.A.U. Parbhani on production of glucose syrup from sorghum starch as well as sorghum flour as starting material, using different hybrid cultivers and the process of liquid glucose production was standardized for enzyme concentration , time of reaction , temperature and pH of reaction . Hence it is necessary to study the conversion characteristics of glucose syrup to high fructose syrup and use of that converted syrup as sweetener.

Keeping this view in mind the programme of research work is carried out

with the following objectives.

1. To analyse selected varieties of sorghum for proximate composition .
2. To standardize the process for preparation of high fructose syrup.
3. To study the properties and composition of Glucose and Fructose syrup .
4. To conduct product application studies using sorghum HFS.

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE.

2.1 Sorghum and it's composition :-

Sorghum grain is the nutritionally poorest and cheapest among the coarse grains. But it is used as human food, because it contains good amount of carbohydrates. Sorghum grains are also low in digestibility when compared with other cereals. Therefore it is necessary to study proximate composition of sorghum.Appreciable yield of starch can be obtained from sorghum. Sorghum starch characteristics are similar to those of corn starch. The composition and different properties of sorghum studied by different workers are as follows.

Miller *et al* (1970) analyzed seventeen varieties and hybrids of grain sorghum for content of total starch , amylose, amylopectin , starch density and starch granule diameter . Total starch with one exception ranged from 65-70% . This agreed with Watson(1959).

The physicochemical properties of sweet sorghum , brown corn and grassy sorghum were studied by Jhon and Muller(1971).They reported that the grain contains 11% protein, 3% fat and 71% starch, protein digestibility may also be reduced by complexing of proteins with tannins.

Viraktamath *et al*(1972) studied varietal differences in chemical composition and reported that the range in protein content was found to be 9-13% .While that in fat was 1.9 to 3.8%.The variation in amylose content was not very marked . It varied only between 23-28% .

Arora and Luthra (1974) studied sixty seven varieties of sorghum for their protein and tannin content and observed that average protein content of yellow endospermic grain , white seeded and brown seeded varieties were 11.64, 11.40, and 12.80% respectively.

Tannin content ranges from 0.33 to 6.33 , 1.0-3.0 & 30.0 - 62.0 mg/gm resply Tannin content has direct effect on digestibility of protein and it is reduced by 6.30 % per unit increase in tannin content.

It was observed that there was an inverse relationship between protein and lysine content (Mali and Gupta , 1974) . When 22 varieties of sorghum were tested for protein and aminoacid composition . The averaged protein was 12.6%, lysine 2.1%., Methionine 1.8% , cystine 1.5% and tryptophan 1.3% presence of tannin content altered the solubility of sorghum protein. Chemical composition of grain sorghum , isolation of starch and its physicochemical properties were reported by Zayate *et al* (1974) . He reported that starch content ranged from 69.9 to 73.6% (in terms of DM) . The amylose content ranged from 16.5 to 24.9% ., similar to that of corn starch.

Tannin content was positively correlated with starch and negatively correlated with protein content . The ranges in value were from 45- 73 mg/100g grain for tannin , 7.3 - 8.93% for protein and 51.2 - 61.5% for starch. (Sisodia *et al.* ,1979).

The chemical composition of different varieties of grain sorghum for proximate analyses were studied by Neucere and Sumrell (1980)of five varieties were moisture in the range of 12.07 to 13.36% protein 9.75 to 14.32% fat 2.66 to 3.49 , ash 1.35 to 1.83 , fiber 1.39 to 2.58 and carbohydrate content was 67.61 to 73.37% .

According to Jadhav and Jogelekar (1982) waxy sorghum starch does not differs markedly from the waxy starch of maize . According to Chang(1982) sorghum starch is being commercially produced in very low amounts, as compared to corn starch .

Goldshtein *et al* (1983) reported that combined action of S02 and lactic acid gave a higher starch yield than two agents used separately . The chemical composition different varieties of sorghum grown in Maharashtra state were studied by Kulkarni (1986).He reported that CSH-9 contained moisture 10.80% protein 8.25% , Carbohydrate 75.41%, crude fat 2.5%,crude fiber 1.67% and ash 1.51%. According to Sangatsaheb (1988) yield of starch from sorghum was 58.87%.

Rooney and Serna (1990) studied the sorghum grain composition which varies significantly , owing to genetic and environmental influences and is similar to that of maize, starch is the major component followed by protein 9-14.1% and oil 2.1-5.0%.

Shear thinning properties of corn and sorghum starches were investigated by Subramanian *et al* (1994). Sorghum starches gave shear thinning more than corn starches, although certain sorghum starches gave low shear thinning ,when hot pastes were sheared at high speed, sorghum starches thinned more than corn starches, Gelatinization characteristics did not appear to be related to shear thinning of sorghum starches Swelling power at 95°C was lower for corn starch than for sorghum starch . Solubility of both corn and sorghum starches at 95°C varied among the cultivers .

Kulkarni (1994) analysed eight Indian hybrid for proximate composition ranged in between,

	Percent
Moisture content	9.29-10.50
Protein	8.44-10.23
Fat	2.40-2.90
Crude fiber	2.30-2.82
Ash	1.38-1.90

Maestri *et al*. (1996) analyzed proximate composition of 11 sorghum cultivers (sorghum bicolor) from Argentina , fatty acids and sterols , oils , protein , carbohydrates and ash content varied between 41 and 66 111 & 156, 670 & 730 & 13.8 to 20.6 gm kg-1 of DM respectively .

2.2 Enzymatic hydrolysis for glucose syrup :-

Glucose syrup is prepared by using two stage enzyme processing.In first stage gelatinization and dextrinization is carried out which is called as liquefaction. In liquefaction alpha-amylases are used. In second stage saccharification is carried out with the glucoamylases at specific temperature and pH range.Enzymatic hydrolysis for glucose syrup is reviewed as follows:

2.2.1 Enzymes properties :-

Madson *et al.* (1973) concluded that ideal conditions for liquifaction with Termamyl are roughly as follows , α thinning stage at 100 to 110°C for approx. 5 min followed by dextrinization at 95-100°C until the required DE has been reached or an isothermal process in which the starch enzyme slurry is held at 105°C for 2 hrs. The effect of substrate concentration on the composition of a 90 -100 DE syrup was described, when amyloglucosidase is used to saccharify liquefied starch at increasingly higher substrates concentrations progressively lower final DE's are obtained on increase in substrate concentration from 30 to 45 % results in 1-2 % decrease in the dextrose content of the final syrup (Novo industries, 1975).

Rosendal *et al* (1979) studied the stability of bacterial alpha - amylase in the starch liquefaction process , depending on temperature , pH, starch , dry substance and salt content . Based on the stability data it was possible to estimate optimum design parameters for the use of the enzyme in industrial processes thus minimizing enzyme costs.

(Novo industries (1984) recommended enzymes for the starch industry . For the liquefaction of starch , alpha - amylases

Termamyl or BAN is recommended depending on the process temperature . And for the saccharification of dextrins , AMG, promozyme and fungamyl are used to obtain a wide range of syrups with different carbohydrate spectra including upto 97% glucose or 60% maltose.)

Novo enzymes and their applications in the main steps of starch conversion are studied (liquefaction, saccharification and isomerization) . Termamyl was heat stable bacterial alpha - amylases produced from a strain of *Bacillus lincheniformis* . Termamyl was applicable in starch solutions at temperatures upto 110°C . AMG (Amylo glucosidase Novo) is a glucoamylase produced from a strain of *Aspergillus niger* . It is used for saccharification of dextrins in the manufacture of glucose and high dextrose syrups. (Novo industries, 1984)

(Brooks and Griffin (1987) studied on liquefaction of rice starch from milled Rice flour using heat stable alpha - amylase Yields of liquified starch in excess of 82% dry flour weight and did not require prior heat treatment to gelatinize the starch liquefied starch yields were highest at 80 -90 and 90°C for the short and long grain respectively .)

Kshirsagar (1988) studied effect of enzyme concentration of pH temperature,DE. There was significant effect of temperature on DE. Higher values for DE are observed at higher temperature. As the enzyme concentration and pH increased the DE values were also increased. There was significant effect of pH on DE. At 5and 5.5 pH, the higher values of DE are observed, the combined effect of enzyme concentration, temperature and pH were significant.It is concluded that there was significant effect of enzyme concentration, temperature and pH on DE of glucose syrup.

Griffin and Brooks (1989) reported that the liquefaction time of 20% rice flour to DE 8.1 and at 95°C by 0.01% heat stable alpha - amylase was 75min .

Lee and Kim (1990) studied gelatinization and liquefaction of starch with a heat stable alpha - amylase . The liquefaction time or a starch slurry to DE 10 by the heat stable alpha - amylase at 95°C was 45 sec for sweet potato starch and 50 sec for corn starch .

Mukna and Elka (1992) made characterization of a heat stable alpha - amylase , produced by thermophillic strain of *Bacillus brevis* , the action pattern of *B.brevis* enzyme on oligosaccharide and polysaccharide substrate was closely similar to those of B.licheniformis , alpha - amylases . Calcium or sodium ions in appropriate concentration were required for enzyme thermostablility.

2.2.2 . **Preparation of Glucose syrup** :-

Sroczynski and Budryn (1974) studied the relation between the degree of (i) initial enzyme hydrolysis of starch and (ii) final saccharifiction with glucoamylase. They concluded that the final degree of sachharification increased with increasing DE values of (i) particularly at, 25 DE, but the concentration o` starch milk and its (i) hydrolysate had little effect on the result of (ii). Detailed carbohydrate composition from (i) and (ii) were dependent on the DE values reached.

Lee *et al.*(1975) reported that glucose production up to maximum with increasing substrate flow rate through the reactor ; above which retrogradation reduced the glucose yield. The standard procedure adopted substrate of 24% DE, temperature 40°C , pH 4.5, flow 0.25 gal/min , residence time 8.9 min, this produced 1000 lb glucose/day , as a product containing 87 - 93 % glucose in the dissolved solids. Bacterial count of the product was very low (30 -50/ml).

Madsan *et al.*(1975) observed that the new amylase Termamyl is stable in starch solution at temperature above 100°C and relatively independent of Ca ions for stabilization . The pH was adjusted with 0.5 M acetate or trismaleate buffer and controlled with a pH meter at the appropriate pH range . The activity of Termamyl is approx. 15% higher and the enzyme is more active over a wider pH range .

Ueda (1978) reported that alpha-amylase had extremely low activity on raw starch , but glucoamylase had high activity on raw starch . When used jointly increase in digestion of raw starch was observed .

Linko *et al.*(1978) studied the suitability of barley starch for syrup production. For liquefaction soluble heat stable Bacillus alpha-amylase at 95°C at pH 6.0 were used to give slurry having DE of 10 - 35 . Saccharification was done by both soluble and immobilized
Asp. niger glucoamylase at 30 - 70°C and pH 4 -5 , glucose syrup having DE 96 was obtained .

