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

María-Teresa Sánchez[a,*], Dolores Pérez-Marín[b,*], Irina Torres[a], Belén Gil[a], Ana Garrido-Varo[b], María-José De la Haba[a]

[a] *Department of Bromatology and Food Technology, University of Cordoba, Campus de Rabanales, 14071 Cordoba, Spain*

[b] *Department of Animal Production, University of Cordoba, Campus de Rabanales, 14071 Cordoba, Spain*

* Corresponding authors: Tel.: +34 957 212576; fax: +34 957 212000.

E-mail addresses: teresa.sanchez@uco.es (M.-T. Sánchez), dcperez@uco.es (D. Pérez-Marín)

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 = 0.83; SECV = 112.44 mg kg^{-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.

Keywords: NIR spectroscopy, summer squash, on-vine, nitrate content, baby food.

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 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 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 kg^{-1}) was measured following Thompson et al., (2009), using an RQFlex reflectometer (Merck, Darmstadt, Germany).

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 180º.

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 105ºC for 24 h (AOAC, 2000); final dry weight was calculated as a percentage of initial wet weight. Soluble solid content (SSC, in ºBrix) 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.

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. Four spectral measurements were made on each summer squash whilst on the vine, at four points located 90º 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 treatment '1,5,5,1', where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (Shenk and Westerhaus, 1995b; ISI, 2000).

After elimination of outlier spectra, calibration models were initially constructed using all the samples available (training set, C1) for all parameters tested (Table 1). After analyzing the accuracy and precision of the models obtained, new models were developed for those parameters for which the best models displayed a predictive capacity sufficient at least to distinguish high, medium and low values for that parameter; later, these were externally validated. For this purpose, and having ordered the sample set by spectral distances (from smallest to greatest distance to the center), the samples forming the validation set were selected by taking one sample out of every four in the initial set, although other alternatives for the selection of this set could have been used. After this procedure, the calibration (C2) and validation (V) sets thus comprised the samples shown in Table 1.

Data were subjected to chemometric treatment using the WinISI software package ver. 1.50 (ISI, 2000).

2.5. Data pre-processing and calibration model construction using a linear regression strategy

NIR calibration models for the prediction of quality parameters (nitrate content, firmness, dry matter, SSC, pH and TA) in intact summer squashes were initially constructed using the training set C1 (comprising all available samples) using modified partial least squares (MPLS) regression (Shenk and Westerhaus, 1995a), with subsequent cross-validation. The calibration set was partitioned into 6 groups; each group was then validated using a calibration developed on the other samples; finally, validation errors were combined to obtain a standard error of cross-validation (SECV).

A number of different pre-processing combinations were evaluated for scatter correction, including SNV and DT. Additionally, a total of four derivative mathematical treatments were tested: 1,5,5,1; 2,5,5,1; 1,10,5,1 and 2,10,5,1.

The statistics used to select the best equations were: the coefficient of determination for calibration (R^2), the standard error of calibration (SEC), the coefficient of determination for cross calibration (r^2), the standard error of cross validation (SECV) and the coefficient of variation (CV), defined as the ratio between SECV and the mean value of the reference data in the calibration set. Furthermore, the Residual Predictive Deviation (RPD) was calculated as the ratio of the standard deviation (SD) of the reference data to the SECV. This statistic, together with the CV, enables SECV to be standardized, facilitating the comparison of results obtained with sets of different means (Williams, 2001).

Having analyzed the predictive capacity of the models obtained using the complete sample set (training set C1), new models were developed to predict the selected parameters using the second calibration set (C2). The best-fitting equations obtained for this new calibration set, as selected by statistical criteria, were subsequently subjected to external validation following the protocols outlined by Windham et al., (1989).

3. Results and Discussion

3.1. Spectral properties

Mean and standard deviation spectrum for summer squash at harvesting are shown in Figure 1.

In the wavelength region 1600-2400 nm, the major absorption peak at around 1920-1930 nm was mainly related to water absorption, as was the peak at around 2200 nm; this was to be expected, since summer squash is around 90% water (Osborne et al., 1993; Williams, 2001). Osborne et al. (1993) reported peaks at around 1780 nm and 2310 nm related to the first sugar-related overtone. Peaks at around 1680 nm may be linked to combination bands of proteins (Williams, 2001).

