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# DECLARATION

The work described in this thesis was carried out by me, under the supervision of Prof E. R. Jansz (Department of Biochemistry, Faculty of Medical Science, University of Sri Jayawardenapura), Dr. H. Peiris (Head Dept. of Biochemistry, Faculty of Medical Science, University of Sri Jayawardenapura) and Prof. A. Bamunuarachchi (Dept. of Chemistry, University of Sri Jayawardenapura) and a report on this has not been submitted to any University for another degree.

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## Declaration of the Supervisors

" We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation."

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# STUDIES ON SOME SRI LANKAN FOODS IN RELATION TO DIETARY FIBRE, DIGESTIBLE CARBOHYDRATE AND HISTAMINE REDUCING CAPACITY

By,

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Thesis submitted to the University of Sri Jayawardenapura for the award of the Degree of Master of Philosophy in Biochemisty

## CONTENTS

## LIST OF TABLES.

## LIST OF FIGURES.

## ABBREVIATIONS

| | | |
|---|---|---|
| DC | - | Digestible Carbohydrate |
| DF | - | Dietary Fibre |
| TF | - | Total Fibre |
| IDF | - | Insoluble Dietary Fibre |
| SDF | - | Soluble Dietary Fibre |
| **NDR** | - | Neutral Detergent Residues |
| **ADR** | - | Acid Detergent Residues |
| **RRR** | - | Raw Red Rice |
| **RWR** | - | Raw White Rice |
| **PRR** | - | Parboiled Red Rice |
| **PWR** | - | Parboiled White Rice |
| **WWF** | - | Whole Wheat Flour |
| **RWF** | - | Refined Wheat Flour |
| **DNS** | - | Di- Nitro Salicylic acid |
| **TLC** | - | Thin Layer Chromatography |
| **HPLC** | - | High Pressure Liquid Chromatography |

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# ABSTRACT

The study comprises three parts: (i) assessment of digestible carbohydrate, (ii) determination of soluble and insoluble dietary fibre of 60 Sri Lankan plant foodstuffs and (iii) the histamine lowering capacity of selected food ingredients.

In Sri Lankan food composition tables the values given are for dietary carbohydrate and not digestible carbohydrate. Dietary carbohydrate includes digestible carbohydrate, non digestible carbohydrate components *e.g.* fibre and even non carbohydrate materials. A further complicating problem is that carbohydrate by difference multiplied by the Atwater factor (4) is used in the calculation of energy content of a food. This would overestimate the energy content of food and mislead dieticians and the general public.

In this study digestible carbohydrate of 60 foodstuffs were determined in an attempt to improve the food composition tables of Sri Lanka. Enzymatic hydrolysis was used to assess digestible carbohydrate as this would more closely resemble in-vivo digestion conditions.

In the case of leafy vegetables, calculating carbohydrate content by difference results in an overestimation of energy of carbohydrate by 5-12 fold . However, in the case of cereals and starchy tubers there was less overestimation. (< 1.2 fold)

The estimation of the unavailable portion of the dietary carbohydrate namely dietary fibre (DF) is as important as the estimation of the available portion. DF is important in being protective against a range of diseases such as coronary heart disease, diabetics, stroke, and large bowel diseases.

However in local food composition tables there is no column for DF. In Indian food composition tables only the crude fibre levels of foodstuffs are given. It is felt that the data on soluble and insoluble dietary fibre would be useful to

nutritionists and clinicians. Therefore there is a case for determining separately more significant data on soluble and insoluble dietary fibre of Sri Lankan plant foodstuffs. From the result obtained it was evident that both soluble and insoluble dietary fibre content of foods are high. In case of leafy vegetables the DF contributed up to 60% of dry weight. Fruits, vegetables and non-starchy tubers also make a significant contribution of dietary fibre to food.

Histamine is an allergen. Certain sea foods are well known for their high histamine content and could cause allergic reactions. Previous studies reported that existing histamine of skipjack fish (*Katsuwonus pelamis*), 200ppm was lowered up to 90% by not only acidic food ingredients such as goraka (*Garcinia cambogia*), tamarind (*Tamarindus indica*), and bilin (*Avverhoae bilimbi*), but also by organic acids. It had been concluded that it was the organic food acids such as tartaric, acetic and lactic which cause the lowering of histamine in fish.

From the present studies it was concluded that at pH–3 histamine forms a complex which is insoluble in methanol, (methanol had been used as an aid in the analytical technique of previous workers) and apparent lowering of already formed histamine in presence of methanol was an artifact.

As histidine is the precursor of histamine it became necessary to study the reaction of histidine to histamine. Therefore, a good analytical technique to separate and quantify both histidine and histamine was required. A TLC method using silica $G_{60}$ and acetone : $NH_3$ (80:22.5) as solvent was developed.

Histidine decarboxylase activity of *Lactobacillus sp.* was reduced by goraka, tamarind and bilin due to the reduction of pH from its optimum. The enzyme activity was maximum between 4.4 - 4.8. Histamine formation at pH 3.2 was >15% of the histamine formed at pH 4.4. Addition of tamarind and bilin resulted in pH values of 4.4 and 4.9 respectively, while goraka reduced the pH of fish

suspensions even in the presence of coconut milk to 3.4. This decrease of pH was the cause of lowering of histamine formation.

Incubation of skipjack fish suspensions (0.5 g $ml^{-1}$) at room temperature resulted in the formation of histamine ($R_f = 0.84$) and two other spots of $R_f$ 0.79 and 0.72. These were suspected to be two other amines. Reduction in histidine levels did not correspond to increase in histamine level suggesting that histamine is not the only product of histidine in fish incubates.

Using surface sterilized skipjack fish indicated that microorganisms were the causative agents for decarboxylation of histidine. It was further observed that the other decarboxylation reactions of fish incubates also could be prevented in the presence of goraka extract. Addition of tamarind and bilin to fish and incubation at room temperature did not inhibit the formation of histamine or other diamines.

Histamine formation in fish depends upon many factors *e.g.* histidine content of fish, microflora of fish and pH of fish suspension . In the case of seer, the low histidine levels were found to be the cause of low histamine formation. The addition of exogenous histidine resulted in significant histamine formation. The study also indicates that histamine may not be the only amine in fish for *e.g.* the so called heaty fish, causing allergic reactions. Hurulla (*Amblygaster sirm*) gave no histamine spot but gave the other diamine spot.

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