Enzymatic conversions of wheat starch to glucose were studied by Linko *et al.* (1979). Various methods of liquefaction and saccharification with several enzymes to maltose and high DE glucose syrup were also studied. He also gave idea about clarification to avoid difficulty during filtration by heat treatment at 140°C after liquefaction . Liquefaction carried out at 95°C with Novo 'Termamyl' alpha-amylase gives end product with better filtering characteristics.

Tegge (1982) processed two starch containing raw materials , Mexican sorghum flour and Pakistan broken rice to various saccharification products without previous isolation. From both raw materials saccharification products of lower purity were obtained than from purified starch but this did not interferes with subsequent food use.

Nonak and Ushiro (1986) described a process for conversion of crude starch containing materials such as broken polished rice and corn grits to starch hydrolyses steeping and milling steps before enzymatic liquefaction and sachharification of the starch . He reported that the process caused minimum solubilization of protein giving readily purified products.

Pickens *et al.* (1986) reported that raw starch hydrolyzing activity isolated from *Humicola grisea* var . *thermoidea* can be used to reduce a substrate such as whole ground kernel corn to high per cent glucose syrup . The process was carried out in 2 stages in which the 1st step allowed a substantial portion , but not all the starch to be solubilized . The solubilized syrup was withdrawn and further starch saccharifying enzyme added to the remaining insoluble fraction so that eventually 95 % of the starch was solubilized . The enzyme extracts from *H. Grisea* were able to solubilise both amylose and amylopectin.

(Kshirsagar (1986) studied the suitability of CSH- 5 sorghum starch for enzymatic production of liquid glucose, he reported that yield of liquid glucose obtained from starch was 29.133 gm/30% starch slurry . He also stated that starch purity does not affect on the yield of glucose syrup .)

(A patent to Jackson and Mccloskie (1987) described the method of producing sugar syrup from sorghum. Starch in the sorghum is partially hydrolyzed using a thermostable alpha - amylase at 90°C . The partially hydrolyze starch is then further treated with exo - 1.4 -alpha - D- glucosidase or endo - 1,3-(4) B-D glucanase , which reduced it to sugars .This aqueous medium can then be filtered and concentrated to produces a sugar syrup.)

2.2.3.Properties of glucose syrup:

Karkalas (1971) gave a description of the commercial process for production of barley syrup from unmalted barley using enzymes (alpha-amylase). He reported that the syrup had the following composition ; TS, 76 - 78 %; DE , 43 - 45 %; total N (Kjeldhal), 0.620 - 0.660 %; alpha-amino N (TNBS method). 0.100 - 0.120 %; ash , 1.2 - 1.5 % and pH 5.5 .

(Madson and Norman (1973) studied the specialty of glucose syrup , including the crystallization properties and carbohydrate composition of the syrups (enzyme concentration , substrate concentration , reaction time , starch liquefaction methods and repolymerisation reactions).

Brzyski and Remiszewaki (1974) improved the procedure for glucose manufacture by acid-enzyme developed in 1972 , in potato industry . The new procedure involved acid liquefaction of potato starch, saccharification by a amyloglucosidase,refining and concentration. The composition of mother liquors from new and old process respectively were , density 62.3 and 65 .8 °B , reducing substance in DM 71.0 and 69.9% , maltose in DM 24.6 and 31.1% glucose in DM 55.0 and 53.3 % ,chloride in DM 1.5 and 6.6 % , copper 2.5 and 22.5 mg/kg and pH 4.3 and 3.9.

Janicki and Czarnecki (1974) gave the procedure for determination of glucose content of starch syrup using glucose-oxidase and peroxidase .These enzymes were dissolved in 100 ml of tris buffer with 1 ml (1%) alcoholic solution . 10 ml of enzyme solution were incubated with 1 ml of sample for 35 min at 37°C and extinction was read at 455 nm.

Ramchander and Feather (1975) studied the mechanism of colour formation in glucose syrup. The compounds like HMF (Hydroxymethy 1-2- furaldehyde) and HAF(Hdroxyacetylfuran) were identified among the products , produced during heat decomposition and colouring of glucose syrup.

The process for production of glucose syrup from degermed maize grits was outlined by Meltzer and Twisk (1975) . Saccharification with a single bacterial enzyme was adapted to commercial scale production .

(Twisk and Meltzer (1976) used maize grits as a raw material for production of glucose syrup and dextrose by using alpha-amylase at pH 6.0 and 85°C . The syrup produced had a DE value of 38 , 0.3 % ash and 0.03% N. The residue (DM basis) contained 44.7% protein , 1.9 % ash , 2.9% crude fiber, 6.3 % fat and 44.2% carbohydrates .)

Woolen (1976) studied the procedure for corn syrup production . Corn was sieved , steeped in acidified water and set milled , during this process starch , germ , barn and protein were separated , the purified starch in suspension was enzymically converted to dextrose and the solution (DE 98) was concentrated by evaporation and decolourized with active carbon . It was used as replacement of sucrose.

Twisk *et al.* (1976) reported that direct enzymatic hydrolysis for producing glucose syrup from maize grits instead of maize starch requires consideration of economic factors, nature of raw material (possible solubles from grits which may complicate filterability and refining) and control of reaction conditions. He reported that hydrolysis for 6 hrs with bacterial + fungal amylase produced (as % of TS) 25 % glucose and 35 % maltose and 24 hrs saccharification produced 43 % glucose and 27 % maltose. Production (%) of glucose , maltose , maltotriose was respectively 17 , 25 ,13 at pH 6.0 ; 23 , 28 , 14 at pH 5.2 and 23 , 19 , 15 at pH 4.8 .

(Kearsley (1978) reported the rapid determination method of Dextrose equivalent of glucose syrup which is based on the fact that osmotic pressure of glucose syrups is directly proportional to the DE of the syrup at constant refractive index

Collins and Yachouch (1980) evaluated viscosity pH, titrable acidity , °B , dry solids contents , colour and sensory attributes of good quality sorghum syrup blended with 0,20, 40, 60 , 80 , or 100 % corn syrup . It was found that corn syrup increased the viscosity of syrup which altered sensory scores and water was added to blends,there by altering percent corn syrup percent.

Dordevic (1982) conducted studies on glucose syrup obtained by starch hydrolysis by acid , acid/enzyme or enzyme / enzyme process . He reported that DE value was directly related to free glucose content for acid hydrolysate, but not for enzymic hydrolysate.

Linko *et al.* (1984) studied the continuous extrusion processing of starchy material for the production of syrups and ethanol . The highest DE values of around 96 (for 30% DM) and 98 (for 10% DM) in glucose syrup after 24 hrs saccharification were obtained after extrusion liquefaction at 135°C , 60% water content with 1.5% (w/w DM) *B. Licheniforms* Termamyl alpha-amylase .

Sangatsaheb (1988) evaluated the liquid glucose produced from sorghum CSH-9 starch and flour . The yield of liquid glucose obtained from starch was 31.5 ml/30% of starch slurry , and that of flour was 24-2 ml/30% of flour slurry.

2.2.4. Uses of glucose syrup:

(Palmer(1975) stated that the increased use of glucose syrup arises from their consistent quality , stable supply and cost advantages. The application of these syrup was described to confectionery , jam and jellies , soft drinks, canning , bakery, infant foods , dietetic, fermentation and ice-creams.)

(Siman (1978) revealed the properties and utilization of glucose syrup and isomerised corn syrup . He pointed out that glucose syrup can increase the degree of super saturation of sugars and control the viscosity and hygroscopic properties of end products . The main areas of application are in the confectionery , bakery, ice-cream industry , soft candies and canned fruits.)

(Cooman and Pelgrams (1980) discussed the importance for use of glucose syrup in manufacture of confectionery with reference to effect on transparency , colour , adhesiveness, undesirable crystallization , structure and density of the product and selection of glucose syrup for optimum quality product .)

(Bussieve (1985) discussed use of glucose syrups in bakery manufacture . Advantages of glucose over other syrups include finer control of dimensional characteristics , improved biscuit texture , improved usefulness of ordinary (Soft) flours and greatly reduced mixing time.)

Jackson (1985) revealed the reasons for use of glucose syrup rather than or as well as sucrose in confectionery manufacture on the basis of microbial spoilage , physical properties and correct physical form. The type of glucose syrup now available are considered as regards, sweetness , viscosity , prevention of graining , hygroscopicity and Maillard reaction.

Effect of glucose -fructose syrup (GFS) on quality of bread and other baked products , on stability and retention of other desired consumer properties was examined (Vasin and Penchenko, 1986) and they reported that the replacement of sugar by GFS was beneficial both economically and technologically .

Kazakova *et al* (1989) described a glucose fructose syrup manufactured from starch (corn , wheat , or potato) and equivalent nutritionally and in sweetness to invert sugar . It can replace upto 50 % sucrose in plain ice-cream and upto 40% in fruit ice-cream . The sensory quality of ice-cream made with syrup was equal to that made with sucrose .

(Anon (1994) discussed the use of glucose syrups in the manufacture of frozen dairy desserts , ice-cream , sherbet(containing milk solids , fruit , sweetener fruit acid and stabilizer) and soft serve ice-cream . Improvement in the functional properties of frozen desserts with addition of glucose syrup .(improvement in flavor, fine crystal formation , increased heat shock stability, elevated freezing point , control of sucrose and lactose crystallization , (reduction in ice crystal and crustation) these aspects are considered .)

2.3 Production of fructose syrup :-

High fructose syrup is prepared by using high DE glucose syrup.Immobilized enzymes play a key role in converting glucose to fructose.Several workers studied the properties of immobilized enzymes, production and uses of high fructose syrup described by several workers is as follows:

2.3.1 Enzymatic conversion | Enzymes used , characteristics :-

Takasaki (1973) disclosed that heating the cells of *Streptomyces* at 65- 80°C for 10 min inactivated the enzymes which caused acetolysis of the cells . These heat treated whole cells , however retained in active form the glucose isomerase bound within the cell wall . These cells could then be used to convert glucose to fructose, with separation of cells by filtration and centrifugation.

(Borach and Nebesny (1980) studied the production of fructose from relatively cheap substrates (hydrol produced by potato starch hydrolysis) by the action of glucose isomerase (GI) . Both single use soluble GE (optisweet A) & repeated use insoluble (GI) (sweetzyme A) were utilized the latter being applied to 14 consecutive batches of each substrate)

Antrim & Auterien (1986) developed a new immobilized glucose isomerase from *Streptomyces rubiginosus* is highly purified and electrostatically absorbed on to a granular DEAE cellulose Productivities >9 t of 42% fructose syrup solid per kg of immobilized enzyme are being achieved on a commercial scale.