3.2. Population characterization.

Calibration (C1 and C2) and validation (V) set details, i.e. number of samples, mean, range, SD, and CV for the parameters analyzed, are shown in Table 1.

It should be stressed that structured selection using only spectral information treatment algorithms such as CENTER proved adequate and useful, since the calibration and validation sets (C2 and V, respectively) displayed similar values for mean, range and standard deviation for all study parameters, and ranges for the validation set lay within the range recorded for the calibration set C2.

As Table 1 shows, the internal quality parameter displaying the greatest variability was nitrate content, with a CV of 70.37% (training set C1) and 69.42% (training set C2); CV for the validation set was 73.73%. Nitrogen fertilization was stopped halfway through harvesting, prompting a marked drop in nitrate content thereafter. The lowest variability was recorded for pH ($CV_{calibration}$ = 2.69% and 2.85% for C1 and C2, respectively; $CV_{validation}$ = 2.54%) and titratable acidity ($CV_{calibration}$ = 11.11% and 11.25% for C1 and C2, respectively; $CV_{validation}$ = 8.89%), values for which displayed little variation over the harvesting period as a whole.

3.3. Prediction of nitrate content and other internal quality parameters in summer squash on vine

Results for the best models developed using training set C1 and various mathematical pretreatments are shown in Table 2. Statistical criteria were used to select the best model for each study parameter.

This study sought to determine whether harvested summer squashes could be used in the production of baby foods, for which nitrate content represents a major constraint. The predictive capacity of the best model for nitrate content (r^2 = 0.83; SECV = 112.44 mg NO3 kg^{-1}) may be considered acceptable in terms of the limits recommended by Shenk and Westerhaus (1996). This result is of particular interest, in that it suggests that using a low-cost, portable NIRS instrument—suitable for use in the field—the industry can rapidly classify fruits as fit or unfit for baby food production on the basis of nitrate content. The European Union Commission Regulation (EC) No 1881/2006 stipulates that summer squashes with a nitrate content of over 200 mg NO_3 kg^{-1} cannot be used for the production of foods for infants and young children, and both producers and processors urgently require a non-destructive technique for measuring nitrate content.

This appears to be the first published report on the use of NIRS technology to measure nitrate content in summer squashes, although a number of authors have tested this technology in other vegetables. Ito et al. (2003) found that NIRS in conjunction with multiple linear regression enabled satisfactory determination of nitrate content in Japanese radishes, although they noted that the RMSE could be improved. Ito and Idezawa (2006) used NIRS and MLR algorithm to determine nitrate content in the leaf stalk of Qing gin cai, reporting a good match between real and Vis-NIR-calculated values for nitrate ion content for the third leaf stalk from outside and for whole nitrate ion content (0.90 and 0.76, respectively). In a 2009 study of nitrate content in spinach, Xue and Yang tested the best PLS model and PCR model in the spectral range 350-2500 nm with an independent dataset, reporting good agreement between predicted and observed values, with a correlation coefficient of 0.94 for the PLS model and 0.95 for the PCR model; the RMSE of prediction was 128.2 mg kg^{-1} for the PLS model and 120.8 mg kg^{-1} for the PCR model. In the only published study in fruit, Srivichien et al. (2015) measured nitrate content in pineapples by Vis-NIR spectroscopy, using a monochromator instrument; the results (RPD = 2.86; CV = 13.84%) were better than those obtained here, perhaps because the spectral range used was 600-1200 nm.

The predictive capacity of the models obtained for predicting dry matter content (r^2 = 0.66; SECV = 0.38% fw), total soluble solid content (r^2 = 0.68; ETVC = 0.33 ºBrix) and pH (r^2 = 0.57; ETVC = 0.11)—parameters crucial for deciding the optimum time for harvesting and determining the shelf-life of summer squashes—enabled high, medium and low values to be distinguished (Shenk and Westerhaus, 1996).

Only one published study has addressed the prediction of dry matter content in summer squashes using NIRS technology: Barnaba et al. (2012) used an AOTF-NIRS spectrophotometer for this purpose, in conjunction with PLS regression. The predictive capacity of the calibration models developed for this parameter (r^2 = 0.81; SECV = 0.46% fw) were similar to those obtained here.

