Evaluation of Quality Changes in Walnut Kernels (*Juglans regia* L.) by Vis/NIR Spectroscopy

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Storage of walnut kernels in light and at room temperature, as is common practice, is detrimental to their sensory quality and shelf life. This study demonstrates that Vis/NIR spectroscopy, in combination with multivariate data analysis (chemometrics), is a most capable rapid method for monitoring the overall quality deterioration of walnut kernels. Spectral predictions of the sensory attributes nutty and rancid tastes by partial least-squares regression (PLSR) resulted in correlations (r^2) of 0.77 and 0.86, respectively, whereas with PLSR prediction of the chemical parameter hexanal content a correlation (r^2) of 0.72 was obtained. The study further establishes that storage in light results in pronounced oxidative changes, especially in walnuts stored at 21 °C, whereas dark storage at 5 °C results in walnuts without any trace of rancid taste during 25 weeks of storage at accelerated storage conditions (50% oxygen).

Keywords: *Walnuts (Juglans regia L.); sensory evaluation; lipid oxidation; light; temperature; NIR; Vis; PCA; PLSR*

INTRODUCTION

Near-infrared (NIR) spectroscopy in combination with chemometrics has become an established method for rapid and nondestructive assessment of quality parameters in the food and agricultural sectors. Increased demands by consumers, legislators, and competitors have been the impetus for development of new quality-monitoring tools in the food industry. On-line noncontact spectroscopic measurements are the only measuring techniques that can meet these demands, and near-infrared (NIR) spectroscopy has proven to be an extremely reliable and informative spectral technique. It was recognized early that the almost holographic vibrational overtone and combination bands residing in the near-infrared spectral region (780−2500 nm) contain an abundance of chemical information comparable to that of the mid-infrared region.

The aim of the present study was to investigate whether Vis/NIR spectroscopy and chemometrics can replace the more traditional chemical analyses (peroxide value (PV) and headspace gas chromatography (GC)) and sensory evaluations of walnut quality, primarily linked to the lipid phase. We have previously demonstrated that Vis/NIR spectroscopy can serve as a good general-purpose technique for monitoring deterioration in frying oils (*1*) with correlations (r^2) of 0.99 and 0.87 to amount of free fatty acids and anisidine value, respectively. And in a study on soybean oils, Yildiz et al. (*2*) demonstrated that NIR spectroscopy was able to measure oxidation levels with predictions of PV and conjugated diene value with correlations (r^2) of 0.99 and 0.89, respectively. NIR spectroscopy has also been proven able to detect minor variations in oil content and quality of more complex food matrixes such as mayonnaise (*3*). In porcine meat, Vis has proven to be able to predict the TBA value with a correlation (r^2) of 0.92 and to predict the sensory rancidity with a correlation (r^2) of 0.76 (*4*). NIR spectroscopy has shown to be applicable for prediction of consistency attributes in cheese, as correlations (r^2) between 0.74 and 0.88 were obtained; whereas the correlations for the flavor attributes were 0.27−0.59 (*5*).

Walnut kernels have a lipid content of about 65%, of which approximately 70% is unsaturated fatty acids. Because of the high content of unsaturated lipids, the level of lipid oxidation is the most important quality parameter in walnuts: high levels result in oxidation products with an undesirable rancid taste. Antioxidants present in the intact pellicle protect the walnut kernels against oxidation (*6*); however, removal of the shell may damage the kernels and thereby expose the kernels to light and oxygen (*7*). Extrinsic factors such as oxygen, light, temperature, and relative humidity all affect the rate of lipid oxidation. Several studies have unambiguously demonstrated that storage at high oxygen concentrations results in more pronounced lipid oxidation than storage at low oxygen concentrations (*8*, *9*, *10*). The effect of relative humidity (RH) on the quality changes in nuts has also been studied: Erickson et al. (*11*) found no consistent difference in oxidative changes for peanuts stored at 55% and 65% RH, but Maté et al. (*8*) found a significant increase in lipid oxidation for walnut kernels stored at 53% RH when compared to those stored at 21% RH. López et al. (*12*) did not observe any effect of storage temperature on walnut quality when comparing unshelled walnuts stored in the range of 3−10 °C. Senesi et al. (*10*) found that almonds could be stored for up to 9 months at 4 °C or 20 °C without a significant quality loss, and the packaging material (low or high oxygen permeability) did not result in differences in the quality changes. Beyond this storage period, quality could be maintained only by using a packaging material with low oxygen permeability and by storage at 4 °C.

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To examine the performance of Vis/NIR spectroscopy to monitor quality changes in walnuts we have chosen an accelerated experimental design in which we simultaneously investigate the effects of storage under the exposure to fluorescent light and to two different temperatures (5 °C and 21 °C) on the quality of walnut kernels during storage. Peroxide value (PV) represents a classical method for quantification of oxidative rancidity in walnuts (*8*, *13*), pecans (*14*, *15*), peanuts (*8*), and Brazil nuts (*9*). Static headspace gas chromatography (GC) has proven to be a reliable method for monitoring oxidative rancidity in walnuts and peanuts (*8*), as well as in pecans (*14*).

