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Research Article

IMPACT OF COOKING ON CHARANTIN ESTIMATED FROM BITTER MELON FRUITS USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT
In this study a validated high performance thin layer chromatographic method was developed for estimating charantin from *Momordica charantia*. Silica gel precoated plates was used as stationary phase and chloroform: methanol: water (1.5:6:2.5%$^{v}/_{v}$) as the mobile phase was developed in a twin trough glass chamber at room temperature. The R_f value was found to be 0.40 at a linearity range of 100-500ng/spot and with a correlation co-efficient 0.9990.The method was validated for accuracy, precision, detection and quantification limits. The limit of detection and quantification was 20ng/spot and 50ng/spot respectively. Bitter melon fruits were cooked using conventional cooking and microwave cooking and the effect of different cooking methods on bitter melon fruits was studied. It was found that charantin content reduction was more in uncovered cooking when compared to other methods of cooking.
Keywords: *Momordica Charantia*, Bitter melon, charantin, microwave, uncovered cooking, high performance thin layer chromatography

INTRODUCTION

Diabetes is a metabolic disorder of carbohydrate, fat and protein metabolism, which is considered as one of the major health problems in the world today. In the year 2000 about 171 million people had diabetes and it is estimated that it will increase up to 215 million in the year 2015 and in 2030 it may increase up to 366 million[1]. Bitter melon (*Momordica charantia*) is a common plant known for its medicinal properties such as anti-inflammatory, antidiabetic, antimicrobial and antitumor[2-4]. It is a valuable food of the gourd family (Cucurbitaceae) which is consumed by many diabetic patients. The active principles of bitter melon are charantin, polypeptide-p, vaccine, momordicine and momordin[5-6]. Apart from this it contains vitamins, folic acid, carotenoids and amino acids. Bitter melon plant is a good source of vitamin C. Charantin is found to have hypoglycemic capacity which is a mixture of two compounds sitosteryl glucoside and stigmasteryl glucoside[7] (Fig:1). Extraction of this compound was done using Soxhlet apparatus and the crude extract was purified[8].Food quality always depends on the production and how it is cooked. The bioavailability of the plant cell wall by disruption and releasing protein complexes can be increased by cooking. Cooking can influence all the parameters like vitamins, amino acids, and charantin etc, both in a positive and negative way.

To date there are clinical studies to determine the efficacy of bitter melon on diabetic patients, but there was no studies to estimate the impact of cooking on bitter melon fruits. Thus, the aim of the study was to find the impact of different domestic cooking methods on unripe bitter melon fruits.

MATERIALS AND METHODS

Plant Materials

Unripe fruits of *Momordica charantia* were collected from the local market of Andhra Pradesh and the seeds were removed and dried.Charantin was extracted and purified from the unripe bitter melon fruits and used for the further studies.

Chemicals

Methanol, Chloroform, Hexane and Ethanol were purchased from Qualigens fine chemicals (Mumbai). HPTLC silica gel 60 F_{254} plates (20 × 20 cm) were obtained from Merck. (Germany).

SOXHLET EXTRACTION[8]

Bitter melon fruit was dried, powdered (1g) and extracted with 200ml of ethanol for 150mins. Charantin remained in the residue sample was extracted repeatedly in 30ml of methanol using ultrasonification. The extract was filtered and evaporated to obtain viscous crude extract and purified.

SAMPLE PURIFICATION[9]

5ml of 50:50% $^{v}/_{v}$ methanol: water was added to the crude extract of charantin, mixture was sonicated for 15 minutes and centrifuged at 3500rpm for 15 minutes to separate the supernatant from the precipitate. To the precipitate add 5ml of 70:30% $^{v}/_{v}$ methanol: water and the mixture were again sonicated and centrifuged. To the precipitate obtained 3ml hexane was added and the above steps were repeated. The precipitate was re dissolved in 200µl of 1:1 $^{v}/_{v}$ chloroform: methanol mixture and then adjusted the volume with methanol and filtered using 0.45µm membrane filter paper.

PREPARATION OF STANDARD SOLUTIONS

Charantin (10 mg) was weighed accurately and diluted with methanol to 10 ml (stock solution, 1 mg/ml). Different dilutions of 100-500ng/spot were prepared using stock solution.