(A patent by Horwarth and Irbe (1986) described the process for preparing fructose from starch *Bacidiomycets* were used as sources of alpha - anylase , glucoamylase and glucose isomerase . The organisms are cultured to give a biomass with alpha - amylase and glucoamylas e being generated in the nutrient medium as extra cellular enzymes . In this form they can be used directly to liquefy starches (e.g. Corn starch) to glucose . Glucose isomerase is present in the mycela and can be utilized for whole cell conversion of glucose to fructose or extracted and used as cell free extract .)

A patent by Saleeb and Mckay (1987) described a method for fixing a water soluble , water dispersible or water emulsifiable food ingredient . It was dried from an aqueous solution of a monobasic , dibasic or tribasic Mg salt , the salt constituting the fixing substrate , Juice solids , flavors , colors , and HFCS can be fixed using the described method .

Gaikwad and Deshpande (1992)studied glucose isomerase produced from *streptomyces* species using wheat bran or bagasse hydrolysate as an inducer was immobilized on an inexpensive anion exchange resin , Indian -48-R . Stability of the enzyme at higher temperature and acidic pH was increased after immobilization. Concentrated glucose syrup (25%) was efficiently converted to fructose by the immobilized enzyme . After the seventh reuse the conversion capacity of immobilized was reduced to 61% with a 35% loss in activity.

Schafhauser and Storey(1992) investigated production of high fructose syrup using immobilized enzymes for the simultaneous hydrolysis and isomerization of polysaccharide substrate. Amyloglucosidase, pullunase and glucose isomerase were co-immobilized . Enzyme ratios showing optimum glucose and fructose production (0.7 : 10 : 22.3 U amyloglucosidase :

pullulanase : glucose isomerase). Reaction pH strongly influenced the yield and ratio of glucose and fructose produced .

2.3.2 Conversion of glucose to fructose :-

Conditions generally recommended for sweetzyme type Q for continuos isomerization of glucose by Novo(1978) depend on source of raw syrup for production of high fructose syrup , enzyme liquefaction of starch and saccharification to 93 - 96 % of dextrose in dry substance. The purified syrup should contain less than 1 ppm Ca.Evaporation to 40-45% w/w of dry substance. Addition of activator 0.1gm/L of $MgSO4.7H2O$ provided the Ca content is bellow 1 ppm. For higher Ca contents the Mg/Ca molar ratio should be above 20, pH 8-2 ±1 temperature 60 - 65°C, preferably 61°C . Treatment of isomerized syrup after isomerization is done by pH adjustments to 4-5 with HCL , carbon treatment, gentle evaporation to 71 % DS . If a higher ash contents can be tolerated ion exchange may be omitted .

(Zittan (1981) studied the enzymatic hydrolysis of inulin which is on alternative way to fructose production . The process parameters for the inulin hydrolysis process using inulinase should be as follows substrate concentration >13% ,temperature 60°C , pH 4.5 , dosage 2 units/gm insulin , the degree of hydrolysis was between 98 and 99% in approx. 48 hrs.)

Asbeck *et al* (1981) evaluated the activated carbon in the purification of starch based sweeteners . It is used for purification and decolourisation of glucose syrup , dextrose and high fructose syrup. The properties of different activated carbons were evaluated.

When the selection criteria were clearly defined it will be possible to determine if steam or chemically activated carbon or a mixture , should be preferred .

The typical process parameters and product composition for fructose syrup.For both continuos batch or reuse process conditions are as follows substrate concentration 35- 45% and 40 -50 % , dextrose content 93 to 97% , inlet pH 7.5 -8.0 and 6.5- 7.0, temperature 55.60°C and 60°C, dosage of $MgSO_4$, $7H_2O$, 0.15 g/lit 1 gm/Lit syrup , enzyme consumption was 0.15 - 0.30 kg/ton DS 1.6 kg/ to DS. And product composition was , fructose 42% Glucose 53% , and oligosaccharides 5% of DS(Novo Industries, 1984).

(Chen and Chang (1984) reported that cassava and corn starch were better raw materials than rice flour, . This 90.8 ± 3.6% glucose syrup was isomerised to fructose syrup by using immobilized glucose isomerase packed in a column . The final high fructose rice syrup contained 50% glucose , 42% fructose and 30% maltose .)

Odorod and Ostrovkaya (1984) studied the conditions for purification and concentration of fructose syrup .Fructose solution with 78.4 % dry matter and optical density of 0.95 was diluted to 45 - 50% dry matter content . The major fructose syrup decolorization occurred in the first 15 min . Activated charcoal was used.Concentration of fructose syrup should be performed at pH 3.5 - 4.5.

A Patent by Llyod (1986) described the process for isomerizing glucose. Glucose is enzymatically isomerized to fructose at approximately 100 -110°C by contact with chemically stabilized glucose isomerase Contact time is sufficient to attain fructose content and degradation time kept below the value calculated with the formula Ed = a log (4300/T + 273)– pH . pH of reaction mixtures was maintained at approximately 5 to 6.5 .

A Patent by Nonaka and Ushiro (1986) described an improved process for conversion for crude starch containing materials such as broken polished , rice and corn grits to starch hydrolytes and high fructose syrups. The process causes minute solubelization of protein giving readily purified products .

A Patent to Rosene (1986) described a fructose or dextrose syrup purification process in which the syrup is passed through a series of adsorption materials , preferably , in the order a cation exchange resin, a microporus adsorbent (e.g. granular or powdered activated carbon which may be regenerated using a caustic wash).and an anion exchange resin. Morgo and Regalo (1988) studied and described the preparation of high fructose, syrup for human consumption from Jerusalem artichoke tubers. Physico - chemical properties of the syrup were determined as T S 82% (w/v), total ash 0.4% (w/w of T S), reducing sugars 81.0% (w/v), fructose 83% w/w of reducing sugar , sulphites 400ppm , density at 25°C 1.26 and viscosity 16.0 cp.

White (1992) reported major steps in high fructose syrup production .(1) Starch liquefaction , saccharifications , controlled enzymatic isomerization of dextrose to fructose (2) Fractionalization of the resulting dextrose fructose mixture to produce enriched syrups up to 90% fructose . These processing steps are linked by a series of operations that maintain the integrity of enzymes , ion - exchange resins and product .

Nakamuta *et al* (1995) described the contineous production of fructose syrups from inulin by immobilized inulinase. It is used to produce syrup with > 75% D- fructose .HFS are used as low calorie sweeteners in the food industry . Volumetric productivity in a bioreactor was 410 g reducing sugars/l/hr. The reaction product was mixture of 97% D- fructose and 3% D-glucose.

Isomerization temperature and pH strongly affects the enzyme activity , stability as well as the byproduct formation . The standard operating conditions were temperature 55 - 60°C, pH , 7.2 - 7.5 and pH was adjusted by using sodium bicarbonate, and feed syrups dry solids levels between 40 and 45% (w/w) . Mg ions are recommended in the syrup at a level depending on the syrup purity ;with < 1 ppm of Ca, the addition of 45 ppm of Mg is sufficient Novo,(1998).

2.3.3 Fructose syrup properties & composition :-

Shallenberger (1963) has reported that fructose has a sweetness of upto 1.8 times that of sucrose . Relative sweetness of fructose is a function of concentration , temperature and pH with the maximum sweetness values , being found in cooled , dilute solution that are neutral or slightly acidic .

Brooks *et al* (1974) showed that in water at concentrations of 11.5% solids , both HFCS(42%) , and sucrose have similar sweeteners characteristics .

Mermelstein (1975) described the properties of Clinton's Isomerose 100 . It is a water white , 71% solid syrup containing approx. 42% fructose and 50% dextrose with minimal amounts of higher saccharides .In addition to high sweetness the syrup also has high fermetability, high humectancy , low viscosity and a clean non-marking taste.

✓ Long (1986) described the composition and regulatory status of 42% high fructose syrup as follows : 71 ± 0.5% solids , 0.05% , maximum ash , 42% minimum fructose , 92% minimum dextrose + fructose , 3.5 minimum pH no objectionable flavor or odor , 3 ppm maximum SO2 4ml or 0.05 N titrable acidity and maximum pH -6 . This product is listed as generally recognized as safe(GRAS) .

✓ Hoppe (1986) discussed the literature data on sweetness of fructose relative to sucrose .Most studies show the sweetness of fructose to be approx. 120% that of sucrose .

✓ Meincke (1987) discussed the advantages of fructose as a sweetener over sucrose or glucose.(For e.g. metabolized differently , up to 70% sweeter, can replace sugar in foods, enhance flavours , used in foods for diabetics).

✓ White (1992) reported physical and chemical properties of high fructose syrup containing dextrose 53% , fructose 42% , higher saccharides 5% ,solids 71% , moisture : 29% , pH-undiluted 4.0, colour RBU, max 25 , ash (sulfated) % 0.03, sweetness 92, density at 20°C as kg/L 1.346, viscosity at 27°C 160 cp.

Byeong *et al* (1995) investigated quality changes in high fructose corn syrup during storage at 60°C for 40 days .Colour characteristics including spectroscopic properties , hydroxymethyl furfural and Hunter colour value increased continuously during storage. Changes were most evident at higher storage temperature and in fructose content .HFCS showed dialatent flow and their viscosity increased slightly during storage. Content of fructose decreased while glucose , maltose and oligosaccharide contents increased during storage .Crystallization was observed in some cases during storage .

2.3.4 Uses and effects of replacement with sucrose :-

✓ Mermelstein(1975) reported that high fructose corn syrup can be used to replace sucrose or medium invert in practically all food applications except those requiring a dry sweetener and those in which medium and total invert give unsatisfactory results .

Koepsel and Hoseney (1980) reported the effects of corn syrups in layer cakes , four corn syrups and glucose were used to replace sucrose in high ratio white layer cakes . At 100% replacement of sucrose only the high maltose corn syrups gave a cake with acceptable volume.

Colemon and Harbers(1983) studied the effect of replacement of sucrose with high fructose syrup (HFS) in Angel cake , Replacement with HFCS of 25,50 or 100% sucrose resulted in foams with lower volume , browner crusts , yellower crumb, firmer texture and decreased sweetness . The higher the levels of HFCS , the lower the estimated volume . The lower volume of cakes containing HFS could be influenced by premature starch gelatinization caused by monosaccharides in HFCS.

Curley and Hoseney (1984) studied the replacement of granular sucrose with high fructose corn syrup (HFCS) in a sugar snap cookie. It affects dough rheology (stickiness), surface cracking , and the characteristic snap associated with this type of cookie . A soft sticky dough results when sucrose is replaced with HFCS . If small amounts of corn syrup are used the typical cracked surface of the baked cookie is lost and a smooth surface results .

Lisakova and Kamneva(1984) studied dietary preserves containing new sweeteners. Fructose syrup in the form of viscous liquid (71-72 % DM) was used to produce dietetic preserves Sucrose was substituted in the ratio , 65:35 , 80:20, and 50:50 with fructose syrup . The products did not differ in their organoleptic properties and also met the safety requirements .

