Monitoring two different drying methods of Kakadu plum puree by combining infrared and chemometrics analysis

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ABSTRACT
The effect of two drying methods (oven and freeze drying) and the addition of maltodextrin to Kakadu plum puree samples (KP) (*Terminalia ferdinandiana*) were evaluated using mid (MIR) and near-infrared (NIR) spectroscopy. Dry powder samples were obtained using the oven and freeze-drying methods and seven levels of maltodextrin. Training (*n* = 32) and validation (*n* = 28) sets were developed for the prediction of moisture (M %), water activity (aw %), hydroxymethylfurfural (HMF) and vitamin C (VITC mg/100 g DM) based on NIR and MIR spectroscopy. Results from this study demonstrated the ability of spectroscopy combined with partial least squares (PLS) regression to monitor these parameters during drying. The standard error in cross validation (SECV) and the residual predictive deviation (RPD) values obtained were of 0.71% (RPD = 4.1) and 0.47% (RPD = 6.1) for M, 0.06% (RPD = 4.4) and 0.02% (RPD = 8.2) for aw, 0.73 (RPD = 3.3) and 0.72 (RPD = 3.3) for HMF, 465.7 mg 100 g DM (RPD = 3.0) and 289.3 mg 100 g DM (RPD = 4.8) for VITC, using MIR and NIR, respectively. The results from this study showed that MIR and NIR spectroscopies are capable of both measuring and monitoring the effect of drying and the addition of maltodextrin as a carrier to KP puree samples.

1. Introduction
The increasing market and consumer demands for high quality and healthy foods have created a need for efficient and accurate analytical methods for the quantification of bioactive compounds (e.g., antioxidants) in raw materials and finished products (Bunaciu et al., 2012; Cozzolino, 2015; Garcia-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). A large group of these bioactive compounds such as antioxidants and secondary metabolites can be found in plants and in many agricultural products having a wide range of biological activities and functions (Bunaciu et al., 2012; Cozzolino, 2015; Garcia-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010).

*Terminalia ferdinandiana* is best known by its common name Kakadu plum (KP) (Brock, 2005; J.T. Gorman et al., 2019). This plant is one of the Australian native species that have the potential to significantly grow into commercial agribusiness (Brock, 2005; J.T. Gorman et al., 2019). The customary use of natural resources, including KP, by Australian Aboriginal people, spans over many thousand years (Brock, 2005; J. Gorman et al., 2016). This plant is utilised as food where the inner bark can be used for medicinal purposes (Brock, 2005; J. Gorman et al., 2016). The fruit of this plant has high levels of vitamin C and it is considered...
an excellent source of natural antioxidants such as phenolic compounds (e.g., gallic and ellagic acid; Brand et al., 1982; Konczak, 2009; Konczak et al., 2014, 2009; Netzel et al., 2007; Williams et al., 2014).

The KP fruit can be consumed directly, as a fresh fruit or kept under frozen conditions for further product development (Brand et al., 1982; Konczak, 2009; Konczak et al., 2014, 2009; Netzel et al., 2007; Williams et al., 2014). The food industry in Australia generally uses this raw material as an intermediate product, either as a puree or powder to be added as an ingredient in products such as beverages and muesli bars (Brand et al., 1982; Netzel et al., 2007; Williams et al., 2014). It is important that this intermediate KP products have consistent quality and retain bioactive compounds for use as a functional ingredient (Brand et al., 1982; Netzel et al., 2007; Williams et al., 2014). The loss of bioactive compounds is particularly modulated by the temperature and the moisture content during the drying processes (Brito de Sousa Lobato et al., 2018; Wu et al., 2020). Freeze drying or lyophilization is considered to be a dehydration method that is mild and can retain the bioactive compounds due to the use of low temperature (< 2°C) and vacuum conditions (Antal et al., 2011). In comparison to oven drying or hot-air drying, which uses hot air with constant flow and elevated temperature (>40°C) to remove moisture, this high temperature can lead to degradation of heat-sensitive compounds (Chua et al., 2019). Carrier agents such as starch and maltodextrin provide protection against oxidation as they form a protective layer around the molecules within the carrier matrix and provide stability during storage (Cai & Corke, 2000). Maltodextrin is a low-cost, odourless and bland compound which is ideal for the formulation of the carrier matrix and has demonstrated the retention of bioactive compounds during the drying process (Rodríguez-Hernández et al., 2005).