MATERIALS AND METHODS

Chemicals. Chloroform, methanol, hexane, and propane-2-ol were purchased from Labscan (Dublin, Ireland), and sodium sulfate, barium chloride dihydrate, iron(II) sulfate heptahydrate, hydrochloric acid, ammonium thiocyanate, and pulverized iron were purchased from Merck (Darmstadt, Germany). Hydrogen peroxide was purchased from Fluka (Buchs, Schweizerland), 2-heptanone was from Sigma Aldrich (Steinheim, Germany), and Whatman filter NR 1 was from Whatman International Ltd. (Kent, England).

Product and Bulk Packaging. The walnuts (*Juglans regia* L.) were harvested in 1996 in France and stored at 5 °C until May 1997 at which time they were cracked and shelled mechanically. In June 1997, they were bulk-packed in paperboard cartons with an inner plastic bag containing 12.5 kg of walnuts and transported to Denmark. Upon arrival the walnut kernels were packed in plastic bags with low oxygen transmission rate (OTR), nitrogen flushed, and stored at 5 °C until the beginning of December 1997. Each bag contained 1 kg of walnut kernels. The walnut kernels had initial water and total fat contents of 3.6% and 52.7%, respectively, and the fatty acid composition was determined by size-exclusion chromatography: palmitic acid 7.1%, stearic acid 2.3%, oleic acid 17.9%, linoleic acid 62.0%, and linolenic acid 10.7%.

Retail Packaging. The walnuts were packed in transparent plastic laminate (OTR = 0.5 μm/m^2/day/atm; light transmission in the visible region = 90%) and in aluminum-coated plastic laminate (OTR = 0.9 μm/m^2/day/atm; light transmission in the visible region = 0.6%) for comparison of storage in light and darkness. To accelerate lipid oxidation in the walnut kernels the bags were flushed with approximately 50% oxygen and 50% nitrogen during packaging (Komet Nirovac x200, Plochingen, Germany). In this experiment the uneven OTRs of the two foils are of no consequence, as the foils have been chosen for their ability to restrain oxygen. In fact, at least 36% of the oxygen remained in the packages after 25 weeks of storage. To obtain equal amounts of headspace in the bags for chemical and sensory analyses two sizes of bags were used. Walnut kernels in the amount of 50 and 100 g were packed manually in 300 cm^3 and 600 cm^3 bags, respectively. The bags containing 50 g were used for chemical analyses and the bags containing 100 g were used for sensory evaluation.

Retail Storage. The samples were stored at either 5 °C (5.0−5.7 °C) or 21 °C (20.8−22.9 °C). All the samples were stored under fluorescent light (Osram 18 W/31, Taastrup, Denmark) with a radiant flux of 1600 lux. Throughout the storage period the samples were randomly interchanged once every second week in order to minimize unequal light exposure. After 0, 3, 8, 12, 16, 20, and 25 weeks of storage samples were withdrawn for chemical and sensory analyses. At each measurement two identical bags were analyzed, and two replicates were made of the chemical analyses and one assessment of each judge of the sensory evaluation. The results of the chemical and sensory analyses are reported as an average of four and 18 values (2 bags × 9 judges), respectively. Between the different chemical and spectroscopic analyses the samples were vacuum packed and stored at 5 °C in darkness, as all the analyses could not be performed on the same day. Until the sensory evaluation the samples were vacuum packed and held at ∼20 °C in the dark to avoid any further lipid oxidation of the walnut kernels.

Chemical Analyses. *Sample Preparation.* Before chemical analyses the walnut kernels were homogenized for 10 s in a coffee mill (Braun, Germany). For the sensory analyses the walnut kernels were chopped (5 × 5 mm) with a knife.

Gas Composition. Before the bags were opened for analyses, the gas composition was determined using a calibrated Gaspace 2 gas analyzer (Systech Instruments Ltd., Thame, UK). The gas composition was used to identify leaky bags. Bags containing 21% oxygen (the oxygen concentration in atmospheric air) were discarded.

Lipid Extraction. The lipid was extracted with a chloroform/methanol (2:1) mixture according to (*16*) with minor modifications. The homogenized walnuts and the solvent were homogenized with an Ultra Turrax for 15 s at 8000 rpm; the solvent was evaporated using vacuum.

Peroxide Value. Peroxide value was determined according to IDF standard 74A:1991 (*17*) with minor modifications. The chloroform/methanol mixture was substituted by a hexane/propane-2-ol/methanol (5:7:2) mixture. The iron(II) chloride solution was prepared by dissolving approximately 0.8 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) and 1.0 g of iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in 50 mL of water. The solutions were mixed according to IDF standard 74A:1991 (*17*). To prepare the ammonium thiocyanate (NH_4SCN) solution, 30 g of ammonium thiocyanate was dissolved in water to a total volume of 50 mL. Iron(III) chloride standard and hydrochloric acid solution were made according to IDF standard 74A: 1991 (*17*).