CHROMATOGRAPHIC PROCEDURE

Densitometric evaluation of the spots was carried out using a CAMAG TLC scanner with Linomat-IV semiautomatic sample applicator at an absorbance wavelength of 536nm. The TLC scanner was combined with Wincats Ats version 1.2.6 software for interpretation of data. TLC plates were pre-washed with methanol, dried with nitrogen gas. Samples and standard solutions (10 µl) were applied as 6mm bandwidth and distance between the bands were 6mm using Camag Linomat IV automatic sample applicator. Chloroform: methanol: water (1.5: 6: 2.5) was used as the mobile phase. The plates were developed in a saturated (15 minutes) twin trough developing chamber at room temperature. The development distance was 85mm and the development time was about 20mins.

CALIBRATION CURVE FOR CHARANTIN

10µl of each concentration of standard solution was applied on the TLC plates from working standard solution for a concentration range of 100-500ng/spot. The plates were dried, developed and analysed.

COOKING METHODS

Unripe bitter melon fruits (5gm) were cleaned washed; seeds were removed and cut into pieces. It was boiled in covered and uncovered stainless steel vessel for 6 and 8 minutes respectively. Blanching in a stainless steel vessel was done for 2 minutes. Microwave cooking was done in a covered microwave container for 3 minutes, without adding water and steaming was done in a covered stainless steel vessel for 8minutes. The samples were cooled and all cooking methods were repeated for five times. An initial and final weight change was done to calculate the mass changes during cooking.

Unripe bitter melon fruits were cooked, extracted and the charantin content was estimated using high performance thin layer chromatography. The impact of different cooking methods is summarized (Table-1).

VALIDATION PROCEDURE

The method was validated as per ICH guidelines in terms of linearity, accuracy, LOD, LOQ, Intra- day precision, Inter-day precision and repeatability of sample application.

Linearity

The linearity of the method was evaluated at a concentration range of 100, 200, 300, 400 and 500ng/spot (Fig:2-6). The solutions were applied on the plate; dried and developed using the above-mentioned mobile phase and analysed. The linear regression data showed a good linear relationship over a concentration range of 100-500ng/spot with a correlation co-efficient 0.9990 (Fig:7).

Accuracy

The accuracy of the method was determined by doing the recovery studies at three levels (in triplicates) after 50%, 100% and 150% drug was added to the standard solutions. The contents were analysed and the % recovery was calculated.

Limit of detection and Limit of quantification

Limit of detection (LOD) was calculated using 3.3σ/s in which "σ" is standard deviation of blank response of the intercept standard deviation and "s" is the slope of calibration curve. The lowest concentration at which the peak was detected was found to be 20ng/spot (Fig:8). Limit of quantification (LOQ) was calculated using 10σ/s expression. The lowest concentration at which the peak was quantified was found to be 50ng/spot (Fig:9).

Precision

Intra-day precision was determined by analyzing the sample solutions of 200ng/spot and 400ng/spot for three times on the same day. Each concentration was applied in triplicates and %RSD was calculated.

Inter-day precision was determined through analyzing the sample solution of 200ng/spot and 400 ng/spot for two consecutive days in triplicates and %RSD was calculated.

The repeatability of sample application was assessed by spotting 200ng/spot and 400ng/spot of standard solution for six times on a plate followed by development of the plate and recording the peak area for the spots.

RESULTS

Unripe bitter melon fruits were extracted using soxhlet extraction and the crude extract obtained was purified to obtain pure charatin. Different mobile phases were tried for which, symmetrical and reproducible peaks was achieved for chloroform: methanol: water at a ratio of 1.5:6:2.5%v/v/v. The R_f value of charantin was found to be 0.40±0.03 at a wavelength of 536nm, and there was no overlapping with other components. The calibration curves were linear at a range of 100-500ng/ spot. Validation of the method was done as per the guidelines (Table-2).

Charantin content in raw and cooked bitter melon was analyzed using high performance thin layer chromatography. Its impact on different cooking methods like boiling, blanching, microwave and steaming was studied. All cooking methods lead to small changes in the charantin content present in bitter melon fruits. The result shows that uncovered and covered boiling leads to high loss of charantin content, while the other methods may cause only small losses. The amount of charantin content observed in case of unripe bitter melon was taken as 100%. In uncovered cooking the content reduced up to 48% and in case of microwave cooked fruits it reduced only to 92%.(Fig-10)

DISCUSSION

The loss of charantin content was less in microwave cooking and steaming when compared to other methods of cooking. The proposed method has been shown to be highly suitable for the analysis of charantin from *Momordica charantia* with respect to the validated parameters: linearity, accuracy, precision and repeatability. The given results let us assume that boiling leads to more loss of charantin content, while through other methods only a small amount of content is lost.

