CHAPTER - VII

INCREASED PRODUCTION OF FREE DIOSGENIN DUE TO GLYCOSIDASE LIKE ACTIVITY IN THE YAM OF *Dioscorea prazeri* Prain & Burkill. SUBJECTED TO HIGH TEMPERATURE TREATMENT

Introduction

The steroid hormone industry has been fast developing during past few years in India. The raw material used by the industry in India is mainly diosgenin. Diosgenin is generally present in most of the *Dioscorea* sp. in two forms, free diosgenin and glycosides of diosgenin or saponin. (Hardman and Fazli 1972). An easy and rapid colorimetric method has already been established and represented in connection with the quantitative estimation of free diosgenin in the yam of *Dioscorea.* The conventional method of isolation of diosgenin, now-a-days in generally done by acid hyrolysis of raw material. But during acid hydrolysis some amount of diosgenin is converted to diene form which is generally regarded as waste not being used is phytochemical industry. Maximum of thirty percent diene form of diosgenin has been observed to be produced during acid hydrolysis.

From preliminary observation it has been observed that during high temperature treatment of yam of *D. prazeri*, a considerable amount of free diosgenin has been observed to be increased.

It is the purpose of this part of work is to isolate enzyme fraction from the yam of *D. prazeri* subjected to high temperature to understand the nature of activity of spaonin to diosgenin.

Materials and methods

Material : Fresh yam of *D. prazeri.*

Methods

Collection and preparation of yam

Fresh yams were collected and were cleaned with water to remove all the soil particles after clipping the roots. They were then cut into small pieces using a chopping knife.

Treatment of yam with high temperature

Small pieces of yams were subjected to 45°C in a hot air oven for 50 mins. After the treatment the pieces of yams were kept at room temperature for 2 hours. Yams were dried in an oven at 100°C for 6 hours. They were ground to powder.

Isolation of free and bound form of diosgenin from powdered yam of *Dioscorea* :

The powdered sample of yam was refluxed with chloroform for 15-20 minutes and filtered. The filtrate contained free diosgenin. The chlorogorm part was concentrated to get free diosgenin.

The residue obtained after filtration of chloroform was hydrolysed with 11.3 (N) HCI for 3 hours. The slurry obtained offer hydrolysis was allowed to attain room temperature and filtered using vacuum in a Buchner funnel. The residue was frequently washed with distilled water till the filtrate was free from acid as indicated by the use of litmus paper. Thefiltered residue was transferred to a petridish and in an oven at 100^{0}C for 6 hours. It was extracted with petroleum ether (b.p. 40-60^{0}C). The extracted solvent was concentrated to a small volume, chilled in ice for sometime and filtered to obtain diosgenin that was present in bound form (Bammi and Randhawa, 1975).

Purification of diosgenin by TLC

Purification of diosgenin has been performed by thin layer chromatography taking silica genl G as adsorbent and chloroform acetone (3:1) as solvent mixture, the details of which has been represented in the chapter four.

Quantitative estimation of diosgenin

The quantitative estiation of free and bound form of diosgenin have been performed following the methodology involving colorimeter and reaction mixture of acetic acid resorcivol and conc. H_2SO_4. The details of the procedure has been represented in the chapter four.

Isolation of saponin from the yam of *D. prazeri*

500 gm of freshly cut yam of *D. prazeri* was crushed in an electrically operated mixture to form a paste that was mixed with 250 ml of water and boiled at 100°C for an hour under reflux condition. It was filtered to obtain water extract of the yam. The extract was concentrated and evaporated more or less to dryness. The solid mass left was treated with methanol and refluxed for 30 min. It was filtered and the methanol extract was concentrated to small volume, chloroform was added dropwise to prodce turbidity. It was kept overnight to obtain crystals in globular form. It was re-crystallised from methanol Chloroform mixture and the solid mass was collected as crude saponin.

Preparation of stock solution of saponin

Saponin was dissolved in distilled water after warming for a short lime till it was dissolved and 1000 ppm of saponin solution was produced.

Preparation of buffer solution :

Acetate buffer was prepared after adding 83 ml of 0.2 M acetic acid and was made upto 100 ml with 0.2 M sodium acetate solution to maintain pH at 4.0.

