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Detection of ochratoxin A contaminated dried vine fruit by FTIR-ATR infrared spectroscopy*

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Abstract

Methodology for screening contaminated sultanas for ochratoxin A (OTA) using mid-infrared spectroscopy/one single reflection ATR Golden Gate is described. A preliminary study involved the acquisition and analysis of the spectra of sultanas from different origins allowing the identification of their principal spectral characteristics. After spectra were recorded, they were subject to principal component analysis (PCA), which showed that samples from various origins had different spectral characteristics, especially in the water content and in the fingerprint region. Samples of sultanas from a specific origin were split into small sub-samples and then were analysed on various days. The repeatability of the spectra was highly improved when the water activity of the sample was set at 0.62. A calibration curve of OTA was built in a range of 10–40 $\mu\text{g OTA kg}^{-1}$. Concentrations of OTA higher than 20 $\mu\text{g OTA kg}^{-1}$ were separated from samples contaminated with a lower concentration (10 $\mu\text{g OTA kg}^{-1}$) and from no contaminated samples. The methodology reported here opens the possibility of the application of a reliable and simple methodology for screening contaminated dried vine fruit.

Keywords: screening, sultanas, origin, ochratoxin A (OTA), FTIR-ATR spectroscopy.

Introduction

Mycotoxin-producing mould species are extremely common; they can grow on a large range of substrates and under a wide range of environmental conditions. Ochratoxin A (OTA) was discovered as a metabolite of *Aspergillus ochraceus* in 1965 and subsequently the toxic effects of (OTA) have been reported (Bennett and Klich 2003). In addition to being a human nephrotoxin, studies involving animals indicated that OTA is a liver toxin, an immune suppressant, a potent teratogen, and a carcinogen. The occurrence of OTA in baby food, barley, corn, beer, coffee and wine as well as the analytical methods used for its detection have been well documented by Gilbert and Anklam (2002). Mycotoxins can enter the food chain in the field, during storage or at later points (Delage et al. 2003). The incidence of Ochratoxin A (OTA) in grapes was correlated with the presence of OTA in wine (Sage et al. 2002).

For the detection of food contamination, rapid, simple and sensitive methods with high degree of automation which can meet the specific necessities of food industries are required. Routinely methods for detection and determination of the levels of mycotoxins make use of various analytical techniques as thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) which provide reliable results but are expensive and time consuming methodologies (Mossoba et al. 1996).

Krska et al (2005) reported the most recent advances in the area of rapid methodologies for mycotoxins detection with their principal applications and their limitations as the 'false positive' or 'false negative' results often occurred with the ELISA method or the overestimation of the contamination when applied in some food matrixes. Until now the main applications reported for the near infrared (NIR) and the Fourier transform infrared spectroscopy (FTIR) in the food industry were for authentication of food products (Defernez et al. 1995, Briandet et al. 1996) and for compositional information (Bruno-Soares et al. 1998). However the possibility of the application of spectroscopic techniques as the near infrared (NIR) and the Fourier transform infrared spectroscopy (FTIR) as a tools for screening fungal infection in agricultural commodities showed promising results (Gordon et al. 1997, Petterson and Aberg 2003). Results of the application of mid-infrared diffuse reflection (DR) and attenuated total reflection (ATR) spectroscopy for the detection of fungal infection in corn were reported by Kos et al. (2004); the outcomes of the ATR were very optimistic

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3 as the performance on the classification of contaminated samples was very high,
4 preceded by a simple methodology for sample preparation, and with the perspective of
5 automatization introducing the likelihood of the on-line control during the process.
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8 For any of the methodologies mentioned previously for the determination of the
9 contamination sample preparation represents a potential problem as the
10 heterogeneous distribution of the mycotoxin in the sample can introduce high variability
11 on the results (Kos et al. 2003, Möller and Nyberg 2003).
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16 In the present work the main objectives pursued were (i) The identification of the main
17 features of sultana's FTIR spectra by screening samples from different origins (ii) A
18 study of the factors that could be involved in the reproducibility of the spectra and (iii)
19 The separation of samples contaminated with OTA at different levels of concentration
20 from the uncontaminated ones.
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25 26 **Materials and methods**

27 28 29 *Samples preparation*

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31 Sultanas of different origins (viz. Chile, South Africa and Turkey) and brands were
32 purchased at the local market in Portugal; most of the samples were bought as
33 packages of 250 g. A total of 5 samples were analysed which included samples from
34 Turkey (N=2), from Chile (N=2) and from South Africa (N=1).
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40 Initially spectra of sultanas from different origins were acquired in order to recover their
41 principal spectra characteristics. A puree of each origin was prepared separately using
42 5 g of fruit; this amount was selected as the heterogeneous distribution of any
43 contamination in the sample could be masked in higher amounts. Spectra
44 reproducibility was studied using only the samples from Chile. Since spectra
45 reproducibility was a concern and most of the samples were under protected packed
46 conditions, the sultanas from Chile were introduced inside four chambers
47 preconditioned with salts of different water activities (a_w) and allowed to get equilibrium
48 for posterior analysis. The salts used for precondition treatments were ammonium
49 nitrate (NH_4NO_3) $a_w = 0.62$, sodium chloride (NaCl) $a_w = 0.75$, potassium chloride (KCl)
50 $a_w = 0.84$ and barium chloride di-hydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) $a_w = 0.90$ all from Sigma
51 Chemicals, St. Louis, MO. Control 1 and Control 2 were samples without any
52 treatment.
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Ochratoxin A (OTA) reference

A standard of 10 μg OTA ml^{-1} (Sigma Chemicals, St. Louis, MO) was used for the preparation of a calibration curve in which the standard was directly added on no contaminated sample, the volumes added in the samples cover a range of $C1 = 10$ $C2 = 20$ and $C3 = 40$ μg OTA kg^{-1} .

