



# The impact of thermal processing on bioactive compounds in Australian native food products (bush tomato and Kakadu plum)

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## ABSTRACT

This study was conducted to investigate the effect of food processing on the survival of bioactive compounds in Australian bush food products. The lycopene, beta carotene, and ascorbic acid were detected from bush tomato sauce, bush tomato ketchup and Kakadu plum chilli and ginger sauce. The finished product samples were collected during real food production line at three interval times; beginning, middle and the end of the real time manufacturing processes. The bioactive contents from the three products were stable throughout the heating process. In another experiment, bush tomato sauce (16% dried bush tomato content), Kakadu plum sauce (70% Kakadu plum filtrate) were prepared in the laboratory. Bioactive contents (lycopene and beta carotene) in lab formulated bush tomato sauce increased by 48 and 14% respectively. In contrast, ascorbic acid content in the Kakadu plum sauce lost by 16.9%. The experiment suggested that heat processing increased the level of lycopene and betacarotene but minimised ascorbic acid content in processed Australian Bush food products.

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## 1. Introduction

Australian native plant (bush food) is considered as a potentially commercial produce due to its ability to provide the basis of a truly Australian cuisine. A more recent trend is the view of Australian native foods as sources of bioactive compounds or antioxidant (Frobes-Smiths and Paton, 2002). Examples of these native foods are: Kakadu plum that is an excellent source of ascorbic acid, bush tomato that is rich in lycopene and Davidson's plum and Illawarra plum that are both rich in anthocyanin (Konczak, Zabar, Dunstan, & Aguas, 2010; McDonald et al., 2006; Netzel, Netzel, Tian, Schwartz, & Konczak, 2006; Netzel, Netzel, Tian, Schwartz, & Konczak, 2007; Tan, Konczak, Sze, & Ramzan, 2010). Since there is growing consumer concern with health, diet and lifestyle particularly in developed countries, there is a need of producing "functional food" (McDonald et al., 2006). Manufacturing of Australian bush food as functional food has been started since 1970 and has been growing continuously since 1970 (Brand, 1989).

Methods of cooking can affect the stability of bioactive compounds in products containing bioactive compounds. Heat processing for example, leads to ascorbic acid degradation but increases the level of phenolic content in vegetables such as tomato (Gahler, Otto, & Bohm, 2003; Nursal & Yucecan, 2000). Moreover, overheated products may also lead to unattractive colour, and off flavour due to oxidation reaction of lipid and bioactive compounds themselves (Rajchl et al., 2010). However, heat treatment can increase lycopene bioavailability by isomerisation

(Shi & Le Maguer, 2000). To manufacture native food products, the processing includes drying and/or freezing of the raw materials to preserve aroma and flavour i.e., drying of lemon myrtle leaves and bush tomato. This may be followed by cooking or heat processing such as pasteurisation. Pasteurisation prolongs shelf life of the products by destruction of spoilage micro-organisms and/or the inactivation of the enzyme (Fellows, 2009).

There are some studies on the stability of bioactive compounds during various cooking methods (Gahler et al., 2003; Perez-Conesa et al., 2009; Sadler, Davis, & Dezman, 1990; Xianquan, Shi, Kakuda, & Yueming, 2005). However, the impact of real time manufacturing processes on bioactive components of native food products has not yet been established. It is probable, based on previous studies of mainstream fruits and vegetables, that there is significant alteration in bioactive contents of bush food products induced by processing. The objective of this study is to determine the effect of processing on the bioactive compounds in native plant contained products. Levels of bioactive compounds were analysed during commercial processing run of native food products. The products tested were also produced in a bench top trial for comparison.

## 2. Materials and methods

### 2.1. Chemicals and sample collection

Standard reagent: ascorbic acid, lycopene and beta carotene were supplied by Analytical Services, the University of Queensland, Australia.

Dried bush tomato and frozen Kakadu plum were sourced from the industry partner (Robins Foods, Braeside Vic). Samples of dried and

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ground bush tomato were randomly taken from boxes stored at ambient temperature in Robins Foods warehouses. The bush tomato which was sent previously from the Northern Territory had been stored for around 6 months in the warehouse. The collected samples were placed in plastic zip top freezer bags and stored below  $-18^{\circ}\text{C}$  until further analysis. Frozen whole fruits of Kakadu plum were also obtained from Robins Foods. About 10 g of fruit was sampled from the top, middle and bottom layers of the plastic bags stored in the company freezer. Samples were collected in plastic zip top freezer bags and stored at below  $-18^{\circ}\text{C}$  until further analysis.

Processed samples, bush tomato chutney, bush tomato ketchup and Kakadu plum chilli and ginger sauce were collected from the manufacturer (Robins Foods, Braeside, Melbourne, Vic). At the relevant processing line, two cartons of finished products were sampled from the beginning, the middle and the end of the processing run. The samples were transported to Brisbane under ambient conditions and then stored in a cold room at less than  $5^{\circ}\text{C}$  until further analysis. Three bottles from the three points in the processing were randomly picked up from those two cartons (total of 9 bottles). For each bottle, the extractions were duplicated.

