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MALTING OF ACHA FOR EFFECTIVE ENZYME AVAILABLE

Dodo, J. D., Shambe, T., and Fali, C. N.
Department of Chemistry, University of Jos, Plateau State. Nigeria

ABSTRACT

The optimum germination time for acha was carried out at room temperature (250C – 280C). Optimum temperature for enzyme activity was determined for malted acha sample and found to be 500C – 650C. The optimum pH condition for amylase activity was also determined to be between pH 5.0 – 6.5 using Na2HPO4 buffers in the range of 3.7 to 8.4 pH values. Time course hydrolysis showed that the concentration of hydrolysed sugar increased with the increase in dilution ratio. The total carbohydrate content in acha grain was determined by L-cysteine sulphuric acid method and found to be 75.50%. Paper chromatography was carried out on the hydrolysed acha sample to determine the type of reducing sugar present and was found to be glucose and maltose mostly.

Keywords: Malting, Acha, Enzymes, Hydrolysis.

1. INTRODUCTION

Nigeria lies between longitude 3^0E – 15^0E and latitudes 5^0N – 15^0N on the West African coast. Nigerians mostly are farmers, producing a wide variety of food crops of which grains form the major part of the agricultural produce. Most of these grains are grown in the northern part of the country. These grains are grown in areas which fall in the climatic zone known as the savannah.

The common types of grains that are grown in the country include:

1. Maize (*Zea mays*)
2. Rice (*oryza sativa*)
3. Guinea corn (*Sorghum spp*)
4. Millet (*Pennisetum typhoids*)
5. Acha (*Digiteria spp*)

These grains are of enormous important use which ranges from industrial to the home as a staple food, in the production of livestock feed, production of beer and other domestic purposes.[1] Grains in general contain cellulose, hemicellulose and starch, of which the percentage composition varies from one grain to another. These components are progressively hydrolysed to simple sugars which contain glucose mainly by the action of enzymes or by the action of dilute mineral acids i.e. (acid hydrolysis).

The production of hydrolysing enzymes in malted grains also is complex and is influenced by factors like genetics, environmental, composition of grain, endosperm structure and texture,

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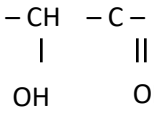
ability to produce desired malting enzymes and the mobility of the enzymes within the endosperm.[8, 9, 10 and 11]

Several studies have been carried out on factors likely to affect quality of enzymes produced by malted grains. The major food component of the grains is a mixture of poly (α–D glucopyranose) polymers of variable high molecular weights called starch. Starch is made up of water soluble, amylase and water insoluble amylopectin all which are progressively hydrolysed to simpler food substances by the action of hydrolytic enzymes or dilute mineral acids.[11] The hydrolytic enzymes which collectively hydrolysed starch molecule include α– amylase, β– amylase, glucoamylase, and limit dextrinase.

Carbohydrates

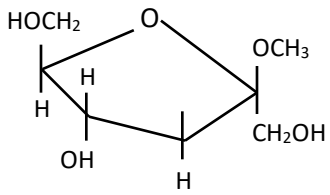
Carbohydrates are the most abundant class of organic components found in living matter. Carbohydrate mainly serves as structural elements in food reserves. Plant carbohydrate in particular represents an enormous store of energy. Large industries process carbohydrate such as sucrose, starch, cellulose, pectin and certain seaweed polysaccharides. Some carbohydrates and their derivatives have been examined and chemo therapeutic drugs for various pathological conditions such as cancer.[13] Carbohydrates are define as compounds of carbon, hydrogen and oxygen containing the saccharose group, or its first reactor product and which usually contain hydrogen and oxygen in the ratio found in water.



Saccharose group[13]

Monosaccharides

Monosaccharides are the simplest carbohydrate. They are polyhydroxyl aldehydes, ketones or derivatives. Treatment of D-glucose with methanol and acid catalyst results in the formation of methyl glycosides commonly as methyl D-glucofuranoside or methyl D-glucopyranoside.



Methyl β – D – glucofuranoside

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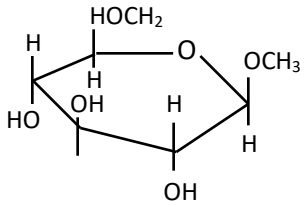
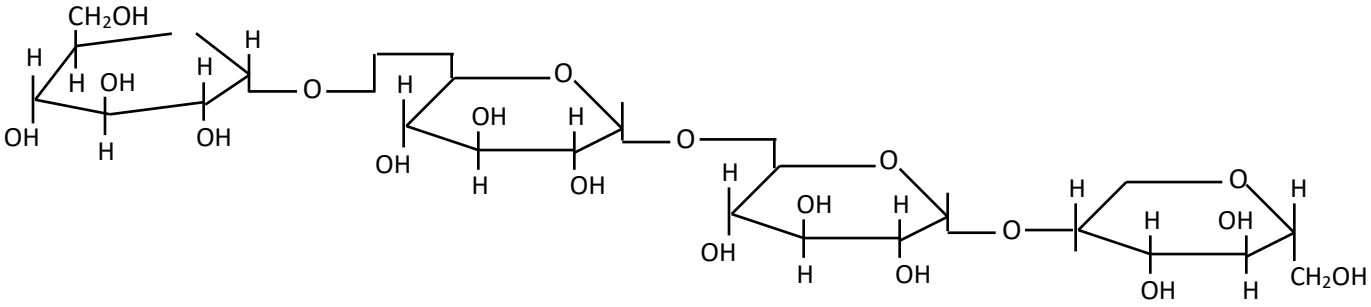