McCullough *et al* (1986) , studied the high fructose corn syrup replacement for sucrose in shortened cakes .Objective measurement showed that cakes prepared with HFCS were lower in volume, darker in crust and crumb colour and no difference in moisture content when compared with sucrose cakes . The 75% HFCS cakes were rated sweeter than the sucrose or the 50% HFCS.

✓(Johnson *et al* (1989) studied the effects of high fructose corn syrup replacement for sucrose on browning , starch gelatinization and sensory characteristics . Increased levels of HFCS lowered the onset of starch gelatinization and resulted in a lower volume cake.

Sensory analysis of cakes prepared with varying levels of HFCS and an acidulant did not show significant differences from the control (100% sucrose) cake for colour , tenderness or flavour.

Johonson and Harris (1989) reported that replacing sucrose in cakes with high fructose corn syrup affects browning , volume, and sensory characteristics . Sensory analysis determined that the cakes containing 100% HFCS and acidulants were significantly different than control cakes containing 100% sucrose and acidulants in crust and crumb, colour, moistness ,tenderness and after taste.

Neidleman and Maselli (1989) produced fructose sweetened cereal products by enzymatically converting a portion of the cereals cellulase fraction to fructose, using preferably cellulase and glucose isomerase . The cereal should be moistened to a m.c of ≥ 25% (w/w) preferably 40-80% .The cereal can be in flour form . These enzymatically treated cooked ,cereal grains are formed in to breakfast cereal shapes and enzymes inactivated to provide a shelf stable cereal product .

Maina and Scarlino (1990) examined replacement of sucrose by fructose in preparation of various bakery products , biscuits, shortbread , products base on whipped cake batters. Results were not organoletically acceptable for biscuits, with fructose adversely affecting flavour and crumbliness . These fructose products had a more aromatic and characteristics flavour , colour was the most affected characteristic with greater browning when fructose was used.

Siddique *et al* (1990) studied the production of wax gourd candy by using high fructose syrup . Candy was prepared by using sucrose and HFS in various proportions. Chemical analysis of the samples showed that TSS, reducing sugars, and non reducing sugars increased , while pH , and moisture decreased with increase in HFS at all brix levels. Cand y prepared with 25% replacement of sucrose with HFS at 75°B proved the best as far as over all sensory quality and storage life of the product were concerned .

Hanover (1992) reported uses of high fructose syrup based on its properties other than sweetness . It can be used as a source of fermentables in baked goods , to retain moisture for maintaining shelf life , provide flavours , and aroma , improve crumb texture and softness . In confectionery HFS helps in grain control and humectancy . It can be used as flavour enhancer.

2.3.4 Future developments / Economics of production oF HFS

Litch (1994) took an overview on the world production of high fructose syrup. There are over 30 countries in which high fructose syrup (HFS) are produced and used . They can be derived from a produced variety of starch sources , including maize, cassava, rice and wheat . There are number of factors which encourage this production , some more important than others .

Litch (1995) reported that HFS can be produced from a variety of feedstocks provided they contain sufficient amounts of starch . Though the availability and the price of sugar form an important determinant for the strategies of the producers of HFS . Five prerequisites are cited which are thought to be conductive for the successful development of an HFS production base in a country (1) high price of sugar . (2) sufficient supplies of starch .(3) A well developed food production and consumption infrastructure . (4) Capital . (5) Support of the government .

Betteravier - Bruxelles (1996) reported that world production and consumption of isoglucose have risen by > 40% in the past 10 years , from 7 to 9.5 mt/year, and continue to rise at 4% per year.

3. MATERIALS AND METHODS

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3.1 Materials

3.1.1 **Substrate** :- Sorghum varieties which are PVK-801 , PVK-400 and CSH-9 were obtained from Sorghum Research Center , Marathwada Agricultural University , Parbhani . These sorghum grains were of kharif season and were blackened because of high rainfall. These grains were stored in a cool and dry place until its use analysis and product preparation.

3.1.2 **Enzyme Preparations** :

Enzyme preparations were obtained from Novo industries , Denmark . Termamyl (trade name of alpha-amylase obtained from *Bacillus licheniformis*) was having activity of 120LS.Glucoamylase i.e. amyloglucosidase enzyme (trade name AMG Novo obtained from selected strain of *Aspergillus*) was having activity of 300LS. Glucose isomerase (Trade named sweetzyme type T granular, immobilized enzyme) was produced from strain of *Streptomyces* having activity 350IGIU/g. These enzymes were stored in refrigerator to maintain the stability of enzymes .

3.1.3 **Control glucose syrup** : It was a commercial sample of liquid glucose obtained from market.

3.1.4 **Mangoes** : Good quality sound and well ripped mangoes of Badami variety were obtained from Parbhani market for the preparation of squash.

3.1.5 **Chemicals** : Chemicals required for different analytical methods were purchased from Glaxo, Sarabhai Chemicals.

3.2 **Millling of sorghum grains** :

Sorghum grains were milled in laboratory milling machine , Milcent Magmum model , by using sieve no.3 upto 70 mesh.

3.3 Physical properties of flour : -

3.3.1 **Bulk density** : 100 ml of flour of each variety ie.PVK-801,CSH-9,PVK-400 were weighed accurately and loose bulk densities were expressed as g/ml.

100ml of flour of each variety were taken in measuring cylinder and after vibrating it was weighed accurately and packed bulk densities were expressed as g/ml.

3.3.2 Colour : It was measured using Lovibond tintometer .

3.3.3 **Viscosity Measurement** :

It was carried out by using Brook field viscometer , 8% flour slurry was prepared by taking 16g of flour in 184ml distilled water taken in a beaker , it was stirred Spindle no-3 was used at several speeds to determine the effect of different rates of shear . The beaker containing slurry was then placed in the boiling water bath and viscosities were found at different temperatures.

Hot Paste Viscosity :

The 8% flour slurry was heated to 90°C and viscosity was measured at different speeds of spindle No.3 of Brookfield viscometer.

Cold Paste Viscosity : After cooling hot paste to room temperature (30°c) viscosity was measured at different speeds of spindle No.3 .

3.3.4 Determination of water soluble material :

For solubility measurements 10 g of each variety flour sample was suspended in distilled water to a total volume of 200ml . The flasks were shaken vigorously after each half hour for 3 hrs . This must be done at constant temperature duplicated for all tests . They were centrifuged and supernatant was filtered , an aliquot of 20ml of the filtrate was evaporated to dryness on steam bath . It was then dried to constant weight, in a oven at 105°C and the residue was weighed . The solubility was reported as % .

3.3.5 Water absorption capacity :

It was measured according to the procedure of Sosulki (1962) .Taking 5gm of sample of each variety flour in centrifuge tubes, 30ml distilled water was added in it and kept for 30min , after that 10ml distilled water was added to wash adhered material , tubes were centrifuged at 3000 Rpm for 15 min . Supernatant was discarded and residue was dried in oven at 50°C for 25 min and weight difference was calculated.

3.4 Proximate chemical composition of sorghum Flour :

3.4.1 Moisture : Moisture was determined by standard AOAC (1975) method by drying the sample at 105°C for 6 hrs, in hot air oven , and moisture content was reported as percent weight loss by the original samples .

$$\% \text{ Moisture} = \frac{\text{loss of moisture}}{\text{wt . of sample}} \times 100$$

3.4.2 Crude fat : It was estimated by standard AOAC method using soxlet apparatus with petroleum ether extraction .

The 5 gm moisture free sample was transferred to an extraction thimble and covered with a cotton wool and extraction with petroleum ether (40 - 60°c) for 6hrs . The extracted , dried , fat was expressed in percent of the original sample.

3.4.3 Protein :

Protein was estimated by using Microkjedhal's method , weighed samples of each variety flours (200mg) was mixed with catalyst mixture (K2SO4,CuSO4,5H2O and Hgo red) , this was added with 5 ml , concentrated sulphuric acid , and digested till the contents were clear colourless and free from black particles . The contents were diluted to 25ml with distilled water and 5ml of this sample was taken for distillation and distilled by adding 10 ml of 40% NaoH solution . Distillation was carried out to collect ammonia in 4% Boric acid solution , containing methyl red and bromocresol green indicators

(1 : 3) . About 50ml of distillate was collected and titrated against 0.1 N HCL till pink colour was obtained . The percent Nitrogen was calculated by using the formula .

$$\% N_2 = \frac{\text{Titration reading} \times \text{Normality of HCL} \times \text{Dilution factor} \times 14 \times 100}{\text{vol. Of sample taken for digestion} \times \text{wt. of sample} \times 1000}$$

% Protein = % N x 6.25

3.4.4 Starch :

It was analysed by standard AOAC method (1975) using prechloric acid and Anthrone reagent .0.2g of ground sorghum flour of each of the three varieties was taken and wet with few drops of alcohol , to this 5 ml of water was added with stirring. With the addition of 25-30 ml. hot 80% ethyl alcohol , extraction or sugar was done , After final extraction residue was made 10 ml with stirring . This was cool down in ice water bath and 13ml of perchloric acid was added with stirring was continued for about 10 to 15 min , during this period it was kept in cold water bath . Again sample was solubelized with addition of water and perchloric acid , in ice water bath and washed contents into 100ml volumetric flask volume was made with 100ml distilled water and filtered . This filtrate was diluted to 500ml or to contain 5-20 μg or starch per ml.

Then 5 ml of the diluted solution was pipetted in a test tube , cooling ice water bath , with the addition of 10 ml of anthrone reagent . It was mixed thoroughly and heated for 7 ½ min in a boiling water bath . Cooling was done rapidly to room temperature and the colour absorbance was observed at 630nm .µg of dextrose were calculated from standard curve

% starch content = % glucose x 0.9

3.4.5 Extractable acidity :

For determination of acidity10 g of sample of each variety of flour was transferred to a container, to this 100ml of distilled water was added at room temperature . It was covered and agitated continuously for 30min . It was then filtered through a filter paper into clean and dry flask. First 25 ml aliquot was discarded 50 ml filtrate was pipetted into a clean flask ad diluted with 50ml of water and 1 ml of phenopthalin indicator was added .

It was titrated immediately with standard 0.1 N. Sod . Hydroxide to the 1st permanent pink colour.

$$\% \text{ Acidity} = \frac{\text{Titer} \times \text{Normality of alkali} \times \text{vol. made-up} \times \text{rq.wt of acid}}{\text{vol. Of sample taken for estimation} \times \text{wt.or sample taken} \times 100} \times 100$$

3.4.6 Total and Reducing Sugars : It was determined by Shaffer -Somogyi micro method. This is an idometric titration of copper sulphate by using starch indicator. Reducing sugars were determined by titrating with sodium thiosulphate solution .

3.4.7 Carbohydrates:

Total Carbohydrates was determined by difference.