Instrumental Setup

All spectra were acquired using a spectrometer (Brüker IFS 55, Germany) set up for mid-infrared measurements equipped with a horizontal one single reflection ATR Golden Gate (Specac, Germany). The software OPUS v. 5.0 (Brüker, Germany) was programmed to record each spectrum between 4000 and 600 cm^{-1} at a resolution of 8 cm^{-1} . Each spectrum was made up of 845 data points. Samples and background measurements were made by adding together 128 scans for each spectrum before Fourier transformation. The interferometer was operated at a laser frequency of 10 kHz and in the single-sided directional mode. Fourier transformation was done with a Mertz phase correction, a Triangular apodization function, with a zero-filling factor of 2. At least fifteen spectrum replicates were recorded for each sample according to their origin, storage precondition and concentration of OTA.

Data analysis

Data analysis was performed using the program CATS 97 (Barros 1999). Principal component analysis (PCA) was used for reducing the dimensionality of the data and to extract the main sources of variability. For calculations all data were arranged in a way that absorbance values were displayed in columns and each row of data represented one sample. Outliers were detected and removed using the Mahalanobis criteria before PCA final analysis, and the datasets were SNV (standard normal deviate) corrected. The PLS1 algorithm was used for modelling the regression. Standard errors of calibration and cross-validation were calculated to assess the quality of the regression.

Results and discussion

Figure 1 shows typical spectra of sultanas from three origins and different brands. The analysis of the whole spectra show small differences specially in the regions between 3600 to 2850 cm^{-1} which correspond to the $-\text{O}-\text{H}$ and $-\text{C}-\text{H}$ groups (I), in the region of 1725 to 1570 cm^{-1} which show bands arising largely from pectins and in the fingerprint region from 1250 to 1000 cm^{-1} (II).

[Insert figure 1 about here]

The PCA analysis of the sultanas shows a separation between the samples according to their origin (figure 2). The loading profile of PC1 (figure 3a) shows that the separation of the sultanas by their origin is due mainly to water, fat and sugar contents with bands located at 3568, 1720 and 1022 cm^{-1} , respectively. The loading profile of PC2 (figure 3b) confirms that samples from South Africa had high water content with a positive PC1 and PC2 (figure 1). Samples from Chile were characterized by a band at 1720 cm^{-1} , negative PC1, corresponding to the fat content. Finally sultanas from Turkey had higher sugar content than those from South Africa; the former ones having a negative PC2, with a band characteristic of fructose at 1049 cm^{-1} (figure 3b).

[Insert figure 2 and 3 about here]

A lack of reproducibility was observed in the spectra of sultanas acquired on different days of analysis. Because some changes could occur once fruit were removed from the package, contributing to the problems of spectral reproducibility, the pre-condition of sultanas with different water activity (a_w) was assessed so that the methodology could avoid or reduce this artefact, allowing the separation of the contaminated samples from the uncontaminated ones. Furthermore, this precondition step may allow the addition of new samples to build the model.

In order to see the effect of the precondition treatment, only samples from Chile were used. The spectral reproducibility was much better in samples pre-conditioned with water activities set at 0.62 and 0.75 than in those with the a_w set at 0.84 and 0.90 and in the control ones, which showed higher degree of dispersion in the cluster formation (figure 4). The PCA analysis also showed a separation of the samples according to the water activity treatment, sultanas from Chile with a_w of 0.75 or higher were separated from those with lower a_w and from the ones used as control (figure 4). The samples with an a_w of 0.75 or higher were characterised by two bands, one located at 3537 cm^{-1} and the other found at 1639 cm^{-1} , corresponding to the $-\text{O}-\text{H}$ group shown in figure 5. On the other hand, the samples treated with ammonium nitrate ($a_w = 0.62$) and the control samples were characterised by the band placed at 1010 cm^{-1} corresponding to glucose (Figure 5).

[Insert figure 4 and 5 about here]

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3 For the PCA analysis of samples pre-conditioned at different a_w various spectral
4 regions were analysed, the 1250-1000 cm^{-1} region was chosen as giving the best
5 results in terms of the introduction of new samples to an existing set of data. As it can
6 be seen in figure 6 the scores for the scatter plot of sultanas from Chile analysed on
7 two different days showed that the precondition of the samples to an a_w of 0.62 allowed
8 their introduction into a set of samples previously analysed (control 1 and 2). It is
9 important to note that in this region the main features arise mainly from coupled C–O
10 modes corresponding to fructose and glucose carbohydrates, the former one is a very
11 hygroscopic compound and by the pre-condition treatment at 0.62 this carbohydrate
12 might be stable (Figure 7a, b).
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26 The PCA analysis of samples from Chile contaminated with different concentrations of
27 OTA showed a separation of the samples with concentrations of OTA of $C_2 = 20$ and
28 $C_3 = 40 \mu\text{g kg}^{-1}$ from those with the lowest concentration $C_1 = 10 \mu\text{g kg}^{-1}$ and from the
29 control samples (Figure 8). The intensities of bands located at 3160 cm^{-1} , and 1550 cm^{-1}
30 and in the *fingerprint* region were very high as is shown in the figure 9.
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35 [Insert figure 8 and 9 about here]
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39 A cross validation was carried out using leave-3-out spectra and PLS1 model was
40 performed using the spectra obtained from the contaminated samples from Chile as an
41 attempt to obtain a calibration curve for OTA. The spectral region chosen which gave
42 the best results for the calibration was located between 1550 and 600 cm^{-1} . The
43 number of latent variables chosen for PLS1 was 4, because the root-mean-square
44 error of cross validation, which is the measure of the quality of predicted values,
45 reached a minimum there. Results of PLS1 model and B coefficients are displayed in
46 the figure 10a and b, which shows a good correlation between the actual
47 concentrations of the contaminated samples and by the FTIR-ATR predicted
48 concentrations (Table 1). The RMSEC and RMSEP (Table 1) were high which could be
49 by the inherent variability of the samples and by the lack of more levels of OTA
50 concentration.
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[Insert figure 10 about here]

