STANDARDISATION AND CHEMICAL ANALYSIS OF TWO COMMONLY CONSUMED RECIPES WITH TUBERS IN RURAL AREAS OF A. P.

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CERTIFICATE

This is to certify that the thesis entitled ''STANDARDISATION AND CHEMICAL ANALYSIS OF TWO COMMONLY CONSUMED RECIPES WITH TUBERS IN RURAL AREAS OF ANDHRA PRADESH'' submitted for the Degree of Master of Science, Home Science, Andhra Pradesh Agricultural University, is the result of bonafide research work carried out by Miss. Madhu Sharma, under my supervision and that the thesis has not formed in whole or in part of the basis for the award of any degree, diploma or similar distinction.

The assistance and help received during the course of investigation has been fully acknowledged.

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

INTRODUCTION

In our country vegetables are commonly consumed by both vegetarians and non-vegetarians. They are often consumed along with other food-stuffs like cereals and pulses. Vegetables are a source of minerals and vitamins in the diet. A regular consumption of all the vegetables is quite impossible; often only a few vegetables become a part of the daily menu in most families. In our country the consumption of vegetables as food-stuff is only 117 gm/day(Annual Report NIN 1976) compared to the recommended allowances of 250 gm/day (Gopalan et al, 1976).

Roots and tubers are consumed more frequently than green leafy and other vegetables due to their high production, availability, low cost and easy storage facility (Chaudhury 1967, Swaminathan 1977). Potato, sweet-potato, carrot, yam, colocasia and cassava are the commonly consumed roots and tubers in India. A balanced diet for an adult man should contain 75 g of roots and tubers per day (Gopalan et al, 1976).

Roots and tubers are very good source of energy and provide higher amounts of carbohydrate than any other nutrients. Some root vegetables like carrot and yellow varieties of yam are rich in carotene. Cassava is produced and consumed in large amounts in Kerala and it also serves as an emergency or famine food in times of cereal shortage (Gopalan et al, 1976).

In India among the various roots and tubers potato tops in production, area of cultivation and consumption. The total amount of protein provided by potatoes is by no means negligible, especially if large amounts are eaten (Chaudhury, 1967).

The sweet-potato comes next to potato in production. Besides the tubers, the leaves of sweet-potato are highly nutritious and are used as a leafy vegetable in some parts of India. The tubers are also used for the manufacture of starch and alcohol (Chaudhury, 1967).

Yam is one of the important vegetables used in tropical areas (Splittstoesser, 1976). Species of greater yam and elephant foot yam are most commonly consumed in India. Some species of yam have acridity and cause irritation to the mouth and throat.

Colocasia is another tuber consumed in some parts of the country. The most commonly used preparation is curry but good chips also can be made. The tender leaves and petioles are cooked like other green leafy vegetables. The tubers of some varities have acridity, which is due to the presence of needle-like crystals of calcium oxalate, which can be destroyed by cooking and addition of acid or tamarind (Chaudhury, 1967).

Pure starch of cassava is called as tapioca and is a staple food in Kerala. The calorific value of cassava

is high, but the protein is of low quality (Ciacco and Appolonia, 1978).

Much work has been reported on the nutritive value of roots and tubers in raw form, but the changes in nutrient composition in the cooked recipe is not available. Cooking brings changes in digestibility and palatibility of foods. Pulusu and chips are the commonly consumed recipes of roots and tubers in Andhra Pradesh (Monica, 1976, unpublished).

Since Pulusu and chips are commonly consumed recipes in Andhra Pradesh, an attempt has been made in the present study to standardise these recipes and to find out the changes in proximate constituents, ash, total starch, amylose, reducing and non-reducing sugars and amylase inhibitor. These recipes were prepared from potato, colocasia, sweet-potato, yam and cassava.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The important root and tuber crops grown in our country are potato, sweet-potato, colocasia, yam and cassava. Owing to their high calorific value, low cost, high yield and easy availability, they constitute an important item of every day diet in low income families(Chaudhury, 1967; Sukhatme and Basu, 1971). Due to the shortage of food, potato and sweet-potato production as a subsidiary food crop has been realised and increased production is recommended (Indian Council of Agricultural Research, 1969).

Production:

Root and tuber production in the world is 506.4 million tons (FAO, 1968). In India it is only 11.0 million tons (Swaminathan, 1972), but 36.6 million tons are required to meet the requirement of roots and tubers in a balanced diet of the population (Gopalan et al, 1976). Therefore, the production of tubers in the country should be improved to meet the recommended allowances.

The world production of potato and sweet-potato is 138 and 84 million tons respectively (FAO, 1968). In our country potato is produced in about 4,17,000 hectares, with an annual production of 39.9 percent of world production. Sweet-potato is produced in about 1,91,500

hectares with an annual production of 26.9 per c.. world production (Chaudhury, 1967).

Consumption:

A food habit survey conducted by the Operation Research Group (1972) showed that the average monthly consumption of vegetables per person in the state of Andhra Pradesh was higher in urban areas than in rural areas.

The work done in the Telangana region of Andhra Pradesh (Monica, 1976) and a survey conducted by the International Development Research Centre Project, A.P.A.U. (1977) showed that Pulusu and chips are commonly consumed recipes of all these roots.

Nutrient Composition of Potatoes:

The nutrient composition of potatoes varies from region to region and also according to the variety. Various values reported for proximate principles in potatoes are: moisture 74.4 to 85 per cent; protein0.6 to 2.17 per cent; fibre 0.3 to 2.1 per cent; ash 0.8 to 1.4 per cent on wet weight basis and 3.42 to 5.46 per cent on dry weight basis; fat 0.1 to 1.0 per cent. The carbohydrate content varied from 22.6 to 22.9 per cent (Appleman and Miller, 1911; MacGillvary, 1953; Duckworth, 1966; Murthyswamy and Krishnamurty, 1972; Gopalan et al, 1976).

The total starch was analysed by many workers and it was found to be less (10.6 to 26.1 per cent) on wet weight

basis than on dry weight basis (12.2 to 28.4 per cent) (Appleman and Miller, 1911; Duckworth, 1966; Sochocka et al. 1974; Mazen and Ciacco, 1976). The total sugar content, both reducing and non-reducing , in potatoes was also studied (Duckworth, 1966; Iritani and Russell, 1973; Iritani and Weller, 1977). The amylose content in potatoes was shown to vary from 14 to 29 per cent (Sochocka et al. 1974; Sinek, 1973 and 1977).

Nutrient composition of sweet-potato:

Proximate composition of sweet-potato was reported by many workers and it also varied from variety to variety. Moisture content was 60 to 80; protein 1.2 to 3.1; ash 0.5 to 1.7; fibre 0.5 to 1.7; fat 0.7 to 1.7 and carbohydrate 27.9 to 31.4 per cent (Kiett, 1911; Chattfield, 1954; Duckworth, 1966; Purcell et al. 1972; Young and Tsai, 1975; Purcell et al. 1976; Gopalan et al. 1976).

Total amylase content was reported to be 18 to 20.0 per cent (Mandemba and Pedro, et 1976). Work was done on total and both reducing and non-reducing sugars (Kiett, 1911; Balls, 1948; Cancan and Joseph, 1965; Duckworth, 1966).

Nutrient composition of Colocasia:

The acridity of colocacia is attributed to the presence of acicular crystals of calcium oxalate (Chaudhury, 1967). Proximate composition of colocacia varied from variety to variety. Moisture content is 54 to 83, protein 0.5 to 3, ash 0.9 to 1.3, fibre 0.4 to 3, fat 0.1 to 0.6 and carbohydrate to be 21.1 to 22.1 per cent (Duckworth,

1966; Gopalan et al,1976; Fredric et al, 1976).

The total starch content in colocasia was 30 to 75.5 per cent (Duckworth, 1966; Purcell et al, 1976). Amylose content varies from 16.01 to 20.15 per cent (Purcell et al, 1976). Total sugar content varied from 0.2 to 1.01 per cent (Duckworth, 1966). The relative amylase inhibitor activity in colocasia was 6.65 mg and recovery was 7 per cent. Colocasia was found to contain water-soluble substances which inhibit salivary amylase. The inhibitor is not dialysable, stable to boiling temperature and is precipitable by the usual protein precipitants (Narayan Rao, 1970).

Nutrient composition of yam:

The proximate principles of yam were analysed, the moisture content varied from 54 to 87, protein 1 to 14, ash 1 to 1.3, fibre 0.4 to 1.5, fat 0.1 to 2.10 and carbohydrates were18.4 per cent (Klett, 1911; Coursey, 1963; Duckworth, 1966; Baquar and Oke, 1976;Splittstoesser, 1976; Gopalan et al, 1976; Ciacco and Appolonia, 1978).