To determine the peroxide value, 0.1500 g of walnut oil was mixed in a glass tube with 9.80 mL of hexane/propane-2-ol/methanol (5:7:2). Ammonium thiocyanate solution (25 μL) was added and mixed. Then, the absorbance of the sample (E_0) was determined at 500 nm and the absorbance of the hexane/propane-2-ol/methanol mixture was subtracted. 25 μL of iron(II) solution was added and mixed. After 5 min the absorbance was determined at 500 nm (E_2). The calibration curve was obtained using standard Fe(III) samples containing 0−40 μg Fe(III). Solutions of 0.00 mL (E_1), 0.05, 1.50, 2.50, and 4.00 mL of the standard iron(III) chloride solution were mixed with 9.90, 9.40, 8.40 , 7.40, and 5.90 mL hexane/propane-2-ol/methanol, respectively. Ammonium thiocynate solution (25 μL) and 25 μL of hydrochloric acid solution (0.2 mol/L) were added and mixed. After 5 min the absorbance was measured at 500 nm and the absorbance of the hexane/propane-2-ol/methanol solution was subtracted. The formulas and methods of calculation are described by IDF standard 74A:1991 (*17*).

Hexanal Content. Hexanal in walnuts was determined by headspace GC according to the method proposed by Shahidi and Pegg (*18*) with minor modifications. Samples of homogenized walnuts were accurately weighed (2.0 g) into 20-mL vials, and 2-heptanone (to yield 100 ppm w/w) was added as an internal standard. The samples were preheated in a HP 7694 headspace sampler (Hewlett-Packard, Palo Alto, CA) for 45 min at 90 °C before the vapor phase was transferred to a 3 cm^3 loop (321−056 HSP) under the following conditions: Carrier ga,s helium; vial pressure, 0.90 bar; pressurization time on the vials, 0.13 min; loop fill time, 0.04 min; loop temperature ,100 °C, transfer line temperature, 110 °C, and loop equilibration time, 0.02 min. Chromatographic separation was performed by using a HP 6890 GC-Headspace (Hewlett-Packard, Palo Alto, CA). From the loop, the vapor phase was injected (injection time, 0.40 min; injection temperature, 200 °C) onto a high-polarity HP19095X-123 HP-wax bonded polyethylene glycol column (30.0 m × 530 μm × 1 μm). Helium was the carrier gas employed at an inlet pressure of 0.66 bar with a split ratio of 7:1. The oven temperature programming comprised three steps: 50 °C (5 min), 115 °C (1 min) after heating at 10 °C/minute, followed by 200 °C (1 min) after heating at 30 °C/minute. The flame ionization detector (FID) temperature was 250 °C. Hexanal content (ppm) in samples was quantified by calculating the peak area of hexanal relative to that of the internal standard.

Table 1. Minimum, Maximum, Mean, and Standard Deviation for the Chemical and Sensory Analyses (all the results are based on 50 samples)

	chemical analysis[a]		sensory evaluation[b]			
	peroxide value	hexanal content	nutty taste	sweet taste	rancid taste	bitter taste
min	0.9	0	25.7	40.6	40.0	6.0
max	12.9	202.1	114.0	98.8	108.4	134.1
mean	5.2	29.9	73.2	75.7	72.7	62.0
SD	2.9	49.3	25.2	16.5	18.3	37.5

[a] The units of peroxide value and hexanal content are meq oxygen/kg oil and mg hexanal/kg walnut, respectively. [b] The descriptors were rated on an anchored line scale 150 mm in length.

Sensory Evaluation. The samples for sensory evaluation were pooled and judged at the end of the storage period by a nine-member trained sensory panel. At each session the panelists were served a control reference sample (stored at ~20 °C) and samples from each preceding storage period. For each panel session approximately 20 g of chopped walnuts were placed in small plastic containers approved for sensory analysis and the containers were tightly capped. The samples were allowed to stand for 2 h prior to the examination to allow equilibration of volatiles in the headspace. To calibrate the panel on taste descriptors the reference sample was used to fix reference points on the scale. In each session eight samples were randomly distributed (one at a time) to each panelist. All sensory evaluations took place in individual taste panel booths in a sensory laboratory. Descriptor terms used to describe the sensory quality of walnut kernels were nutty, sweet, bitter, and rancid tastes. The descriptors were rated on an anchored line scale 150 mm in length, with "low intensity" and "high intensity" being the anchor points. Low and high intensity correspond to, for instance, no and much rancid taste, respectively. Values for nutty, sweet, bitter, and rancid tastes for the reference sample were anchored at 114, 96, 40, and 6 mm, respectively. The samples were served in red light to avoid differences in darkness of the walnuts and were evaluated at a temperature of 20 °C. Between each serving the judges rinsed their mouths with sparkling water (room temperature), cucumber, and natural-tasting crackers.

Visible/Near-Infrared Spectroscopy. Visible/near-infrared (Vis/NIR) spectroscopic data in the range from 400 to 2498 nm were collected using a NIRSystems spectrophotometer (model 6500, NIRSystems Inc., Silver Spring, MD). The spectrophotometer uses a split detector system with a silicon (Si) detector between 400 and 1100 nm and a lead sulfide (PbS) detector from 1100 to 2500 nm. The angle of incident light was 180°, and reflectance was measured at a 45° angle. The Vis/NIR reflection spectra were recorded using a rotating sample cup with a quartz window and a compressive paper disk to ensure constant pressure. The collected spectral data were converted to log(1/*R*) units.

