DOI : 10.31357/fapsmph.2004.00611

STUDIES ON THE PECTIC POLYSACCARIDES OF WATTAKKA CULTIVAR (*Cucurbita spp.*) AND SOME APPLICATIONS IN FOOD FORMULATIONS

By

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Thesis submitted to the University of Sri Jayewardenepura for the award of the degree Master of Philosophy in Chemistry on Studies on the pectic polysaccharides of Wattakka Cultivar (*Cucurbita spp.*) and some applications in food formulations.

May 2004

The work described in this thesis was carried out by me under the supervision of Dr. Jaanaki Gooneratne and Prof. A.M. Abeysekera and a report on this thesis has not been submitted to any University for another degree.

.....

H. M. Theja Herath

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

Dr. Jaanaki Gooneratne

Prof. A.M. Abeysekera

Dedicated

To

My loving parents, husband, son Hansaka

and

daughter Amanda.

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ABBREVIATIONS

η	Viscosity of solution
η_I	Intrinsic Viscosity
η_s	Viscosity of solvent
η_{sp}	Specific Viscosity
AEC	Anion Exchange Chromatography
AIR	Alcohol Insoluble Residue
Ara	Arabinose
CDTA	Cyclo-Hexane-trans –1-2-diamine –NNN'N' tetra acetate
DF	Dietary Fibre
DMSO	Di- Methyl Sulphur Oxide
EC	Emulsifying Capacity
EDTA	Ethylene Diamine Tetra Acetate
ES	Emulsifying Stability
F/C Yogurt	Full Cream Yogurt
FAU	Formazine Attenuation Unit
Gal	Galactose
GC	Gas Chromatograph
Glu	Glucose
HM	High Methoxyl
Kcal	kilo calorie
KPa	Kilo pascal

LM	Low Methoxyl
Man	Mannose
N	Newton
N/F Yogurt	Non Fat Yogurt
NSP	Non Starch Polysaccharides
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SR	Stabilizing Rate
UA	Uronic acid
Xyl	Xylose

ACKNOWLEDGEMENT

I wish to express my most sincere appreciation and gratitude to my supervisor, Dr Jaanaki Gooneratne, Senior Research Officer, Industrial Technology Institute, for the generous advice and guidance given, in conducting this investigation. Her unhesitant help and co-operation at all times has contributed immensely to make this study a success. It is my bounden duty to thank her most sincerely.

I especially, wish to express my deepest sense of gratitude to my supervisor, Prof. A. M. Abeysekera, Dean, Faculty of Applied Sciences, Professor of Chemistry, University of Sri Jayewardenepura, for his valuable advice, kind guidance and professional expertise throughout my study.

My deep sense of gratitude is expressed to Dr. Nanadani Ediriweera, Head, Agro and Food Technology Division, and Dr. A. M. Mubarak, Director, Industrial Technology Institute, for granting me the opportunity to carry out this study.

I extend my gratitude to National Science Foundation (NSF) for providing financial assistance to carryout my work successfully.

I wish to acknowledge the help of the staff, Dept. of Taxonomy, Plant Genetic Resources Centre (PGRC), Gannoruwa, for identification of cultivars and their assistance.

I wish to thank my colleagues for their kind help in various ways and their friendly co-operation. Especially Ms. Siryani Kumari for her kind support given during my laboratory work.

I extend my thanks to Ms. Chamari Hettiarachchi and Mr. M.Yoheshwaran for their fullest support during finalizing of the thesis. I extend my thanks to Mr. T. Wimalasena, Laboratory Assistant for his kind help in various ways.

I wish to thank Mr. K .S. Wijenayake, Technical Officer, Dept of Zoology, University of Sri Jayewardenepura, for photographing of raw materials and Mr. Rangoda, Technical Officer for his kind help in many ways.

Last but not the least, I am extremely grateful to my husband Gamini and son, Hansaka and daughter, Amanda for their patience and support during the progress of this work

Studies on the pectic polysaccharides of Wattakka cultivar (*Curcubita spp.*) and its applications in food formulations.

H.M.T Herath.

ABSTRACT

In this study, pectin from the fruits of *Cucurbita spp.*, namely, Wattakka, Meemini and Butternut cultivars, was investigated as a potential source for use in food industry. Towards this end, basic and applied research on the following work was carried out. (a) Isolation and characterization of the cell wall polysaccharides of *Cucurbita moschata*, Watakka cultivar, using non- degradative methods. The native pectin was purified and characterized to obtain its monomer sugar composition. (b) A comparison was also made on the non-starch polysaccharide compositions of the three cultivars of *Cucurbita spp.* as components of dietary fibre. (c) Extraction of pectin from the three cultivars of *Cucurbita spp.* using an industrial process and determination of chemical and physical characteristics and (d) a study of the applications of pectin from the Wattakka cultivar in some food formulations.