The starch content in yam varied from 43 to 70.9 per cent (Klett, 1911; Duckworth, 1965; Ciacco and Appolonia, 1978). The total sugar reported in yam is 2.23 per cent (Keitt, 1911).

Nutrient composition of Cassava:

The proximate principles in the tuber were analysed by many workers. The moisture content varied from 50 to 74, protein 0.5 to 0.7 on wet weight basis and 3 on dry weight basis, ash 0.5 to 1.0, fibre 0.6 to 4.66, fat 0.1 to 0.7 and carbohydrate 38.7 per cent (Duckworth, 1966; Gopalan et al. 1976; Yeoh and Chow, 1977; Ciacco and Appolonia, 1978).

Studies on total starch content in cassava revealed that it varies from 80 to 88.4 per cent (Duckworth, 1966; Ciacco and Appolonia, 1978).

Effect of cooking on roots and tubers:

Jacob (1957) observed that there is no change in the total nitrogen content in potatoes even after boiling. Chaudhuri et al. (1963) studied the effect of frying, baking, and canning on the nutritive value of potatoes and found that on moisture and fat-free basis there is no change in the ash content but the mean protein content slightly changed by canning than by other methods. Splittstoesser (1976) reported very low net protein utilisation for potato starch and amylose. Mondy and Muller (1977) found that the crude lipid content of potatoes was significantly lowered by all methods of cooking. In boiled potatoes the loss was the least and there was no

alteration in the fatty acid composition after cooking.

The starch and dextrin content of tuber were found to influence the textural and organoleptic properties of the tubers due to the high water binding capacity and their interaction with each other and other food constituents (Galinskaya, 1977).

Cooking quality of the potato is based on the dry matter content of the potato. The yield of potato chips was more when the dry matter content was high (Ordonez, 1972).

The colour of potato chips was correlated with reducing sugar content of the tuber. Light colour chips were obtained when the reducing sugar content was 7 per cent and the colour was too dark with lower values (Thornton, 1942).

The specific gravity of the potato was found to influence fat absorption in the preparation of chips. Fat absorption increased with decrease in specific gravity and vice versa (Hunkel et al, 1951; Johnson, 1957).

The protein content of sweet-potato was 2 to 3.5 per cent and there was no significant difference even after cooking (Jacob, 1957). There was marked softening of

the flesh and increase in the sweetness of sweet-potato on boiling. During boiling a part of the starch is transformed to dextrin and sucrose; thus it alters the starch- moisture ratio and increases maltose conversion, which is responsible for consistency and sweetness. In cooking sweet-potatoes there was a marked increase in reducing sugar and maltose content and, decrease in alcohol and insoluble solids (Jacob, 1957).

The quality changes in sweet-potatoes were observed by Hoover and Harmon (1967) after 45 minutes cooking at 75°C. Increased maltose content in cooked sweet-potato provides a reliable guide to the conversion time necessary for the production of high quality flakes (Deobald, 1968, 1969).

During cooking a large amount of starch and dextrin is available for hydrolysis by amylase thus increasing the conversion process (Galinskaya, 1977).

Sarhan et al, (1975) found an increase in sugar and decrease in starch and total solids of sweet-potatoes after blanching for 30 minutes and there was increase in total solids after baking.

Francis et al, (1975) observed a slight increase in the protein content of yam after cooking. Splitts-

toesser (1976) studied the protein and total amino acid content before and after cooking the yam. There was a loss of non-protein nitrogen after boiling (25 to 59 per cent) but there was no change in the nitrogen of protein origin of the yam.

MATERIALS AND METHODS

MATERIALS AND METHODS

In the present study commonly consumed recipes chips and Pulusu of potato, sweet-potato, yam , colocasia and cassava were standardised according to the method reported by Monica (1976). The recipes were analysed for:

a) The proximate principles: (protein, ash, moisture, fibre, and total carbohydrate).

b) Total starch.

c) Amylose.

d)Reducing and non-reducing sugars.

e) Amylase inhibitor.

The tubers were obtained from the local vegetable market for the present study.

Standardised recipes:

Pulusu and chips with potato, sweet-potato, yam, colocasia, and cassava were standardised (Appendix-I).

Chemical Analysis:

The standardised recipes were analysed with raw tubers for proximate principles, amylose, total starch, reducing and non-reducing sugars, and amylase inhibitor.

For chemical analysis raw samples were dried and powdered finely by an electric grinder. The chips were dried at room temperature and powdered. Puluan (along with added water) was also dried and ground into fine powder.

Proximate principles:

The moisture content was estimated in all the samples by an infra-red moisture balance. Ash and fibre were estimated by the standard (AOAC, 1965) method. Fat was estimated by the Soxhelet method in all the samples (AOAC, 1965). Protein was estimated by colorimetric method in all the samples (Alers, 1955). (Appendix II— V).

Amylose content:

It was determined by a standard procedure based on colour development with iodine and measurement of the colour transmittance at 600 nm (Bhattacharya, 1968). (Appendix-VI).

Estimation of total starch:

This was done by the Hassid and Newfeld (1963) method. Soluble sugar-free solution is treated with perchloric acid for liberating starch. The intensity of colour developed due to the addition of anthrone is

determined colorimetrically (Appendix-VII).

Estimation of reducing and non-reducing sugars:

The estimation was done by the standard A.A.C.C. method (1975) and computed as mg of maltose per 100 gm of sample (Appendix-VIII - IX).

Amylase inhibitor:

The Jaffee (1973) method was used for all the samples. (Appendix-X).

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Two commonly consumed recipes of tubers in Andhra Pradesh are Pulusu and chips. These two recipes are standardised and analysed for their nutrient composition.

TABLE - I: Amount of water absorbed, time taken for boiling and preparing the recipes with different tubers.

Tuber	Initial weight of the tuber (g)	Water absorbed by the tuber (ml)	Time taken for boiling (min)	Time taken for cooking the Pulusu+ (min)
Potato (Solanum tuberosum)	50	10	10	20
Sweet-potato (Ipomea batata lam)	50	12	10	15
Colocasia (C.Esculenta.L)	50	6	10	22
Yam (Ammorphophalus compamulutum)	50	12	15	20
Cassava (Manihot esculenta)	50	8	12	15

+1 cup (200 ml) of Pulusu.

The data given in table-I shows that in the preparation of Puluasu with tubers, water absorption is less in the case of colocasia and cassava, even though the time taken for boiling the tuber is same for all the tubers. In the preparation of Puluasu, colocasia took more time than other tubers.

The difference in the weight, time and temperature of frying the chips of different tubers in refined groundnut oil is given in table-II.

TABLE - II: Per cent loss of weight, yield, time and temperature required for frying chips of different tubers.

Tuber	Initial weight of the tuber (g)	Weight of edible portion (g)	Weight after frying 6g)	Loss of weight due to frying (o/o)	Yield of chips (Number)	Frying time (min)	Frying Temperature (^{o}C)
Potato	50	43	40	7	25-26	4-5	80
Sweet-Potato	50	45	32	29	27-30	3-4	85
Colocasia	50	45	37	14	25-30	5	90
Yam	50	50	27	46	35-37	3	85
Cassava	50	43	28	35	25-27	4	90

The percentage loss of weight due to frying was more in yam and cassava and less in potato chips. There was no variation in time taken for frying, except for colocasia chips. The frying temperature, and yield of chips was the same for all the tubers. Chips with sweet-potato were crisp within four minutes at 85°C but Hoover and Harmon (1967) reported that crisp flakes were obtained at75°C after cooking for 10 minutes. The higher temperature used in the present study might have reduced the cooking time.

Analysis of the proximate composition of recipes along with raw tubers was done and the results are given in table-III.

TABLE - III: Percentage composition of proximate principles of different tubers before and after cooking.