Total carbohydrates = 100 – (% moisture + %fat +% protein +%ash +%crude fiber)

3.5.1 Enzymatic Hydrolysis of flour to glucose syrup and conversion to fructose syrup.

(The conversion of flour to liquid glucose using enzyme preparations,termamyl 120L(alpha-amylase) and AMG 300L(amyloglucosidase) was done as follows :)

Milling of sorghum grains to flour

↓

Determination of dry solids

↓

30% w/w flour slurry was prepared with distilled water

↓

pH of the slurry was adjusted to 5.5 to 6.0

↓

Gelatinization

↓ **at 150°C for 5-10 min**

Addition of enzyme Termamyl (120L) at 1.0% level

↓

Dextrinization

↓ **For 2 hrs at 95 to 100OC**

Liquified starch

↓

Allowed to cool at room temperature

↓

PH was adjusted to, 4 to 4.5

↓

Addition of AMG (300L) at 1% level

↓

Saccharification

↓ **at about 60^{O}C For 72 hrs.**

Crude glucose

↓

Decolourization with 2% active carbon at 60^{O}C for 50 min.

↓

Vacuum Filtration

↓

Concentration to 45% DS

↓

Fortification of glucose syrup with $MgSO_4$, $7H_2O$, 0.05 g/100ml

↓

pH adjustment at about 7.2 -7.5 pH

↓

Addition of sweetzyme T at 1% level

↓

Isomerization at 55 to 60^{O} C

↓

Isomerised syrup (Fructose syrup)

↓

Purification and filtration

↓

Concentration

After milling of sorghum grains of each variety into flour, dry solids were determined by subtracting moisture. Depending on dry solids content of each variety flour, weight of flour samples were taken and flour slurry was prepared with the addition of distilled water. For eg.

	Flour		Water (ml.)
CSH-9	34.32g	+	65.68
PVK-801	33.63g	+	66.37
PVK-400	33.78 g	+	66.22

pH of these flour slurries were adjusted in between 5.5 to 6 pH with the help of acetate buffer and liquefaction was carried out with 5 to 10 min. thinning at 105^{O}C followed by a longer

dextrinization period i.e. for about 2 hrs at 95-100°C, till required DE of 16 to 17% was obtained . These were allowed to cool to room temperature , and pH was adjusted to 4 to 4.5 pH with the help of 1N acetic acid . Use of acetate buffer for pH adjustment were helpful because it prevents pH change during reaction . Addition of enzyme AMG 300L at 1.0% level ,and saccharification was carried out at 60°C for about 72 hrs.

After saccharification crude glucose was obtained ,which was passed through muslin cloth and then decolourised with 2% activated carbon at 60°C for 30 min. Vacuum filtration was done . Physical and chemical properties of liquid glucose were studied .

The liquid glucose DS were adjusted to 45% and fortified with MgSO4 , 7H20, 0.05 g/100ml .This was added for the stability of enzyme throughout the reaction . Then feed syrup pH was adjusted to 7.2 to 7.5 by using carbonate buffer . Enzyme preparations were carried out , the enzyme was soaked in 5-10 times of its weight of feed syrup whose pH was previously adjusted . This was left at atmospheric pressure and ambient temperature for 1-2 hrs with gentle stirring or shaking . Isomerization reaction was carried out at 60°C. The isomerised syrup is finally purified and carbon treated . After filtration pH was adjusted to 3.0 to 4 pH and concentrated to 71% DS.)

The composition and properties of high fructose syrup and glucose syrup were determined .

3.5.1 **°Brix** : It was determined by hand refractrometer .

3.5.2 **Viscosity :** It was determined by Brookfield viscometer .

3.5.3 **Refractive Index** : It was determined by using Abbe's refractrometer.

3.5.4 **Dextrose Equivalent** : DE of glucose syrup was calculated by using DNSA method . Absorbance was read at 540 nm and calculating mg of dextrose from standard curve . percent total solids were determined by heating the sample at 130 ± 2°C for constant weight .

$$\text{Dextrose equivalent} = \frac{\%\ \text{Reducing sugars}}{\%\ \text{Total solids}} \times 100$$

3.5.5 **Chemical composition** :

Proximate composition like protein , ash , monosaccharide composition , and acidity was determined by using previously described methods.

3.6 **Preparation of Mango squash :**

Mango squash of Badami mangoes was prepared by replacing sucrose with high fructose syrup prepared from sorghum flour . The replacement levels with sucrose were 0% , 50% , 75% and 100% , with the following recipe.

3.6.1 **Product Preparation :** Mango squashes were prepared by using following recipe.

Level of replacement		Mango pulp(g)	Sucrose (g)	HFS (g)
A.	0	100	130	0
B.	50	100	65	65
C.	75	100	32.5	97.5
D.	100	100	0	130

3.6.2 Method of Preparation of Squash :

Fully ripe , good quality sound mangoes were washed , cleaned with water . Peeling of mangoes with knife, cutting them into vertical slits and pulping was done with the help of mixer . °B and acidity of pulp was calculated and according to that all ingredients like citric acid , sugar were calculated required for the final squash . Addition of sugar syrups and citric acid to the pulp potassium metabisulphate (350 ppm) was added for preservative action . Then it was filtered through muslin cloth to remove fibrous material . This was stored at refrigerated temperature .

3.6.3 **Organoleptic evaluation :**

Organoleptic evaluation of the product was checked for colour , mouthfeel , taste , aroma and overall acceptability , by using 9 point hedonic scale by a panel consisting of 10 semitrained judges and average values were tabulated . For organoleptic evaluation 3:1 dilution of squash was prepared and served cold squash .

9 point hedonic scale was as follows .

I	-	Like extremely	-	9
II	-	Like very much	-	8
III	-	Like moderately	-	7
IV	-	Like slightly	-	6
V	-	Indenscive	-	5
VI	-	Dislike slightly	-	4
VII	-	Dislike moderately	-	3
VIII	-	Dislike very much	-	2
IX	-	Dislike extremely	-	1

3.6.4 **Properties of Squash** :

°B , acidity and pH of the mango squash was determined with previously described methods.

4. RESULTS AND DISCUSSION

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The present project of research was undertaken to study the feasibility of production of glucose syrup and fructose syrup from sorghum flour by using genotypes CSH-9 , PVK-801 and PVK-400. Various physicochemical tests were applied to study the properties of sorghum flour , glucose syrup and high fructose syrup, and its application in Mango squash . Different properties of Mango squash were also studied and its overall acceptability was tested . These tests are analysed statistically by using analysis of variance for significance test. The results prevented discussed in this Chapter.

4.1 Physical properties of Sorghum Flour (CSH-9 PVK-801 , PVK-400) :

Physical properties like bulk density ,water absorption capacity, water soluble fractions, % acidity , are reported in table 1.

4.1.1 Bulk density :

Loose bulk density :

The results from table. 1 show no significant difference among the loose bulk density of flours . In case of

pvk-801 and pvk-400 it was 0.48 31gm/ml and 0.4981gm/ml respectively .

Loose bulk density obtained for CSH-9 flour was higher (0.5009gm /ml) than the results obtained by Sangatsaheb(1988) 0.4364 gm/ml. Chidrawar(1987) and Kshirsargar(1986) reported

0.5010 gm/ml Loose bulk density for CSH-9 starch and 0.5126 gm/ml loose bulk density for CSH-5 .

Table - 1 Physical properties of sorghum flour :

Name of variety	Bulk density(gm/ml)		water absor- -ption capacity (%)	water soluble fraction (g/100gm)	Acidity ml of 0.1N NaOH/ 100g
	Loose Bulk density	Pack Bulk density g/100g			
PVK-801	0.48317	0.7314	25.23	18.06	15
PVK-400	0.4981	0.7691	22.16	19.13	15
CSH-9	0.5010	0.7694	23.53	19.13	12
SE	2.6088	0.7589	0.4464	0.0666	0.2081
CD	NS	NS	2.2944	NS	0.3456

4.1.2 **Packed bulk density :**

The results showed , that there was no very much variability among the packed bulk densities of flours . PVK-801 and PVK-400 flours have packed bulk densities 0.7314 g/ml and 0.7691 g/ml respectively . The results obtained for CSH-9 flour 0.7694 g/ml ,which was higher than the results obtained by Chidrawar(1987) for CSH-9 sorghum (0.760 g/ml) and CSH-9 Flour was 0.7400 g/ml. By Sangatsaheb(1988) .

It is important to study loose and packed bulk density of starches and flours used in the food industry . It plays role during handling mixing , and pumping or the starch or flour paste. Bulk density also plays important role in viscosity of starch or flour paste, during production , of syrup , paste is prepared , for this purpose equipment , time and cost is reduced when starchy material has higher bulk density.

4.1.3 **Water absorption capacity** :

It was seen from table 1. that percent absorption capacity of flours of PVK-801 , PVK-400 and CSH-9 have significant differences. PVK-400 have significantly lower 22.16% water absorption capacity as compared to CSH-9 23.53 % and PVK-801 have significantly higher water absorption capacity 25.23 % than CSH-9 and PVk-400.

Sangatsahab(1988) reported that water absorption capacity of both flour and starch increase with increase in the temperature . The water absorption of capacity of sorghum flour was more than that of sorghum starch , while above 70°C it was reversed . (Desikachar and Chandrashekhar,1984).

Water absorption capacity helps in finding out the water holding power of that particular type of sorghum cultiver, starch/ flour when used in foods like soups or used as thickener it also reflects on how starchy material swell and solubalize during consumption.

4.1.4 **Water soluble fractions :**

It was observed from table that percent water soluble fraction not significantly different for CSH-9 and PVK-400 (19.13%) and PVK-801 flour (18.06%)

The results obtained for CSH-9 flour were lower (19.131%) than that obtained by Kulkarni (1986),20.21%. Determination of the amount of water soluble material is of importance in starchy flours because it gives an indication of the presence of added materials. In dextrinized product it is also useful as a means of determining roughly the degree of conversion , for the more highly converted dextrins and gums contain larger amounts of soluble constituents.

4.1.5 **Acidity :**

Acidity as 0.1 N NaOH per 100 gm flour is reported in table 1. PVK -801 and PVK-400 , (15 ml/100 gm) showed no variability, but CSH-9 (12 ml 100 gm) was having significantly lower acidity . The acidity of all the three flours were lower than ISI standard (40.0 ml/100 gm). The results for CSH-9 (12 ml/100gm) were higher than that obtained by Sangatsaheb(1988) , 8.6 ml/100gm.

The acidity of sorghum flour is chiefly related to the amount of amylo-phasphoric acid as hydrolysable salt (Tryller 1967) .Acidity also plays important role during enzymatic production of glucose syrup . For the production of glucose syrup for enzyme stability and activity acidic pH is very important.Lower acidity indicates longer storage life of the product.

4.2 **Viscosities:**

Viscosity is important parameter of starch containing raw materials .Hot and cold paste viscosities were observed and results are presented in table 2.

