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Characterisation of the arabinose-rich carbohydrate composition of immature and mature marama beans (*Tylosema esculentum*)

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ABSTRACT

Marama bean (*Tylosema esculentum*) is an important component of the diet around the Kalahari Desert in Southern Africa where this drought resistant plant can grow. The marama bean contains roughly 1/3 proteins, 1/3 lipids and 1/3 carbohydrates, but despite its potential as dietary supplement little is known about the carbohydrate fraction. In this study the carbohydrate fraction of "immature" and "mature" marama seeds are characterised. The study shows that the marama bean contains negligible amounts of starch and soluble sugars, both far less than 1%. The cell wall is characterised by a high arabinose content and a high resistance to extraction as even a 6 M NaOH extraction was insufficient to extract considerable amounts of the arabinose. The arabinose fraction was characterised by arabinan-like linkages and recognised by the arabinan antibody LM6 and LM13 indicating that it is pectic arabinan. Two pools of pectin could be detected; a regular CDTA (1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid) or enzymatically extractable pectin fraction and a recalcitrant pectin fraction containing the majority of the arabinans, of which about 40% was unextractable using 6 M NaOH. Additionally, a high content of mannose was observed, possibly from mannosylated storage proteins.

1. Introduction

Marama bean (*Tylosema esculentum* Burchell A. Schreiber) commonly called morama bean, tsin bean or gemsbok bean is an important component of the diet in settlements around the Kalahari Desert (National Research Council, 1979). It is a desiccant-tolerant plant with an ability to grow in high temperatures and dry environments such as the Kalahari area. Raw mature seeds of marama beans store well and remain edible for years (National Research Council, 1979). Leguminous seeds are an important part of the diet of rural communities in developing countries as they provide proteins, lipids and carbohydrates (Ketshajwang et al., 1998).

The mature seeds of *T. esculentum* are encapsulated in woody pods with 1–2 seeds and the pods open at maturity. Their seed coats, which are removed before consumption, are reddish to brownish black in colour (National Research Council, 1979). Previous work of mature marama seeds (Bower et al., 1988; Amarteifio and Moholo, 1998; Holse et al., 2010; Mosele et al., 2011) has indicated that they are a rich source of proteins and lipids, both above 30%, making them comparable to soya bean and peanut. They also have a considerable content of dietary fibre (19–27%), and mineral content is similar to that of peanut and approaching that of soya bean (Holse et al., 2010). The immature seeds are rich in proteins containing around 21% (w/w) (Mosele et al., 2011). Other studies have focused on the quality of marama bean oil (Mitei et al., 2008) and characterisation of the fatty acids, phytosterols and vitamin E compounds in the oil (Mitei et al., 2009).

Despite the potential of marama bean as a healthy nutritive crop for developing countries none of its carbohydrates have been thoroughly studied. Potential use of the carbohydrate fractions in food applications could yield valuable income for rural communities gathering marama beans. Studies on the use of pressed marama oil have been initialised and the defatted press rest might be readily available for potential extraction of useful carbohydrates.

Carbohydrates, especially polysaccharides are important in the food industry because they can be used as thickeners, stabilisers, texturisers and gelling agents (Viñarta et al., 2006; Khurana and Kanawjia, 2007). A number of studies have investigated the

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composition of carbohydrates in legume seeds, with special emphasis on starch and soluble sugars. Lentils and chickpeas contain around 50% (w/w) starch and approximately 20% (w/w) dietary fibre (Aguilera et al., 2009a). In soya bean, which compares to marama bean in nutritional quality, the carbohydrate components include 5.3% oligosaccharides, 2.4–5.5% polysaccharides (crude fibre) and 0.2–0.9% starch (Reddy et al., 1984). The main soluble sugar in soya bean is sucrose, followed by stachyose (Hou et al., 2009; Saldivar et al., 2011). The total carbohydrate content (by difference) of marama bean has been reported to constitute between 18.9% and 24.1% but has never been studied in detail (Bower et al., 1988; Amarteifio and Moholo, 1998; Mosele et al., 2011).

Structure elucidation of complex polysaccharides can be challenging due to the inherent heterogeneity and due to the differences in extractability. Analysis of legume seed cell wall is no exception and high protein content in the cell wall fraction does not make its structure elucidation any easier. Holse et al. (2011) characterised the bulk carbohydrate content of intact mature marama bean using different spectroscopic techniques. However a detailed characterisation of the cell wall polysaccharides was not possible without fractionation.

Dicot cell walls are generally considered to consist of three fractions, namely pectin, hemicellulose and cellulose. Pectin can be fractionated into homogalacturonan (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II) and xylogalacturonan (XGA). Apparently, these polymers are covalently linked to each other but it has proven very difficult to obtain unambiguous information on how the different pectic polysaccharides are connected wherefore several models exist (Vincken et al., 2003). Recent reviews have tried to elucidate the relationship of these pectic polysaccharides (Caffall et al., 2009; Scheller et al., 2007). RG I is built up of a backbone containing the disaccharide (α-1→4-GalA-α-1→2-Rha) as the basic repeating unit. The rhamnosyl residues can be substituted with galactan, arabinan or arabinogalactan side chains. The galacturonic acid (GalA) residues can furthermore be acetylated as in homogalacturonan. In some species the arabinose and galactose residues in RG I side chains can be substituted with ferulic and coumaric acid esters (Fry, 1982).