Methyl β – D – glucopyranoside

Oligosaccharides

Oligosaccharides are water soluble polymers consisting of 2 – 10 monosaccharides units. Examples are starchyrose, or natural occurring tetrasaccharide. Maltopentaose, a five cyclic polysugar, (schardinger dextrin), consisting of six monosaccharide units.[13]



Structure of Starchyose[13]

Starch

Starch $(C_6H_{10}O_5)_n$ is a mixture of linear amylase and branched amylopectin polymers of α-D-glucopyranosyl units. It is principally a reserve polysaccharide in plants and constitutes a substantial portion of the human diet. Starch occurs in plants in the form of granules. In hot water irreversible swelling occurs, producing gel. Starch contains two types of D-glucopyranose polymers. Amylose is essentially a linear polymer of 1-4 linked α-D glucopyranosyl units. Amylopectin is a highly branched polymer of α-D glucopyranosyl units containing $1 \rightarrow 4$ linked with $1 \rightarrow 6$ linked at branch points.[15]

Hydrolysis of starch is an important industrial reaction which is accomplished by enzymes, acids or both. The action of acids produces glucose, and other products may also be formed. α-amylase, hydrolyses starch to a mixture of D-glucose, maltose and dextrin obtained

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from amylopectin, β-amylase removes maltose units successively until, the reducing end of the molecule is encountered or in the case of amylopectin an α (1→ 6) branch point is met. Glucoamylase hydrolyzes both amylose and amylopectin yielding D-glucose.

Hydrolysis of starch

The breaking down of complex molecules to simple ones by the addition of water molecules is termed hydrolysis. Polysaccharides are not easily accessible to acids and enzyme hydrolysis thereby preventing reasonable degradation into monosaccharides because of the ordered structures of polysaccharides. For adequate conversion of the starch to its simpler units, it needs to be cooked or gelatinized.

Enzyme hydrolysis

Under suitable conditions of temperature and amylase hydrolyses starch molecule into simple sugars. Most food industries utilize enzyme hydrolysis because of the non-poisoning effect compared to acid hydrolysis[12]. Enzyme hydrolysis is also more economical since enzymes can be produced from sprouted grains unlike acid which need to be bought. Because of the poisoning effect of the acid, food treated with acids cannot be eaten. The simple hydrolysis reaction of starch is as follows:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{enzyme}} 2C_6H_{12}O_6$$

Complex sugar — Simple sugar

The activities of hydrolytic enzymes during hydrolysis are greatly influenced by factors like temperature, pH, substrate concentration and time of hydrolysis.

Acid hydrolysis

The treatment of starch with acid under appropriate conditions of temperature and time, results in random hydrolysis. Analysis of the end products of this hydrolysis shows that the major product is glucose. A large number of other minor carbohydrates are found in such products and are refer to as reversion products, because they are formed by the recombination of glucose molecules under the conditions of hydrolysis. Most of the reversion products are dissacharrides, the commonest being Iso maltose 6–0 – (α–D – glucopyranosyl) – D – glucose and its anomer gentio biose 6 – 0 – (β – D – glucopyranosyl) – D – glucose.

Industrially acid hydrolysis was used for the production of crystalline glucose before the advent of cheap industrial enzymes.[16]

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Enzymes

Enzymes are a catalytic protein produced by living cell. All enzymes are protein and can act or function within the cell that produces them in which case they are known as intracellular enzymes.[17, 18] Enzymes catalysed reactions proceed only when accompanied by a decrease in free energy like other chemical reactions, at equilibrium the concentration of reactants and products are the same in the presence of an enzyme as in the absence. An enzyme can catalyse an indefinite amount of chemical change without itself being diminished or altered by the reaction. However, because most isolated enzymes are relatively unstable, they often gradually lose activity under the conditions employed for their study. T

Enzyme activity is influenced by factors like temperature, pH, substrate concentration and enzyme concentration. Temperature changes affect the initial activity and the stability of the enzyme. Increase in temperature increases the activity of the enzyme with a decrease in rate of enzymes activity with increasing temperature proceeds steadily to a point where the enzymes attain their maximum activity. Beyond this point known as optimum temperature, the activity of the enzyme decreases with further increase in temperature[17, 18, 20]- The increase in temperature increases kinetic energy thereby causing rotation about the hydrophobic ionic and electrostatic bonds. Important R-groups are then shifted from their normal positions resulting into a break in stability.