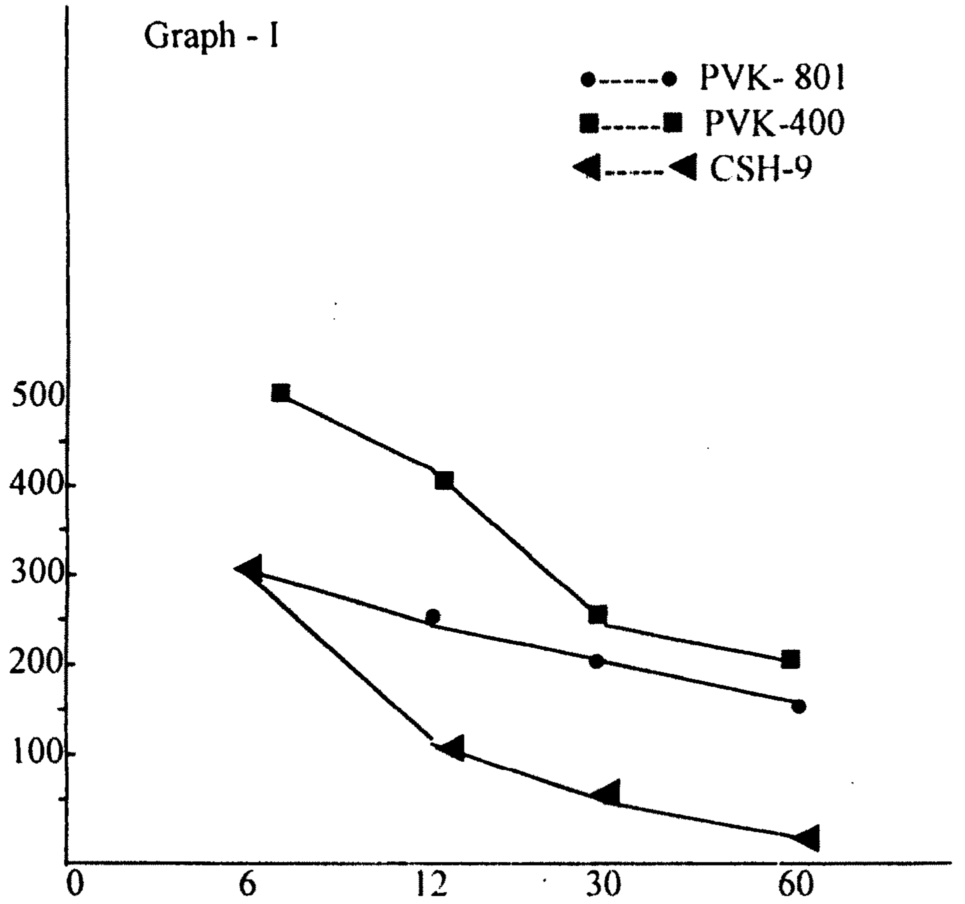
4.2.1 **Hot paste and cold paste Viscosities** :

The results obtained from table 2 for hot paste viscosities (90°C), and cold paste viscosities on cooling this paste to room temperature (at 35°C), for flour at different shear rates. PVK -801 and PVK-400 sorghum flour showed similar hot paste viscosities and cold paste viscosities behavior as compared to CSH-9 flour .

The observed values of hot paste viscosities and cold paste viscosities of flours were lower than values reported by Sangatsaheb (1988) for CSH-9 flour . Sorghum flour viscosity was lower than that of sorghum starches.

2. **Hot paste and Cold paste viscosity of flour slurry (8%).**

Name of variety	Shear rate	Hot paste viscosity(cp.) 90°C	Cold paste viscosity(cp.) 35°C
PVK-801	6	300	1100
	12	220	700
	30	200	540
	60	160	356
PVK-400	6	500	1200
	12	450	800
	30	240	600
	60	200	380
CSH -9	6	300	400
	12	120	300
	30	60	280
	60	44	190



Shear rate (RPM).

Fig 1- Viscosity of flours at different shear rates.

Flour samples show pseudoplastic behavior as shear rate was increased the hot paste and cold paste viscosities were decreased and cooling caused tremendous increase in viscosity . As the shear rate was increased resistance due to hydrogen bonding reduced and showed the low viscosity values with increased shear rate . During cooling retrogadation occurs and thick gel consistency is formed with more viscous mass.

Hot paste and cold paste viscosities play important role in food industry where mixing and pumping are followed when starch or starch containing raw materials are used in food products. Cold paste viscosity gives idea about stability of starch when used as a thickener.

4.2.2 **Viscosity of flours :**

Data presented graphically in Fig 1. shows the viscosities of 8% flour slurry of PVK-801 , PVK-400 , CSH-9 at different shear rates. From Fig 1 it is With the increase in shear rate there was decrease in viscosity of flour.

4.3 **Proximate composition of Sorghum flour** :

Table 3 reveals the percent chemical composition of sorghum flour with respect to protein , moisture crude fat , Ash ad total carbohydrates .

Table -3 Percent Proximate composition of sorghum flour .

Name of variety	Moisture(%)	Protein(%)	Crude fat (%)	Ash(%)	Total-carbo-hydrates(%)
PVK-801	10.82	8.77	2.14	1.52	75.89
PVK-400	11.40	10.67	2.41	1.22	70.66
CSH-9	11.13	10.89	3.02	0.98	71.84
SE	0.35	0.0156	0.0027	0.01	0.51
CD	NS	0.054	0.0095	0.036	1.78

4.3.1 **Moisture :**

Moisture content of PVK-400 , PVK-801 and CSH-9 from table 3 were not having very much variation . Moisture content for varieties PVK-801 (10.82%) and PVK-400(11.40%) was higher than obtained by Kulkarni (1986) for varieties SPV-297 (9.70%) and SPV-475(9.50%), moisture content of CSH-9(11.13%) was higher than obtained by Kulkarni (1986) and Kulkarni (1994) 10.80% for the same CSH-9 flour .

Moisture contents were higher in case of our flour samples may be due to the poor drying conditions. And this may be the effect of climate at drying time and poor storage facilities. Moisture content is responsible for the storage quality of product . If there is high moisture content then this variety will be susceptible to insect , pest and microbial attack.

4.3.2 **Protein (%)-**

The results show that CSH -9 have significantly higher protein content (10.80%) and PVK-801 significantly lower protein content (8.77%) .The protein content of CSH-9 (10.89%) and PVK-400 (10.67%) showed slight difference . The results obtained for CSH-9 flour protein (10.89%) was higher than reported by Kulkarni (1987) 10.80% and 9.10% by Sangatsaheb (1988) . Generally protein content ranges from 9.75 to 14.32% (Nucere and Sumrell ,1980) .

4.3.3 **Crude fat(%) :**

The results from table 3 shows that there was significant differences among the fat content of CSH-9,PVK-400 and PVK-801 .Fat content of CSH-9 was significantly higher (3.02%) than PVK-801(2.14) and PVK-400 (2.417%).

The results shows that fat content of CSH-9 was higher than obtained by Kulkarni (1986) 2.5% and 2.65% Sangatsaheb (1988). Fabre *et al.* (1981) reported that fat content ranges for sorghum as 2.59 to 4.02% whereas Nucere and Sumrell (1980) reported the range of fat 2.66% to 3.49%.

Table - 4 Percent Carbohydrate composition of sorghum flour :

Name of variety	Starch	% Total sugar	% Reducing sugar	% Non reducing sugar
PVK-801	75.40	1.42	1.27	0.16
PVK-400	59.82	1.20	1.08	0.11
CSH-9	57.59	1.19	0.97	0.21
SE	0.089	0.0042	0.0018	0.0048
CD	0.307	0.014	0.006	0.016

4.3.4 **Ash :**

It was found that ash content of PVK-801 (1.524%) was significantly higher than that obtained from CSH-9(0.9868%). Ash content from flours of PVK-801 (1.524%) and PVK-400(1.22%) are also significantly different . Ash content of CSH-9 flour (0.9868%) was very low as compared to results obtained by Kulkarni (1994) 1.5% and 1.85 % by Sangatsaheb (1988) for the CSH-9 . Ash content of sorghum is generally in between 1.35 to 1.83% (Neucere and Sumrell,1980). Ash content is important from the enzymes stability point of view during enzymatic production of glucose ,because minerals like Zn,Fe, etc.affects the stability of enzymes.

4.3.5 **Carbohydrates :**

Carbohydrates content of PVK-801 sorghum flour recorded by difference was significantly higher (75.89%) than both PVK-400(70.66%) and CSH-9(71.84%) .

Carbohydrates content of CSH-9 sorghum flour 71.84% was very lower than value reported by Sangatsaheb(1988),Kulkarni (1986) 75.853% and the value reported by Chidrawar (1987) i.e. 84.50%.

4.4.1 **Starch** :

Results from table 4 shows that PVK-801 was having significantly higher (75.40%) starch content than CSH-9(57.59%) and PVK-400(59.82%).

The values obtained from table 4 for CSH-9 57.59% were lower than the values reported by Kulkarni (1986) 72.85% and 71.68% by Sangatsaheb (1988) . The starch content obtained for PVK-801 variety (75.4%) was higher than the values reported by Kulkarni(1986) for SPV-297(74.60%) and SPV-351(73.95%).

Zayate *et al.*(1974) reported starch content in range of 69.9 to 73.6%. The CSH-9 sorghum flour starch content was lower than the values reported by Fabre and Torre(1981) i.e. 68.42% and by Deshpande(1983) for CSH-1 sorghum (66.16%) . This may be due to the sorghum grains get affected by rain.

From results obtained for starch content PVK-801 is one of the good raw materials for the production of starch , and starch based products starch content is very important during the production of glucose syrup , for the good yield of glucose syrup, because starch is the only raw material which is converted into the glucose sugar during enzymatic production.

4.4.2 **Total sugars :**

Total sugar is a main source of energy . The result from table 4 showed the total sugar of PVK-801 was significantly higher (1.425%) . Total sugar is formed by reducing and non-reducing.sugar. In PVK- 801variety reducing sugar content was significantly higher . The total sugar was significantly lower in CSH-9,(1.1013%) and also reducing sugar was significantly lower(0.9745%). Total sugars content of CSH-9(1.1913%) from table 4 was very lower than the value obtained by Kulkarni(1986) 1.666% for same cultiver.