No reports have been found in the literature regarding the determination of SSC in summer squashes using NIR spectroscopy. However, Sánchez et al., (2013) measured SSC in intact mandarins using a Phazir 2400 instrument in the 1600-2400 nm spectral region, reporting results (RPD = 1.49; CV = 6.06%) very similar to those obtained in the present study.

Portable MEMS-based NIRS instruments have not hitherto been used to determine pH in summer squashes, but Sánchez et al. (2013) report poorer results for pH prediction in intact mandarins (RPD = 1.11; CV = 2.59%), highlighting the fact that pH is difficult to predict if training sets are insufficiently varied.

The predictive capacity of the models developed to predict firmness (r^2 = 0.45; SECV = 1.46 N) and titratable acidity (r^2 = 0.44; SECV = 0.01 % citric acid) enabled high and low values to be distinguished for these parameters, thus meeting the criterion recommended by Shenk and Westerhaus (1996).

The results obtained for firmness underline the difficulty in correlating a destructive measurement made at a puncturing depth of 10 mm with a non-destructive measurement; as Peirs et al. (2002) have noted, NIRS light only penetrates to a useful depth of between 1 and 5 mm, depending on the wavelength, the instrument used and the maturity of the fruit tested.

The predictive capacity of the models developed to predict titratable acidity reflects the fact that all summer squashes were harvested at commercial maturity, and thus displayed very uniform acidity values. González-Caballero et al. (2010) report that this parameter cannot be predicted using NIRS technology and MPLS regression if the training sets have low standard-deviation values, as was the case here.

Portable MEMS-based NIRS instruments have not been used to date for the prediction of firmness in summer squashes, but Pérez-Marín et al. (2010) reported a predictive capacity for firmness in intact plums (RPD = 1.18; CV = 53.10%) slightly lower than that obtained here, using the same instrument. This highlights the difficulty of correlating measurements when using an instrument working in the 1600-2400 nm spectral region.

Although there are no reports in the literature regarding the *in situ* measurement of titratable acidity in outdoor-grown summer squashes, Sánchez et al. (2013), studying quality measurements in on-tree mandarins using the Phazir 2400 instrument, obtained models whose predictive capacity (RPD = 1.68; CV = 11.93%) was better than that obtained here, perhaps because the training sets contained greater variability, since measurements were made throughout ripening.

3.4. New calibration process and external validation

Once the predictive capacity of the models using the training set C1 and cross-validation had been analyzed, only those models (nitrate content, dry matter, SSC and pH) for which $r^2 > 0.5$ were subjected to external validation.

The aim was, in the first instance, to validate the best calibration models using a sample set not included in the calibration, but similar to the calibration set. Validations of the best calibration models obtained with training set C2 were performed using a set comprising 34 samples (Table 3).

Models constructed for predicting nitrate content in intact summer squash using the MEMS instrument met the validation requirements in terms of r^2 ($r^2 > 0.6$), while the SEP(c), bias and slope were within confidence limits: the equation thus ensures accurate prediction, and can be applied routinely (Windham et al., 1989). As Table 3 shows, two samples in the initial validation set (nitrate content of 1,030 and 1,065 mg kg^{-1}) were eliminated since the samples were unrepresentative of the calibration set (Fig. 2), thus hindering their correct prediction.

Using the monitoring procedure, the prediction statistic values obtained for dry matter fell short of the limit recommended for routine application ($r^2 > 0.60$). However, it should be stressed that the SEP(c) and slope values were close to confidence limits and bias was below confidence limits, suggesting that the NIRS equation for this internal parameter can be regarded as a useful preliminary trial for obtaining accurate on-vine quality predictions for intact summer squashes. Likewise, the values for bias in the models constructed for predicting SSC and pH in intact squashes using a handheld MEMS spectrophotometer lay within confidence limits, although r^2, SEP(c) and slope results did not always attain recommended minimum values, indicating that the NIRS equations constructed may be considered as a first step in the fine-tuning of NIRS technology for the on-vine monitoring of internal quality parameters in summer squashes.

These results highlight the importance not only of ensuring a sufficient number of samples in the calibration set, but also of guaranteeing the adequate distribution and structure of the sample set.