[Insert table 1 about here]

Conclusions

The main outcomes of the methodology presented here show the possibility of use the FTIR-ATR as a simple procedure for screening OTA in dried sultanas samples: As for any methodology applied for the detection of contamination in food matrices there are parameters that need to be optimised in order to get meaningful results; as in this case the inherent variability of the samples due their origin and the way they were preserved were shown to be critical points that influenced the reproducibility of the spectra.

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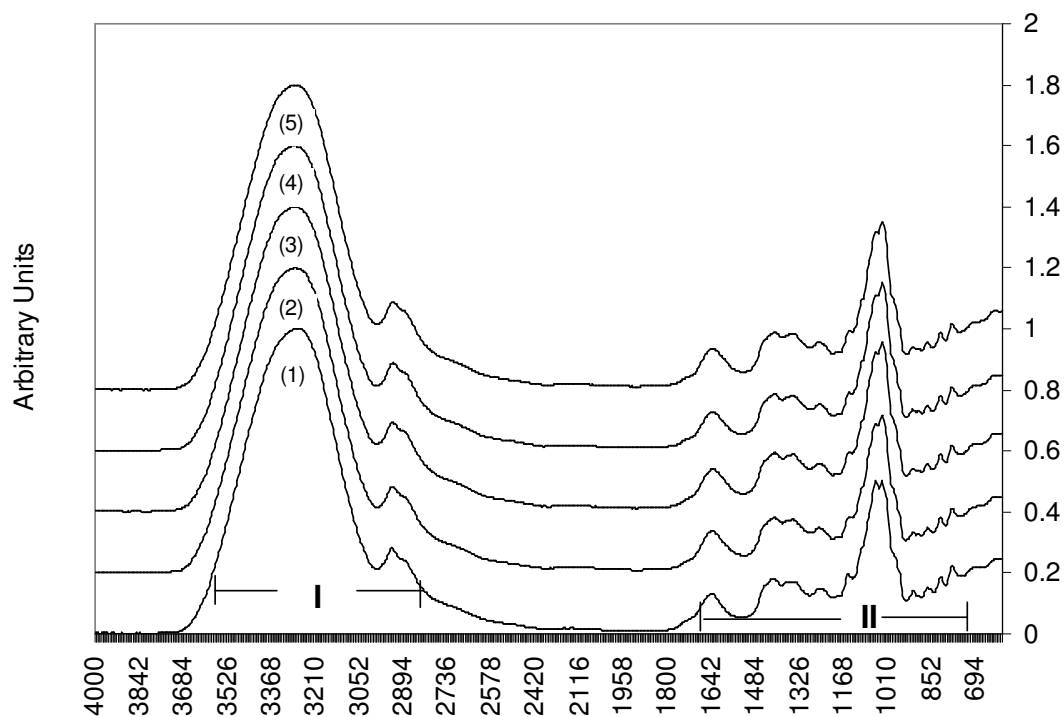


Figure 1. Typical spectra of sultanas from different origins and brands: (1) S. Africa, (2) Turkey A, (3) Turkey B, (4) Chile A and (5) Chile B.