ACKNOWLEDGEMENTS

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Table-1 Analysis of charantin after various cooking modes

Cooking methods	Cooking time(min)	Concentration of charantin (ng/spot)	% charantin
Unripe bitter melon		410.15	100
Boiling, covered	6min	267.81	65.29
Boiling, uncovered	8min	196.52	47.91
Blanching	2min	362.17	88.30
Microwave	3min	376.34	91.75
Steaming	8min	355.24	86.61

Table-2 Summary of Validation Parameters

Parameters	Values
Linearity range **Correlation coefficient(r)**	100-500 ng/spot 0.9990
LOD	20 ng/spot
LOQ **Accuracy**(n = 6) **Precision** i) Intra-day(n = 3) ii) Inter-day(n = 3) iii)Repeatability of sample application(n = 6) iv) Repeatability of measurement(n = 6)	50 ng/spot 99.43±0.42% % RSD 0.65 0.66 0.98 0.59
Stability Studies	3hrs

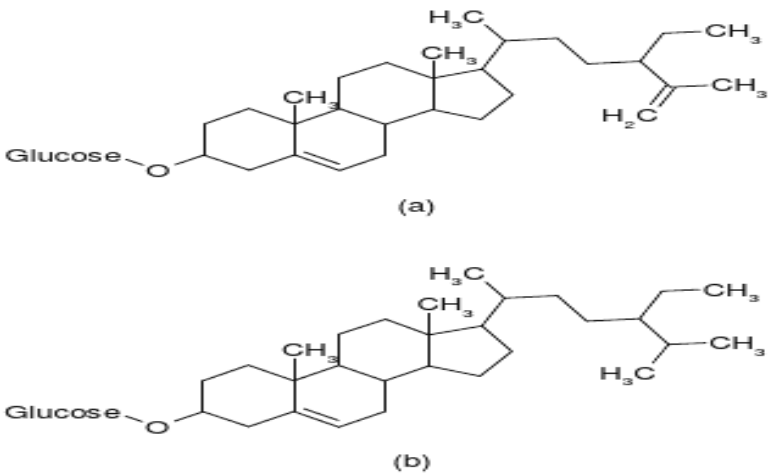

Fig-1 Charantin-(a)sitosteryl glucoside (b)stigmasteryl glucoside

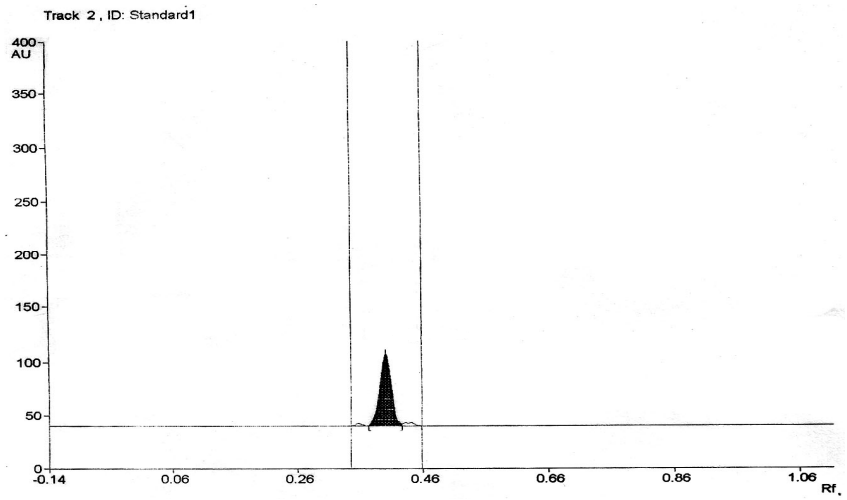

Fig-2 Chromatogram of charantin (100 ng/spot)