Substrate concentration also increases the rate of enzyme activity positively but at a certain substrate concentration, no significant changes occur i.e. when the enzymes are saturated with the substrate. Rate of enzyme catalysed reaction is directly proportional to concentration of enzyme in practically all cases.

Enzymes are sensitive to changes in pH of the medium in which they act. Enzymes exhibit maximum activity over a narrow pH range within which the range is known as optimum pH. The effect of pH on enzyme activity is of importance, since it produces valuable information on the nature of enzyme and in particular the nature of the active site.

The prevention of an enzymic process as a result of the interaction of some substance with an enzyme so as to decrease the rate of the enzymic reaction is known as inhibition. The substance causing such an effect is termed an inhibitor. Enzyme inhibitors are important as chemotherapeutic agents as regulators in normal control of enzymic processes in living organism and as useful agents in the study of biochemistry.[21]

Malting

Malting is essentially the same process that occurs when seeds are sown in the ground, moistened by water and germinate. During germination, root-lets (sprouts) and nascent stem (acrospire) emerge, simultaneously. Enzymes are produced or activated and the cellular structure and composition are modified resulting in a product that can be used as a substrate for fermented

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beverages and as food adjunct. The terms malts and malting can be apply to any germinated grain, in our own case acha, however nearly all commercial malting involves barley.[22]

This is because barley is superior to all our local grains used in brewing. Barley is superior because the quality of malt produced or the enzyme produced is barely during malting is better than that produced in the local grains. Also the brewing process and finished beer characteristics are a function of malt properties and malting is considered to be part of the brewing process.

Malting is designed to provide fermentable carbohydrates, hydrolyzing enzymes, assimilable nitrogen as well as precursors for beer flavour. The process consists of 3 steps, steeping, germination and kilning. [22, 23]

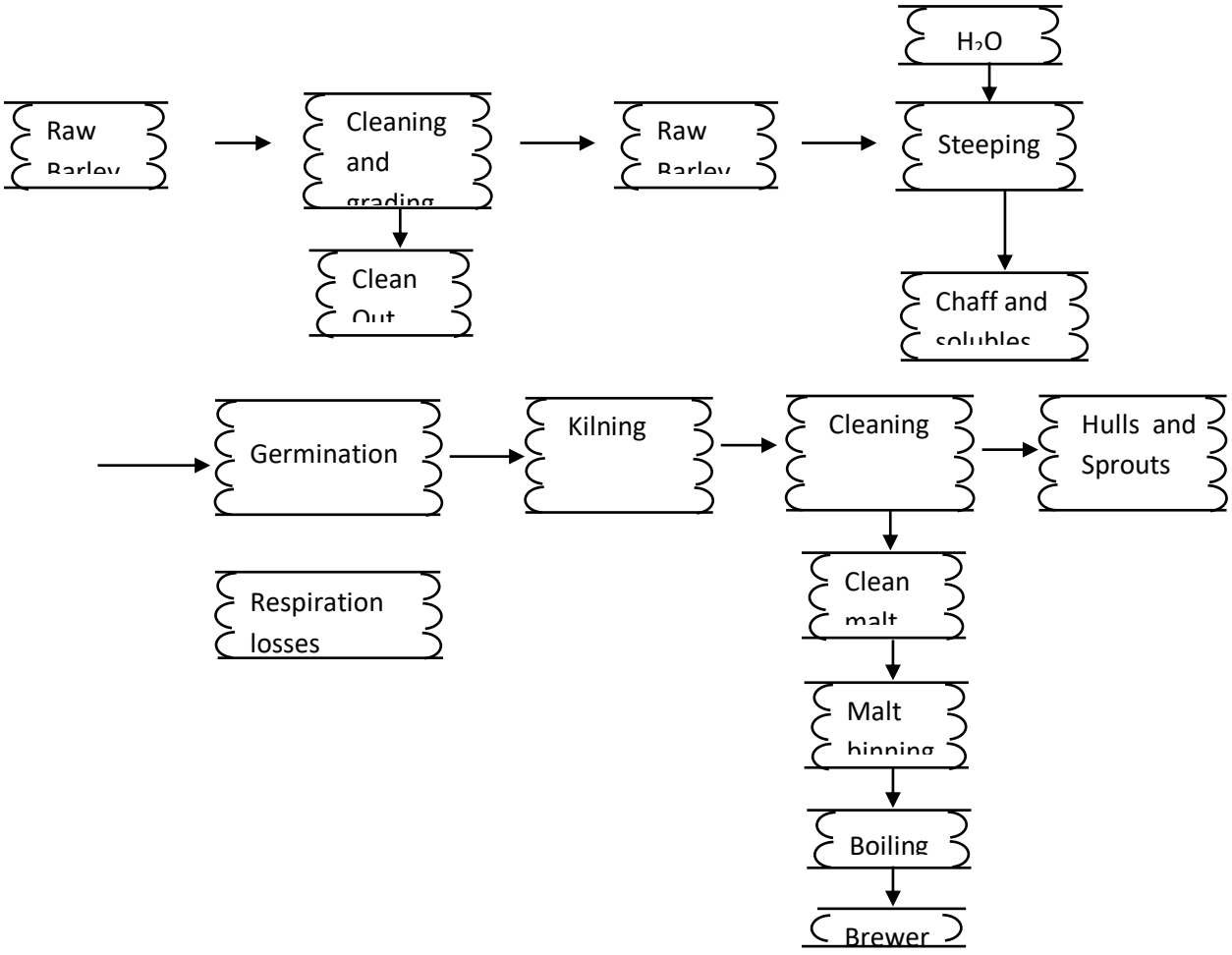

Figure 1: Malting process as apply in the brewing industry.[22]