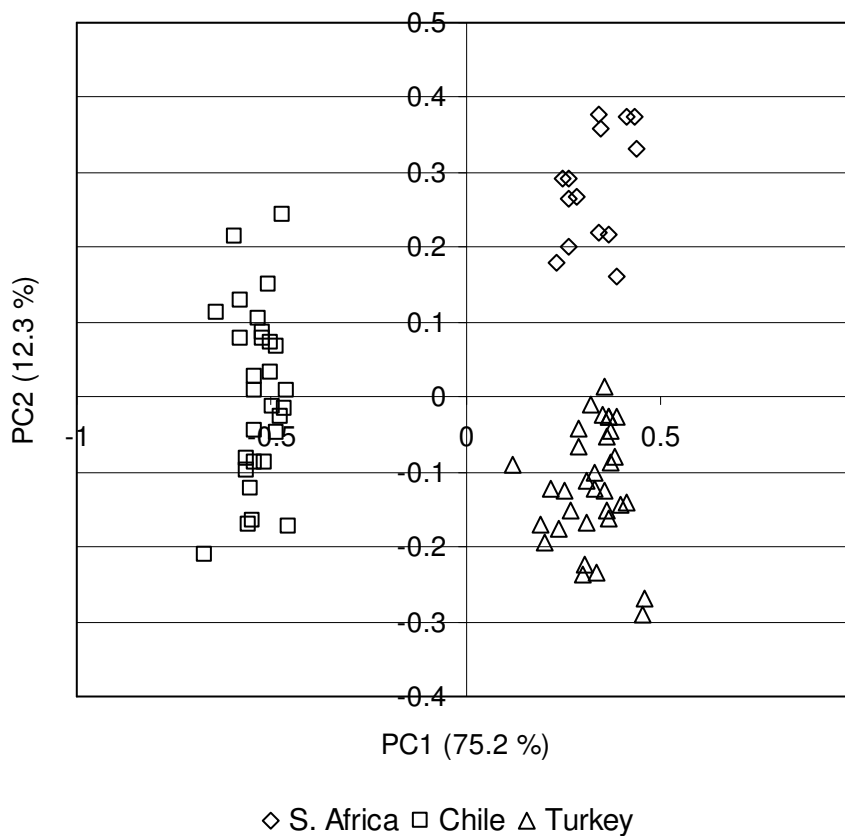
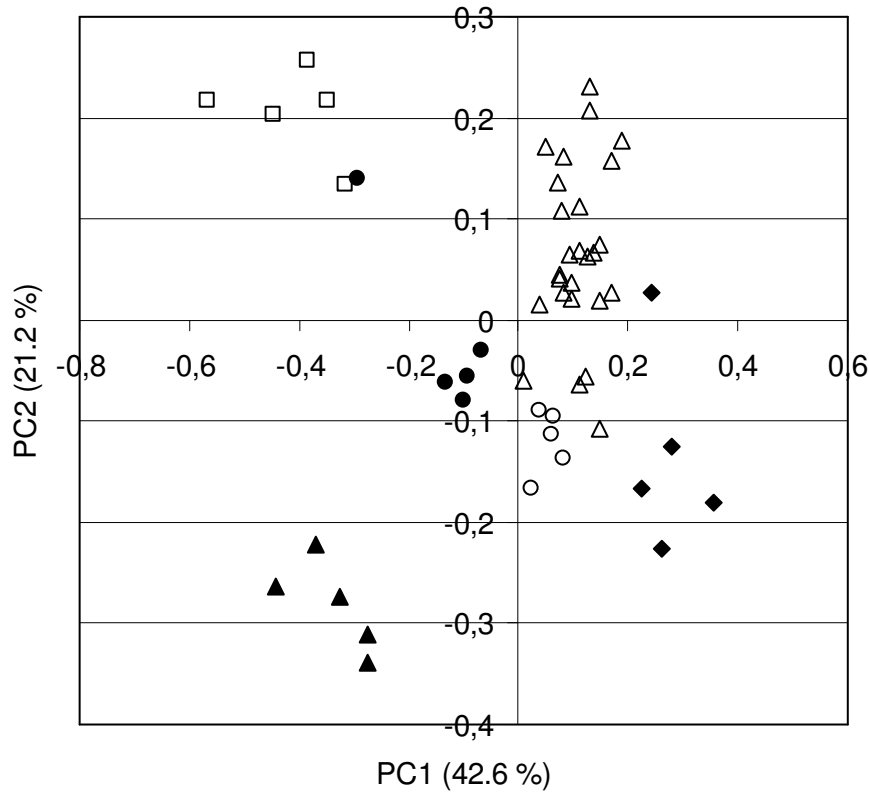


Figure 2. Scores scatter plot (PC1 vs. PC2) of sultanas according their origin.



\square $A_w=0.90$ \blacktriangle $A_w=0.84$ \bullet $A_w=0.75$ \circ $A_w=0.62$ \triangle Control 1 \blacklozenge Control 2

Figure 4. Scores scatter plot (PC1 vs. PC2) of sultanas from Chile according to the water activity.