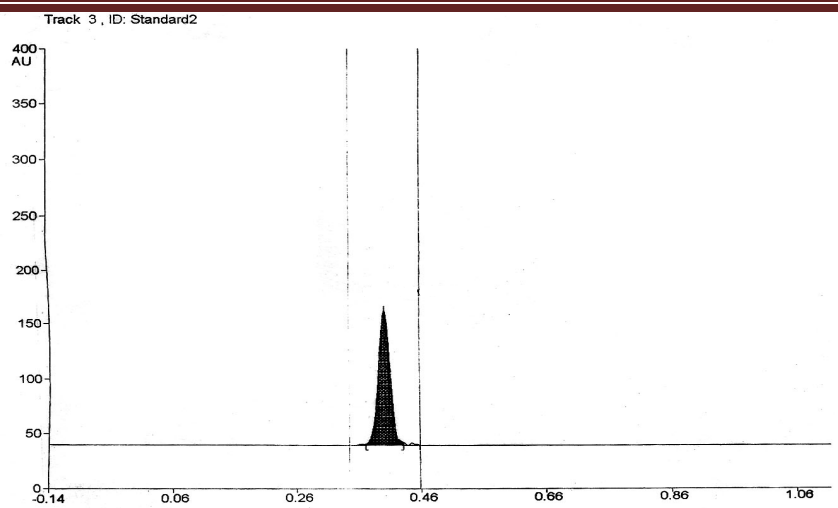

Fig -3 Chromatogram of charantin (200 ng/spot)

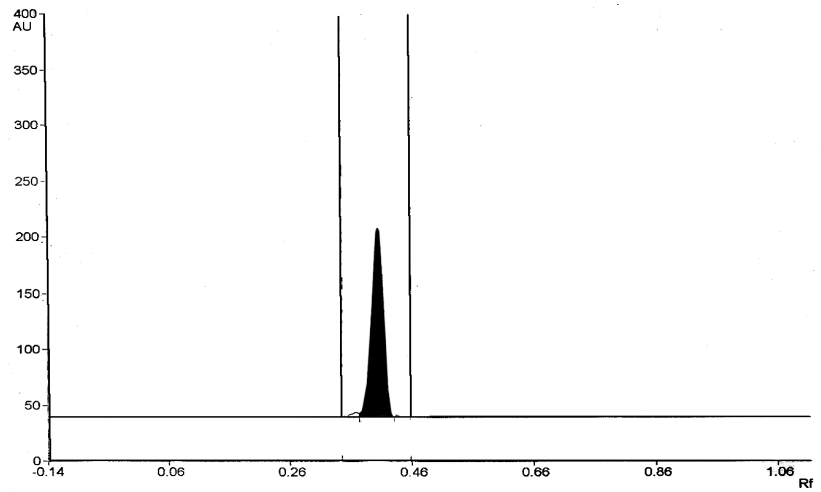

Fig -4 Chromatogram of charantin (300 ng/spot)

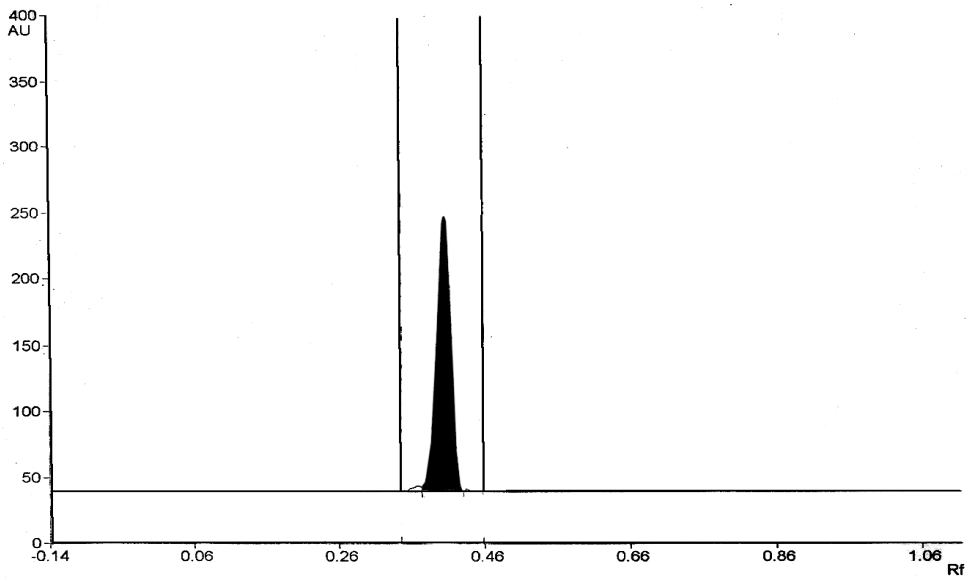

Fig -5 Chromatogram of Charantin (400 ng/spot)