Rapid, low cost and reliable analytical methods will be of benefit in order to determine and quantify the nutritional and compositional value of the sample, as well as to evaluate the contribution to the overall bioavailability of different compounds and to allow the identification of specific biomarkers (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). In recent years, both mid-infrared (MIR) and near-infrared (NIR) spectroscopy with its intrinsic benefits such as non-invasive, rapid, almost no necessary sample preparation, ability to perform on-/inline measurements, have been able to determine a wide range of physical and chemical parameters in a wide range of foods. These techniques have become widely used as analytical techniques in the so-called field of phyto-analytics and they have been included in pharmacoepia (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). These methods provide a range of tools that can be used in a wide range of biological samples without the need for extraction, which can often lead to degradation of the antioxidant components (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). These methods can also provide a high degree of precision when applied to the analysis of nutraceutical and antioxidant compounds as well as to monitor antioxidant activity in foods (Ignat et al., 2001; McGoverin et al., 2010; García-Cañas et al., 2010; Lu & Rasco, 2012; Bunaciu et al., 2012; Dong et al., 2013, 2014; Cozzolino, 2015). Other authors have also reported the use of vibrational spectroscopy (e.g., MIR and NIR) techniques to evaluate and monitor both the effect and the process of drying in different pharmaceutical ingredients and food matrices (Brito de Sousa Lobato et al., 2018; Pielesz, 2012; Watanabe et al. 2006; Wu et al., 2020; Zubak et al., 2020).

In this study, the effect of the drying method (oven and freeze drying) and the addition of maltodextrin as a carrier agent to Kakadu plum (KP) puree samples were evaluated using mid-infrared (MIR) and near-infrared (NIR) spectroscopy. Calibration models were developed using partial least squares regression to predict some of the routine parameters used to monitor the drying process.

2. Materials and methods

2.1. Samples

Commercial frozen Kakadu plum (KP) puree samples (ca. 15 Kg) were purchased from Traditional Homeland Enterprises Holding Co Pty Ltd (Morwell, Victoria, Australia), thawed at 4°C overnight and used for KP powder production. Maltodextrin (MALTO, food grade) with DE = 17–19 from Manilda Group (Gladesville, NSW, Australia) was completely pre-dissolved in water before adding to KP puree at different levels from 5 to 25% (w/v). The blend mixtures (approx. 500 g) were homogenized for 4 min at maximum speed using a high-speed homogeniser (Ultra-Turrax® T25, IKA, Germany) and the homogenous mixtures were smeared into stainless steel trays (50 x 30 cm) and subsequently subjected to either freeze dry (FZD) (Lab Gear SCANVAC, QLD, Australia) at −48°C for 7 days or conventional oven dry (OD) (Steridium, Brisbane, Australia) at 45°C for 3 to 4 days depending on the levels of added MALTO levels. The KP puree sample without MALTO was included as a control sample. After drying, the samples were ground into a fine powder using a Laboratory blender (Waring®8010/8011, NSW, Australia). Powder samples were sieved through a 200 μm sieve to obtain uniform particle size and stored in air-tight containers at −80°C for further analysis. All experiments were conducted in duplicate. A total of 60 samples were generated in this study (two drying methods and maltodextrin addition).