4.5 **ENZYMATIC PRODUCTION OF LIQUID GLUCOSE** :

Glucose syrups are defined by the Codex Alimentarius as aqueous mixtures of nutritive saccharides obtained from starch. Glucose syrup was prepared from sorghum flour of three different cultivers PVK-801 ,CSH-9 , and PVK-400 , where liquefaction was carried out by using alpha-amylase and saccharification was carried out by amyloglucosidase enzyme for 72 hrs at the level of 1% of each enzyme concentration.) Madsan(1974) produced glucose syrup by using alpha amylase or fungal amylase . In our study heat stable alpha -amylase is used which is produced from a genetically modified strain of

Bacillus licheniformis for liquefaction and glucoamylase (AMG) obtained from a selected strain of *Aspergillus niger* . Microbial glucoamylases have been reviewed by many researchers , including Fogarty(1983), Saha and Zeikus (1989) . Vinhinen and Mantsala(1989) and Pandey(1995). Norman(1982) used debranching enzymes such as pullulanase from Bacillus species or isoamylase from *Pseudomonas amyloderamosa* in the production of dextrose syrup from maize starch . He stated that pullulanase was more heat stable than isoamylase and dual enzyme saccharification with glucoamylse + pullulanase was highly efficient .)

4.5.1 **Physical properties** :

The physical properties of liquid glucose produced from sorghum flours were calculated for refractive index,yield and colour in table 5 .

4.5.1.**Yield of liquid glucose:**

Yield of liquid glucose from table -5 shows the yield of liquid glucose from sogthum flours of PVK-801,CSH-9 , PVK-400 sorghum starch . The observed values for liquid glucose obtained from three cultivers are significantly different from each other.

The observed values from Table-5 shows that yield of liquid glucose from starch was significantly higher 35.167 ml/30% w/v starch slurry . Yield of liquid glucose from PVK-801 flour and PVK-400 flour was significantly lower. (33.86 ml/30% w/v flour slurry, a d 33.16 ml/30% w/v flour slurry).

The observed values for liquid glucose obtain by CSH-9 flour was highers (31.2 ml/30% w/v flour slurry)than values reported by Sangatsaheb(1988)24.2ml/30% w/v flour slurry and Kshirsagar (1986) , 29.133 gm/30% w/v flour slurry from CSH5 Yield of liquid glucose from sorghum starch observed from Table -5 was higher than values reported by Sangatsaheb (1988) for CSH-9 starch (31.5 ml/30% w/v starch slurry)and 29.13 ml/30% w/v starch slurry reported by Kshirsagar (1986) from CSH-5.Deshpande(1983) reported the yield of liquid glucose from CSH-1 starch was 29.0 ml/30% w/v starch slurry.Low yield of liquid glucose in case of flour may be due to the presence of nonstarchy materials.

Table -5 Physical properties of liquid glucose :

Sr.No.	Variety	Yield	Refractive index	Color
1.	PVK-801	33.867	1.3837	Straw
2.	PVK-400	33.267	1.3917	Lightstraw
3.	CSH-9	31.167	1.3947	"
4.	Starch	35.167	1.4007	Whitish Yellow
	SE	0.109	0.006	
	CD	0.355	0.0019	

in the flour which interfere during conversion of starches to glucose syrup . The starch purity does not affect on the yield of glucose syrup.

4.5.1.2 Refractive index:

It was observed from table -5 that there were significant difference in refractive index.

The observed value of 1.4007 for liquid glucose from starch was comparable with the value reported by Sangatsaheb(1988) 1.4065 for liquid glucose from starch and by Kshirsagar (1986) for CSH-5 starch based liquid glucose .

The observed values for liquid glucose from flours of PVK-801 CSH-9 and PVK-400 were comparable with the values reported by Sangatsaheb(1988) 1.3935 .

The lower refractive index for liquid glucose obtained from sorghum flour is due to the low soluble solid content .

Refractive index is a function of temperature, enzyme concentration and DE, but it will be also affected by concentration of minerals present . It is widely used in the food industry as a quick method of determining the solids concentration and degree of conversion .

4.5.1.3 Colour :

It was observed from table -5 that colour of liquid glucose obtained from sorghum starch and flours were significantly different .

Colour of liquid glucose was improved by the treatment of activated charcoal and vacuum filtration.

Colour of liquid glucose was darker than liquid glucose from starch may be get affected due to temperature and time.

Ramchander and Feather (1957) studied the mechanism of colour formation in glucose syrup. The compounds like HMF(Hydroxy Methyl-2 Furaldehyde) and HAF(Hydroxy acetyl Methyl-2) were identified among the products , produced during heat decomposition and colouring of glucose syrup.

4.6.1 Viscosity :

Viscosity of liquid glucose at different shear rates is reported in table 6.The results for viscosity of liquid glucose showed Newtonian behavior, as there was no change in viscosity with change in shear rate. Higher value for viscosity of liquid glucose obtained from sorghum starch was due to the presence of more solids and higher DE values as compared to liquid glucose obtained from sorghum flour.

Table -6 Viscosity of liquid glucose at different shear rates :

Sr.No	Name of variety	Shear rates	
		12	30
1.	PVK-801	11.5	11.5
2.	PVK-400	11.5	11.5
3.	CSH-9	10.5	10.5
4.	Starch	12.5	12.5

Viscosity of glucose syrup is a function of temperature , concentration of solids and molecular distribution . Viscosity values are of importance in food formulations. Low viscosity syrups are used to produce a superior product with good chewiness , body and flavour release properties .

Dordevic(1984) reported that viscosity of starch hydrolysates is directly related to the degree of hydrolysis of the starch and decrease considerably with increasing temperatures. Sangatsaheb (1988) reported that viscosity of starch glucose syrup is higher than that of viscosity of glucose syrup made by sorghum flour.

4.7 **Chemical composition of liquid glucose** :

Chemical composition of liquid glucose viz. dextrose equivalent , protein , ash,°B and acidity are reported in table -7

4.7.1 **Protein content** :

Protein content of liquid glucose samples from flours and starch showed significant differences. Liquid glucose from PVK-801 was having significantly higher protein content 0.85% than PVK-400(0.833%) and CSH-9(0.80%) . These values are comparable with liquid glucose from flour of CSH-9 (0.87%) reported by Sangatsaheb(1988).

Protein content of liquid glucose obtained from sorghum starch was 0.3466% which was comparable with the values obtained by Sangatsaheb(1988) 0.33% for starch based liquid glucose.

4.3.1 **Moisture :**

Moisture content of PVK-400 , PVK-801 and CSH-9 from table 3 were not having very much variation . Moisture content for varieties PVK-801 (10.82%) and PVK-400(11.40%) was higher than obtained by Kulkarni (1986) for varieties SPV-297 (9.70%) and SPV-475(9.50%), moisture content of CSH-9(11.13%) was higher than obtained by Kulkarni (1986) and Kulkarni (1994) 10.80% for the same CSH-9 flour .

Moisture contents were higher in case of our flour samples may be due to the poor drying conditions. And this may be the effect of climate at drying time and poor storage facilities.

Moisture content is responsible for the storage quality of product . If there is high moisture content then this variety will be susceptible to insect , pest and microbial attack.

4.3.2 **Protein (%)-**

The results show that CSH -9 have significantly higher protein content (10.80%) and PVK-801 significantly lower protein content (8.77%) .The protein content of CSH-9 (10.89%) and PVK-400 (10.67%) showed slight difference .

The results obtained for CSH-9 flour protein (10.89%) was higher than reported by Kulkarni (1987) 10.80% and 9.10% by Sangatsaheb (1988) . Generally protein content ranges from 9.75 to 14.32% (Nucere and Sumrell ,1980) .

4.3.3 **Crude fat(%) :**

The results from table 3 shows that there was significant differences among the fat content of CSH-9,PVK-400 and PVK-801 .Fat content of CSH-9 was significantly higher (3.02%) than PVK-801(2.14) and PVK-400 (2.417%).

The results shows that fat content of CSH-9 was higher than obtained by Kulkarni (1986) 2.5% and 2.65% Sangatsaheb (1988). Fabre *et al.* (1981) reported that fat content ranges for sorghum as 2.59 to 4.02% whereas Nucere and Sumrell (1980) reported the range of fat 2.66% to 3.49%.

Table - 4 Percent Carbohydrate composition of sorghum flour :

Name of variety	Starch	% Total sugar	% Reducing sugar	% Non reducing sugar
PVK-801	75.40	1.42	1.27	0.16
PVK-400	59.82	1.20	1.08	0.11
CSH-9	57.59	1.19	0.97	0.21
SE	0.089	0.0042	0.0018	0.0048
CD	0.307	0.014	0.006	0.016

91.68%, DE from starch . DE values obtained for PVK-801 liquid glucose significantly higher DE than that of PVK-400 (72.13%) and CSH-9(83.58%) , which was comparable with the values obtained by Sangatsaheb (1988) 84.21% , DE for CSH-9 flour.

Kshirsagar(1986) reported that there was significant effect of enzyme concentration , temperature and pH on DE of glucose syrup . Linko *et al* (1984) reported 96 to 98 % DE value for glucose syrup in 22 hrs. Saccharification while Scroczynsk *et al* (1978) reported 55 to 70 DE from saccharified potato starch by using glucose amylase.

In order to increase DE level in glucose syrup sample from flour slurry it is essential to increase enzyme concentration and duration of reaction . The liquid glucose based on flour find it's applications in confectionery bakery products, jam, jellies but limits it's use in soft drinks and canning because of high protein content. Twisk *et al*(1976) reported that glucose syrup obtained from corn grits contained a high percent of oligomers which rendered it very suitable for confectionery use.

4.7.4 **°Brix** :

Higher ° B value (45°B) was reached in case of liquid glucose from sorghum starch . There is significant difference for °B in the glucose samples . °B for liquid glucose from starch was highly significant than the flour samples . °B values for liquid glucose obtained from PVK-801 was highly significant different than the liquid glucose from PVK-400 (38°B and 37°B) for CSH-9 liquid glucose .

These values were comparable with liquid glucose from sorghum starch (45°B) reported by Sangatsaheb(1988) . Low value may be due to the interference of nonstarchy materials with starch hydrolysis .

Kshirsagar(1986) reported that there was no significant effect of different temperature at different pH on °B . The observed values were higher than reported by Kshirsagar(1986) for CSH-5 sorghum starch liquid glucose (28°B).

4.7.5 **Acidity :**

Acidity from Table-7 shows significant difference among the liquid glucose obtained from flours and starch . Liquid glucose from starch have significantly higher acid content (0.08%) from that of liquid glucose from PVK-801(0.06%) , PVK-400(0.05%) and
CSH-9(0.06%) .

This value of liquid glucose from starch is comparable with the value obtained by Sangatsaheb(1988) 0.08%.

Acidity is useful parameter in the assessment of the quality of liquid glucose as the acidity has a bearing on colour formation in storage.

4.7.6 Dry solids :

Dry solid content of liquid glucose samples were significantly different . Higher DS in case of PVK-400(39.0%). Lowest DS in case of liquid glucose from starch .

The DS content of liquid glucose affects the rate of fructose formation too high DS level results in a lower apparent enzyme activity . On the other hand too dilute syrup will lead to lower than optimum substrate enzyme ratio and increased risk of microbial infection.

4.8 Enzymatic conversion of glucose to fructose :

Enzymatic conversion of glucose to fructose was done by using immobilized enzyme glucose isomerase at 0.15% enzyme concentration and pH 7.5 and temperature 60°C for isomerization of glucose to fructose.