The pectic polysaccharides of the cell walls of Wattakka were isolated as alcohol insoluble residue using a modified method described by Ng *et al.* (1998). This alcohol insoluble residue, was sequentially extracted, under non- degradative methods, using CDTA (0.05 M, pH 6.5) at 20°-22 °C, for 6 h (CDTA-1) and 2 h (CDTA-2), respectively. The residue thus obtained was further extracted with Na_2CO_3 (0.05 M) at 8° C, for 24 h (Na_2CO_3-1) and at 20°-22 °C for 2 h (Na_2CO_3-2), respectively. The extracts of all fractions were filtered, neutralised and dialysed exhaustively and freeze-

dried. The monomer sugar compositions of the fractions were determined after 12 M H_2SO_4 hydrolysis, neutralization and derivatization to alditol acetates and quantified by GC techniques. The uronic acid content was determined calorimetrically.

The yield of the cell wall material of the edible portion of the Wattakka fruit was 29.6 %, (as alcohol insoluble material), consisting of 91 % of carbohydrates, mainly pectic polysaccharides. The total CDTA and Na_2CO_3 fractions accounted for 24.7 % and 3.7 % respectively, of the original material. The monomer sugar composition of the fractions, showed that the branching points of pectic material (as indicated by the ratio of rhamnose; uronic acid) were high in CDTA-1 (1:38) and CDTA-2 fractions (1:30), while the Na_2CO_3-1 (1:22) and Na_2CO_3-2 (1:25) fractions contained less branching points. The major neutral sugar in all fractions was galactose, which was a significant characteristic of *Cucurbita spp.*

The main fraction of the cell wall CDTA-1, was subjected to anion exchange chromatography (DEAE- Trisacryl column), more or less a homogenous individual polymer was obtained, when eluted with 0.25 M NaCl. This polymer contained 84 % of uronic acid, with a rhamnose to uronic acid ratio of 1: 44. The major neutral sugar of the polymer was galactose, with substantial amounts of arabinose and mannose.

The non-starch polysaccharides (NSP) of all three cultivars were determined as dietary fibre. Wattakka contained 11.8 g /100 g of NSP, of which 22 % was soluble fibre. The NSP of the other two cultivars was less, Meemini containing 9.1 g/100g (20 % soluble

fibre) and Butternut containing 8.9 g / 100 g (18 % soluble fibre). NSP of *Cucurbita spp.* may be of importance in terms of its physiological role in human systems.

The pectin from all three cultivars of *Cucurbita spp.* was extracted under industrial conditions (in HCl at pH 1.3 for 2h, followed by alcohol precipitation), after pre-processing using three different methods. The yields of the pectin were in the range of 7.4 to 28.8 % for all three cultivars and was found to be dependent on the method of pre-processing. These yields are in the same range of values reported for citrus (25 %) and apple (15-18 %).

The chemical characterization of the industrially extracted Watakka pectin was classified as a high methoxyl (HM) pectin, as it had methoxyl content of over 7 %. However, the Wattakka pectin showed weak gelling properties (gel grade 100) as compared to citrus pectin (gel grade 150), with a low rupture point (0.7 K Pa) and a low compression ability (18 N). The presence of higher concentrations of neutral sugars in the pectic polymer may hinder the gel forming ability. Both other cultivars, Meemini and Butternut, did not demonstrate gel forming characteristics, probably due the high acetyl content (over 2 %). Hence it could be concluded that the use of these two cultivars in food applications are limited.

Wattakka pectin had an average molecular weight of 2927 daltons and its viscosity properties increasing markedly at a pH of 4.6 at a concentration of 1% (w/v).

Wattakka pectin was applied to several food formulations and its technological characteristics were investigated as compared to that of citrus pectin.

Wattakka pectin was incorporated into a dietetic fruit juice, showed a higher viscosity values (18-30 cP) as compared to citrus pectin (12-17 cP), at concentrations of 0.15 - 0.25 % (w/v), imparting a body and texture to the product.

Wattakka pectin when added to a formulation of stirred yoghurt (non-fat), demonstrated the formation of a stabilized milk-pectin complex by showing an increase in the viscosity, ranging from 2200 to 3800 cP, at pH 4 - 4.5 and at concentrations ranging from 0.15 - 0.25 % (w/v). These stabilizing properties, however, were not demonstrated when Wattakka pectin was used in fat containing stirred yoghurt formulations.

When Wattakka pectin was used in emulsions of oil-in-pectin solutions, an emulsion stability of 100 % was obtained at a concentration of 2 % (w / v), as compared to citrus pectin which showed a 40 % stability, under similar conditions.