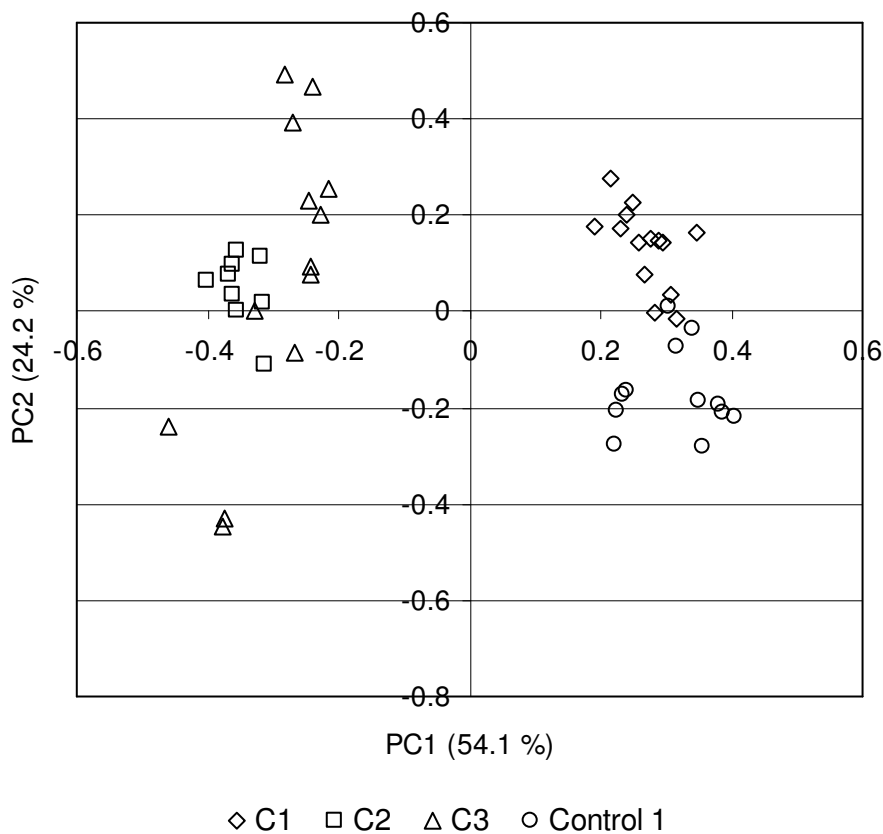


Figure 8. Scores scatter plot (PC1 vs. PC2) of sultanas from Chile contaminated with OTA were C1 = 10, C2 = 20, C3 = 40 $\mu\text{g kg}^{-1}$ and the control 1 = no contaminated samples.

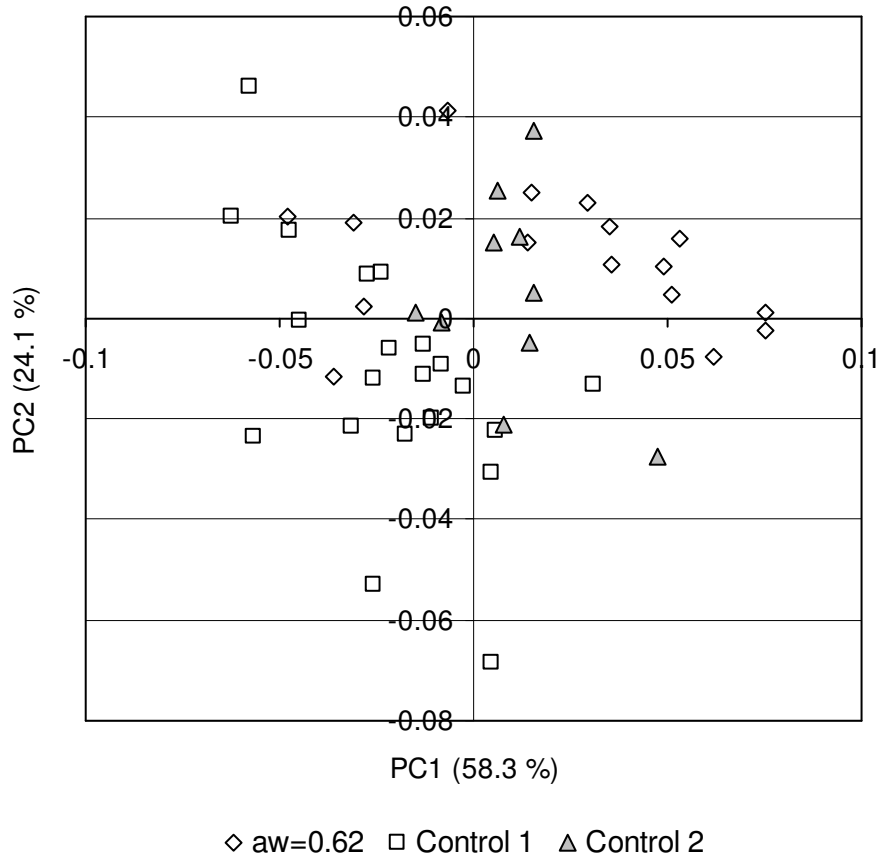


Figure 6. Scores scatter plot (PC1 vs. PC2) of sultanas from Chile preconditioned at a_w of 0.62 (\diamond) and samples without any treatment control 1 (\square) and control 2 (\triangle).