2.2. Reference methods

Water activity (aw) was determined by weighing approximately 2 g of the dry powder samples placed in a standard measuring cup for measurement of water activity using a LabTouch-aw water activity meter (Novasina AG, Labchen SZ, Switzerland) at a constant temperature of 25 ± 1°C and average stable scanning mode. The moisture content of the powder products was determined according to AOAC method 934.01 (AOAC, 2019). Approximately (3–4 g) of the dry powder samples were weighed into stainless steel dishes covered by lids and dried in a vacuum oven (Heraeus GmbH, Hanau, Germany) for approximately 16 h at 70°C under 250 mBar pressure to a constant weight where moisture content was expressed in percent. Extraction and analysis of 5-HMF were conducted followed the method previously published (Korb et al., 2013), with modifications. Briefly, 200 mg KP powdered sample was
homogenized with 50% methanol (v/v) using a vortex. The homogenate was subsequently placed in an ultra-sonication bath for 30 min at room temperature, followed by centrifugation at 1800g for 10 min (EppendorfCentrifuge5804, Hamburg-Eppendorf, Germany). Supernatants were retained, while residues were re-extracted twice following the procedure described above. The supernatants were combined and subjected to UHPLC-PDA analysis employed a Waters UPLC-PDA system and a Waters HSS-T3 column (150 x 2.1 mm i.d.; 1.8 μm; 25 °C), with 95% aqueous acetonitrile containing 0.1% formic acid (v/v) as the mobile phase (0.3 mL/min) and isocratic elution. These compounds were identified and quantified at 285 nm based on an external calibration curve of HMF standard (HPLC grade) from Sigma-Aldrich (Castle Hill, NSW, Australia). HMF was expressed as mg/100 g DW. Extraction and analysis of vitamin C [Ascorbic acid (L-AA) and dehydroascorbic acid (DHAA)] in KP pure and KP powder products were conducted as previously reported by Phan et al. (2019) adapted from Campos et al. (2009). Briefly, 200 mg KP powder sample was extracted with 3% meta-phosphoric acid (w/v) containing 8% acetic acid (v/v) and 1 mM Ethylenediaminetetraacetic acid (EDTA). The reduction of dehydroascorbic acid (DHAA), which was also present in the extracts/samples, to L-AA was performed following the method of Spinola et al. (2012), prior to UPLC-PDA analysis. Total vitamin C (L-AA + DHAA) was determined using a Waters UPLC-PDA system and a Waters HSS-T3 column (150 x 2.1 mm i.d.; 1.8 μm; 25 °C), with aqueous 0.1% formic acid as the mobile phase (0.3 mL/min) and isocratic elution. An aliquot of 2 μL of sample was injected into the UPLC system and L-AA peak was detected at 245 nm, identified and quantified by comparison to a commercial standard (Williams et al., 2014). The LOD and LOQ for the method were 1.0 and 3.0 mg/L, respectively. An external calibration curve of L-AA was used for quantification and vitamin C was expressed as mg/100 g DW. The standard error for each of the reference methods was 0.003% for aw, 0.20% for moisture, 0.23 mg/100 g DW for HMF and 92.1 mg/100 g DW for Vitamin C.

### 2.3. Infrared measurements

The MIR spectra of dry samples was acquired using a Bruker Alpha spectrophotometer fitted with an attenuated total reflectance platinum diamond single reflection cell (Bruker Optics GmbH, Ettlingen, Germany). The MIR spectra were recorded using OPUS software version 8.5 (Bruker Optics GmbH, Ettlingen, Germany). Measurements were recorded in the spectral region, 4000 to 400 cm⁻¹. Each spectrum was computed using the average of 24 interferograms at a resolution of 4 cm⁻¹. Air was used as the reference background spectra and reset every 10 samples. The FT-NIR spectra of the dry powder samples were collected using a Bruker Tange-R spectrophotometer with a gold-coated integrating sphere (diffuse reflection). Samples were placed in a borosilicate-glass cuvette 10 mm diameter (Bruker Optics GmbH, Ettlingen, Germany). The reflectance spectra were recorded using OPUS software (version 8.5, Bruker Optics GmbH, Ettlingen, Germany) with 64 interferograms at a resolution of 4 cm⁻¹ in the wavenumber range of 11,550 to 3950 cm⁻¹. Cuvettes were cleaned with 70% ethanol and dry with paper wipes between samples.