Tuber	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
1. Potato						
a) Raw	76.0	0.37	1.21	0.10	2.07	20.26
b) Pulusu	82.2+	0.39	0.67	4.30	1.76	18.82
c) Chips	61.2	0.48	1.00	16.08+	1.86	19.39
2. Sweet-Potato						
a) Raw	68.0	0.46	0.99	0.09	1.48	28.98
b) Pulusu	74.6+	0.52	0.89	4.20	1.28	30.02
c) Chips	66.3	0.52	0.84	14.56+	1.24	16.64
3. Colocasia						
a) Raw	64.0	0.87	2.31	0.10	2.65	30.13
b) Pulusu	78.0+	0.93	1.21	6.20	2.41	21.25
c) Chips	43.6	1.10	1.62	25.00+	2.07	26.61
4. Yam						
a)Raw	70.0	0.39	1.20	0.09	1.25	27.07
b) Pulusu	74.4+	0.47	0.84	4.70	0.94	19.70
c) Chips	40.1	0.63	1.10	19.80+	0.94	27.44
5. Cassava						
a) Raw	74.0	0.46	1.20	0.10	1.70	22.54
b) Pulusu	80.2+	0.54	0.91	4.20	1.49	13.00
c) Chips	61.6	0.71	0.93	16.30+	1.48	20.25

+ Statistically significant ($P < 0.05$)

The moisture, fibre, fat and protein contents of raw potatoes observed in the present study are the same as the values reported (Keitt, 1911; Duckworth, 1966; Gopalan et al, 1976; Mondy and Muller et al, 1977). Ash content is lower than the value reported (Wegner, 1972) and the carbohydrate content is also lower than the value observed by Gopalan et al, (1976). Though there is slight change in the nutrient composition after cooking the Pulusu and chips, it is not significantly different (Jacob, 1957; Chaudhury, 1967) except for moisture and fat content.

In the preparation of Pulusu, tubers are boiled first in water. Water is also added while cooking. This might cause an increase in the moisture content of the tubers. In the case of chips there is a decrease in moisture content and an increase in fat content, which may be due to the deep fat frying used for the preparation.

The proximate composition of raw sweet-potato is similar to the reported values (Keitt, 1911; MacGillvary, 1953; Chattfield, 1954; Wall and Merril, 1954; Duckworth, 1966; Young and Tsai, 1976; Gopalan et al, 1976). In the case of Pulusu and chips there was a significant increase in moisture and fat content respectively.

There was no change in other nutrients as observed by Jacob (1957).

In colocasia and yam the proximate composition in the raw tuber was same as reported (Keitt, 1911; Coursey, 1963; Duckworth, 1966; Baquar and Oke, 1976; Gopalan et al, 1976; Splittstoesser, 1976; Fredric et al, 1977; Ciacco and Appolonia, 1978) except for carbohydrate, which was higher than the value reported by Gopalan et al, (1976). In the case of other tubers there is a significant increase in the moisture content of Pulusu and the fat content of chips. No change in other nutrients were observed.

The protein content of cassava is higher than the values observed by Duckworth (1966), but the carbohydrate content is lower than the values observed by Gopalan et al, (1976). Other nutrients are the same as the values reported (Duckworth, 1966; Gopalan et al, 1976; Yeoh and Chew, 1977; Ciacco and Appolonia, 1978). There was no change in the proximate composition even after cooking, except for moisture contents in Pulusu and fat content in chips, which are significantly higher.

The total starch, amylose, reducing and non-reducing sugar contents were estimated, and the results are given in table - IV.

TABLE - IV: Percentage of starch, amylose, reducing, and non-reducing sugar contents of different tubers before and after cooking.

Tuber	Starch	Amylose	Reducing Sugar	Non-reducing sugar
1. Potato				
a) Raw	20.60	18.20	0.14	0.14
b) Pulusu	16.24	20.18	0.14	0.14
c) Chips	16.70	24.95	0.20	0.79
2. Sweet-potato				
a) Raw	26.84	23.45	0.22	1.96
b) Pulusu	24.56	26.36	0.30	2.10
c) Chips	20.60	27.92	0.41	2.23
3. Colocasia				
a) Raw	30.24	17.10	0.28	0.23
b) Pulusu	27.00	19.19	0.20	0.25
c) Chips	29.50	19.28	0.31	0.63
4. Yam				
a) Raw	26.75	10.75	0.11	0.35
b) Pulusu	21.01	13.62	0.11	0.37
c) Chips	20.40	16.26	0.23	0.68
5. Cassava				
a) Raw	27.92	16.26	0.18	1.10
b) Pulusu	20.10	18.45	0.19	1.24
c) Chips	23.00	17.85	0.21	1.68

In all the tubers there was a decrease in starch content and an increase in amylose, reducing sugar and non-reducing sugar after cooking but the change is not significantly different. Cooking makes the starch available for ensyme hydrolysis. This may be the reason for the slight increase in the amylose content of tubers after cooking as observed by Galinskaya (1977). The reducing sugar and non-reducing sugar content of the raw potatoes observed in the present study are lower than the values observed by Iritani and Russell, (1973);Iritani and Weller (1977).

Starch and amylose content are the same as the values reported (Appolonia and Miller, 1911; Duckworthh, 1966; Masem and Ciacco, 1976; Sochoka et al, 1974) and the amylose content is with in the range of values reported (Sinek, 1973 and 1977; Sochocka et al, 1974). There was a significant increase in reducing and non-reducing sugar in the case of potato chips. Thornton (1942) observed dark colour chips if the reducing sugar content was low but in the present study, eventhough the reducing sugar content was low, the colour of the chips was not so dark.

There was a significant increase in non-reducing sugars after cooking the sweet-potatoes. Jacob (1957) also observed a similar increase in non-reducing sugars

after cooking. The amylose contents of raw sweet potato observed in the present study are higher than the values reported by Mandamba and Pedro et al. (1976).

The starch content of colocasia, yam and the amylose content of colocasia observed in the present study are within the range of values reported (Keitt, 1911; Duckworth, 1966; Purcell et al, 1972; Ciacco and Appolonia, 1978).

The starch content of cassava observed in the present study is far lower than the values reported (Duckworth, 1966; Ciacco and Appolonia, 1978). The reducing sugar content is expressed in the present study on dry weight basis and is lower than the reported values which are expressed on a fresh weight basis.

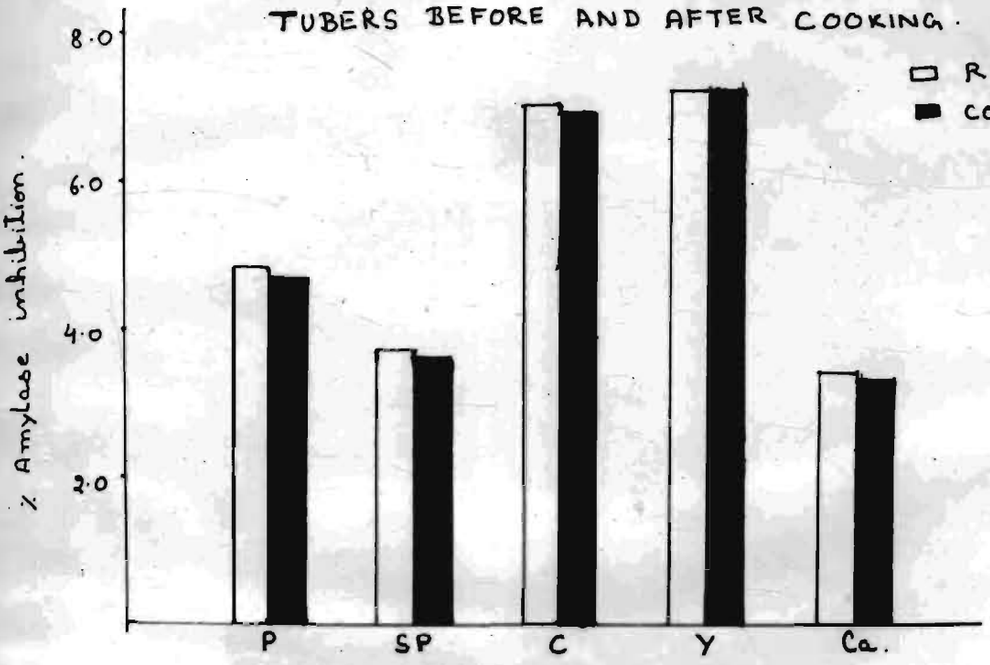
The percentage of amylase inhibition was analysed in all the tubers before and after cooking, and the results are given in table-V.

TABLE -V: Percentage amylase inhibition in different tubers before and after cooking.

Tuber	Maltose released from the samples (g)	Amylase inhibition (o/o)	pH of the substrate
1. Potato			
a) Raw	0.402	4.8	7.00
b) Pulusu	0.403	4.7	7.00
c) Chips	0.405	4.5	7.00
2. Sweet-potato			
a) Raw	0.413	3.7	7.00
b) Pulusu	0.414	3.6	7.00
c) Chips	0.414	3.6	7.00
3. Colocasia			
a) Raw	0.380	7.0	7.00
b) Pulusu	0.381	6.9	7.00
c) Chips	0.381	6.9	7.00
4. Yam			
a) Raw	0.378	7.2	7.00
b) Pulusu	0.378	7.2	7.00
c) Chips	0.379	7.1	7.00
5. Cassava			
a) Raw	0.416	3.4	7.00
b) Pulusu	0.417	3.3	7.00
c) Chips	0.418	3.3 - 3.4	7.00
Standard pure starch	0.450	—	6.80

Fig-1

PERCENT AMYLASE INHIBITION IN DIFFERENT TUBERS BEFORE AND AFTER COOKING.