Chen and Chang (1984) used sweetzyme for the isomerization of glucose to fructose from rice flour glucose syrup concentration was 40-50% and fortified with 0.1% MgSo4, 7H20 and pH adjustment of 8.2 was done . The same condition were applied for the production of high fructose syrup from sorghum flour .

More starch containing variety and having more glucose yield was taken for the further preparation of fructose syrup from glucose syrup of PVK-801 flour slurry .

The production of HFS from starch comprises three enzymatic process stages i.e. Liquefaction ,saccharification and isomerization at the above recommended operating conditions like pH 7.5 , 40-45% DS of feed syrup , temperature 60-62°C , $MgSo_4$ content 0.55gm/lit and enzyme concentration 0.1% . The productivity of sweetzyme to produced from a selected strain of *Streptomyces murinus* is the combined effect of stability and activity and is defined as the weight in kg of 42% fructose syrup (dry substance) produced per kg of enzyme through out its life time.

Different physical and chemical properties of high fructose syrup from sorghum flour and control glucose were calculated in the table -8

Table -8 Physical and chemical properties of fructose syrup :

Name of variety	Refractive Index	Colour	Dry solids (%)	Moisture (%)	Ash (%)	Acidity (%)	°Brix
PVK-801 Control	1.4530 1.4553	Yellowish Whitish yellow	67.93 70.00	32.06 30.00	0.08 0.050	0.11 0.090	58.33 60.00
SE	0.00047	--	0.04714	0.04714	0.0041	0.0062	0.1178
CD	NS	--	0.18477	0.1847	0.01600	NS	0.4619

4.8.1 **Refractive Index :**

The results observed from table 8 were not significantly different . The refractive index for HFS from PVK-801 (1.4853) flour was same as that of for HFS from glucose sample .(1.4530) Refractive index is a function of the temperature , DE and concentration of enzyme but it will be also affected by the concentration of minerals present.

4.8.2 **Colour** :

Colour of the syrup prepared from flour was yellowish and the colour of the HFS prepared from control sample was whitish yellow Long(1986) and White (1992) reported colour of HFS fructose syrup containing 42% fructose was white or colorless liquid. It tends to develop straw colour at higher temperature .

4.8.3 **Moisture content and dry solids** :

Moisture content for flour was significantly different from that of control syrup sample . Moisture content for flour was 32.067 % and that of control was 30.0%. Moisture content is related to the dry solids content of the high fructose syrup .Control glucose HFS have significantly higher DS content than that of flour(PVK-801) sample syrup,White (1992) reported DS content of HFCS was 71% and moisture content was 29% High moisture contents increase the chances of microbial growth . The osmotic pressure of monosaccharides at high dry solids is prevent microbial growth.

4.8.4 **Ash content** :

The results from table -8 showed variability of significant differences into the ash content of the high fructose syrup. Ash content of HFS from PVK-801 flour was significantly higher than (0.08%) control sample HFS i.e.

0.05% , White (1992) reported 0.03% ash in high fructose corn syrup. Higher ash% may be due to absence of ion exchange refining Long(1986) 0.05% ash in high fructose syrup.

4.8.5 **Acidity :**

Acidity of high fructose syrups shows no significant difference it was in the range of (0.09 to 0.13) . The little buffering capacity maybe remained after carbon treatment .

4.8.6 ° **Brix :**

Table 9 reveals that °B of HFS from sorghum flour was significantly different from control sample syrup i.e. (60°B) . Low °B in case of HFS from control may due to the interference of non-starchy materials with starch hydrolysis , °B is the measure of the total sugar % present in the syrup.

4.8.7 **Viscosity of high fructose syrup :**

Viscosity of high fructose syrup at different shear rates were calculated in table - 9.

The results showed that high fructose syrup prepared by using sorghum flour was having higher viscosities as compared to the high fructose syrup prepared from control liquid glucose sample.

9. **Viscosity (cp.) of High fructose syrup at different shear rates :**

	Shear rate(RPM)			
Name of variety	6	12	30	60
PVK-801	200	170	150	100
Control	180	160	120	90

Viscosities of high fructose syrup were in the range 140 to 200 , Viscosities of HFS are similar to that of total invert sugar (White 1992). It is clear from table -9 that as the rate of shear increased there was decrease in the high fructose syrup viscosity .

4.10 **Carbohydrate composition of high fructose syrup viscosity :**

Carbohydrate composition of high fructose syrups were calculated for fructose , glucose and higher saccharide contents and revealed in table - 10 .

Table -10 Carbohydrate composition of High fructose syrup :

Name of variety	Fructose (%)	Glucose (%)	other Carbohydrates (%)
PVK-801	39.85	54.71	5.8000
Control	42.33	51.83	4.9667
SE	0.3776	0.1185	0.1433
CD	1.4804	0.4648	0.5619

The percent carbohydrate composition for fructose , glucose and other saccharides was significantly different .

Fructose content of HFS obtained from flour was significantly lower(39.85%) than from control glucose sample (42.33%) White (1992) reported maximum content of fructose in HFS was 42% , glucose-55% and other saccharides 5 to 6% .

Long (1986) reported 42% fructose , 52% glucose and 6% others in the HFS.

From table 10 it is clear that glucose and other saccharide content was significantly higher in case of fructose syrup from flour (54.71% and 5.80%) than the HFS from control glucose syrup sample (51.83% and 4.9668) .

Long (1986) reported that on energy value of HFCS approximately 4 calories per gm DS . This is same as major component fructose and glucose .

4.11 Product formulation by using High fructose syrup :

Commercially sucrose is usually marketed as sucrose or medium invert sugar which is liquid and contains 50 parts sucrose , 25 parts dextrose and 25 parts fructose . HFCS is similar to total sucrose invert , therefore it has replaced sucrose on pound for pound basis.

Due to the refining to remove non-sweetener components and because of excessive microbiological control HFCS is a very high quality liquid sweetener . The predominant user of HFCS is the soft drink industry . High fructose syrup prepared from flour and control glucose were blended with sucrose in the production of squash at different levels , 0% HFS, 50% HFS, 75% HFS and 100% HFS.

Table 11. **Mango squash Properties:**

Mango squash :

	0% HFS	50% HFS	75% HFS	100% HFS
1. ° Brix	45	45	44	42
2. Acidity (%)	1.2%	1.2	1.0	0.9
3. pH	4	4	4.5	5

°Brix and acidity values were higher in case of 0% and 50% HFS replacement level . very low °Brix and acidity values were obtained for squash prepared from 100% fructose syrup .

4.12 **Organoleptic evaluation of Mango squash :**

Organoleptic evaluation of Mango squash prepared with HFS by using different blends like 0,50,75 and 100% with sucrose was carried out by the 9 point hedonic scale by the 10 panel members for color , aroma, taste, mouthfeel and over all acceptability. The mean values of the products for different characters e.g. colour , aroma , taste and overall acceptability were calculated .And superiority of the products was decided ,

Table - 12 Organoloptic score for samples :

	A (0%)	B (50%)	C (75%)	D (100%)
Colour	7	7	8	7.5
Aroma	7	7	7	7.0
Taste	7	7	8	6.0
Mouthful	8	7	8.5	6.0
Overall acceptability	8	7	8.5	6.0

The results obtained from table 12 showed that the sample C has higher organoleptic score , hence it was superior sample among all the four (A,B,C,D) samples for overall acceptability . taste , mouthful and colour .

The sample D(100%HFS) was having lower scores among the all four samples, product B was slightly acceptable . The results from table 12 showed that overall acceptability or the product C is good . It is superior blend among all the blends . 75% high fructose syrup can be effectively used to replace sucrose in squash .

5. SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The production of liquid glucose from three cultivers of sorghum CSH-9 , PVK-801 , PVK-400 and enzymatic conversion of the glucose into fructose syrup was studied which was produced form flour.

Various tests were employed to determine the physicochemical properties of sorghum flour. Liquid glucose prepared from sorghum flour and starch and high fructose syrup prepared from flour and control glucose syrup whose DS was standardized to 40 to 45% .

For the production of liquid glucose flour slurries of 30% w/w dry solids were prepared followed by controlling pH to 5.5 - 6 pH, 1% addition of alpha-amylase and liquefying at 100°C for 2 hrs. Saccharification was carried out with 1 % amyloglucosidase at pH 4.5 and temperature 60°C for 72 hrs to obtain higher DE values.

Physicochemical properties of flours and liquid glucose were analyzed statistically and compared with other workers, our values of chemical composition and physical properties of flour were in the range reported by other workers. Physicochemical properties of liquid glucose were compared with the ISI standards.(The values of liquid glucose samples for ,DE, protein , ash , °Brix , were significantly different, yield and DE of liquid glucose obtained from flour was lower than that of starch), which may be due to presence of higher amount of nonstarchy materials in the flour i.e. ash, fat, protein and fiber. Liquid glucose from PVK-801 flour which was filtered and purified was chosen for the production of high fructose syrup. Among all the cultivers sorghum flour of PVK-801 was having good content of starch . Yield and DE of liquid glucose from PVK-801obtained was higher as compared to other flours PVK-400 and CSH-9 . This was compared with the high fructose syrup produced from control glucose syrup, and compared with the works of other workers. The 40-45% DS liquid glucose having higher DE was fortified with MgSo4 and 0.15% enzyme concentration of glucose isomerase (sweetzyme T) and enzyme slurry was prepared pH was adjusted to 7.5 with carbonate buffer to control pH drop , and reaction was carried out at 60°C . The syrup is purified and analyzed for moisture , ash , °Brix , fructose , glucose and higher saccharide content our values of physical and chemical properties of HFS were in the range reported by different workers.

The values of high fructose syrup sample for ash, moisture, fructose ,glucose and other saccharide contents were significantly different . Higher percentage of ash may be due to the avoidance of ion exchange treatment . Low percentage of fructose in case of sorghum flour as compared to the high fructose syrup from control glucose syrup, which may be affected due to the feed syrup purity.

Minor differences were observed among the syrups obtained from control sample and flour . Use of flour instead of starch reduces the cost of purification and isolation of starch .

Low purity of glucose syrup may not affect the quality of bread, biscuit , confectionery etc. so sorghum flour can be employed on industrial scale for the production of liquid glucose .

But low purity of glucose syrup affects the yield of fructose syrup and enzyme activity ,hence it is need to improve feed purity, by applying different techniques .

Mango squash was prepared replacing sucrose at 0,50,75 and 100% level ,with high fructose. It was evaluated by organoleptic evaluation for overall acceptability , mouthfeel , colour , aroma , and taste . The blend of 75:25 was superior among all the blends .This blend can be used in different squash preparations.

(Further it is necessary to carry out research in the direction of increasing content of fructose in the syrup and suitability of the high fructose syrup in different types of product applications .)

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