Steeping[23]

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Steeping is the process of soaking the grain in water for a particular time and temperature. The soaked grains absorbs water and the water quickly becomes brown. The water is often changed and the grain washed as well to improve the circulation of air within the grains. Air supplies oxygen for proper germination which begins during the steeping process.

Germination[23]

Germination is achieved by the passage of cooled air through the grains. The air flow and moisture are required for the grains to germinate optimally and the temperature also has to be controlled. Germination normally continues for about 3 to 5 days depending on the variety of grain used.

Kilning[23]

Completion of germination is judged by the soft consistency of the contents of the grain and by the growth of shooth. Kilning therefore is the process of removal of moisture absorbed by the grain during steeping. Kilning is done under controlled temperature and time to preserve the enzymes present in the malt and also to induce good malt flavour. The modified starch also renders it more readily soluble in water. During this germination process, the starch splitting enzyme α-amylase is created (β-amylase exist in barley) and substantial protein breakdown to amino acid is achieved.

Mashing[24]

In mashing, the adjunct is gradually heated to boiling to gelatinize the starch of the adjunct as apply in the U.S. for corn or rice. For the separate malt mash, malt is milled, mixed with hot water and stirred at about 38^0C for about 10 to 20 minutes. The boiling adjunct mash is then mixed into the malt mash and the temperature of this combined mash is 70 to 71^0C. The temperature is critical because it is here that the α- and -β amylases of the malt intensively breakdown the starch of the malt and adjunct to produce simple fermentable sugars (such as maltose) and unfermentable dextrins. This conversion takes 15 to 30 minutes. Many other compounds essential to fermentation and beer production including amino acids, vitamins and minerals are dissolved in this process.

Milling

Milling is the crushing or grinding of malted grain to a uniform or near uniform degree of fineness. The aim of milling is to expose the endosperm by crushing the husk, completely disintegrate the endosperm and make its content assessable to enzymatic action and finally to prevent the formation of substances that will cause dough in the mash.[25]

The grain acha (*Digitaria exilis*)

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Digitaria is a large genus of about 300 species of annual or perennial grasses in the warmer parts of both hemispheres belonging to the tribe of paniceae and two cultivated West African species are.[26, 27]

D. *exilis*

D. *ibura*

D. *scalarum* - African couch grass

D. *decumbens* - Pangola grass

D. *exilis* is said to be grown as a cereal throughout the savannah zone of West Africa, from Senegal to Cameroon, on the Fouta-Djallon in Guinea and Bauchi Plateau in Nigeria. It is a staple food crop, but not in the Sudan zone. It is widely grown as a complementary cereal where the rainfall exceeds 400mm. The cereal is an annual of 1½ to 3 feet high, with many small long grains, usually yellow but sometimes dark. Its varieties are classified by the density of the spikelet's and the number of racemes,[27] the tiny seeds are said to be 5300 ounce. It is used in making porridge or may be ground to mix with the mean of other cereals. It also makes a good substitute for semolina for Europeans. Beer is also brewed from acha.

Table 2: Nutrient Value of Acha[26]

Component	**Percentage**
Water	6.0
Protein	8.7
Fat	1.1
Carbohydrate	81.0
Fibre	1.1
Ash	2.1

Acha grow in areas with 40 to 50 inch of rain on poor, sandy or rocky soil. It gives a reasonable yield on shallow, infertile soils where other crops fail or survive only with difficulty and on rocky places. The grain is sown in the first rains the soil being cultivated to a fine tilt, although it does not appear to benefit from being planted deeper than 2 inch. In east Dagomba, Ghana, it follows yam in rotation and is planted in the hollows made by the breaking down of the mounds. In Sierra Leone, it follows rice or is used as a substitute when rice fails or the season is poor. In northern Nigeria, where it is sometimes grown with millet, it is sown on terraces or open fields.

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Seeds are sown by broadcasting and then hoed in lightly. They usually germinate in 3 to 5 days and cover the ground so quickly that little weeding is necessary. Most varieties mature within 3 to 4 months but some are quick maturing. In Sierra Leone, one variety matures in 40 days and another in 2½ months. In Gambia, the crop is late maturing being sown in the first rain in June and harvested with groundnuts in January.