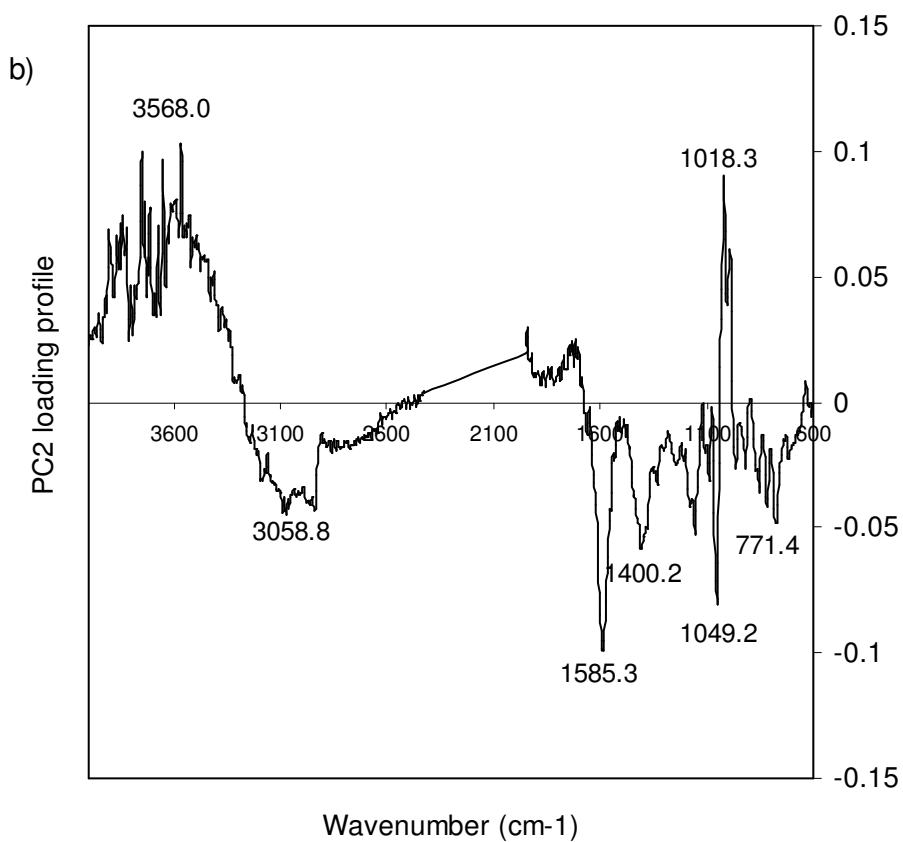
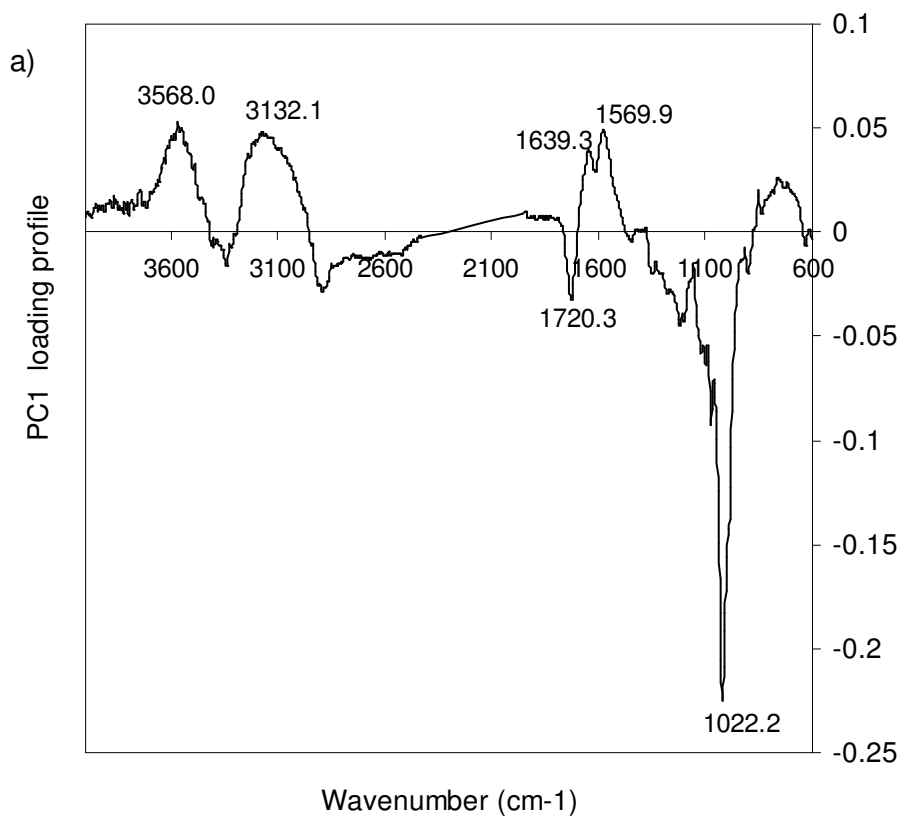


Figure 3. PC1 (a) and PC2 (b) loading profile plots of sultanas according their origin.

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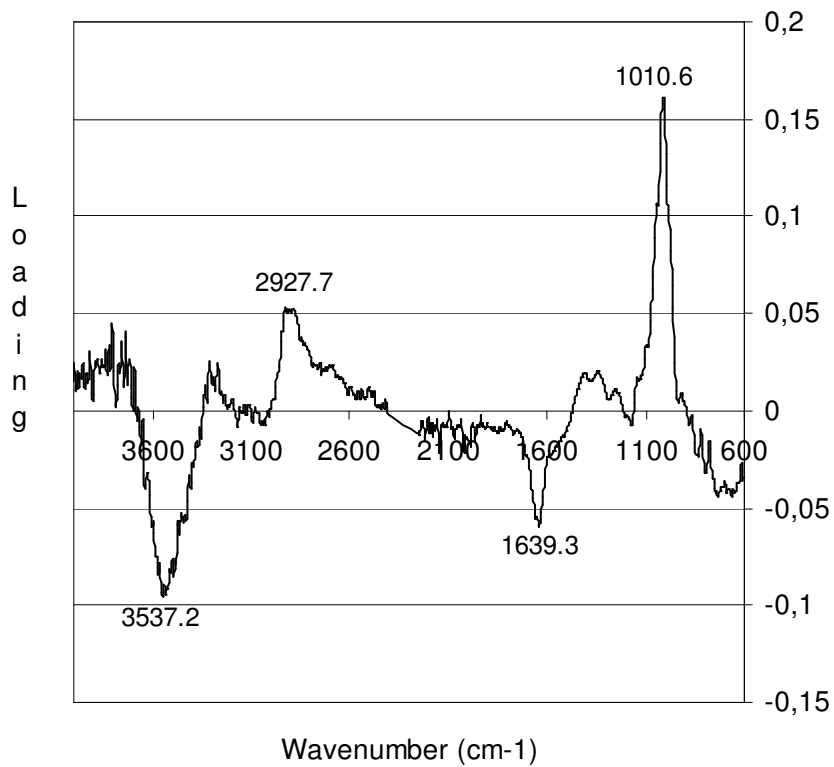


Figure 5. PC2 Loadings plot of sultanas from Chile according to the water activity.