3.5. Analysis of sensitive wavelengths for the prediction of nitrate content

The x-loading and regression coefficient plots for the best model obtained for predicting nitrate content in intact summer squash are shown in Fig. 3. These plots show the areas across the spectral range in which variance has influenced the computing of the model to a greater or lesser degree, and the direction (positive or negative).

For the prediction of nitrate content using the Phazir 2400, representation of the six latent variables (LV) used in constructing the calibration equation and the regression coefficients shows that the areas of the spectrum exerting greatest weight on model fitting were 1712, 1776, 1850, 1920, 1984, 2008, 2100, 2152 and 2264 nm. Their influence was either positive or negative, depending on the latent variable in question (Fig. 3).

4. Conclusions

These results suggest that NIRS is a very promising and useful sensor for the non-destructive quantification of changes in nitrate content and other internal quality parameters in summer squashes in the course of on-vine ripening, enabling decisions to be taken regarding the optimum harvesting time. Harvested fruit can thus be swiftly streamed, allowing batches with different nitrate contents to be processed separately.

Findings also show that the use of portable MEMS-based NIRS instruments enables nitrate content to be measured, rapidly and *in situ*, during on-vine ripening, thus providing the industry with a means of establishing the final destination of the product, since if nitrate content exceeds the maximum levels stipulated under current legislation, the fruits cannot be used in the production of baby foods.

Acknowledgements

This research was under the Research Project 'Quality determination of summer squash grown on an open-air plantation in Santaella (Córdoba)', funded by Gelagri Ibérica, S.L. The authors thank to Ms. Mª Carmen Fernández her technical assistance.

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Table 1.

Number of samples (N), range, mean, standard deviation (SD), and coefficient of variation (CV) for different calibration sets (C1 and C2) and for the validation set (V)

	Parameters																	
	Nitrates (mg kg^{-1})			Firmness (N)			Dry matter (% fw)			SSC (ºBrix)			pH			Titratable acidity (% citric acid)		
	C1	C2	V	C1	C2	V	C1	C2	V	C1	C2	V	C1	C2	V	C1	C2	V
N	150	116	34	150	116	34	150	116	34	150	116	34	150	116	34	150	116	34
Range	30.00-1074.00	30.00-1074.00	55.00-1068.00	0.25-9.81	0.25-9.81	1.35-9.20	3.48-6.74	3.48-6.74	3.71-5.83	3.60-6.70	3.60-6.70	3.70-6.35	5.86-6.76	5.86-6.76	5.99-6.60	0.07-0.11	0.07-0.11	0.07-0.10
Mean	410.25	403.49	433.34	5.76	5.67	6.05	4.87	4.92	4.67	4.73	4.75	4.65	6.31	6.31	6.29	0.09	0.08	0.09
SD	288.68	280.13	69.43	2.09	2.14	1.94	0.67	0.68	0.62	0.61	0.60	0.66	0.17	0.18	0.16	0.01	0.009	0.008
CV	70.37	69.42	73.73	36.28	37.74	32.07	13.76	13.82	13.28	12.90	12.63	14.19	2.69	2.85	2.54	11.11	11.25	8.89

Table 2.

Calibration statistics for NIR-based models for predicting internal quality parameters in intact summer squash. Training set C1.

Parameter	Math treatment	N	Range	Mean	SD	SECV	r^2	CV (%)	RPD
Nitrates (mg kg^{-1})	2,5,5,1	139	30.00-1074.00	386.64	271.82	112.44	0.83	29.08	2.42
Firmness (N)	2,10,5,1	145	1.30-9.81	5.88	1.98	1.46	0.45	24.83	1.36
Dry matter (% fw)	2,10,5,1	145	3.48-6.74	4.84	0.65	0.38	0.66	7.85	1.71
SSC (ºBrix)	1,5,5,1	144	3.70-6.00	4.71	0.58	0.33	0.68	7.01	1.76
pH	1,5,5,1	144	5.86-6.62	6.30	0.17	0.11	0.57	1.75	1.55
Titratable acidity (% citric acid)	2,5,5,1	148	0.07-0.11	0.09	0.01	0.01	0.44	7.54	1.35

Table 3

MPLS regression statistics for NIR-based models for predicting internal quality parameters in intact summer squash.