P= Potato , SP= Sweet potato, C= Colocasia, y= yam , Ca= Cassava.

The data presented in table -V show that the amount of maltose released from the tuber is less in the case of colocasia and yam compared to other tubers. This may be due to the presence of amylase inhibitor. There was 7 per cent amylase inhibition in colocasia and yam (fig.1) which is higher compared to the other tubers. There was no change in maltose content or in amylase inhibition observed even after cooking. The amylase inhibitor may be a heat stable compound as that observed by Narayan Rao (1978) in colocasia. The per cent inhibition in colocasia observed in the present study is in accordance with the values reported by Narayana Rao (1970).

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

Roots and tubers are commonly consumed vegetables. Due to their easy availability and low cost that becomes part of the daily menu in many families. A survey conducted in Andhra Pradesh had shown that these are consumed after cooking. Chips and Pulusu are the commonly consumed recipes of these tubers. Cooking of tubers will bring about some changes in their nutritive value as well. In the present study an attempt has been made to study the proximate principles, ash, total starch, reducing, and non-reducing sugars, and amylase inhibition of potato, sweet-potato, colocasia, yam, and cassava before and after cooking the recipe.

The results obtained in the present study show that there is an increase in the moisture content of tuber in the preparation of Pulusu and there is an increase in the fat content of chips. This may be due to the cooking method used for the preparation of the recipe and no significant change in other nutrients was observed even after cooking.

After cooking the tubers, though there was a decrease in the starch content, and an increase in the amylose and reducing sugar contents in all the tubers, it was

not significant. The non-reducing sugar contents of all the tubers increased in the preparation of chips.

There was no change in the per cent amylase inhibition even after cooking in all the tubers and the per cent amylase inhibition was higher in colocasia and yam.

At present only the changes in the proximate principles are studied. A study of the changes in the contents of the vitamins and minerals after cooking also might give a better picture. Therefore, further studies including the analysis of these are suggested. The increased inhibition of amylase in yam and colocasia needs further analysis.

BIBLIOGRAPHY

BIBLIOGRAPHY

Alers, R.(1955) — The method of foliar diagnosis as applied to sugar-cane, Capo University of Puerto Rico, Bulletin No.125, 65.

American Association of Cereal Chemists(1975) — Approved Methods, Published by the American Association of Cereal Chemists, 88-60; 2,p.1.

Annual Report, National Institute of Nutrition (1976) — N.N.N.B. data on roots and tubers composition in different states, p.149.

Appleman, C.C. and Miller, E.V.(1911) — Sugars in sweet-potato varieties, J.Agri.Res.,33, p.569.

Association of Official Agricultural Chemists (1965) (A.O.A.C.) — Official and Tentative methods of Analysis, Association Official Agricultural Chemist, Washington.

Balls, A.K.(1948) — A crystalline β-Amylase from sweet-potato, J.Bio.Chem.,9, p.1175.

Baquar, S.R. and Oke, O.L. (1976) — Protein in Nigerian yams, Nutr. Rep.Inter., 14, p.234.

Bhattacharya, K.R.(1968) — Curing of freshly harvested rice by heat treatment, Ind.J.Tech. 2, pp.372-380.

Can Can, F. and Joseph, A.A.(1965) — Quantitative estimation of sugars in the tubers of several varieties of sweet-potato, Ser.Biol.,3, p.87

Chattfield, F.(1954) — Nutrient Composition of some varieties of sweet-potato, p. 1210, vegetable production in India, Ed.Chauhan, D.V.S., Ram Ram Prasad and Sons, Agra.

Chaudhuri, R.N.R., Joseph, A.A., Daniel, U.A., Rao, M.N., Swaminathan, M.S., Srinivasa, A.A., and Subramanyam, V. (1965) — Effect of cooking, leaching, and canning on the nutritive value of potato, J.Food Sci., 12, p.253.

Chaudhury, B.(1967) — Vegetables, p.149, Dr. B.N. Keskar, National Book Trust, New Delhi.

Ciacco, C.F., and Appolonia, B.L.(1978) — Biochemical composition of Cassava and Yam flour, Cere. chem., 55, p.402.

Coursey, E.J. (1965) — Shelf life study of farm Libian yam, Amer.J.Agri., 57, p.196.

Deobald, H.J.(1968) — Control of sweet-potato amylase for producing optimum quality precooked dehydrated flakes, Food Tech.,22, p.93.

Deobald, H.J.(1969) — Relationship of Sugar formation and sweet-potato α-amylase activity during processing, Food Tech., 23, p.118.

Duckworth, R.B.(1966)	Fruits and Vegetable, p.145 and 279, Ed.J.Hawthorn, Pergamon Press, London.
Francis, B.J., Halliday, D. and Ralaison, J.M. (1975)	Yam as a source of edible protein, Trop.Sci., 17, p.103.
Fredwrick, I., Oputete and Osagie, V. (1976)	Identification and quantitative determination of the lipids of Alocasia Macrorealisa tubers, J.Sci.Food. Agri.29, p.1002.
Galinskaya, L.A.(1977)	Determination of level of starch gelation in potato products. Cited from Nutr.Abst.,10, p.102 (1978).
Gopalan, C.(1976)	Growing Population and rising aspirations, Selected Popular lectures of Dr.C.Gopalan, NIN Publication, p.94.
Gopalan, C., Ramashastri, B.V., and Balasubramanium, S.C.(1976)	Nutritive value of Indian Foods, p.33, NIN, Citizen Press, Hyderabad.
Hassid, W.Z. and Newfield, E.F.(1963)	Methods in carbohydrate chemistry, IV, p.32, Ed. Whistler, Roy.L and Wolform, Acad.Press, INC.NY.
Hoover, N.W. and Harmon, S.J.(1967)	Carbohydrate changes in sweet-potato flakes by enzyme activation technique, Food Tech., 21, p.115.

Indian Council of Agricultural Research, Hand Book of Agriculture (1969) — Vegetables, p.50, ICAR, New Delhi.

International Development Research Centre(1977) — IDRC project, College of Home Science, A.P.A.U., unpublished data.

Iritani, W.M. and Russell, L.(1973) — Relative differences in sugar content of basal and apical portions of Russet Burbank potatoes, Amer.Potato J., 50, p.24.

Iritani, W.M. and Weller, L.(1977) — Changes in sucrose and reducing sugar content of Kennebec and Russett Burbank tubers during growth and post-harvest holding temperature, Amer. Potato J.54, p.395.

Jacob, M.B.(1957) — Technology of foods and food products, vegetables and mushrooms, p.1299, Interscience publishers, Inc. New York.

Jaffee, G.(1973) — J.Food Sci.Tech.,3,p.44.

Johnson, E.(1957) — Fat absorption in white potato chips, Amr.Potato J., 54, p.117.

Kiett, T.E. (1911) — Nutrient composition of some varieties of potato and sweet-potato, So.Carolina.Agri.Expt. Sta., 156, p.47.

Kunkel, R., Gressoy and Birkly, A.M.(1951) — Mechanical separation of potatoes into specific gravity group, Amer.Potato J., 28, p.690.

MacGillvary, J.M.(1953) — Vegetable production, p.47, Blakistan Co.Inc. New York.

Mandamba, L.S.P. and Pedro, E.L.(1976) — Chemical composition of sweet-potato flour, Phill. Agri., 9, p.350.

Masen, T. and Ciacco, Z. (1976) — Influence of fertilization and planting density on tubers yield and quality of potato, Field Crop, 31, p.49.

Mondy, N.L. and Muller, T.O. (1977) — The effect of cooking methods on lipid composition of potatoes, Amer.Potato J., 54, p. 203.

Monica, M.K.R.(1976) — A study on consumption patterns, method of storage, standardi-sation and ascorbic acid analysis in some common recipes with non leafy and root vegetables in selected rural areas of Telang-ana region in Andhra Pradesh, (M.Sc.Thesis presented to A.P. A.U.).

Murthyswamy, P.and Krishnamurthy, M.K.(1972) — Nutritive value of some promising varieties of potato tubers, Andhra Agri. J., 19, p.157.

