DEPARTMENT OF FOOD SCIENCE
FACULTY OF SCIENCE
UNIVERSITY OF COPENHAGEN

Invitation to PhD defense

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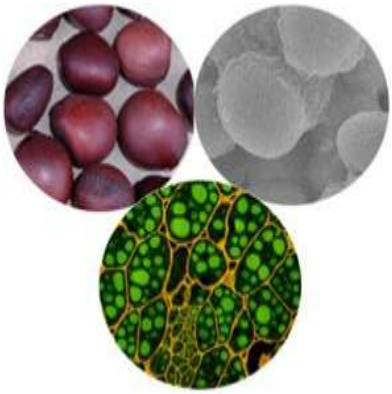
Chemical and Structural Characterisation of Marama Bean (*Tylosema esculentum*) Carbohydrates

PhD Thesis by
Minah Mmoni Mosele · 2012



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ABSTRACT

The aim of this thesis was to characterise the chemical and structural composition of carbohydrates in marama bean (*Tylosema esculentum*) at two developmental stages of immature and mature. Marama bean is a wild growing legume indigenous to the Kalahari Desert and neighbouring sandy semi-arid regions of Namibia and South Africa. Despite its potential as a nutritional food, little is known about the carbohydrate composition. Most of the results reported herein focus on mature seeds, though immature seeds were used in some of the earlier work of this project because they were unavailable for further analyses.

In this study, various methods were used to elucidate the nature of the marama bean carbohydrates. Physicochemical (proximate composition) and histochemical (microscopy) methods gave a general overview, where the carbohydrate content was calculated at 19-24% (mature seeds), constituted by cell wall polysaccharides, mainly pectin and cellulose. There seemed to be a positive relationship between carbohydrates increase and cell wall thickening, in relation to maturation, i.e. during maturation, carbohydrates increased with cell wall thickening as noticed under observation with fluorescent microscopy.

To measure the actual amounts of marama bean carbohydrates, high performance anion exchange-chromatography (HPAEC) with pulsed amperometric detection (PAD) was used. Immature and mature seeds had mannose and arabinose as the most abundant cell wall monomers, respectively, followed by galactose and glucose. The arabinose was recalcitrant to extraction with strong alkali and was highly branched. It was also characterised by arabinan-like linkages recognised by the arabinan antibody LM6 and LM13 indicating pectic arabinan. The pectin amount was 4.2% in mature seeds, with a low degree of esterification, constituted mainly by homogalacturonan. The mannose was possibly from mannosylated proteins, because the linkages observed are typical of protein mannosylation. Starch was negligible at 0.2% in both immature and mature seeds, which explains why the iodine stain test in light microscopy was negative.

Fourier transform Raman spectroscopy (FT-Raman) revealed α-anomeric carbohydrates, observed mainly as pectin and galacturonic acid by nuclear magnetic resonance (NMR). The extracted carbohydrates used for spectroscopy analyses showed residual proteins, suggesting a strong linkage of carbohydrates and proteins, already assumed as protein mannosylation. This also suggests the existence of glycoproteins in marama bean. However this assumption needs to be further investigated. Since only about 5% of the 24% total carbohydrate content of marama bean could be quantified, the rest of the carbohydrate is considered to be non-starch polysaccharides and glycoproteins.

The chosen combination of methods has gone a long way in the characterisation of marama bean carbohydrates, with some limitations. Therefore this study forms a strong basis for further research in marama bean with great lessons for the next torchbearer.