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Use of NIRS technology for on-vine measurement of nitrate content and other internal quality parameters in intact summer squash for baby food production

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ABSTRACT

This study sought to assess the feasibility of using NIR spectroscopy to predict the physico-chemical composition of summer squash during on-vine ripening, with a view to deciding on its possible use in baby food production depending on nitrate content at harvesting. NIR calibration models were developed using a set of 157 samples scanned *in situ* in the 1600–2400 nm region, using a portable handheld MEMS-NIR spectrophotometer working in reflectance mode. Modified partial least squares (MPLS) regression was used to interpret spectra and develop calibrations for summer squash composition. Results (R^2_{cv} = 0.83; SECV = 112.44 mg L^{-1}) showed that NIRS technology has great potential for measuring nitrate content and also other quality parameters in intact summer squashes during on-vine ripening. In addition, suitable wavelengths for nitrate content determination were identified by x-loading weights and regression coefficients. These findings suggest that NIRS may be a valuable tool for the rapid, accurate and non-destructive measurement of nitrate content, with a view to ascertaining the suitability of individual fruits for use in the production of baby foods.

1. Introduction

Over recent years, consumers have become increasingly aware of the risks involved in excessive consumption of nitrates and nitrites in water and foods. Vegetables are a major source of nitrates in the human diet, while nitrites are ingested mainly through canned foods. In response to growing public concern, the European Union passed Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs; the maximum level for nitrates in processed cereal-based foods and baby foods for infants and young children was set at 200 mg NO_3/kg (OJEU, 2006).

Summer squash is a common ingredient in processed vegetable-based baby foods. It is rich in polysaccharides, active proteins, essential amino acids, vitamins, carotenoids and minerals, and provides a moderate amount of dietary fiber; interest in this vegetable has increased considerably in the last few years due to its nutritional properties and health benefits (Reiss et al., 2012).

Nitrate levels at harvesting are a key issue, particularly if the summer squash is to be processed for the production of baby food. Toxicity occurs due to the conversion of nitrate to nitrite, which may lead to methemoglobin due to the oxidation of Fe^{+2} in hemoglobin. The impaired capacity of methemoglobin to deliver oxygen to tissues may lead to severe toxic effects, and may even prove fatal where methemoglobin accounts for over 70% of total hemoglobin. This occurs almost exclusively in infants and very young children, due to: lower stomach acidity (favoring the growth of bacteria able to convert nitrate to nitrite); the presence of fetal hemoglobin (which is more easily oxidized by nitrite); and lower levels of NADH-dependent methemoglobin reductase, an enzyme capable of reducing methemoglobin, which is very efficient in adults (Santamaria, 2006). In recent years, a number of studies have highlighted a possible link between nitrate exposure and childhood type 1 insulin-dependent diabetes mellitus (Van Maanen et al., 2000).

All this has prompted greater attention to squash quality and safety concerns; as a result, producers are increasingly anxious to provide consumers with assurances regarding the quality and

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provenance of this product. Nitrate accumulation in squashes depends not only on type and genetic variety, but also on a number of other factors, including temperature, sunlight, available nitrogen and growing method (Blom-Zandstra, 1989).

There is a clearly need for non-destructive sensors that can be used in the field to measure squash nitrate content as well as other internal quality parameters (firmness, dry matter and soluble solids content, pH and titratable acidity); on the basis of the values obtained, decisions can be taken regarding optimum harvesting times and possible industrial uses.

Near-infrared reflectance spectroscopy (NIRS), in conjunction with the application of multivariate analysis strategies, is a valuable tool with great potential for the agrifood sector, ensuring rapid and reliable measurement of these parameters; over recent years, the field implementation of NIRS techniques has been helped by the development of compact, portable instruments, which may be hand-held or tractor-mounted, and can thus be readily used in the field.

There are no reports in the literature regarding the use of MEMS-NIRS instruments for the pre-harvest monitoring of summer squashes with a view to establishing the optimum time for harvesting depending on their potential destination in the industry, since research to date on the use of NIRS technology for summer squash quality control has focused only on the measurement of dry matter, hue angle h* and firmness using a NIR-AOTF spectrophotometer (Barnaba et al., 2012), and on the determination of antioxidant compound content (Blanco-Díaz et al., 2014) and mineral and carotenoid content (Martínez-Valdivieso et al., 2014a,b) using a monochromator instrument to analyze lyophilized, ground product.

Several authors have highlighted the viability of NIRS technology for the non-destructive measurement of nitrate content in various fruits and vegetables, including Japanese radishes (Ito et al., 2003), leaf stalk of Qing gin cai (Ito and Idezawa, 2006), spinach leaves (Xue and Yang, 2009), and pineapple (Srivichien et al., 2015).