Direct manuring has no effect on the yield. Infact manuring is said to cause logging, but as the crop is harvested by hand, this is not important. As the crop matures within 3 to 4 months, harvest starts immediately by cutting in handfuls with knives or curved sickles and tied into small sheaves which are dried before storage. The grains are removed by beating or tramping and after sun-drying, the husks are removed, then the grains winnowed.

Early varieties yield more than late varieties since the later are affected by the drying harmathan winds. [26, 27] .The grain forms a staple food as among the semi-Bantu tribes of northern Nigeria, in Guinea and Portuguese, Guinea. It can be boiled and eaten with fish, meat or spinach or used in soup. In Plateau, among the Beroms, it is made into porridge (called Tere), Tuwo and jellof acha (called gusgus). Local beer is made from the grain like in the Zaria area of northern Nigeria. The straw from the crop is used for stuffing mattresses or for bedding for livestock. It is also burnt and ash extracted with water to make potash.[27]

Acha is not commonly used as wheat, rice, maize and sorghum for the production of beer industrially but is used locally in the production of local beer and as a staple food in areas found. The end products of acha hydrolysis are simple sugars. It is these simple sugars that are particularly important in the production of alcohol. The enzyme hydrolysis is governed by certain parameters like temperature, pH, malting time, moisture content and substrate concentration. These parameters determine to a large extent, the effectiveness of enzyme activity.

The aim of this research is to determine optimum conditions for the activity of the hydrolytic enzymes produced from malted acha.

2. MATERIAL AND METHODS

Acha grains were gotten from Jenta Adamu in Jos North Local Government Area of Plateau State. All chemicals were of analytical grade obtained from British Drug House (BDH) and were used without further purification.

MOISTURE CONTENT OF ACHA GRAINS

5g of ground acha grain (non steeped) was weighted accurately into two different beakers and dried in an oven for 24 hours at 110^0C. The dried sample and the beaker were cooled, and then reweighed to obtain the weight of the dried sample. The loss in weight is expressed as moisture content of the grain.

STANDARD CURVE FOR D-GLUCOSE BY MILLER METHOD

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Reagent for use was prepared by dissolving a mixture of accurately weighed 0.117g $K_3Fe(CN)_6$ and 1.95g Na_2CO_3 and was made up to 100ml with distilled water. It is prepared fresh each time it is required for used.

Solutions of vacuum dried D-glucose (BDH) of the compositions 600 μ g/ml, 500 μ g/ml, 400 μ g/ml, 300 μ g/ml, 200 μ g/ml, 100 μ g/ml, 50 μ g/ml and 25 μ g/ml were made in distilled water.

0.4ml of each of these solutions were taken in separate test tubes and immersed in an ice bath. 2ml of the reagent was added and 1.5ml of buffer solution v/v was also added to the mixture while still in the ice cooled bath. Aluminum foil was used to cover the test tubes and held over boiling water for 5 minutes after which it was cooled to room temperature then diluted with 4ml of distilled water. Absorbances of these mixtures were taken with reference to the blank solution at 430nm and the concentration of sugar deduced from the difference in absorbance. Blank solution was distilled water which has zero absorbance.

DETERMINATION OF OPTIMUM GERMINATION TIME

5.0g of acha grains were weighed into 19 different Petri dishes each. They were steeped drained after 24 hours and allow germinating for different periods, at intervals of 8 hours. The sample were milled at the end of 8 hours, 16 hours, 24 hours, 32 hours, 40 hours, 48 hours, 56 hours, 64 hours, 72 hours, 80 hours, 88 hours, 96 hours, 104 hours, 112 hours, 120 hours, 128 hours, 136 hours, 144 hours, and 152 hours, respectively. The milled samples were made up to 100ml mark with distilled water, filtered by suction and hydrolysed for 4 hours. The hydrolysed samples were centrifuged and assayed for reducing sugars by miller method.[28] The grains were germinated at room temperature ($25^0C – 28^0C$).

DETERMINATION OF OPTIMUM TEMPERATURE FOR ENZYME ACTIVITY

ON ACHA GRAINS

5.0g of acha grains were steeped, drained off and allow to germinate for 100 hours. The germinated grains were milled, made up to 100ml mark with distilled water and filtered. The filtrate was hydrolysed for 2 hours at temperature of 25^0C, 30^0C, 35^0C, 40^0C, 45^0C, 50^0C, 55^0C, 60^0C, 65^0C, 70^0C, 75^0C, 80^0C, 85^0C, 90^0C respectively. Each of the hydrolysed samples was centrifuged and assay for reducing sugars by Miller's method.[28]

DETERMINATION OF OPTIMUM pH FOR ENZYME ACTIVITY ON ACHA

GRAINS

5.0g of acha grains each were weighed into 9 different Petri dishes and steeped, drained off and allowed to germinate for 100 hours. Each of the germinated grains were milled at the end of 100 hours made up to 100ml with solutions of phosphate buffers of different pH values, filtered and

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hydrolysed for 2 hours. The hydrolysed samples were centrifuged and assayed for reducing sugars by Miller's method.[28]

