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Quantification of nitropropanoyl glucosides in karaka nuts before and after treatment

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ABSTRACT

A high performance liquid chromatography (HPLC) method was developed to assay nitropropanoyl glucosides in the nuts of karaka (*Corynocarpus laevigatus*) a traditional food of New Zealand Māori. Levels of glucosides, measured as 3-nitropropanoic acid, ranged from 50.25 to 138.62 g kg^{-1} (5.0–13.9% w/w) and were highest in nuts from unripe drupes; these levels are higher than any previously reported. Other parts of the drupe also contained nitropropanoyl glucosides but at lower levels than the nut. Treatment procedures to remove the glucosides from the nuts varied in their efficacy with soxhlet extraction removing 98.7% and prolonged boiling and cold water extraction both removing 96%. These findings confirm the traditional methods for preparation of these nuts for consumption.

1. Introduction

Karaka (*Corynocarpus laevigatus*) is indigenous to New Zealand; it is thought to have spread widely throughout both the North and South Islands as well as the Kermadec and Chatham Islands because of planting by Māori around their settlements as karaka "nuts" were a valued food source (Leach & Stowe, 2005). The nuts were still consumed until the 1950's and they have been recently reviewed as a potential food crop (Klinac, Benton, & Rentoul, 2009). The tree is of environmental interest because the fruit is an important food source for birds, especially the New Zealand pigeon (*Hemiphaga novaeseelandiae*) or keruru which may also have played a part in its spread (Sawyer, McFadgen, & Hughes, 2003). Corynocarpus is the only genus in the family Corynocarpaceae which is distributed from Papua-New Guinea to New Zealand and in Western Pacific Islands such as New Caledonia and Vanuatu (Klinac et al., 2009); karaka is an introduced species in Hawaii (Little and Skolmen, 1989).

Karaka is an evergreen tree with large dark green glossy leaves and which bears copious bright orange drupes from December to March (Salmon, 1980) (Supplementary Figs. 1 and 2). Trees produce an average of 20–30 kg of drupes per tree and up to 50 kg for individual trees (Klinac et al., 2009). The drupes are variable in size and can range from 2 to 5 cm in length (Salmon, 1980).

The part of the karaka drupe that is of most nutritional interest is not the fruit, but the elliptically shaped kernel/nut which is enclosed inside a fibrous shell under the fruit (Klinac et al., 2009) (Supplementary Fig. 3). Karaka nuts were second only in dietary importance to the kumara tuber, meaning that the nuts were a staple part of the Māori diet, especially in southern regions where kumara does not flourish (Klinac et al., 2009). The nuts were a highly valuable food source as treated nuts could be stored and eaten during winter when other food was in short supply; they were also important in traditional ceremonies, banquets, funerals and in formal exchanges between tribes. Preparation methods have been reported as variations on steaming the drupes in earth ovens for several hours followed by soaking for days to weeks in stagnant or running water (Klinac et al., 2009). Treated nuts are gluten-free (Klinac et al., 2009) and contain 58% carbohydrate, ~11% protein and 15.5% fat, they also contain six essential amino acids and are thus still a potentially valuable food source (McCurdy, 1947).

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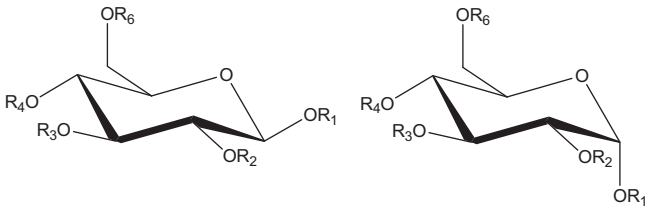

1 R_1=R_2=R_6= NP, R_3=R_4=H
2 R_1=R_6= NP, R_2=R_3=R_4=H
4 R_1=R_4=R_6= NP, R_2=R_3=H
7a R_6 = NP, R_1=R_2=R_3=R_4=H
8 R_1=R_3=R_6= NP, R_2=R_4=H
9 R_1=R_2=R_3=R_6= NP, R_4=H
10 R_1=R_3=R_4=R_6= NP, R_2=H
11 R_1=R_2=R_4=R_6= NP, R_3=H

3 R_2=R_6= NP, R_1=R_3=R_4=H
5 R_2=R_3=R_6= NP, R_1=R_4=H
6 R_3=R_4=R_6= NP, R_1=R_2=H
7b R_6= NP, R_1=R_2=R_3=R_4=H

NP = $COCH_2CH_2NO_2$

Fig. 1. Nitropropanoyl esters of D-glucopyranose found in karaka drupes.