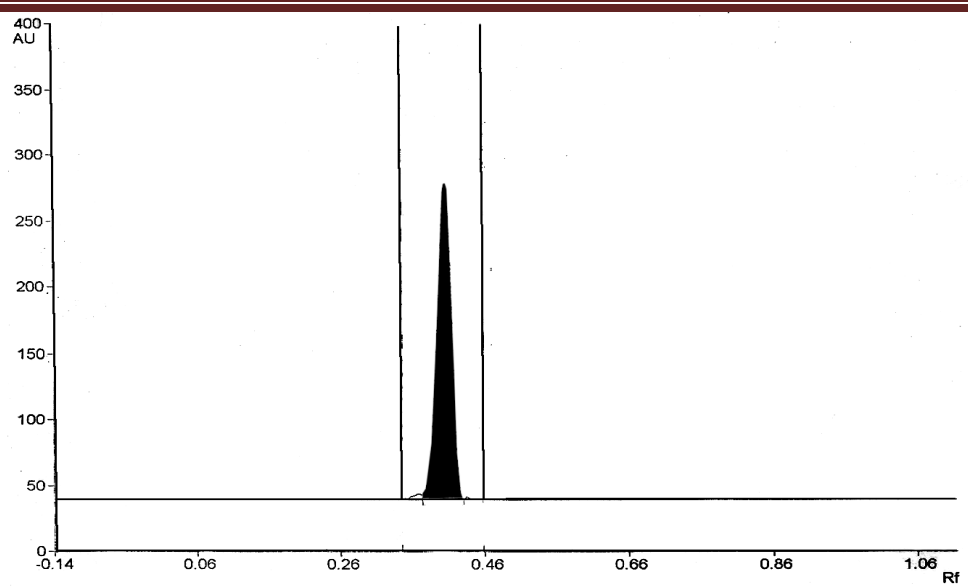

Fig-6 Chromatogram of charantin (500 ng/spot)