TIME COURSE HYDROLYSIS OF ACHA GRAINS

5.0 of acha grains were weighed into 4 different Petri dishes. The grains were steeped drained off and allow germinating for 100 hours after which they were milled and made up to 50ml, 100ml, 200ml, 300ml mark with distilled water. The solutions were filtered and hydrolysed for 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours and 13 hours. The hydrolysed solutions were centrifuged and assayed for reducing sugars by Miller's method.[28]

STANDARD CURVE FOR TOTAL CARBOHYDRATES BY L-CYSTEINE

SULPHURIC ACID METHOD

Solutions of vacuum dried D-glucose in distilled water were made of the following concentrations: 0.25mg/ml, 0.5 mg/ml, 0.7 mg/ml, 1.0 mg/ml, 1.25 mg/ml, 1.5 mg/ml, 1.75 mg/ml and 2.0 mg/ml respectively. To each of the solutions was added L-cysteine sulphuric acid reagent, while the test tubes were immersed in ice cooled bath. The reaction mixtures were then cooled to room temperature. Their absorbance were taken and plotted against the corresponding values of concentration.

TOTAL CARBOHYDRATE DETERMINATION

A 0.4 g of ground Acha grain was taken into a test tube and immersed in an ice cooled bath. To this sample was added 2 ml of 86% H_2SO_4. The test tube was sealed and the contents hydrolysed for 6 hours in boiling water. The test tube was cut open at the end of 6 hours and the content filtered quantitatively and diluted with 100ml distilled water. The total carbohydrate was then determined by L-cysteine sulphuric acid method.[29, 3]

PAPER CHROMATOGRAPHY

0.4 of milled germinated acha grain was taken in two different test tubes, 2ml of trifluroacetic acid added to each test tube. The test tubes were sealed and hydrolysed in the oven at 105^0C for 6 hours. The test tubes were cut open at the end of 6 hours and content filtered using suction pump and diluted to 100ml with distilled water. 10ml of this solution was taken in a test tube and deionised with a mix 1R-120 (H^+) and 1R – 145 (OH^-) resins for 30 minutes.

The solution was used for paper chromatography using Whatman No. 1 filter paper in ethyl acetate-pyridine-water (10:4:3) ratio for 14 hours. The paper was sprayed with phthalic anhydride (1.67g) in acetone (100ml) and HCl (1ml) and dried in an air oven at 105^0C for 10 minutes to make visible the various spots.

3. RESULTS AND DISCUSSION

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Table 1. Moisture Content of Unsteep and Steeped acha grain

Samples of Acha.	Weight of acha taken (g)	Weight of acha flour after drying (g)	Loss in weight	Moisture Content (%)
Unsteep (Flour)	5.000	4.1343	0.8657	17.314
Steeped (Grain)	5.000	3.4966	1.5033	30.066

Table 2. Standard Curve for D-glucose by the Miller's Methods

D-Glucose concentration µg/ml	Absorbance at 430nm
25	0.280
50	0.260
100	0.240
200	0.188
300	0.180
400	0.100
500	0.080
600	0.040

Table 3. Standard Curve for D-Glucose by L-Cysteine Sulphuric Acid Mehod

D-Glucose Concentration mg/ml	Absorbance at 430nm
0.25	0.09
0.50	0.16
0.75	0.28
1.00	0.32

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1.25	0.39
1.50	0.48
1.75	0.52
2.00	0.64

Table 4. Optimum Malting Time for Acha Grains

Malting Time (hours)	Absorbance at 430nm	Concentration of hydrolyzed sugars µg/ml	Percentage hydrolyzed sugars
8	0.226	135	0.27
16	0.220	150	0.30
24	0.215	170	0.34
32	0.210	175	0.35
40	0.200	200	0.40
48	0.196	210	0.42
56	0.194	215	0.43
64	0.185	240	0.48
72	0.176	260	0.52
80	0.170	275	0.55
88	0.168	280	0.56
96	0.155	310	0.62
104	0.155	310	0.62
112	0.155	310	0.62
120	0.155	310	0.62
128	0.155	310	0.62

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136	0.160	300	0.60
144	0.155	310	0.62
152	0.155	310	0.62

Table 5. Optimum Temperature for Enzyme Activity on Acha.