Data Handling. The results were evaluated by multivariate data analysis, namely principal component analysis (PCA) and partial least-squares regression (PLSR) (*19*). Prior to the PCA and PLSR analyses, the Vis/NIR spectra were converted to second derivatives in order to remove offset and linear trends in the spectra. PCA and PLSR models were performed on mean-centered and autoscaled (divided by the standard deviation) data and evaluated using segmented cross-validation. Each segment contains data from one storage time only, except one segment which contains data from weeks 0 and 3. Only validated results are reported. Multivariate data analysis was performed using The Unscrambler, version 7.5 (CAMO, Trondheim, Norway).

Statistical analyses of the effects of treatment (storage in light and darkness; 5 °C and 21 °C) and storage time were evaluated as a 2 × 2 full-factor experiment by general linear model using SAS, version 6.12 (SAS Institute Inc., Cary, NC). The analyses were performed for the response variables peroxide value, hexanal content, and sensory evaluation. Significant treatment effects were further classified by LSD ($p \leq 0.05$).

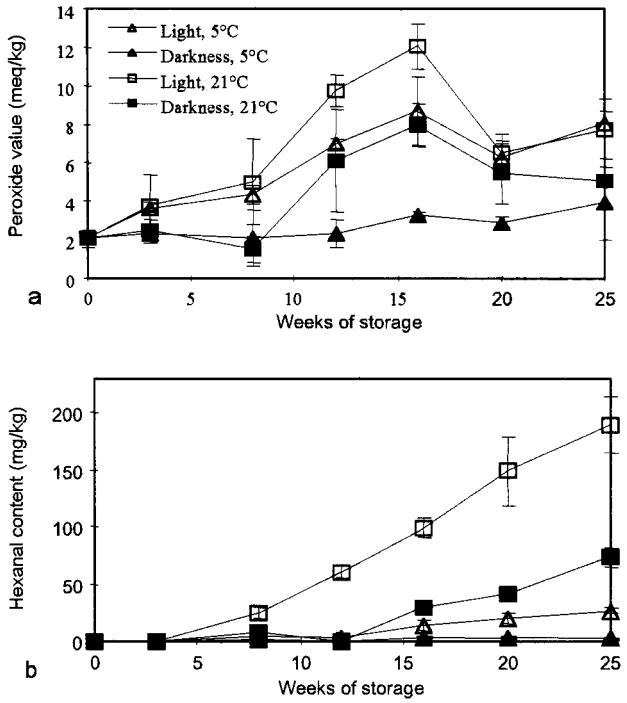

Figure 1. Chemical assessment of walnut lipid oxidation measured as (a) peroxide value (meq/kg lipid) and (b) hexanal content (mg/kg). Bars indicate the standard deviation.

RESULTS

Chemical and Sensory Characterization. To establish spectral calibration models of walnut quality from the Vis/NIR spectra, a thorough chemical and sensory characterization of the samples is required. In this work we have chosen to use peroxide value, hexanal content, and the sensory descriptors nutty, sweet, rancid, and bitter tastes in order to evaluate the samples. Table 1 gives an overview of the chemical and sensory evaluation of the walnut samples. From the table it can be seen that, e.g., the range for the hexanal content is 0−202 mg/kg. In addition, the mean and standard deviation (SD) are 30 mg/kg and 49 mg/kg, respectively, indicating that most of the samples have a low hexanal content; i.e., that most of the data for the walnut samples analyzed are within a range that is realistic for commercially stored walnuts.

Figure 1a, which displays the PV as a function of storage time, exhibits the classical pattern trend of primary oxidation products. The initial increase in PV is followed by a decrease for three of the samples (storage in light at 5 °C and in light and darkness at 21 °C) when the peroxides subsequently are decomposed to form secondary oxidation components, e.g. hexanal, during storage. After 16 weeks of storage walnuts stored in light and darkness at 21 °C and in light at 5 °C reached a maximum, whereas walnuts stored at 5 °C