### 2.4. Data analysis

The Unscrambler X software (v11, CAMO ASA, Oslo, Norway) was used for multivariate analysis. Both MIR and NIR spectra were pre-processed using the second derivative (Savitzky & Golay algorithm with a second polynomial order and a smoothing window size of 10 points; Savitzky & Golay, 1964). The second derivative was applied as it has been reported to be effective at correcting for baseline effects and slope of a spectrum (Savitzky & Golay, 1964). Principal component analysis (PCA) was performed to visualise the structure of the data, identify dominant features in the spectra and variation in the samples for further investigation (Bureau et al., 2019; Cozzolino et al., 2019; Naes et al., 2002). Calibration models between the spectra (MIR and NIR) and reference data were developed using partial least squares regression (PLS) using cross validation (Bureau et al., 2019; Naes et al., 2002). The optimal number of factors for the calibration model was selected based on the minimal value of the predicted residual sum of squares (PRESS) and the highest correlation coefficient (R²) between actual and predicted values. The PLS models were evaluated in terms of the number of factors, standard error of cross-validation (SECV) and correlation coefficient. The residual predictive value (RPD) was used to evaluate the accuracy of the models (Bureau et al., 2019; Williams, 2001; P. Williams et al., 2017). Please note that the calibration models developed were only used to test the ability of spectroscopy to monitor the drying process.

### 3. Results and discussion

#### 3.1. Spectra interpretation

The second derivative of the absorbance values originated from the dominant features in the MIR range is presented in Figure 1. It was observed that the main peaks were associated with the O-H regions mainly associated with the water content of the samples. These regions were more dominant in the OD samples compared with the FZD ones (Figure 1 panel A). In the MIR second derivative spectra, peaks were observed between 3500 and 3000 cm⁻¹ predominantly associated with stretching vibrations of hydroxyl groups (O-H) of water (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). Peaks between 3000 and 2700 cm⁻¹ might be also associated with asymmetric (2917 cm⁻¹) and symmetric (2859 cm⁻¹) vibration of C-H bonds of aliphatic CH₂ groups, mainly related to the presence of lipids (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). In addition, peaks

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**Table 1. Descriptive statistics for the parameters measured in the Kakadu dry powder samples.**

| Parameter | Mean | Minimum | Maximum |
|-----------|------|---------|---------|
| M (%)     | 3.03 | 2.9     | 7.2     |
| aw (%)    | 0.27 | 0.22    | 0.05    |
| HMF (mg/100 g DW) | 8.66 | 2.40 | 5.8 | 13.4 |
| Vitamin C (mg/100 g DW) | 19,041.4 | 1384 | 16,846 | 20,938 |

M: moisture, aw: water activity, HMF: hydroxymethylfurfural, DW: dry weight, SD: standard deviation.

M: humedad, aw: actividad del agua, HMF: hidroximetilfurfural, DW: peso seco, SD: derviación estándar.
related to both lipid structures and esters groups were identified at around 1730 cm\(^{-1}\) (Stuart, 1996). The region between 1694 cm\(^{-1}\) and 1441 cm\(^{-1}\) is mostly related to the amide groups corresponding to proteins as well as with the presence of polysaccharides in the KP powder fruit samples analysed (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). The amide I was assigned with the frequency around 1645 cm\(^{-1}\) (N-H bending) while the amide II was associated with 1542 cm\(^{-1}\) (Stuart, 1996). The region between 1240 and 800 cm\(^{-1}\) has been reported with the H-O-C stretch vibrations of the saccharide ring, polysaccharide molecules and cellulose structures (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). In addition, frequencies between 1040 and 800 cm\(^{-1}\) might be related to polysaccharides (e.g., starch) (Stuart, 1996).