Temperature (°C)	Absorbance a 430nm	Concentration of Hydrolyzed Sugars µg/mg	Percentage Hydrolyzed Sugars.
25	0.180	250	0.50
30	0.175	260	0.52
35	0.154	310	0.62
40	0.145	315	0.68
45	0.154	315	0.63
50	0.143	350	0.70
55	0.143	350	0.70
60	0.143	350	0.70
65	0.143	350	0.70
70	0.156	310	0.62
75	0.10	300	0.60
80	0.176	260	0.52
85	0.186	240	0.48
90	0.200	200	0.40

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Table 6. Optimum pH for Enzyme Activity on Acha.

pH	Absorbance at 430nm	Concentration of Hydrolszed Sugars µg/ml	Percentage Hydrolysed Sugars
3.7	0.185	240	0.48
4.5	0.156	310	0.62
5.0	0.130	375	0.75
5.5	0.138	352	0.704
6.0	0.122	398	0.796
6.5	0.130	375	0.75
7.0	0.136	360	0.72
8.0	0.200	200	0.40
8.4	0.264	40	0.08

Table 8. Time Course Hydrolysis of Acha at Different Dilution Ratio

Time (Hours)	ABSORBANCE at 430nm.				Concentrations of Hydrolyzed Sugars. (µg/ml				Percentage Hydrolyzed Sugars.			
	50	100	200	300	50	100	200	300	50	100	200	300

0.5	0.270	0.226	0.164	0.108	25	140	290	430	0.025	0.28	1.16	2.58
1	0.260	0.220	0.162	0.096	50	150	300	460	0.05	0.30	1.20	2.76
2	0.260	0.215	0.156	0.088	50	160	310	480	0.05	0.32	1.24	2.88
3	0.255	0.206	0.148	0.084	60	190	330	490	0.06	0.38	1.32	2.94
4	0.255	0.206	0.148	0.076	60	190	330	510	0.06	0.38	1.32	3.06
5	0.248	0.200	0.142	0.076	80	200	350	510	0.08	0.40	1.40	3.06
6	0.248	0.196	0.136	0.070	80	210	360	525	0.08	0.42	1.44	3.15
7	0.240	0.196	0.136	0.071	100	210	360	525	0.10	0.42	1.44	3.15
8	0.240	0.184	0.128	0.064	100	240	380	540	0.10	0.48	1.52	3.24
9	0.235	0.184	0.122	0.056	110	240	400	560	0.11	0.48	1.60	3.36
10	0.226	0.180	0.116	0.050	140	250	410	575	0.14	050	1.64	3.45
11	0.226	0.180	0.116	0.050	140	250	410	575	0.14	0.50	1.64	3.45
12	0.226	0.180	0.116	0.050	140	250	410	575	0.14	0.50	1.64	3.45
13	0.226	0.180	0.116	0.050	140	250	410	575	0.14	0.50	1.64	3.45

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4. DISCUSSION.

The moisture content of unsteeped acha grains was found to be 17.31%, while that of the steeped acha grain was 30.065%. This is a direct measure of the water uptake by the acha grains and is the maximum moisture required for effective germination of acha grain.

The percentage hydrolysed sugars increased with increase in malting time until a maximum value was reached at 96 hours after which the percentage hydrolysed sugars became constant at 96 hours for the successive malting time (Fig. 3.3).

The observation shows that the optimum malting time for acha is 96 hours and this is almost in agreement with the time taken (which is 4 days) by the natives to germinate their grains for the purpose of making alcoholic drinks.

Similar work has been done on sorghum, millet and acha and the result also show that the optimum time for germination is 96 hours for this grains.[31, 32, 33]

The percentage hydrolysed sugars increased with increase in temperature of hydrolysis until a maximum value was reached at a temperature of 50-55^{0}C. The values of percentage hydrolysed sugars remained constant up to a temperature of 65^{0}C was reached after which it started decreasing. The observation here shows that the optimum temperature for enzyme produced from malted acha is within the temperature range of 50^{0}C – 65^{0}C (Fig. 3.4).

The temperature ranges for β-amylase, α-amylase and glucoamylase are 52+^{0}C – 62+^{0}C – 67+^{0}C and about 40^{0}C respectively.[34] These are major enzymes produced from malted grains, acha inclusive. Therefore the temperature range observed for enzyme produced from malted acha fall within the optimum temperature range reported. The decrease in percentage hydrolysed sugars with increase in temperature above the optimum temperature range shows that, the enzyme does not tolerate very high temperature and are therefore denatured. The decreased in percentage hydrolysed sugars at temperatures below the optimum temperature range is also due to the fact that enzymes do not tolerate very low temperature and therefore the enzyme activity is suspended.

The percentage hydrolysed sugars increased steadily with increase in pH, at low pH values until the maximum value was reached at pH5 and then it became constant within pH value of 5 – 6.5. Above this range, the percentage hydrolysed sugars decreased with increase in pH. This shows that the optimum pH for enzyme activity in acha grain falls within 5.0 – 6.5 (Fig. 3.5) which is almost close to the neutral region.

The effect of pH on enzyme activity can be explained by the phenomenon of charge distribution on an enzyme. The distribution of charge on an enzyme determines the extent to which the enzyme reacts or interacts with other substances. The distribution of charge is affected by pH of the medium. At the optimum pH, the enzymes exist in their proper charged form. At pH values

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other than the optimum, however, there is an alteration in the distribution of these charges, here, a decrease in the extent of enzyme interaction with substrate molecules.[34]

Time course hydrolysis of acha for different times at dilutions of 50ml, 100ml, 200ml, and 300ml shows an increase in percentage hydrolysed sugars with increase dilution up to 300ml, and a gradual increase in percentage hydrolysed sugars with increase in time of hydrolysed sugars. Above 10 hours, the percentage hydrolysed sugar is constant, therefore optimum time for hydrolysis is 10 hours.