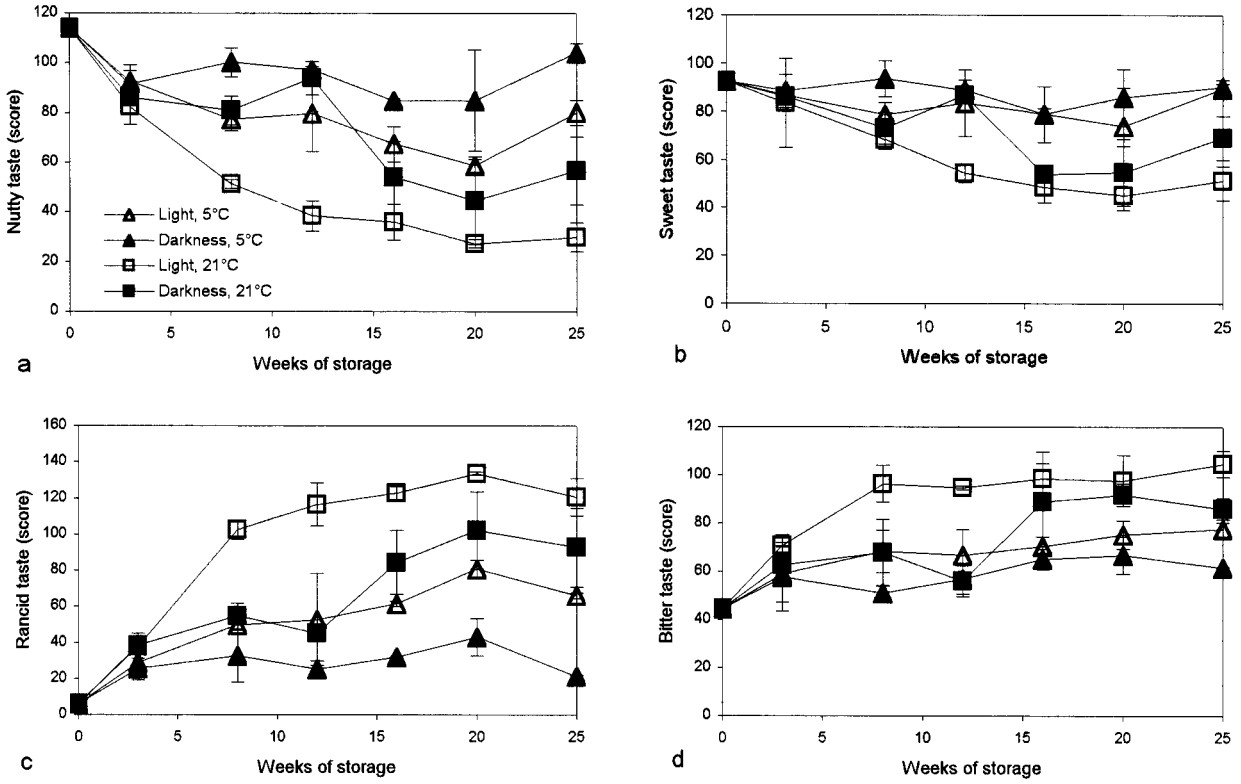

Figure 2. Sensory assessment of walnut lipid oxidation expressed as (a) nutty, (b) sweet, (c) rancid, and (d) bitter tastes where high scores correspond to high intensity of taste. Bars indicate the standard deviation.

and in darkness did not reach a maximum during the 25 weeks at accelerated storage conditions (50% oxygen). Storage in light results in significantly ($p < 0.001$) higher peroxide value than storage in darkness.

Formation of secondary oxidation products was followed by determination of the hexanal content (Figure 1b). The interaction between storage time, light, and temperature found in the variance analysis indicates that the effects of light and temperature vary independently of each other during the storage period. After 8 weeks of storage the hexanal content increased significantly for walnut kernels stored in light at 21 °C, and thereafter the hexanal content was significantly ($p \leq 0.05$) higher than the content in walnuts stored under the three other storage conditions. The walnuts stored in darkness at 21 °C did not display a significant increase in hexanal content until after 16 weeks of storage. The hexanal content in walnut kernels stored in darkness at 5 °C did not increase significantly ($p > 0.05$) during the 25 weeks of storage. After 25 weeks of storage. the walnuts stored at 21 °C (in light and darkness) had significantly ($p \leq 0.05$) higher hexanal content than the walnuts stored at 5 °C (in light and darkness); and storage in light resulted also in a higher hexanal content than storage in darkness ($p \leq 0.05$) irrespective of the storage temperature.

The changes in walnut quality as followed by the chemical analyses naturally also resulted in changes of sensory attributes. As can be seen from Figure 2, the sensory scores for (a) nutty and (b) sweet tastes decreased, whereas the (c) rancid and (d) bitter tastes increased from week 0 to week 25 for walnut kernels stored at 5 °C in light and at 21 °C in light and darkness; i.e., the walnuts' sensory profiles changed from nutty and sweet tastes to include also rancid and bitter tastes with storage. Because sweet and nutty tastes, and bitter and rancid tastes, in this study correlate strongly, only the changes in nutty and rancid tastes are described in detail below. Walnut kernels stored in light at 21 °C had a significantly ($p \leq 0.05$) less nutty taste than walnuts stored under the three other storage conditions after 8, 12, 16, and 25 weeks of storage. Already after 8 weeks of storage the rancid taste of walnuts stored at 21 °C and in light was significantly ($p \leq 0.05$) higher than that of the walnuts under the other storage conditions. With respect to the nutty taste, there was a significant ($p \leq 0.05$) difference between all four different storage conditions after 25 weeks. Comparison of rancid taste after 16 and 25 weeks showed that the four storage conditions differ significantly ($p \leq 0.05$). It can be concluded that storage at 21 °C or in light resulted in more rancid and less nutty-tasting walnuts than storage at 5 °C or in darkness. For walnuts stored at 5 °C in darkness the nutty and rancid tastes remained at their initial levels during the 25 weeks of storage. Similarly, a combination of storage in light and at 21 °C resulted in walnut kernels with a very high degree of rancid taste.

Principal Component Analysis. To analyze the relationship between chemical and sensory analyses a principal component analysis (PCA) was performed. The loading plot (Figure 3) shows the first two principal components (PCs), which describe 90% (81% + 9%) of the total variance in the data material. The plot clearly illustrates that the chemical and sensory (sweet, nutty, bitter, and rancid tastes) analyses are strongly intercorrelated (lying on an axis passing through origo). Only the peroxide value appears to contain independent (orthogonal) information. Nutty and sweet tastes are negatively correlated to bitter and rancid tastes, hexanal, and peroxide value. In addition, the first PC shows that the hexanal content is positively correlated to bitter and rancid tastes. The second PC almost entirely describes the variation in peroxide value.