Parameter	Math treatment	Calibration								Validation				
		N	Range	Mean	SD	SECV	r^2	CV (%)	RPD	N	r^2	SEP	SEP (c)	Bias
Nitrates (mg kg^{-1})	2,5,5,1	115	30.00-1074.00	398.82	276.8	145.04	0.73	36.37	1.91	32	0.67	163.75	165.55	16.25
Dry matter (% fw)	1,5,5,1	111	3.48-6.74	4.90	0.66	0.38	0.67	7.69	1.75	34	0.50	0.48	0.47	-0.09
SSC (ºBrix)	1,10,5,1	107	3.70-6.00	4.73	0.50	0.32	0.59	6.82	1.56	34	0.35	0.53	0.54	-0.02
pH	2,5,5,1	113	5.86-6.62	6.31	0.17	0.11	0.56	1.81	1.50	34	0.33	0.14	0.14	-0.01

Fig. 1. Mean and standard deviation spectrum for summer squash

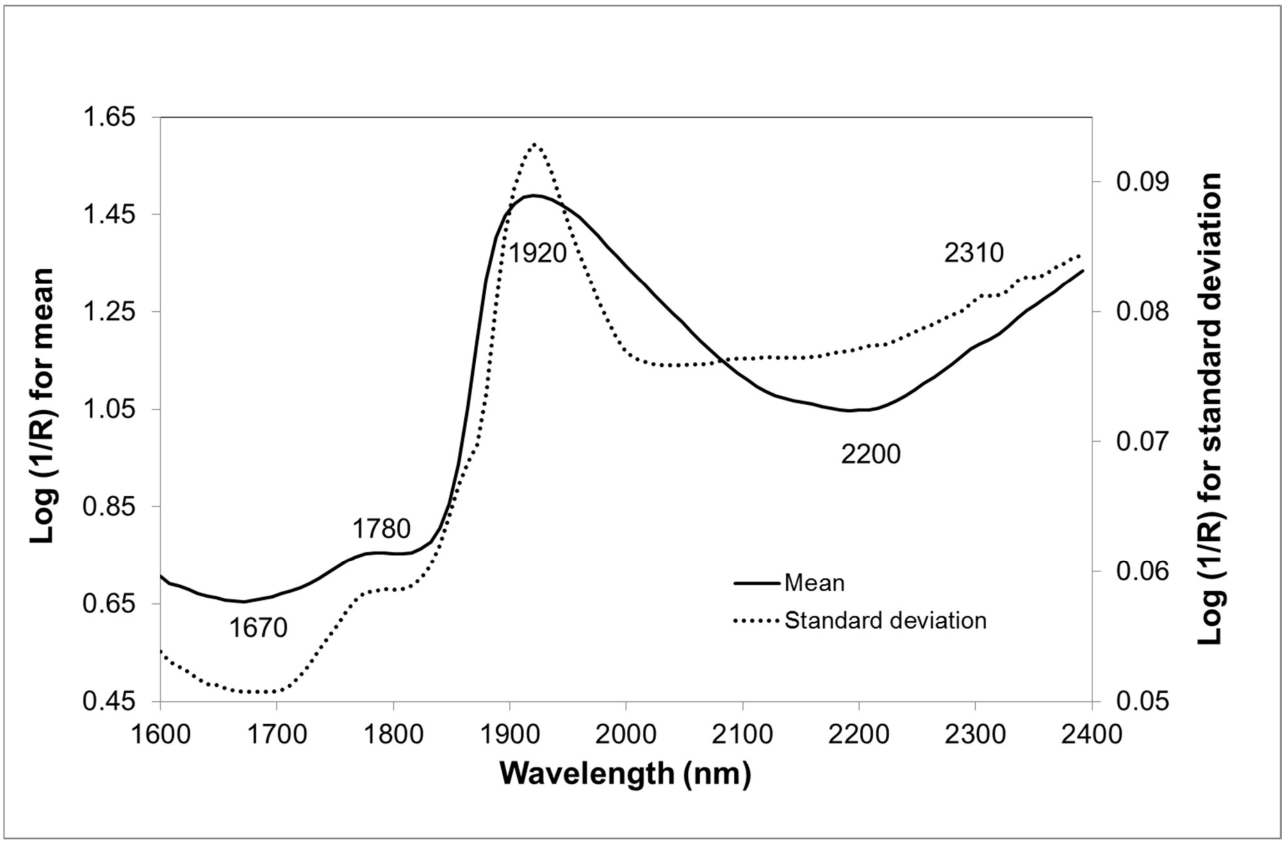

Fig. 2. Distribution of nitrate content for intact summer squashes during on-vine ripening.