Narayan Rao, M. (1970)	Purification of amylase inhibitor from colocasia tubers, Ind. J. Biol.Chem., 7, p.241.
Operation Research Group (1972)	Food habit survey conducted on Southern India, (PFNDAI), Mahalakshmi Chambers, Bombay.
Ordonez, C.R. (1972)	Potato chips; dry matter and specific gravity of potato tuber and yield on processing into chips, Tech.Agri., 6, p.37.
F.A.O. Compendium (1968)	The need for nutritional guidelines in Agriculture, 2, p.3-3-2/8, Ed.Moshe, Y. Sachs, World Mark Press Ltd., Halsted Press Division, New York.
Purcell, A.E., Walter, W.M., Swaisgood, T. and Daniel, T. (1972)	Protein and amino acid content of Sweet-potato, J.Agri.Food Chem., 26, p.699.
Purcell, A.E., Marald, E., Swaisgood, T. and Daniel, T. (1976)	Changes in amyloid carbohydrate flakes, J.Food Sci., 41, p.7.
Sarhan, M.A.I., Wakeil, E.L. and Morris, M.E.W. (1975)	Chemiscal changes in sweet-potato during cooking, J. Food Sci., 3, p.25.
Sinek, J. (1973)	The problem of increasing the amylasecontent of potatoes by hybridisation, Cited from: Field Crop Abst.,27,p.1238 (1974).

Sochoka, J.Federowicz, T., Palasinski, M. and Warchal, M. (1974) — Characteristic of the tuber of certain solanum specifies in aspect of potato industry, Cited from: Field Crop Abst., 31, p.81 (1975).

Splittstoesser, W.E.(1976) — Protein and total amino acid content before and after cooking of yams, Hort.Sci.,11, p.611.

Sukhatme, P.V. and Basu, M.(1971) — Food production and consumption, the efficiency of human food chains and nutrition cycle, p.14, ICAR, New Delhi.

Swaminathan, M.S.(1972) — Agricultural growth in India, Proc.Nutr. Soc. India., 16, p.4.

Swaminathan, M.S.(1977) — Food production technology and achievement of Social Goals, Proc.Nutr.Soc.India., 17, p.15.

Wegner, M.L. (1976) — Tissue selection and its effect on solid content and amylase/amylopectin ratios, Amr.Potato J., 55, p.73.

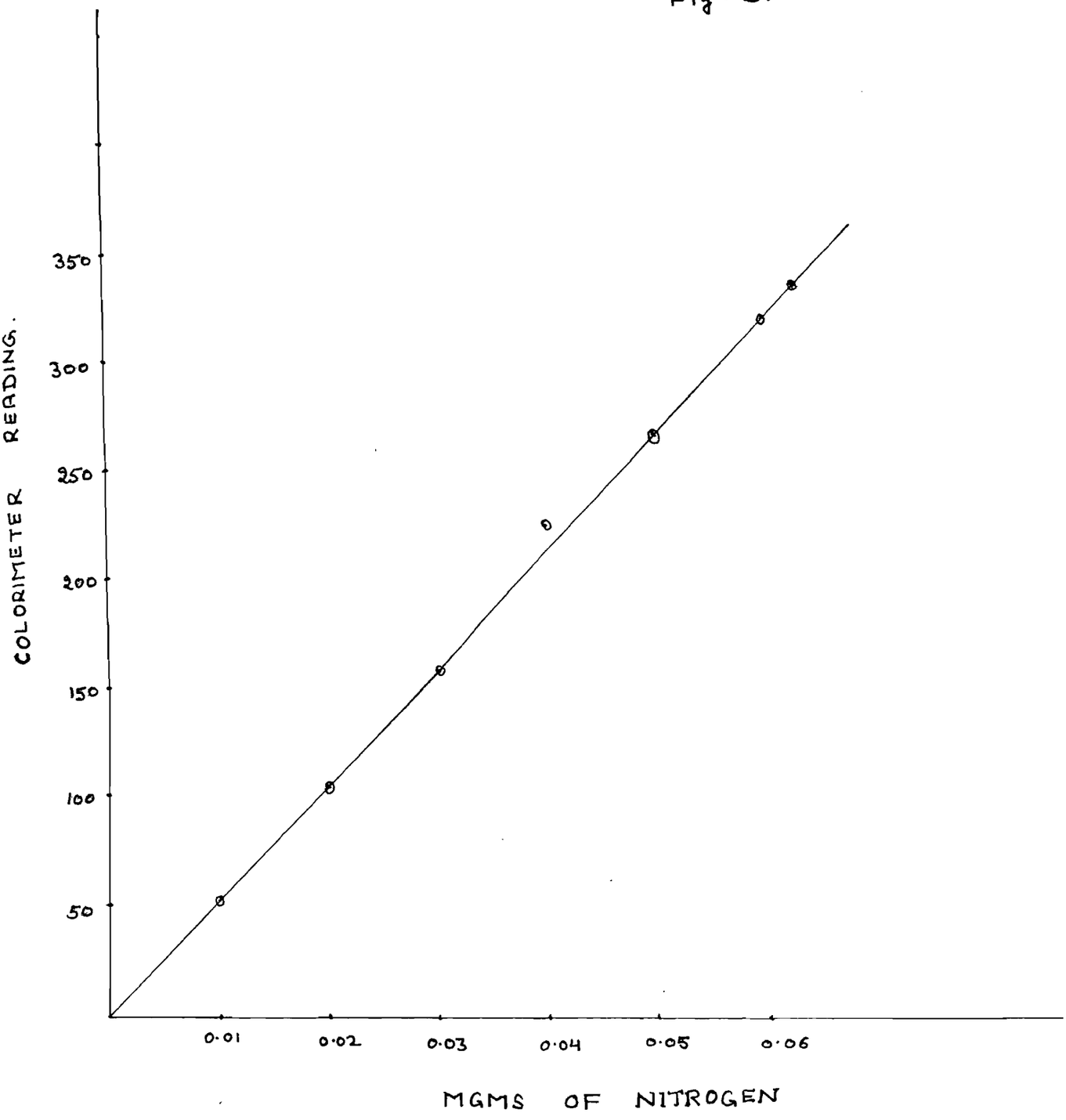
Yeoh, H.M. and Chew, M.Y. (1977) — Protein and amino acid composition of cassava seeds and tubers. Malaysian Agri. J., 51, p.1.

Young, T.H. and Tsai, Y.C. (1975) — Studies on protein nutrition of the main foods of Taiwan, Cited from: Field Crop abst., 31, p.49, (1978).