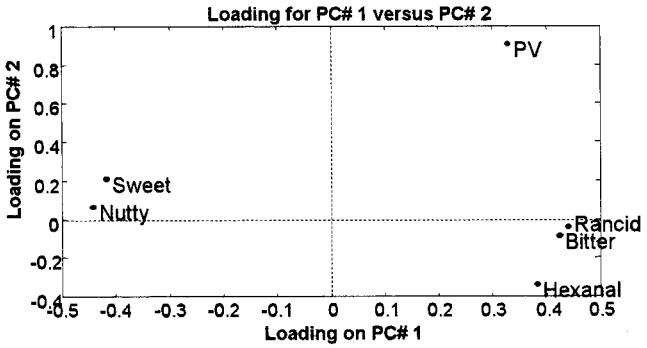

Figure 3. Loading plot (X-loadings) from principal component analysis (PCA) on chemical (peroxide value (PV) and hexanal content) and sensory (sweet, nutty, bitter, and rancid tastes) data. The two first principal components (PC) describe 81 and 9% of the total variation, respectively.

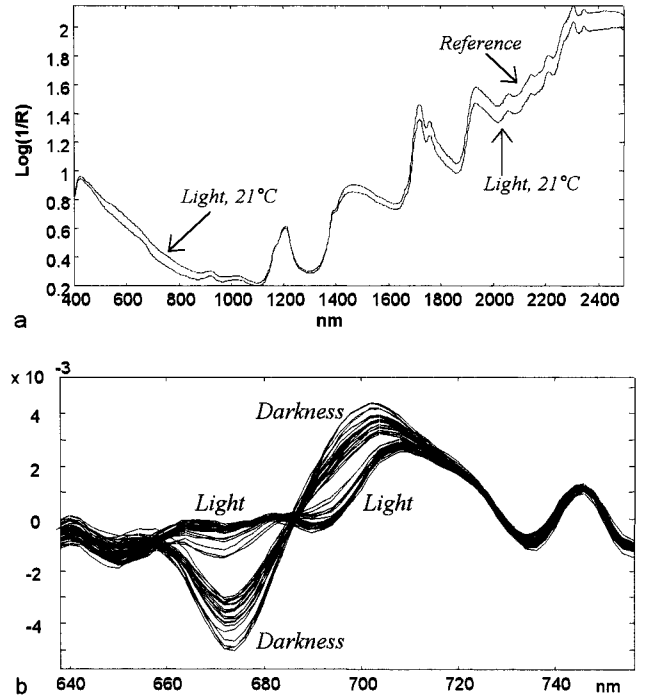

Figure 4. Line plot of (a) vis/NIR spectra of the reference and walnut stored for 25 weeks in light at 21 °C and (b) the second derivatives of the interval 650−750 nm of the vis/NIR spectra of all the samples.

One of the main aims of this study was to investigate whether the rapid and noninvasive Vis/NIR spectroscopy in combination with chemometrics could describe the overall quality changes in walnut kernels. The difference in Vis/NIR spectra between a rancid and a nonrancid walnut is shown in Figure 4a where Vis/NIR spectra for the reference walnut and walnuts stored for 25 weeks in light at 21 °C are shown. Several different pretransformations of the Vis/NIR spectra were investigated by principal component analysis (PCA). The best classification of the samples was achieved by using the second derivatives of the spectra in the intervals 650−750 nm (Vis) or 1850−1980 nm (NIR). Figure 4b shows the spectral difference between light and dark storage of the second derivatives in the interval 650−750 nm. Figure 5a shows a score plot for this relatively narrow spectral region as a function of the first two PC's, which explain 99% (98% + 1%) of the total variance. The plot illustrates how the Vis data from the spectral interval 650−750 nm can classify the samples as being stored

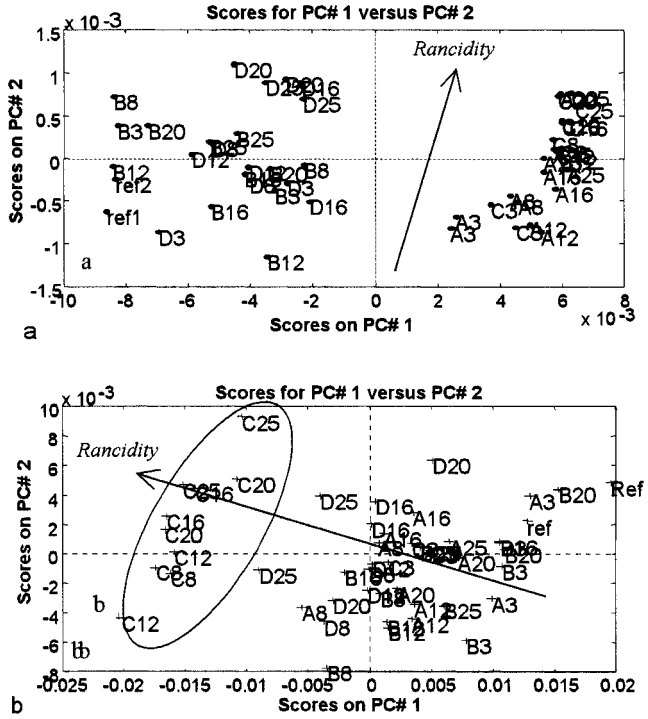

Figure 5. Score plot from principal component analysis (PCA) on second derivatives of (a) 650−750 nm (X-expl. 98%, 1%) and (b) 1850−1980 nm (X-expl. 84%, 11%) of the vis/NIR spectra. Walnuts have been stored at 5 °C in light (A), 5 °C in darkness (B), 21 °C in light (C), and 21 °C in darkness (D), respectively (ref = reference). Numbers correspond to weeks of storage.