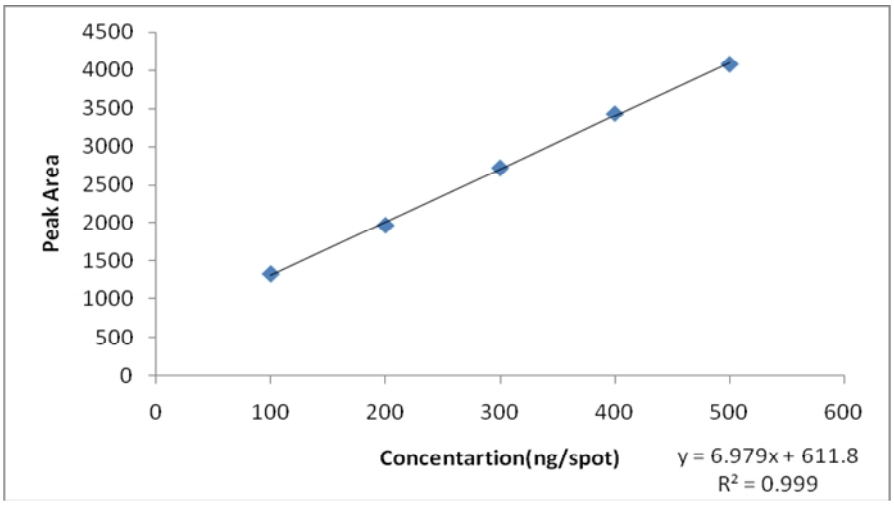

Fig-7 Linearity graph of charantin

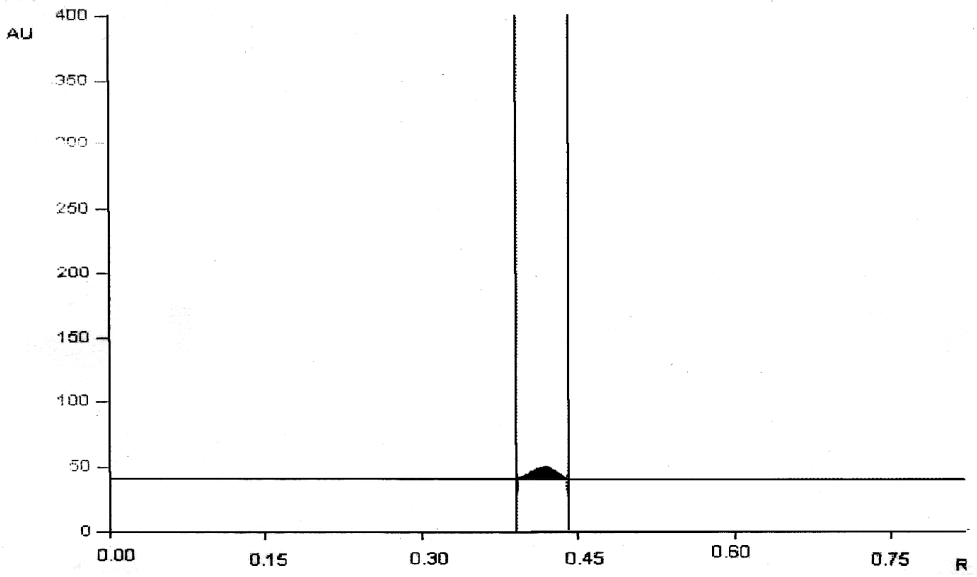

Fig-8 Limit of Detection (LOD) (20ng/spot)

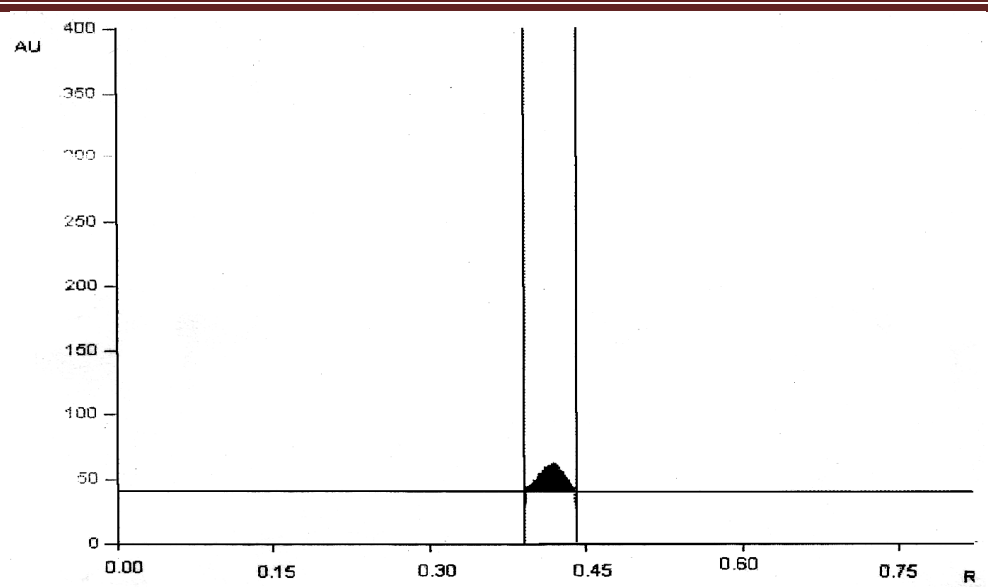

Fig-9 Limit of Quantification (LOQ) (50ng/spot)

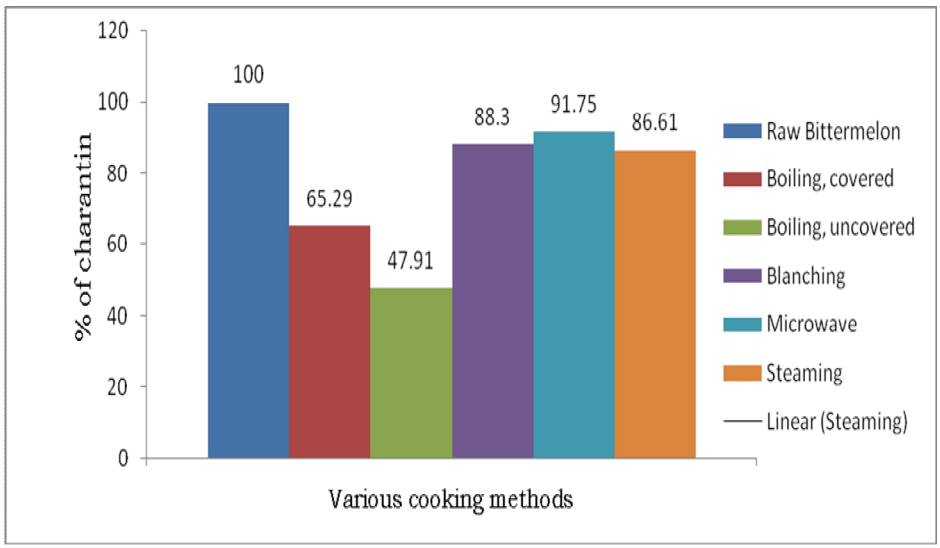

Fig -10 Percentage of charantin after various cooking modes

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