The protracted preparation was required because it was recognised that the nuts were toxic and because it removed a bitter taste, which was probably also attributable to the toxicity. The toxic agent is a series of eleven nitropropanoyl esters of D-glucopyranose (NPG), **1**–**11** (Fig. 1), (Majak & Benn, 1994; Moyer, Pfeffer, Valentine, & Gustine, 1979), with the presence of free 3-nitropropanoic acid also likely. With the exception of karakin, **1** (Fig. 1), information on the toxicities of all the NPGs found in karaka nuts or their relative distributions is not available in the literature; most studies report quantification based upon crystallisation of isolated compounds or by reaction to yield NO_2 (Moyer et al., 1979). A study by Greenwood (1984) on the metabolism of karakin by larvae of grass grubs, found that the esters themselves appear more toxic than the equivalent amount of free acid, both on a weight and NPA-molar equivalent basis. That is taking into account the different esters yield different moles of 3-nitropropanoic acid, karakin, for example, gives three times the concentration of 3-nitropropanoic acid on a molar basis. Thus, the reason esters themselves are most likely more toxic is because they are able to be more readily absorbed than 3-nitropropanoic acid.

Thus, although karaka presents as a possible crop that might be exploited as a food, it is necessary to establish the required procedures for pre-treatment to render the nuts safe for consumption and to have a simple method for monitoring the success of the development process for this procedure. This paper describes the development of a liquid chromatography method to quantify nitropropanoyl esters of D-glucopyranose as 3-nitropropanoic acid in karaka nuts, and its application to the fruit and to the nuts, both raw and under a variety of treatment regimes.

2. Experimental

2.1. Materials

Analytical grade sulphuric acid (95–98%) and acetic acid (99.7%) were sourced from Ajax Finechem Pty Ltd., malonic acid (99%) and 3-nitropropanoic acid (>97%, see Safety note) were obtained from Sigma Aldrich. MilliQ water was collected from a Barnstead E-Pure water filter at a resistivity ~17.9 MΩ.

The trees for drupe and nut collection were chosen with assistance from a local NZ Treecrop member and were located in central Hamilton. Drupes or nuts were collected at various stages of ripeness some from the tree and some from the ground, with and without berry flesh. The collection dates, stage of ripening, quantities and location of all samples collected were recorded. An additional single sample was collected from a tree at Whatawhata, which was reported to be over one hundred years old. Upon collection, the drupes and nuts were stored in air tight zip lock bags and stored in a freezer at −17 °C, until analysis.

2.2. High performance liquid chromatography (HPLC)

HPLC was carried out using a Waters pump (model 515), a Waters photodiode array (PDA) detector (model 2996), a Rheodyne 20 μL injector loop and a BIO-RAD Micro-Guard® cation H guard cartridge. Separation was performed on a BIO-RAD Aminex® HPX-87H ion exclusion column (300 mm × 7.8 mm), with sulphuric acid (0.5 mL min^{-1}; 5 mM). 3-Nitropropanoic acid, malonic acid and acetic acid eluted at ~23.2, ~12.1, and ~18.1 min, respectively (Supplementary Fig. 4) and were quantified against external standards. The total run time was 51 min because of two small late-eluting unidentified peaks. For detection of NPA alone the run time was reduced to 35 min whereupon the two peaks carried over to the beginning of the subsequent chromatogram but did not interfere. Detection was carried out between 190 and 400 nm. The chromatograms at 210 nm were extracted and integrated using Empower Pro® software. Limit of quantification (LOQ) for 3-nitropropanoic acid was 2.98×10^{-3} mg mL^{-1} and 0.08 mg mL^{-1} for malonic acid.

2.3. Preparation of samples

The fruit was removed, the shell cut open and the nut with the pellicle removed. The pellicle was peeled off the nut and discarded.

Nuts were freeze dried (−80 °C) overnight and ground until as homogeneous as possible. Sulphuric acid (0.9875 mol L^{-1}, 10 mL) was added to the homogenate (~1 g), the sample was stirred (10 min) and heated (100 °C, 1 h). The solution was centrifuged (3000 rpm, 1 min) and the supernatant was removed and filtered. A sub-sample (100 μL) was diluted (1:4) with MilliQ water and analysed by HPLC.

2.4. Kinetics of degradation of 3-nitropropanoic and malonic acids

2.4.1. The degradation of 3-nitropropanoic and malonic acid

Four different concentrations of 3-nitropropanoic acid and malonic acid were made using sulphuric acid (0.9875 mol L^{-1}). Aliquots (n = 14, 3 mL) were placed in individual vials for each concentration and heated (100 °C) for the desired times (t = 0–180 min). At the appropriate times the vials were cooled and the contents analysed by HPLC.

2.4.2. Determination of 3-nitropropanoic acid and malonic acids activation energies

A solution of each acid was made using sulphuric acid (0.9875 mol L^{-1}). Aliquots (n = 14, 3 mL) were placed into vials for each of the three heating temperatures (60, 100 and 120 °C). Each batch of vials was heated for various times (t = 0–180 min), cooled and analysed by HPLC.

2.4.3. The pH dependence of 3-nitropropanoic acid degrading to malonic acid

Solutions of 3-nitropropanoic acid (~0.5 mg mL^{-1}) were made using 1 M and 0.1 M sulphuric acid. Aliquots of equal volume (n = 14, 3 mL) were placed in vials and heated (100 °C) for the desired times (t = 0–180 min). Each sample was diluted (1:4) with MilliQ water and analysed by HPLC.

2.5. Moisture content of the nuts

Moisture contents were determined by freeze drying (−80 °C) overnight to a constant weight.