in light or darkness: samples to the right are stored in light and samples to the left are stored in darkness. The score plot also displays a trend in rancidity: samples in the top of the score plot are more rancid than samples in the bottom of the plot. Figure 5b shows the score plot for a part of the second derivatives of the NIR spectra (1850−1980 nm) as a function of PC1 and PC2, which explain 95% (84% + 11%) of the variance. In the score plot the walnuts stored at 21 °C in light (C) are separated from the other samples, which are not divided clearly. As in Figure 5a, a trend with respect to the degree of rancidity is shown.

Partial Least-Squares Regression Models. PLSR models between the chemical, sensory, and Vis/NIR data were also investigated. Table 2 shows the number of PLSR factors (which is the PLSR analogue to principal components), the prediction error (RMSECV), and the correlation coefficients (r^2) for the models. Correlations between peroxide value and sensory scores result in low correlation coefficients, i.e., peroxide value does not describe the variation in the sensory evaluation very well (from 28% to 56% of the variation). On the other hand, the hexanal content gave a much better explanation of the sensory scores, as hexanal can describe 61−68% of the variation in the sensory evaluation. A weak but significant synergistic effect between hexanal and peroxide values was detected, as a combination of peroxide value and hexanal resulted in better predictions (between 75 and 78%) of the sensory changes than by hexanal or peroxide value alone.

The PLSR models in Table 2 are constructed with either unchanged Vis/NIR spectra or (if indicated) second derivative Vis/NIR spectra. The models are both constructed using the whole spectra (400−2498 nm) and for selected intervals which resulted in the best separa-

Table 2. PLSR Correlations (r^2) between Chemical, Spectroscopic, and Sensory Analysis

		sensory evaluation				chemical analysis	
		nutty taste	sweet taste	bitter taste	rancid taste	peroxide value	hexanal content
peroxide value	PC (number)[a]	1	1	1	1		1
	RMSECV[b]	17.7	13.2	10.7	24.5	-	45.0
	$r^{2,\,c}$	0.46	0.28	0.56	0.49		0.19
hexanal content	PC (number)	1	1	1	1	1	
	RMSECV	14.4	9.1	9.1	18.8	2.5	-
	r^2	0.61	0.66	0.66	0.68	0.28	
PV + hexanal	PC (number)	2	2	2	2		
	RMSECV	11.5	9.1	8.4	16.5	-	-
	r^2	0.75	0.65	0.72	0.78		
				vis/NIR			
400−2498 nm	PC (number)	3	4	2	4	3	5
	RMSECV	11.7	7.8	8.8	13.4	1.9	26.2
	r^2	0.77[d]	0.76	0.75	0.86[d]	0.55[d]	0.72
650−750 nm	PC (number)	2	2	5	2	3	2
	RMSECV	13.1	10.3	7.7	17.1	1.5	30.7
	r^2	0.72[d]	0.60[d]	0.82[d]	0.78[d]	0.68[d]	0.51[d]
1850−1980 nm	PC (number)	3	2	4	4	1	3
	RMSECV	13.0	9.6	8.5	15.2	2.5	27.8
	r^2	0.71[d]	0.63[d]	0.76[d]	0.83[d]	0.28[d]	0.69[d]

[a] The number of principal components (PC) providing the optimal PLSR fit. [b] RMSECV = root-mean-square error of cross-validation. [c] The fully cross-validated correlation coefficient r^2. When multiplied by 100, it provides the percentage of explained variance. [d] Second derivation of vis/NIR.

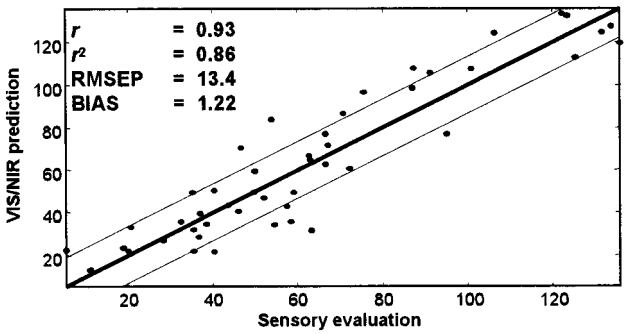

Figure 6. Predicted vs measured for the PLSR model constructed between vis/NIR (400−2498 nm) and rancid taste. The correlation coefficient (r^2) for the model is 0.86.

tion of the samples in a PCA. These intervals were found to be 650−750 and 1850−1980 nm. The best descriptions of nutty (0.77), sweet (0.76), and rancid tastes (0.86) were obtained using the second derivatives of 400−2498 nm, whereas bitter taste (0.82) and peroxide value (0.68) were best described with the second derivatives of 650−750 nm (Vis). Figure 6 shows the PLSR model constructed between Vis/NIR spectra (400−2498 nm) and rancid taste. A PLSR model constructed between unchanged Vis/NIR spectra (400−2498 nm) and hexanal content resulted in an explanation of 72%. Although the interval 1850−1980 nm contained information to separate walnuts stored in light at 21 °C from the other samples, this interval does not describe the sensory and chemical changes as well as the intervals mentioned above.