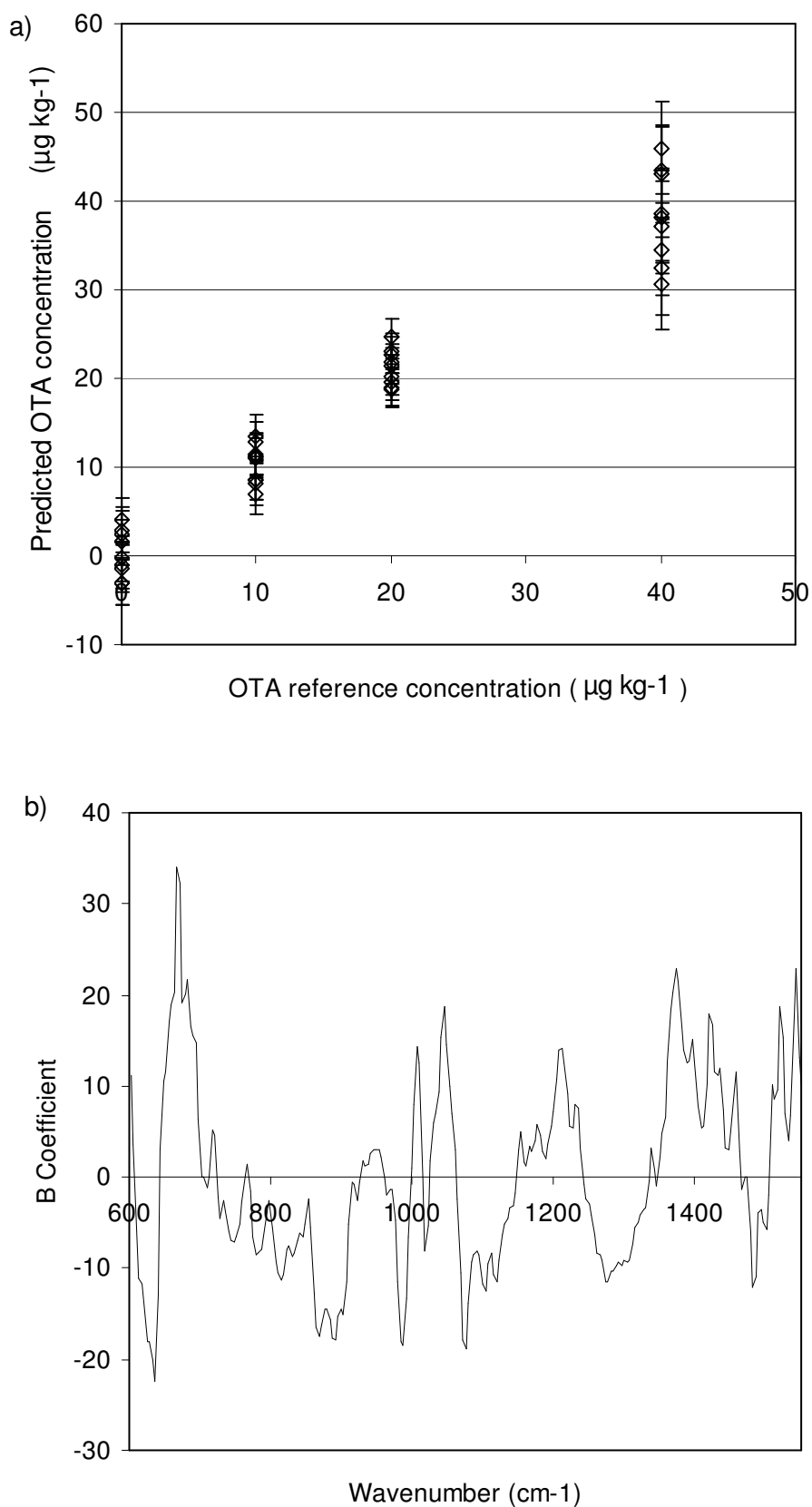


Figure 10. PLS1 regression (a) and B coefficients (b) of sultanas from Chile contaminated with reference concentrations ranging from 10 to 40 $\mu\text{g kg}^{-1}$ OTA. Bars represent the standard deviation.

Table 1. PLS1 regression statistics of sultanas from Chile for an 4 Latent Variable OTA ($\mu\text{g kg}^{-1}$) model.