APPENDIX

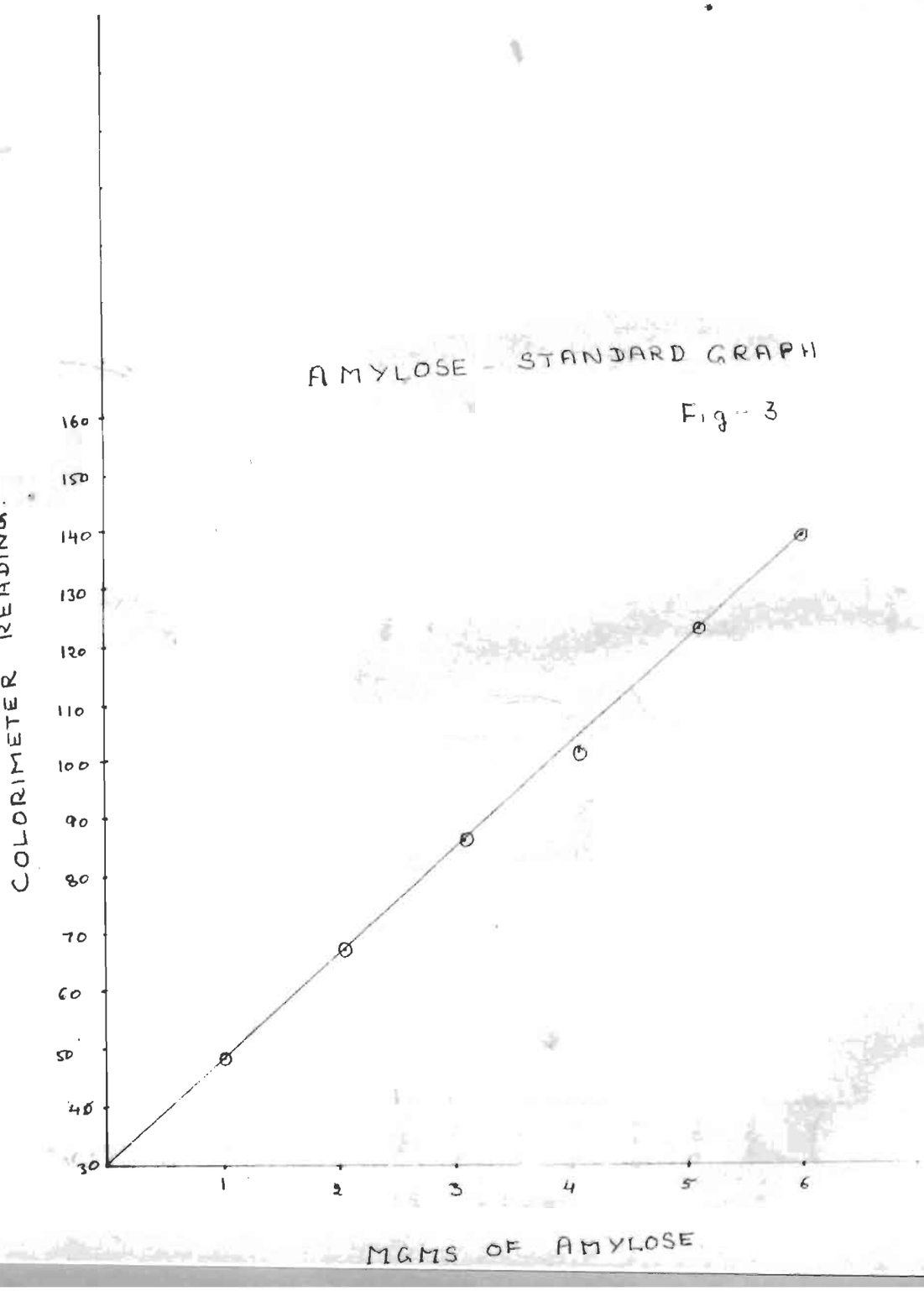
PROTEIN - STANDARD GRAPH

Fig - 2.



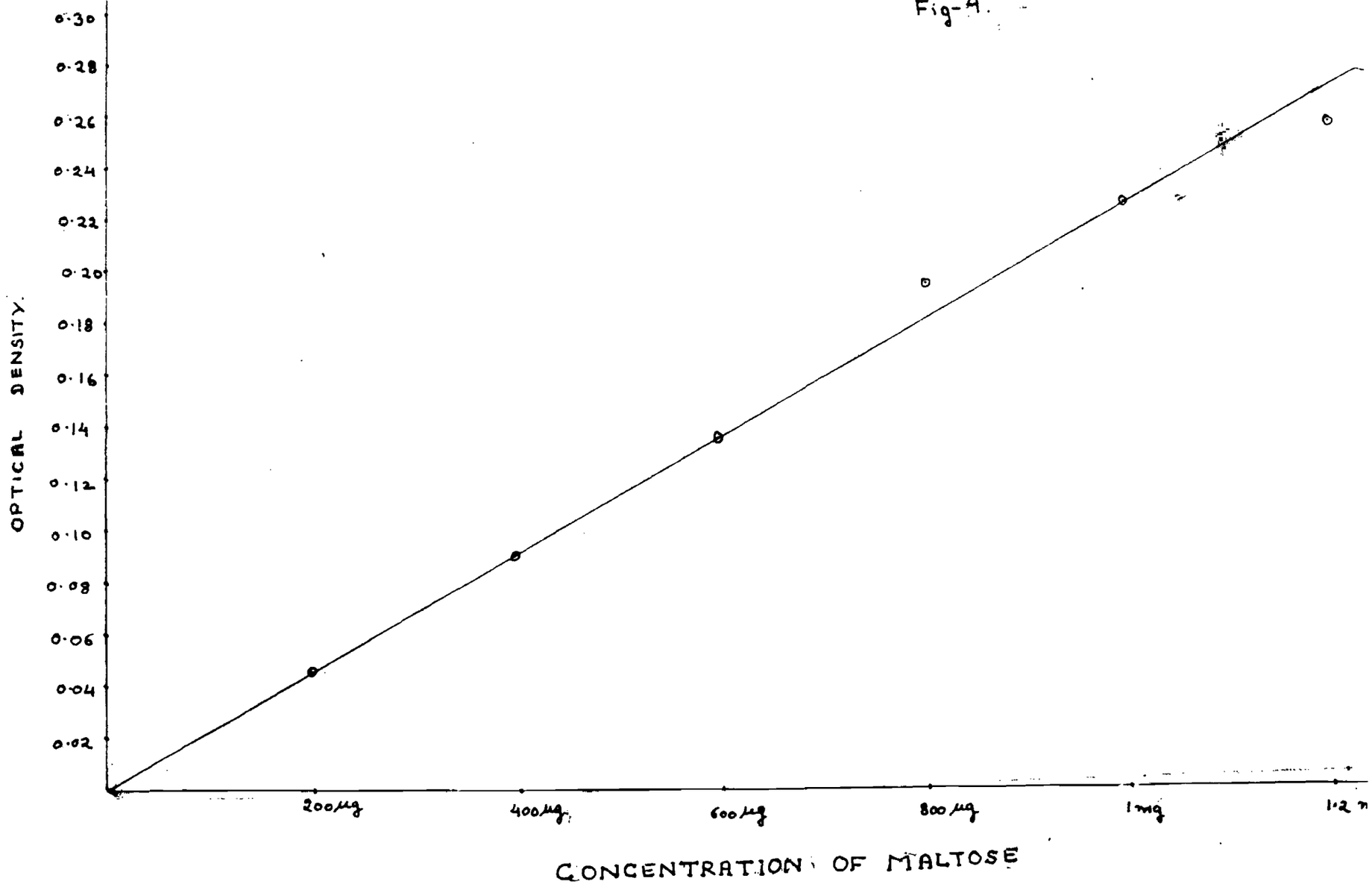
AMYLOSE - STANDARD GRAPH

Fig - 3



MALTOSE - STANDARD GRAPH

Fig-4.



APPENDIX - I

STANDARDISED RECIPES

a) Potato Pulusu

Potatoes (edible portion)	180 g
Oil	20 ml
Water	650 ml
Seasoning	½ tsp
Tamarind	1 lime size (10 g)
Turmeric powder	¼ tsp
Chilli powder	2 tsp

METHOD:

Potatoes are semi boiled, peeled and cut. Tamarind water is prepared by boiling tamarind for 10 minutes in 400 ml of water and the juice is squeezed after cooling and kept aside. After heating the oil, seasonings, chilli powder, turmeric, salt and potatoes are added. After a few minutes tamarind water is added and cooked till it becomes a little thick and removed from the fire.

b) Potato Chips

Potatoes	50 g
Oil	To fry

METHOD:

Potatoes are peeled and chips are made by chip maker and deep fried till it becomes crisp.

e) Sweet-potato Pulusu

Sweet-potatoes (edible portion)	87 g
Oil	20 ml
Water	500 ml
Seasoning	1/4 tsp
Tamarind	One lime size (14g)
Turmeric powder	1/8 tsp
Chilli powder	1 tsp

METHOD:

Same as potato Pulusu

d) Sweet-potato chips

Sweet-potatoes	50 g
Oil	To fry

METHOD:

Same as potato chips.

e) Colocasia Pulusu

Colocasia (edible portion)	92 g
Oil	15 ml
Water	400 ml
Seasonings	1/4 tsp
Turmeric powder	1/8 tsp
Chilli powder	1 tsp

METHOD:

Same as potato Pulusu

f) Colocasia chips

Colocasia	60 g
Oil	To fry

METHOD:

Same as potato chips

g) Yam Pulusu

Yam (edible portion)	100 g
Oil	20 ml
Water 50	500 ml
Seasoning	1/4 tsp
Tamarind	15 g
Turmeric powder	1/8 tsp
Chilli powder	1½ tsp

METHOD:

Same as potato Pulusu.

h) Yam chips

Yam	50 g
Oil	To fry

METHOD:

Same as potato chips.

i) Cassava Pulusu

Cassava (edible portion)	96 g
Oil	20 ml
Water	600 ml

seasoning	1/2 tsp
Tamarind	15 g
Turmeric powder	1/8 tsp
Chilli powder	1½ tsp

METHOD:

Same as potato Pulusu.

3) Cassava chips

Cassava	50 g
Oil	for frying

METHOD:

Same as potato chips.

APPENDIX - II

ESTIMATION OF ASH CONTENT

Single Incineration Method:

2 g of the air dry sample in a porcelain flat bottomed dish at a heat below redness to a white or gray ash was burned.