DISCUSSION

The walnut kernels used in this experiment had high initial quality. This can be seen from the low content of peroxides and hexanal (Figure 1). After the accelerated storage period of 25 weeks the quality of the walnuts was still excellent when stored at 5 °C and in darkness.

In most of the storage period (from weeks 8 to 25) walnut kernels stored in light at 21 °C were of lower quality than walnuts from the three other storage conditions, when evaluated on the basis of the hexanal content, and nutty (except the 20th week) and rancid tastes (Figures 1b and 2). The very strong relationships between lipid oxidation (hexanal content) and nutty and rancid tastes, respectively, observed in the present study suggest that lipid oxidation is the primary parameter determining the sensory quality of the product. This relationship is very pronounced for walnuts stored in light at 21 °C. The high correlations between hexanal content, and nutty and rancid tastes, respectively, support this relationship, because variations in hexanal content can explain 61% and 68% of the total variation. The comparison of chemical and sensory analyses showed a positive correlation between hexanal and rancid/bitter tastes, i.e., as the hexanal content increases, the walnuts taste more rancid and bitter. As expected, the comparison showed a negative correlation between nutty/sweet tastes and rancid /bitter tastes, i.e., when walnuts become more rancid and bitter, they taste less nutty and sweet. A PCA on the reference measurements showed that only the peroxide value provided information independent of (orthogonal to) the other chemical and sensory analyses. This may be explained by the fact that there is an initial increase in the formation of peroxides followed by a decrease, whereas the hexanal content and the rancid and bitter tastes increase during the entire storage period. The peroxide value, and thereby the primary oxidation product, is therefore a good descriptor of lipid oxidation only in the initial process. This initial information may explain why the combination of peroxide value and hexanal (peroxide value + hexanal) results in an improvement of the explained variance (up to 78%).

A PCA model of the second derivatives of the visible (650−750 nm) part of the spectra showed a classification between samples stored in light and samples stored in darkness including a vertical trend with respect to rancidity. This part of the spectra gives an excellent description of bitter and rancid tastes, as the explanations are 82% and 78%, respectively. During the storage experiment it was clear that walnuts stored in light became darker than walnuts stored in darkness (the sensory evaluation took place in red light). Erickson et al. (*11*) and Forbus et al. (*14*) showed that Hunter L

and b values decreased, and Hunter a value increased, during 12 weeks of storage of pecan kernels; i.e., the surfaces of the kernels became darker. After 19 weeks of storage the changes in Hunter L, a, and b values became inconsistent (*11*). Comparison of sensory scores and Hunter L, a, and b resulted in correlations (r^2) of 0.85, 0.50, and 0.81, respectively (*14*). There is no doubt that the change in darkness during storage for walnuts stored in light is due to the chromophore at approximately 660−700 nm. However, we have not been able to assign this to the substance responsible for the change.

The second derivatives of the near-infrared part (1850−1980 nm) of the spectra (Figure 5) showed a diagonal degree of rancidity in a score plot and separated the walnuts stored at 21 °C in light (C) from the other samples. Whereas 1400−1450 nm indicates changes in the aliphatic C−H bonding patterns, 1850−1980 nm contains no such information and should rather be assigned to changes in the second overtone of the carbonyl (C═O) bonding, perhaps the creation of secondary oxidation products such as aldehydes, ketones, and free fatty acids. However, it should be kept in mind that this part of the spectra is not able to predict the sensory and chemical changes as well as the second derivatives of the whole spectra (400−2498 nm). The second derivatives of the whole spectra were, through PLSR, able to describe 77% of the total variation in the nutty taste and 86% of the rancid taste. These results are of approximately the same precision as NIR correlations obtained for other sensory attributes such as warmed-over flavor of processed meat (*4*) and to flavor attributes in cheese (*5*).

In conclusion, we have demonstrated the excellent potential of Vis/NIR spectroscopy for monitoring quality changes in walnut kernels. Vis/NIR spectroscopy evaluated by chemometrics was able to predict the four sensory scores simultaneously with higher precision than the two chemical descriptors of peroxide value and hexanal content. However, because of large biological differences between walnut species, the developed models may be valid only for walnuts similar to those used in this study.

Walnuts are traditionally considered to have long shelf life, even when stored at room temperature, and very often commercial walnut kernels are packed in plastic materials with a low barrier to both oxygen and light. These packaging materials allow almost unlimited access of oxygen and light to the walnuts. This study clearly demonstrates that significant sensory quality improvements can be obtained if walnuts are stored at 5 °C with no exposure to light. On the contrary, the combination of room temperature (21 °C) and transparent packing materials will result in a very short shelf life of walnuts. To keep lipid oxidation at the lowest possible level, and thereby prevent rancid taste, it is recommended that walnuts be stored at 5 °C in darkness.

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