Latent Variables	4
R^2	0.95
RMSEC (%) ^(a)	18.8
RMSEP (%) ^(b)	43.4

(a) Root Means Square Error of Calibration

(b) Root Means Square Error of Prediction

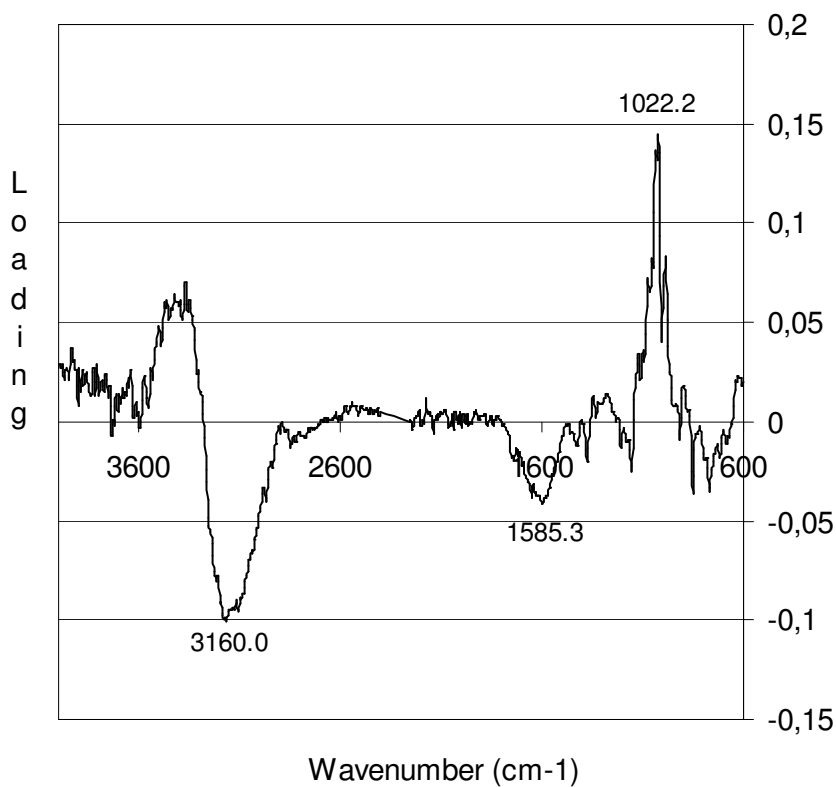


Figure 9. PC1 Loading plot of sultanas from Chile contaminated with OTA.

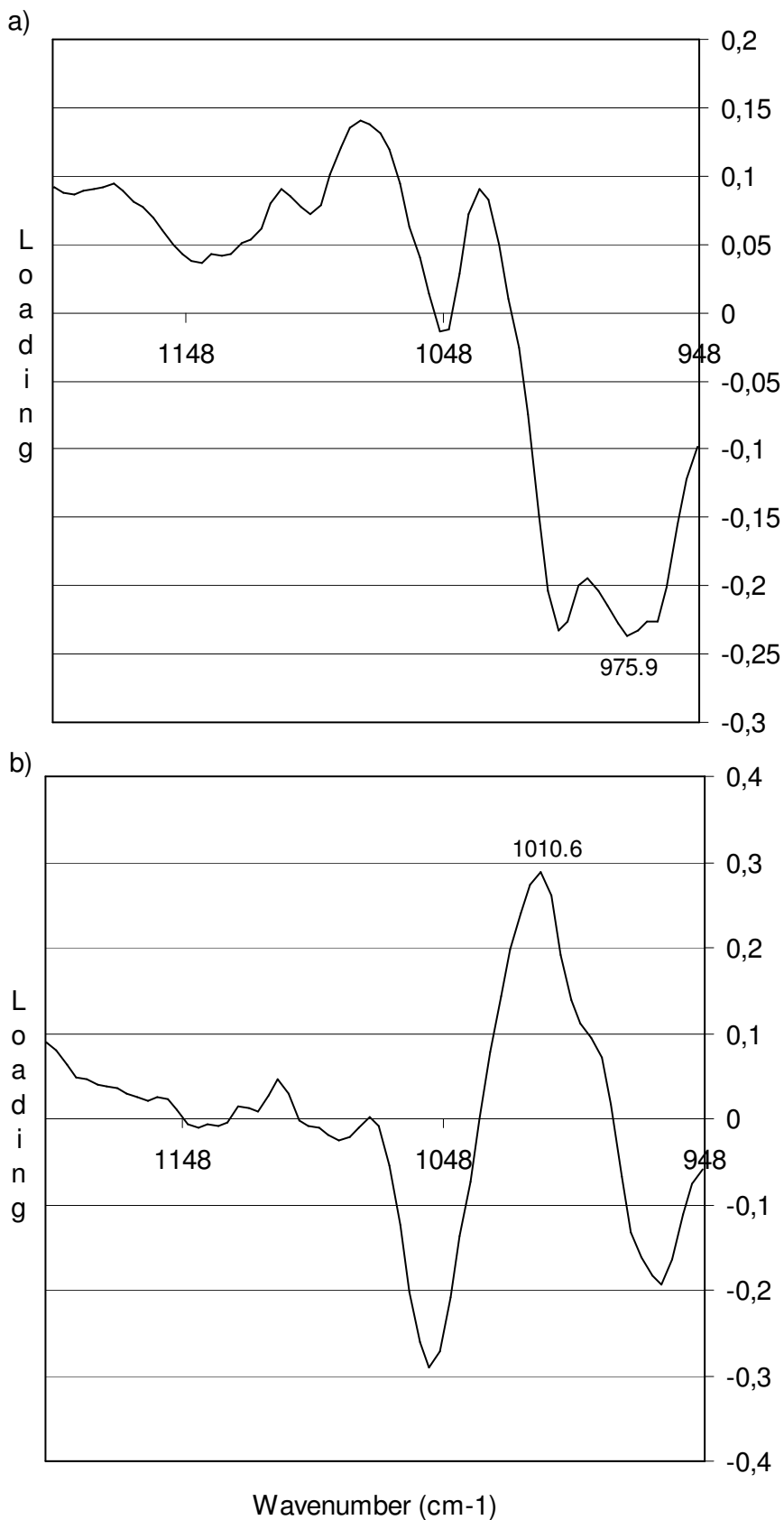


Figure 7. Loading plots of (a) PC1 and (b) PC2 of sultanas from Chile preconditioned at a_w of 0.62 and without any treatment.