The NIR second derivative spectra of the KP powder samples analysed showed specific bands around 8550 cm\(^{-1}\) (C–H, second overtone) around 5800 cm\(^{-1}\), the C–H stretch first overtone CH\(_2\) bond vibrations (C–H, stretch the first overtone) appearing at around 5680 cm\(^{-1}\) (Figure 1 panel B) (Schoenbichler et al., 2014; Workman & Weyer, 2012). Lipid structures might be also associated with absorbance around 4330 cm\(^{-1}\) (C–H bending the second overtone; Schoenbichler et al., 2014; Workman & Weyer, 2012). In addition, around 4855 cm\(^{-1}\), protein vibrations are located due to the amide combination band of CONH\(_2\) (Schoenbichler et al., 2014; Workman & Weyer, 2012). The broad absorbance band in a range of 7100–6100 cm\(^{-1}\) is associated with the N–H stretching (first overtone) of proteins and the absorbance associated with water content (O–H symmetric and asymmetric stretching combination, first overtone). Furthermore, a combination band of water appears at a wavenumber around 5155 cm\(^{-1}\) due to the O–H stretching and H–O–H bending combination tones (Schoenbichler et al., 2014; Workman & Weyer, 2012). In the NIR spectra, the OD samples showed higher absorbance values compared with the FZD.

Figure 1. Second derivative of Kakadu plum dry powder samples analysed using either mid- or near-infrared spectroscopy.

Figura 1. Segundo derivado de las muestras de polvo seco de la ciruela de Kakadu analizadas mediante espectroscopía de infrarrojo medio o cercano.

Figure 2. Principal component score plot for the analysis of Kakadu plum dry samples analysed using mid- and near-infrared spectroscopy.

Figura 2. Gráfico de puntuación del componente principal para el análisis de las muestras secas de ciruela de Kakadu analizadas mediante espectroscopía de infrarrojo medio y cercano.
3.2. Principal component analysis

To determine the similarities and differences in the infrared spectra of the samples dried using the two drying methods a PCA analysis was applied. Figure 2 (Panel A) shows the scores plot of the KP powder samples analysed using both MIR and NIR spectroscopy. Using the MIR spectral data, the first two principal components explained 95% of the variability in the dataset. The samples were clustered based on the drying method along with the first principal component while PC2 explained the changes in the MIR spectra associated with the addition of maltodextrin to the powder samples. Figure 2 (Panel B) shows the scores plot for the KP powder samples analysed using NIR spectroscopy. Similarly, the first two principal components explained 99% of the variability in the data. A similar trend as the one obtained using the MIR data was observed for the samples analysed using NIR spectroscopy (e.g., PC1 was associated with water content while PC2 was associated with the addition of maltodextrin).

3.3. Partial least squares regression models

Table 1 shows the descriptive statistics (average, range, standard deviation) for the moisture, water activity, hydroxymethylfurfural and vitamin C measured in the KP powder samples used to develop the PLS calibration models. These results indicated a wide range of variation in the data set due to the different drying methods and levels of maltodextrin added to the KP puree samples. The variability in the data set was considered adequate to develop calibrations for this bioactive compound using either MIR or NIR spectroscopy.