Extraction of enzyme from the yam

100 gm of high temperature treated yam, kept at room temperature for one hour was taken in a chilled mortar pastle containing chilled buffer solution (100 ml) and macerated thoroughly. The concentrated mass was then centrifused in cold and supernatent was decanted off, strained through four layers of cheese cloth and the homoginate was taken for further purification through dialysis.

Activity of enzyme of saponin to yield diosgenin

5 ml of saponin solution was taken in a number of conical flask and 2.5 ml of enzyme extract was added to each of the conical flask and kept in incubator at 35±1°C. Every ten minutes after, a flask was taken out and enzyme activity was stopped after direct heatng. It was concentrated under reduced pressure and spotted on the glass plate with a layer of silica gel. G. in the form of streak, developed in the solvent system of chloroform. Acetone (3:1) along with the authentic diosgenin. The developed chromatogram having diosgenin (Rf 0.54) and Saponin (Rf 0.00) was air dried. The zone of diosgenin, detected under UV light was scraped off and treated with glacial acetic acid, warmed for 2-3 minutes and centrifuged. The final volume was made upto 1 ml and diosgenin was estimated taking the reaction mixture of resorcinol,acetic acid and conc. H_2SO_4 following the procedure represented in the chapter four.

Table - 32: Production of free diosgenin in heat treated and untreated yam of *D. prazeri*.

Treatment	Total weight yam (g)	Yield of free diosgenin (g)	Percentage on dry weight basis
Heat treatment	100	1.20	1.20
Untreated	100	0.60	0.60

Results and Discussion

The table 32 shows the yield of diosgenin in heat treated and untreated yam of *D. prazeri*. The yield of free diosgenin in the yam untreated has been observed to become 0.60% on dry weight basis but increase of free diosgenin content (1.20%) has been observed in the yam when it was subjected to high temperature (45°C) for the period of 50 minutes.

Elujoba et al (1993) observed high yield of monohydroxy sapogenin in the seed of *Trigonella foenumgraecum* using a temperature of 45°C. Bhusari et al (1982) also observed an increase in diosgenin content during heat treatment of