Calculation

$$\text{Per cent ash} = \frac{\text{Wt of the ash}}{\text{Wt of the sample taken}} \times 100$$

APPENDIX - IV

ESTIMATION OF CRUDE FIBRE

Reagents:

H_2SO_4 - 0.255 N

NaOH - 0.313 N

METHOD:

Two grams of the mixture fat-free sample was weighed into a 500 ml beaker and 200 ml of boiling 0.255 N (1.25 o/o W/V) sulphuric acid was added. The mixture was boiled for 30 minutes, keeping the volume constant by the addition of distilled water at frequent intervals. A glass rod was inserted into the beaker to help smooth boiling. At the end of the period, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N (1.25 o/o) sodium hydroxide was added. After boiling for 30 minutes, keeping the volume constant as before, the mixture was filtered through muslin cloth, the residue washed with hot water till it was free from alkali followed by washing with some alcohol and ether. It was then transferred to a crucible, heated in a muffle furnace at 600°C for 2 to 3 hours, cooled and weighed again. The crude fibre per 100 g of the moisture

was calculated using the formula below.

$$\text{Crude fibre per cent} = 100 \times \frac{(\text{wt of ash + fibre}) - \text{wt of ash}}{\text{wt of moisture and fat-free sample taken.}}$$

APPENDIX - XI

ESTIMATION OF FAT

REAGENT:

Solvent Ether

METHOD:

Dry the food sample in a hot air oven at 60°C. Make a stout thimble with filter paper. Take a known weight (about 2 g) of the food sample in the thimble. Transfer the thimble into the glass cylinder provided with a siphon. Fill the flask to half with solvent ether. Keep ether in the cylinder also. Boil the solvent ether gently. The boiling ether rises through the side tubes and condenses in the reflux condenser. Thus a constant stream of pure solvent drops into the thimble and the solution of the extracted matter siphons back periodically into the flask. Repeat the process 4-5 times, i.e., till the food sample is free from fat. Remove the thimble, dry in hot air oven. Note the weight.

Calculations:

Weight of the thimble W_1 g

Weight of the thimble + food (before extraction) W_2 g

Weight of the thimble + food (after extraction) W_3 g

Weight of the sample $(W_2 - W_1)$ g

Weight of the fat present in the sample $(W_2 - W_3)$ g

$$\text{o/o of the fat in the sample} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

APPENDIX - V

ESTIMATION OF PROTEIN

REAGENTS:

Nitrogen Mixture

Nessler's reagent

Standard Ammonium Sulphate

Digestion:

The chemicals used were sulphuric acid, Hydrogen peroxide, 100 vol (30 o/o), Ammonium Molybdate, Stannous chloride or Stannous oxalate powder, Pottasium dihydrogen orthophosphate.

METHOD:

Weighed samples (0.1 g) of the powder were transferred to dry test tubes. 1 ml of concentrated H_2SO_4 was added thoroughly mixed. Exactly 0.5 ml of H_2O_2 was added and the test tube shaken well. A vigorous reaction took place. Shaking was continued till the reaction slowed down and the test tube were set aside for 2-3 minutes for the colour to become almost clear. They were then warmed gently on a small flame until very fine bubbles appeared. The tubes were set aside for the bubbling to stop and the process was repeated until the size of the bubbles were appreciably bigger and the colour turned brown again, while hot drops of H_2O_2 were added into the tubes and shaken immediately. The colour was almost discharged, and again,

the tubes were heated until bubbles evolved. Heating was continued until the colour was completely discharged and did not appear again. The digestion should be completed within 4-6 drops of H_2O_2. The extract was cooled with water and made upto 50 ml. This was filtered and used for nitrogen analysis.

Nitrogen Analysis:

Two ml of the extract was pipetted into a 25 ml volumetric flask, and diluted with water to about 20 ml. 1 ml of the nitrogen mixture was added and mixed, and 15 drops of Nessler's reagent was added. The volume was made up and the intensity of the colour was read in the colorimeter, using a blue filter (450 nm). The nitrogen content was calculated by using the standard graph. The total protein was obtained by multiplying the total nitrogen by 6.25.

Nitrogen Mixture:

To 100 ml of Sodium acetate dissolved in 100 ml of water, 4 ml of glacial acetic acid, 6 g of Sodium hydroxide dissolved in 100 ml water and 2 g of sodium silicate paste dissolved in 100 ml of water and the volume was made upto 500 ml.

Nessler's Reagent:

Weigh 7 g of Mercuric chloride and dissolve in 100 ml of water, warming not more than 55°C to dissolve completely. Cool thoroughly. Prepare 2.5 g of Potassium iodide in 10 ml of water and pour the mercuric chloride solution into this stirring until the red precipitate which forms and disappears on stirring just persists. Prepare a solution of 40 g of NaOH in 100 ml of water and cool thoroughly. Add 30 cc of this NaOH solution to the solution of mercuric chloride in potassium iodide, taking care that the temperature does not rise . A pale straw liquid should result. If there is any turbidity in the solution add a few crystals of KI and the colour will be discharged. Stand overnight and decant the supernatant liquid into a brown flask and store away from ammonia fumes.

Standard Ammonium Sulphate:

0.4716 g of Ammonium Sulphate was dissolved in one litre of distilled water. 1 ml of this solution contains 0.0001 g of Nitrogen or 0.1 mg of Nitrogen.

For the working standard 10 ml of the above solution is taken and diluted to 100 ml. Therefore, 1 ml of this working standard contains 0.01 mg N_2.

Calculation:

50 ml of filtrate has 'A' mg or ml of sample.

'P' ml is used for colour development.

'P' ml filtrate has $\frac{'PA'}{50}$ mg of sample; $\frac{'PA'}{50}$ mg sample contains 'Y' mg nitrogen.

100 g sample will therefore contain = $\frac{100 \times Y \times 50}{PA}$ g of nitrogen, or $\frac{100 \times Y}{1000} \times \frac{50}{PA}$ mg of nitrogen.

APPENDIX - VI

ESTIMATION OF AMYLOSE

Reagents:

Sodium Hydroxide 1 N (approximately)
HCl 1 N (approximate)
Iodine solution - 0.2 o/o in 2o/o KI
Phenolphthalein - 0.1 o/o in 95 o/o alcohol
Distilled ethyl alcohol
Boiled and cooled distilled water
Standard amylose solution: 100 mg amylose in 10 ml sodium hydroxide was dissolved accurately by shaking, diluted to about 50 ml, and about 3/4 of the calculated amount of HCl needed to neutralize the solutions was added, made up to 100 ml and stored in the refrigerator.

Method for sample preparation:

The recipe was fully dried and powdered to pass through a 30-40 mesh screen. For routine and approximate work, amylose was directly estimated in this flour and the results multiplied by 1 - 6. For accurate work, the flour was defatted by Soxhelet extraction, first with methanol and then with petroleum ether for 6-8 hours each, and then dried in air. It is convenient to expose the flour (along with standard amylose) to a definite atmosphere in a flat dish for about a day, so that moisture need not be determined in each sample. For example at 28°C and 65 o/o R.H., the sample

will attain approximately the following moisture contents: Undefatted rice flour 12.5 per cent, defatted rice flour 11.5 per cent, standard amylose (N.B.C.) 13.5 per cent.

METHOD:

10 mg of flour in 100 ml of volumetric flask were weighed accurately. One ml of alcohol was added and mixed, 10 ml of sodium hydroxide solution were slowly added and left overnight at room temperature. Alternatively the mixture was intermittently heated in a boiling water bath for a few minutes till the flour was dispersed and then cooled and was shaken thoroughly and diluted to 100 ml. The solutions were analysed immediately, but it is preferable, if timepermits, to have it for a day at room temperature as the reading often increases slightly next day.

It was mixed thoroughly and 5 ml were pipetted into a 100 ml volumetric flask, 3 drops of phenolphthalein and about 50 ml distilled water added and dilute HCl was added dropwise with shaking until the colour was discharged. 2 ml of Iodine solution was added and made up to volume with boiled distilled water. The standard amylose solution was treated similarly starting with 1 ml of the standard solution. All Iodine blanks were prepared by making up to

2 ml of Iodine solution to 100 ml with distilled water. The solutions were read after 30 minutes in a photoelectric colorimeter with 600 nm filter against the blank.

Calculation:

$$\text{Amylose content o/o dry basis} = \frac{\text{unknown reading}}{\text{std. reading}} \times \frac{\text{mg dry amylose in 1ml std.}}{5 \times \text{dry solids in sample (g/g)}}$$

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APPENDIX - VI

ESTIMATION OF TOTAL STARCH

REAGENTS:

1) 80 o/o ethanol: 1680 ml of 95 o/o ethanol is diluted with water to make two litres.

2) Perchloric acid: (53 o/o) - 270 ml of 72 o/o perchloric acid is diluted with 100 ml of water. Solution was stored in a glass stoppered container.