This study sought to assess the feasibility of using NIR spectroscopy, with a low-cost, miniaturized, handheld, near-infrared device based on MEMS technology, for characterizing internal quality variations – particularly nitrate content – in intact summer squashes during on-vine ripening, with a view to optimizing harvesting times and enabling staggered harvesting by quality, thus allowing certain harvested squashes to be used in the production of baby foods.

2. Material and methods

2.1. Sampling

A total of 157 summer squashes (*Cucurbita pepo* subsp. *pepo* var. Mirza), grown on an open-air plantation in the district of La Montiela, Santaella (Córdoba, Spain), were harvested between May and July 2015.

2.2. Reference data

Nitrate content (mg NO_3 L^{-1}) was measured following Thompson et al. (2009), using an RQFlex reflectometer (Merck, Darmstadt, Germany). The reflectometer which measures the colour intensity of Reflectoquant ® test strips (Merck, Darmstadt, Germany) is based on a colorimetric method. For NO_3^- analysis, 50 g of sample from the equatorial part of fruit were cut into very small pieces and blended with 100, 150 and 200 ml of deionised water in a blender, depending on NO_3^- concentrations in the sample. After that, the solution was filtered using a coffee filter and let to settle for 5 min. Subsequently, a test strip was dipped in the supernatant for 2 s, and then the colour was allowed to develop for 1 min. The test strip was then inserted into the reflectometer and the amount of light reflected from the test strip was measured and converted to concentration by a standard calibration previously introduced into the equipment through a bar coded plastic strip. The dilution factor was also taken into consideration.

Firmness was measured as the maximum force required to penetrate the summer squashes to a puncturing depth of 10 mm using a 3-mm cylindrical tip. Summer squashes were arranged with the stem-calyx axis horizontal; the first measurement was made at a point on the equator, and the second after turning the fruit through 180°. Texture measurements were made using a Universal Instron Texturometer (Model 3343, single-column, Instron Corporation, Norwood, MA, USA), with a head speed of 0.0008 m/s (50 mm/min) and a 1000 N load cell.

Dry matter content was determined by desiccation at 105 °C for 24 h (AOAC, 2000); final dry weight was calculated as a percentage of initial wet weight. Soluble solid content (SSC, in °Brix) was measured as the refractometer reading for summer squash juice, using a temperature-compensated digital Abbé-type refractometer (model B, Zeiss, Oberkochen, Würt, Germany). Values for pH and titratable acidity (TA) were measured using an automatic titrator (Crison Micro TT 2050, Crison, Alella, Barcelona, Spain); TA was measured by titration with 0.1 mol L^{-1} NaOH to an end point of pH 8.1. Results were expressed as % citric acid. All the samples were taken from the equatorial zone of the fruits and analyzed in duplicate. All measurements were performed immediately after NIRS measurements.

2.3. Spectral data acquisition

NIR spectra of intact summer squashes were collected in reflectance mode (log 1/R) using a handheld micro-electromechanical system (MEMS) instrument (Phazir 2400, Polychromix, Inc., Wilmington, MA, USA).

The Phazir 2400 is an integrated near-infrared handheld analyzer that incorporates all the essential components to deliver on-vine applications. The spectrophotometer scans at a non-constant interval of around 8 nm (pixel resolution 8 nm, optical resolution 12 nm), across the NIR wavelength range of 1600–2400 nm, with a scan time per sample of 3 s. Instrument performance was checked every 10 min, following the diagnostic protocols provided by the manufacturer, and white reference measurement was carried out using Spectralon as reference.

Four spectral measurements were made on each summer squash whilst on the vine, at four points located 90° from each other in the equatorial region of the fruit. The four spectra were averaged to provide a mean spectrum for each fruit.

2.4. Data analysis: definition of calibration and validation sets

Prior to carrying out NIRS calibrations, the CENTER algorithm included in the WinISI II software package ver. 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) was applied to ensure a structured population selection based solely on spectral information, for the establishment of calibration and validation sets (Shenk and Westerhaus, 1991). This algorithm performs an initial principal component analysis (PCA) to calculate the center of the population and the distance of samples (spectra) from that center in an *n*-dimensional space, using the Mahalanobis distance (GH); samples with a statistical value greater than 3 were considered outliers or anomalous spectra.

The CENTER algorithm was applied in the spectral region 1600–2400 nm. Mathematical treatments SNV (Standard Normal Variate) and DT (De-trending) were applied for scatter correction (Barnes et al., 1989), together with the mathematical derivation