Table 2 shows the cross-validation statistics for moisture, water activity, hydroxymethylfurfural and vitamin C analysed...
Table 2. Cross-validation statistics obtained for the prediction of chemical parameters in Kakadu dry powder samples analysed using either mid- or near-infrared spectroscopy.

| R² | SECV | Bias | Slope | RPD | LV |
|----|------|------|-------|-----|----|
|    |      |      |       |     |    |

MIR (n = 28)

M (%) 0.95 0.71 0.03 0.96 4.1 3
aw (%) 0.92 0.73 −0.05 0.91 3.3 4
HMF (mg/100 g DW) 0.88 465.7 25.9 0.88 3.0 4
NIR (n = 28)

M (%) 0.97 0.47 0.007 0.97 6.1 2
aw (%) 0.98 0.02 0.0004 0.98 8.2 2
HMF (mg/100 g DW) 0.92 0.72 −0.06 0.87 3.3 6
Vitamin C (mg/100 g DW) 0.96 289.3 2.1 0.97 4.8 2

R²: coefficient of determination; SECV: standard error of cross validation; RPD: residual predictive deviation; LV: latent variables; M: moisture; HMF: hydroxymethylfurfural; aw: water activity; n: number of samples.

using both MIR and NIR spectroscopy. The Standard error in cross validation (SECV) and the residual predictive deviation (RPD) values obtained were of 0.71% (RDP = 4.1) and 0.47% (RDP = 6.1) for M, 0.06% (RDP = 4.4) and 0.02% (RDP = 8.2) for aw, 0.73 (RDP = 3.3) and 0.72 (RDP = 3.3) for HMF, 465.7 mg 100 g DM (RDP = 3.0) and 289.3 mg 100 g DM (RDP = 4.8) for VITC, using MIR and NIR spectroscopy, respectively. The RPD values obtained for moisture, water activity and vitamin C measured in the KP powder samples were equal or higher than 4, indicating that these calibrations can be used for the quantitative determination of these parameters using either MIR or NIR spectroscopy (Bureau et al., 2019; Williams, 2001; P. Williams et al., 2017). However, a semi-quantitative (low, medium or high HMF) calibration model was obtained for the measurement of HMF using either MIR or NIR spectroscopy (RPD≥ 3) (Bureau et al., 2019; Williams, 2001; P. Williams et al., 2017). The low performance for the HMF calibrations might be related to the low SD for this parameter or the lack of information about compounds derived from the Maillard reaction contained in the infrared spectra.

The PLS loadings for the optimal calibration models developed for the measurement of the chemical parameters in KP powder samples are shown in Figure 3. Similar IR spectral regions as described in Figure 1 were used by the PLS algorithm during calibration development using either MIR or NIR spectroscopy. In both MIR and NIR calibration models, PLS loadings for moisture and water activity were almost identical, corresponding with frequencies or wavelengths associated with O-H groups (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996; Workman & Weyer, 2012). The PLS loadings for the measurement of vitamin C and HMF were different from those observed for water.

4. Conclusions

The results from this study showed that MIR and NIR spectroscopies are capable of both measuring and monitoring the effect of drying and the addition of maltodextrin as a carrier to KP puree samples. The use of these methodologies offers considerable advantages compared with the use of routine methods of chemical analysis, as the sample preparation is simple. The practical implications of this study demonstrated that vibrational spectroscopy provides valuable benefits for the food industry allowing for the rapid monitoring of the drying process of a native food like Kakadu plum puree. The utilization of these techniques also offers the possibility to develop calibrations between spectra and the reference methods in order to measure several parameters simultaneously and therefore, reducing the time and cost of analysis. The utilization of rapid and low-cost tools for measuring quality can be implemented throughout the food value chain and they will provide with huge benefits to the natural food industry.

Acknowledgments

The authors acknowledge the Traditional Owners of the lands on which the *Terminalia Ferdinandiana* fruits were harvested respecting the knowledge and experience the Traditional Owners hold regarding the care, harvest and use of these plants.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Funding support from CRC for Developing Northern Australia Limited Project AT.2.1718031 – Improving the efficiency of Kakadu plum value chains to grow a robust and sustainable industry and the Australian Research Council (ARC) Industrial Transformation Training Centre (ITTC) for Uniquely Australian Foods (Grant number: IC180100045); Cooperative Research Centre for Developing Northern Australia [AT.2.1718031;ARC [IC180100045].

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