This shows that at low dilutions (high-substrate concentration) the percentage hydrolysed sugars was low indicating substrate inhibition, while there was an increase in percentage hydrolysed sugars at high dilutions (low substrate concentration) (Fig. 3.6).

At low dilutions however, at any given time, all the enzymes are saturated with the substrate. The rate of substrate interaction with the enzymes will be less compared with the intra-molecular interaction of the substrate molecule. This therefore bring about an inhibition of enzymes activity in the mixture.[34]

The total carbohydrate content of acha grain was found to be 75.50%. This shows that the bulk of acha is made up of carbohydrate. This high carbohydrate content is the source of starch which is hydrolysed to simple reducing sugars.

From the paper chromatography carried out, it was observed that acha which is malted produce simple reducing sugars especially glucose and maltose.

CONCLUSION

From the work done so far on enzyme hydrolysis of acha, it is very necessary to germinate acha grain for 96 hours and to have a good control over factors like temperature, pH, substrate concentration, time for hydrolysis for effective enzyme activity. It is a fact that acha grain has a high carbohydrate value which is the source of starch that is hydrolysed to simple sugars.

Acha, as can be seen from this work is important in both homes and industries and because of its low cost of production. The researcher recommends an increase in the production of this grain.

Because of its high carbohydrate content also, and low cost of production, acha can serve as a good substitute for imported barley for the purpose of beer production in our industries.

APPENDIX I

PREPARATION OF BUFFER SOLUTION OF CH_3COOH AND CH_3COONa

Molecular weight of CH_3COOH (liquid) = 60.05gm/mole 1ml of CH_3COOH weights 1.0495gm

% purity of CH_3COOH = 99.8%

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Density of CH_3COOH = 1.0495

Volume of CH_3COOH =

$$\frac{\text{Molecular weight}}{\text{Density}} \times \frac{100}{\text{Purity}} \times \frac{\text{Concentration}}{}$$

$$= \frac{60.05gm}{1.0495gm} \times \frac{100}{99.8} \times 0.05m$$

= 2.86/100ml ∴ 1n 100ml = 0.286ml

To prepare 0.05m $CH_3COOONa$

$$\text{Molarity} = \frac{gm/dm}{\text{Mole - wght}}$$

gm/dm = molarity x mole-wght

= 0.05 x 82.03

= 4.1015gm

= 4.1015gm _____ 100ml

x _____ 100ml x = $\frac{4.1015 \times 100}{1000}$

= 0.41015gm

0.286ml of CH_3COOH was taken and diluted to 100ml with distilled water. 0.41015gm of CH_3COONa was weighed and dissolved in distilled water, then made up of 100ml mark. These two solutions gave the buffer solution.

APPENDIX II

CALCULATION OF PERCENTAGE HYDROLYSED SUGARS

The percentage hydrolysed sugars is calculated using the relation.

$$\frac{\text{Concentration of hydrolysed sugars } (\mu g/ml)}{\text{Concentration of sugars in 5gm of sample of } 100{,}000 \mu g} \times \frac{\text{Dilution factor}}{100}$$

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APPENDIX III

PREPARATION OF 86% H_2SO_4 AND L-CYSTEIN SULPHURIC ACID

Volume of 86% H_2SO_4 needed = 100ml

Percentage purity of H_2SO_4 = 98%

From the relation

86 x 100ml = 98 xx

Where x = volume of 98% H_2SO_4 needed

$$\therefore \ x = \frac{86\% \times 100ml}{98\%} = 87.7ml$$

This volume is then made up to 100ml with distilled water. L-cystein sulphuric acid was made by dissolving 35mg of L-cystein hydrochloride acid monohydrate in 50ml 86% H_2SO_4.

CALCULATION OF TOTAL CARBOHYDRATE CONTENT OF ACHA GRAIN

Absorbance of hydrolysed sample = 0.08

Concentration of hydrolyzed sample in mg/ml = 0.250

0.4gm of ground acha grain yields = 0.250mg/ml

In 100ml distilled water 0.4gm of acha flour give 0.250mg/ml

Moisture content of acha grain = 17.314%

Sugar content of dry acha flour is given by

0.4gm – (0.1731 x 0.4gm)

= 0.4gm – 0.06924gm = 0.33076gm

= 0.331gm

In 5gm of acha, amount of sugar is given as

5.0gm – (0.1731 x 5.0fm)

5.0gm – 0.8655gm

= 4.1345hm

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= 4.13gm

Amount of hydrolysed sugar in 5gm is given as

$$\frac{4.13gm}{0.331gm} \quad x \quad 0.250gm$$

= 12.477 x 0.250 = 3.12gm

Percentage carbohydrate:

$$= \frac{3.12gm}{4.13gm} \quad x \quad 100\%$$

= 75.5%

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