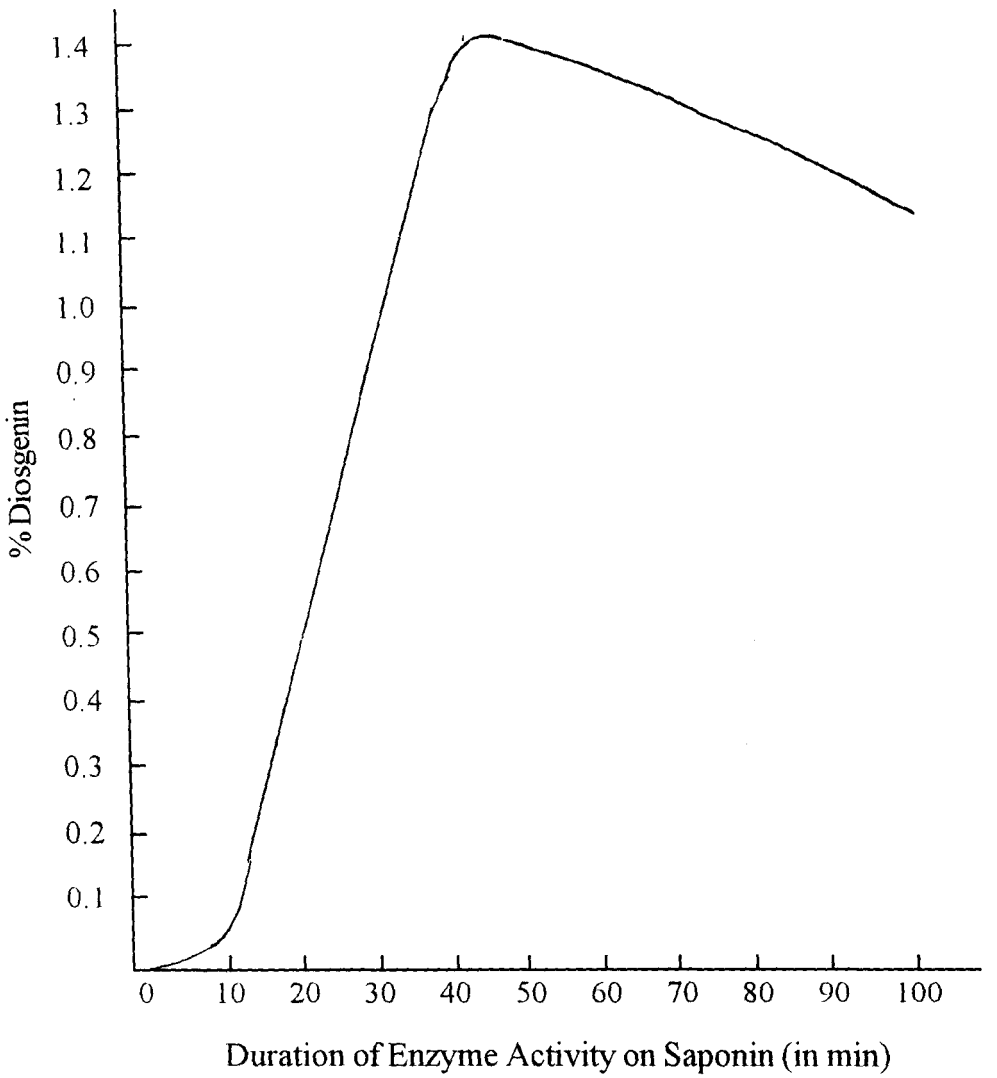

Fig. 45 : Acitivity of crude enzyme isolated from yam of *D. prazeri,* subjected to high temperature (45^{0}C) on saponin to yield diosgenin

seed of the plant. All these observations suggest the involvement of temperature effect on the activation of certain enzymes which may accelerate diosgenin production in the plant material. The result of the present investigation also supports the involvement of temperature in connection with the accumulation of diosgenin content. That the enzyme is a glycosidase and acts on saponin, is evident from the observation represented in the Fig.45. It has been observed that along with the increase of duration of enzyme activity, the free diosgenin has been observed to increase. Yield of free diosgenin becomes maximym of 1.40 percent on dry weight basis upto the limit of 50 minutes of enzyme activity after that the yield becomes low.

In the study on the chemical basis of temperature response in plants Ketellaper and Bonner (1961) observed cellular injury caused by unfavourable temperature and which might affect specific biochemical events related to diosgenin yield. Although this citations does not pin point any particular aspect of metabolism, it is equally possible that the activity of glycosidase becomes activated to supply the respiratory substrate obtained from glycoside during enzyme mediated hydrolysis. The released carbohydrate may in turn can be used as energy source by the plant material to cope with the adverse situation of accelerated aging of tusue caused by high temperature.

It has been pointed out earlier (Fig. 6, Chapter I) that during acid hydrolysis of saponin a considerable amount of diosgenin is lost due to its diene formation (Harborne, 1973). But no such loss of diosgenin occur during enzyme hydrolysis and which has been varified with the help of Thinlayer Chromatography. Thus there is a possibility of utilisation of enzyme hydrolysis for the production of diosgenin on industrial scale. This will result higher production. Besides it is expected that enzymatic process involve much less financial expenditure as compared to that involved during chemical process because the latter one always involve energy as well as huge chemical consumption.

Further research is very necessary to study enzyme kinetics and other problems related to the commercial production of diosgenin involving endogenous enzymes in the yam of *Dioscorea* sp.

SUMMARY

Small pieces of yams of *Dioscorea prazeri* has been subjected to high temperature of 45°C in a hot air oven for 50 minutes and kept at room temperature for 2 hours.

High temperature treated yam has been crushed to make powder from which free diosgenin has been extracted wtih chloroform.

Free diosgenin has been purified by TLC and estimated quantitavely by colorimetric method taking reaction mixture of acetic acid, resorcinol and conc. H_2SO_4 and measured at 510nm.

Heat treated yam showed higher amount of 1.20 percent as compared to that 0.60 percent in the untreated yam on dry weight basis.

It has been expected that the higher percent of free diosgenin in the yam treated wtih high temperature is due to enzyme hydrolysis of glycoside of diosgenin.

The glycoside of diosgenin has been identified to be dioscin after comparing mp. chromatogaphic behaviour and IR spectrum of isolated product with those of authentic sample of dioscin.

Enzyme has been extracted from yam treated with high temperature with the help of acetate buffer of acetic acid and sodium acetate maintained at pH 4.0.

Activity of crude enzyme on dioscin, the saponin during the period of 100 minutes to yield free diosgenin has been studied. Enzyme activity for 50 min has been observed to be optimum for yielding maximum percentage of free diosgenin.

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