3) Iodine-Potassium Iodide solution: 7.5 g Iodine and 7.5 g of potassium iodide were ground with 150 ml of water, the resulting solution diluted to 250 ml and filtered if necessary through a Whatman No.3 filter paper with suction.

4) Ethanolic sodium chloride solution: 350 ml of ethanol, 80 ml of H_2O, 50 ml 20 o/o aqueous sodium chloride solution are diluted to 500 ml with water.

5) 20 o/o aqueous sodium chloride solution: 20 g of NaCl is dissolved in 100 ml of water.

6) 0.25 N ethanolic NaOH solution: 350 ml of ethanol, 100 ml of water and 25 ml of 5N NaOH are diluted to 500 ml with water.

7) Anthrone reagent: (0.1o/o / 72 o/o V/V H_2SO_4) 1 g Anthrone was dissolved in 1 litre of cooled H_2SO_4

containing 760 ml of concentrated H_2SO_4 with a specific gravity of 1.84, and filtered through fritted glass. The solution is stable at 4°C for several days, but must be discarded when it turns green.

8) Glucose solution (0.01 o/o): A stock solution is prepared in 0.1 o/o sodium benzoate solution.

METHOD:

The sample was dried rapidly at 90-95°C. It was ground to pass a 50-80 mesh screen. 50-250 mg sample was placed in 50 ml centrifuge tube. A few drops of80 o/o ethanol were introduced to wet the ground material so as to prevent clumping, 5 ml of water was added and the mixture was thoroughly stirred. The soluble sugars are extracted as follows:

The tube was immersed in a hot water bath. 5 ml of hot 80 o/o ethanol was added and mixture was stirred for several and centrifuged. The ethanolic solution was decanted and discarded. The extraction was repeated three more times to remove soluble sugars.

The sugar-free sample was heated for about 15 minutes with 5 ml of H_2O in a water bath in order to gelatinize the starch. The suspension was cooled to room temperature and 6.5 of 52 per cent perchloric acid was added while stirring. The tube was kept cool by immersion in water bath at 25°C.

Stirring was continued for 5 minutes and ocassionally during 15 minutes. 20 ml of water was then added and the mixture was centrifuged. The aqueous starch solution was decanted into a 50 ml volumetric flask and extraction with perchloric acide was repeated without preliminary heating. The tube and rod were washed with water. The combined extract were diluted to the mark and centrifuged, and the supernatant was decanted through glass wool.

A 10 ml aliquot of starch was transferred to a 50 ml conical centrifuge tube, 0.5 ml 20 per cent NaCl and 2 ml I_2/KI solution was mixed and let stand for 20 minutes. This was centrifuged and the supernatant liquid was removed with extreme care to avoid any loss of the precipitate. The precipitated starch-iodine complex is then suspended in 5 ml of ethanolic NaCl solution and centrifuged. The supernatant fluid was discarded and the washing process was repeated at least one more time. To the precipitate 2 ml of 0.25 N ethanolic NaOH were added and gently shaken and topped until the blue colour was discharged. A stirring rod must not be used as the gummy precipitate would stick to it and ample time given for the decomposition of complex.

The liberated starch was then centrifuged and washed several times with 5 ml portions of ethanolic NaCl solution as before. About 5 ml hot water was added to the precipitate

starch in the tube and the mixture was stirred with glass rod until the starch had dissolved. The solution was quantitatively transferred to a 50 ml volumetric flask, the tube and rod were thoroughly washed with warm water and the wash was added to the contents of the flask which were then diluted to volume and mixed thoroughly. The concentration of starch was determined by the method of Fairbrain.

Aliquot of starch solution expected to contain 20 to 200 µg of starch were pipetted into acid-washed test tubes and diluted with water to 2 ml. At the same time a series of tubes containing 0-200 µg D-glucose in 2 ml of solution were prepared. The tubes were immersed in cold water. 10 ml of anthrone reagent was added and mixed well and placed in a boiling water bath for 15 minutes, cooled rapidly and read at 620 nm. In calculating the amount of starch from the standard curve of percentage D-glucose, the D-glucose reading must be multiplied by 0.90.

APPENDIX - VIII

ESTIMATION OF REDUCING SUGAR

REAGENTS:

Ethyl alcohol - 95 o/o

Acid buffer solution

Sodium tungstate - 12 o/o

Alkaline ferricyanide solution - 0.1 o/o

Acetic acid salt solution

Soluble starch-(Potassium Iodide solution)

Thiosulphate solution - 0.1 o/o

Preparation of extract:

5.675 g of flour were introduced into a 100 ml flask. The flask was tipped so that all the flour is on one side and wetted the flour with 5 ml alcohol. The flask was tipped to keep the flour on top, 50 ml buffer solution added keeping away from coming in contact with flour till all had been added. It was shaken to bring flour into suspension. 2 ml of sodium tungstate solution was added immediately and mixed thoroughly, and filtered (Whatman No.4) discarding first 8-10 drops.

METHOD:

5 ml of extract pipetted into a test tube and 10 ml alkaline ferricyanide reagent added, mixed and immersed in a vigorously boiling bath 3-4 cm below the surface and boiled

for 29 minutes. The test tube was cooled under running water and poured immediately in 200 ml flask. The test tube was rinsed with 25 ml acetic acid, salt solution, the rinsed added to the flask and 1 ml of the starch KI solution added and titrated with 0.1 N thiosulphate solution till the blue colour disappeared.

Calculation:

X ml ferricyanide reduced was calculated by subtracting 4 ml than required from the equivalent of ferricyanide reagent. Reducing sugar as mg maltose/100 g flour was computed from the table.

APPENDIX - IX

ESTIMATION OF NON-REDUCING SUGAR

REAGENTS:

Reagents are the same as used for reducing sugar.

METHOD:

5 ml of filtered clarified extract was taken into a test tube and immersed in a vigorously boiling water bath for 15 minutes. The test tube was cooled under running water and exactly 10 ml of alkaline ferricyanide reagent was added. Reduction and titration was done as for reducing sugar.

Calculation:

The volume of ferricyanide reduced (ml) calculated and subtracted from this value, the ml ferricyanide reduced in procedure for reducing sugars above expressed the difference as sucrose per 10 g from the table.

APPENDIX - I

ESTIMATION OF AMYLASE INHIBITOR

REAGENTS:

Hydrochloric acid - 1 N

Sodium Hydroxide - 1 N

Phosphate buffer - 1 M, pH 6.8

Soluble starch solution in buffer - 1 o/o

3,5-Dinitrosalicylic acid reagent

standard maltose solution

Enzyme solution(0,1 o/o extract of potato in buffer)

METHOD:

Extracts were prepared by suspending 20 g of finely ground sample in 100 ml distilled water. After magnetic stirring for two hours at room temperature the suspensions were centrifuged and the clear supernatant solutions adjusted to pH 4 with 1 N HCl and heated at 60°C for 30 minutes in order to inactivate any amylase present. The solutions were clarified by centrifugation and neutralized with 1 N NaOH.

For measurement of amylase activity the method of Bernfeld (1955) was adapted as follows: Known amounts of enzyme preparation were dissolved in buffer; 0.5 ml aliquots

were incubated with 1 ml of 1 o/o solution of soluble starch prepared in the same buffer as the enzyme. After three minutes at 25°C, 2 ml of a 1 o/o 3,5-dinitrosalicylic acid solution was quickly added. The mixture was heated for five minutes in a boiling water bath. After cooling, the optical absorbance at 556 nm was measured, with maltose solution serving as the standard. One enzyme unit is defined as the amount of enzyme liberating 1 mg of maltose under the above conditions. A blank was prepared in each case by first adding 3,5-dintrosalicylic acid reagent to the starch solution and then the enzyme. In the inhibition experiment the amounts of amylase corresponding to 1 enzyme unit was employed.

Amylase inhibition was determined by preincubating 0.5 ml of purified sample extract with 0.5 ml of the enzyme solution for 8 minutes at 25°C, previous to the assay of hydrolytic activity. The results were compared with the activity of the same amount of enzyme diluted with 0.5 ml of water. A blank with the corresponding sample extract heated in a boiling water bath for eight minutes was seen simultaneously. This treatment abolished the inhibitor activity of pancreatic amylase.

One inhibitor unit is defined as the activity that decreases the liberation of the reducing sugars from starch

by one unit of pancreatic amylase to 50 o/o of the amo... produced in the controlled experiment without inhibitor. For comparing inhibition of different amylase, the amount sol-uble sample inhibition of different amylase, the amounts of soluble proteins causing a 50 per cent reduction of enzyme activity were calculated.

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