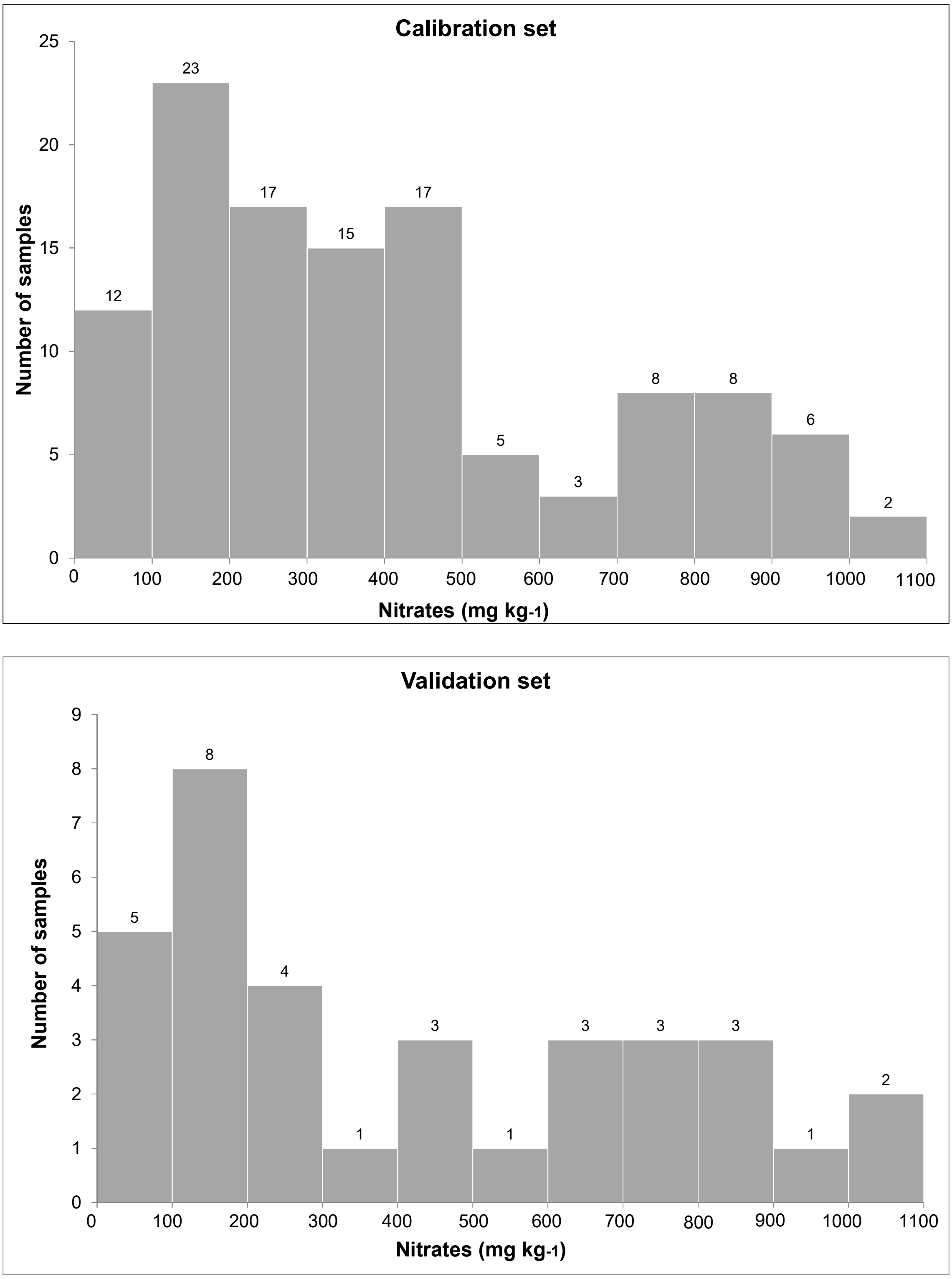

Fig. 3. x-Loading weights (a) and regression coefficients (b) for summer squash nitrate content during on-vine ripening.