The aim of this study is to characterise the carbohydrate composition of marama bean seeds at the two developmental stages: immature and mature, corresponding to the stages utilised for consumption.

2. Results and discussion

2.1. Starch

Marama bean is virtually devoid of starch in both immature and mature seeds (Table 1). On average, the starch content is 0.2% dry mass, similar to that of mature soya bean as reported by Reddy et al. (1984) at 0.2–0.9%; Karr-Lilienthal et al. (2005) at 0.5%, and Saldivar et al. (2011) at 0.2–1.0%. Holse et al. (2011) also found starch signals to be absent in the infra red and NMR spectra of mature marama seeds. Carob seeds (*Ceratonia siliqua*), which is in the same family as marama bean also has a low starch content of 0.1% (Avallone et al., 1997). Other legumes such as pea, lentil and chickpea have a starch content of more than 40% (Dalgetty and Baik, 2003). Aguilera et al. (2009a) showed that total starch is 53.4% in chickpea (*Cicer arietinum* L.) and 46.3% in lentil (*Lens culinaris* L.).

The negligible amount of starch made detailed characterisation of starch, e.g. amylose/amylopectin ratio, unwarranted.

Table 1
Starch content of marama bean cotyledon from immature and mature seeds (% dry mass).

Sample	Starch content %
Immature seeds	0.19 (0.003)
Mature seeds	0.17 (0.003)

Values in parentheses indicate standard deviations, $n = 3$.

2.2. Soluble sugars

In general the content of soluble sugars is very negligible (less than 1% dry mass) in both immature and mature seeds. The main soluble sugar in immature seeds is glucose, followed by myoinositol and fructose (Table 2). Mature seeds had a higher amount of sucrose, followed by myoinositol and raffinose. The presence of raffinose in the mature seeds confirm the observations by Holse et al. (2011) using ^{1}H HR-MAS NMR.

In a study conducted by Aguilera et al. (2009b) the main soluble carbohydrate found in white beans and pink-mottled cream beans was also sucrose. However, other legumes contain raffinose family oligosaccharides (RFOs) as the main soluble sugars. The main soluble carbohydrate in soya bean (*Glycine max*), dolichos (*Lablab purpureus*) and cowpea (*Vigna unguiculata*) is RFOs (mainly stachyose), followed by sucrose, while jack bean (*Canavalia ensiformis*) has RFOs raffinose and ciceritol, followed by sucrose (Martín-Cabrejas et al., 2008).

A pattern of accumulation characterised by glucose, myoinositol, fructose, trehalose and ribose was observed at the immature stage, which disappeared at the mature stage as these sugars further decreased or were no longer detectable. The content of arabinose, raffinose, sucrose and maltose increased with maturation. Overall, a marked difference was observed between the two samples, with immature seeds having a higher amount of soluble sugars. However, soluble sugars represent a negligible percentage of total carbohydrates, as also observed for other legumes (Reddy et al., 1984).

2.3. Alcohol insoluble residue (AIR)

The composition of the non-cellulosic monosaccharides in AIR of mature marama seeds was characterised by a high content of arabinose, and with lower levels of mannose and galactose (Fig. 1). The immature seeds had a high content of mannose, followed by intermediate amounts of glucose and galactose. The mannose in the linkage analysis described below only show linkages characteristic for protein mannosylation indicating that the mannose in both samples originates from protein glycosylation (Lis and Sharon, 1978). As an abundance of cytoplasmic protein bodies have been visualised in marama seeds, possibly containing the majority of the protein associated mannose, the mannose detected in the sugar composition analysis of the non-cellulosic monosaccharides in AIR should not be considered part of the cell wall (Mosele et al., 2011). High mannose content has previously been reported in legume seeds with no evidence for its origin (Mullin and Xu, 2000).

Generally, legume seed cell wall contains high levels of galactose and arabinose, supposedly from long side chains of RG I (Huisman et al., 1998; Dalgetty and Baik, 2003). The presence of xyloglucan, mannan and callose (β-1,-3 glucan) was confirmed in CoMPP analysis (Fig. 2). However, based on the composition analysis of AIR the quantities of these polymers are minor compared to pectin. Both arabinogalactan protein (AGP) and extensin were also detected in the CoMMP analysis and with similar extraction profile as seen in Arabidopsis (Fig. 2) (Moller et al., 2007). Xylan could not be detected in this analysis and xylogalacturonan as recognised by the LM8 antibody, known to recognise an epitope present in a subpool of xylogalacturonans (Jensen et al., 2008; Nakamura et al., 2002) was not detected using CoMPP.