## 2.2. Quantitative determinations of bioactive compounds

### 2.2.1. Lycopene and beta-carotene

The method of extraction by Eitenmiller, Ye, and Landen (2008) was followed with a slight adaptation. Ground bush tomato (10.0 g) was added into a 50 mL, round bottom, plastic centrifuge tube followed by 10 mL of hexane–acetone–ethanol (50:25:25) and 5 mL of water. The mixture was mixed by rotating machine for 30 min. Thereafter, it was centrifuged at  $12,000\times g$  for 10 min using SS34 head by super speed refrigerated centrifuge RC-5 SORVALL<sup>®</sup>. Two layers of the solution appeared in the tube and the hexane layer which contained lycopene and beta carotene was collected. The extraction was done twice and the supernatant was combined. After that, the extraction solvent was evaporated off under  $\text{N}_2$  stream to dryness and stored in the fridge for further analysis. All procedures were done in subdue light conditions and samples were triplicates. After redissolving with 2.0 mL hexane the sample was filtered through 0.45  $\mu\text{m}$  nylon filter, the hexane layer was injected in to HPLC (Agilent 1100) with a UV detector using methanol/tetrahydrofuran/water, 67:27:6, v/v/v as mobile phase on a Hypersil column (250 $\times$ 4.6 mm, Agilent) with a flow rate of 2 mL/min as per procedure developed at Analytical Services Laboratory, School of Land, Crop and Food Sciences, The University of Queensland. Lycopene and beta carotene were qualified at 475 nm based on standard lycopene (20  $\mu\text{g}/\text{mL}$ ) and beta carotene (10  $\mu\text{g}/\text{mL}$ ).

For processed bush tomato sauces (bush tomato chutney and bush tomato ketchup), the excess sample was homogenised using a high speed electrical blender. During homogenisation,  $\text{O}_2$  was eliminated from the sample by placing a cover over the blender and purging with  $\text{N}_2$ . Homogenate (10.0 g) was extracted accordingly. Finally dried extract was redissolved with hexane (2 mL for bush tomato ketchup and 4 mL for tomato chutney) prior to HPLC injection.

### 2.2.2. Ascorbic acid

Frozen Kakadu plums were pitted and cut into thin pieces. Sample slices were placed onto a tray and kept frozen prior to freeze drying (Alpha 1–4 LSC, Christ<sup>®</sup>) over night. Dried sample was then ground to powder with an electrical blender.

Kakadu plum powder (0.5 g) was added to a 50 mL centrifuge tube followed by 15 mL 0.05 N phosphoric acid ( $\text{H}_3\text{PO}_4$ ). The mixture was then mixed for 30 min and centrifuged at  $4820\times g$  for 15 min. The supernatant was transferred to 100 mL volumetric flask. The extraction was repeated using the same extract residue twice and the supernatant was combined. The supernatant was made up to volume with the addition of 500  $\mu\text{g}/\text{mL}$  of sodium metabisulphite, purified by passing through a  $\text{C}_{18}$  cartridge which was preconditioned by flushing with

methanol followed by deionised water, and a 0.45- $\mu\text{m}$  Millipore filter. The extraction was triplicate. The method of Epriliati, D'Arcy, and Gidle (2009) for detection of ascorbic acid on HPLC was followed. A Prevail column (250 $\times$ 4.6 mm, Alltech) was used with 25 mM  $\text{KH}_2\text{PO}_4$  pH 2.25 as a mobile phase. The detection of the ascorbic acid was performed at 235 nm using UV detector. Ascorbic acid (mg/100 g) was calculated from ascorbic acid mixed standard (20  $\mu\text{g}/\text{mL}$  of ascorbic acid and iso-ascorbic acid) chromatogram.

For Kakadu plum sauce, three bottles of each were randomly picked up from those two cartons. For each bottle, the extraction was duplicated. Excess sample was homogenised using a hand held blender. While blending,  $\text{O}_2$  was eliminated by purging the mixture with  $\text{N}_2$ . Homogenised sample (10.0 g) was extracted with 15 mL of extraction solvent accordingly.

## 2.3. Formulated bush tomato sauce

Because of the confidential nature of the commercial formulations, the laboratory scale samples were formulated using the ingredient lists and product specifications of the above products as a guide. The percentage of ground bush tomato was doubled to ensure that there was enough lycopene content that could be detected after processing (Table 2). There are no other ingredients other than bush tomato which may affect the amount of lycopene from bush tomato such as tomato, or chilli. Flavouring ingredients were not incorporated into formulations as the processing and formulation parameters of interest were related to pH and holding and filling temperatures. The major ingredients used were water, thickener, food acid and the ground bush tomato. The formulation was balanced to a pH range of 3.5–4.0. The mixture was brought to  $80^{\circ}\text{C}$ , hot filled into jars and inverted to sterilise the inside of the lid. The jars were then left to cool at an ambient temperature and the bioactive content analysed.

## 2.4. Formulated Kakadu plum sauce

The formulation of Kakadu plum sauce was carried out in a similar manner to the bush tomato products. The form in which the Kakadu plum is used as an ingredient for sauces is called Kakadu plum filtrate. This was made from a kilogramme of fresh Kakadu plum boiled in 12.5 kg of water for 30 min. This filtrate contained 8% of whole Kakadu plum (including stone) stone. The major ingredients used were water, thickener, food acid and the Kakadu plum filtrate and formulation was balanced to a pH range of 3.5–4.0 (Table 2). Sauce was processed as described previously for bush tomato sauce. Forty jars were made and three of them were used for the ascorbic acid content analysis.

**Table 1**

The processing of native plant sauce (Jensen's choice Foods, Huntingdale, Vic, Australia).

Processing steps	Operation
Raw materials	
↓	
Cooking	Bring to boil and hold for 5 min
↓	
Holding tank	Holding at $95^{\circ}\text{C}$ for 30–40 min
↓	
Filling	Hot fill the product at $95^{\circ}\text{C}$
↓	
Washing filled jars/bottles	
↓	
Pasteurising	Steam pasteurisation tunnel at $95^{\circ}\text{C}$ for 20 min
↓	
Cooling	Products are cool and dried at the end of the tunnel
↓	
Labelling	
↓	
Storage	At room temperature in the ware house

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