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Fourier-transform infrared spectroscopy discrimination for wheat and rice pasta

Simelda E. Zippenfening [1,2], Dina Gligor (Pane) [1], Adrian Rivіş [1,2]*, Nicoleta Gabriela Hădărugă [1,2]*, Andreea Annemarie Kiss[1,4], Alexandru Nicolae[1], Daniel Ioan Hădărugă [1,3]

[1] Doctoral School "Engineering of Vegetable and Animal Resources", Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara, Calea Aradului 119, 300645-Timişoara, Romania

[2] Department of Food Science, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara, Calea Aradului 119, 300645-Timişoara, Romania

[3] Department of Applied Chemistry, Organic and Natural Compounds Engineering, Polytechnic University of Timişoara, Carol Telbisz 6, 300001-Timişoara, Romania

[4] DORU & MARI KISS SRL, Deta, Aleea Revoluţiei, Timiş, Romania

Abstract

Pasta is a commonly consumed food products all over the world. Principally, it is made by durum wheat (*Triticum durum* Desf.), but the high content on gluten determined to be replaced by other cereal flour. One of this is rice flour (*Oryza sativa* L.), which can be used solely or in combination with durum wheat flour for obtaining pasta. The goal of the study was to discriminate between various types of pasta by a simple, rapid and non-destructive combined technique, named attenuated total reflectance – Fourier-transform infrared spectroscopy coupled with principal component analysis (ATR-FTIR-PCA). Pasta products were obtained by classical recipes using durum wheat or rice flour, egg yolk and water. The dried products were finely ground and subjected to ATR-FTIR using a Bruker Vertex 70 FTIR equipment, with a spectral range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1}. The wavelengths and intensities corresponding of the characteristic ATR-FTIR bands for polysaccharides and proteins were used as input variables for PCA multivariate discriminant analysis. The wheat and rice pasta were well discriminated especially by means of FTIR intensities of C-O-H deformation of the starch glucose ring and ring vibrations overlapped by stretching vibrations of C-OH side groups and the C-O-C glycoside bond vibration (at 1644-1648 cm^{-1} and 996-1003 cm^{-1}, respectively). Moreover, the unprocessed and processed (boiled and dried) samples were clearly discriminated by PCA. On the other hand, better classifications were obtained for pasta with egg yolk, which were discriminated from the control samples by the FTIR intensities of the above-mentioned bands that also includes C=O stretching from lipids and amide I and II bands at 1733-1748 cm^{-1} for $\nu_{C=O}$ and 1538-1541 cm^{-1} for $\nu_{amide\text{-}II}$. In conclusion, the ATR-FTIR-PCA coupled technique allows discriminating pasta products and can be a useful technique to evaluate the quality of such type of food products.

Keywords: pasta products, wheat flour, rice flour, *Triticum durum* Desf., *Oryza sativa* L., attenuated total reflectance – Fourier-transform infrared spectroscopy, ATR-FTIR, principal component analysis, PCA, discriminant analysis, coupled techniques

1. Introduction

Pasta is one of the most consumed food product, especially in South Europe, but also on both American continents. Both the production technology and preparation for consumption are simple [1]. The technology involves wheat semolina, water and salt.

Sometimes, eggs and other vegetables are added in order to enhance the pasta quality [2]. The pasta stability is enhanced by adding additives such as cysteine hydrochloride, monoglycerides, or ascorbic acid [3-5]. The mixing are performed under vacuum using an extruder.

Corresponding authors: adirianrivis@usab-tm.ro; nico_hadaruga@yahoo.com

After that, the dough in pressed through another extruder in order to obtain pasta strings. The last step is drying by a specific temperature program [1, 6].

The high quality pasta is made from durum wheat flour (*Triticum durum* Desf.), but other cereal flour types or cheaper common wheat flour (*T. aestivum* L.) are also used [1]. Rice (*Oryza sativa* L.), beans (e.g., *Phaseolus vulgaris* L.) and lentils (*Lens culinaris* Medikus) are examples of other cereals and legumes used for obtaining pasta, even alone or in combination with wheat semolina [7-10]. High quality pasta is sometimes subjected to adulteration, even by replacing of durum wheat or rice flour by common wheat flour [11]. There are many techniques used for determination the adulteration of cereal flour and pasta products, which involves the determination of proteins by electrophoretic, immunological or chromatographic techniques, the determination of DNA profile by PCR, or by spectroscopic techniques for profiling the composition [2, 3, 11]. Infrared spectroscopy techniques such as FTIR (Fourier-transform infrared spectroscopy), MIR and NIR (middle or near infrared spectroscopies), coupled with multivariate statistical techniques are rapid and non-destructive methods used for detection and discrimination of pasta types adulteration [11].

The goal of the study was to discriminate between various types of pasta by the attenuated total reflectance – Fourier-transform infrared spectroscopy coupled with principal component analysis (ATR-FTIR-PCA).

2. Materials and Method

2.1. Materials

The pasta samples were obtained by classical recipes, by mixing common wheat (*T. aestivum* L.) or rice (*O. sativa* L.) flour with water at a mass ratio of 7.5:1, as well as chicken egg yolk (one medium size for 300 g of flour) for egg-based pasta. Cereal flour samples and chicken eggs were purchased from the local market. Pasta products were extruded using an *in house* equipment and dried in a dark and cool place for 24 h. Isopropanol (IR grade, Merck&Co Inc.) was used for cleaning the ATR-FTIR equipment.

2.2. Attenuated total reflectance – Fourier-transform infrared spectroscopy (ATR-FTIR) analysis

ATR-FTIR analysis was performed on a Bruker Vertex 70 apparatus, equipped with a DLaTGS detector (Bruker Optik GmbH, Ettlingen, Germany). The spectral range was 4000-400 cm^{-1}, with a resolution of 4 cm^{-1} and 128 scans for every sample. Air was used for acquiring the background spectra. Raw and processed (boiling for 3 min and drying) pasta samples were finely ground at particles having diameters <0.2 mm. The ATR surface was cleaned using isopropanol, followed by complete drying. OPUS ver. 7.2 software (Bruker Optik GmbH 2012, Ettlingen, Germany) was used for the acquisition and handling of the ATR-FTIR data.

2.3. Principal component analysis (PCA) and classical statistical analysis

Pasta samples were obtained as triplicates (if otherwise stated) and the ATR-FTIR wavelengths and intensities corresponding to the specific bond stretching and bending were recorded as mean ± standard deviation (SD). On the other hand, principal component analysis (PCA) was applied for discriminating between samples. The stretching and bending wavelengths (ν and δ) and intensities ($I(\nu)$ and $I(\delta)$) of all bond vibration types have been used as input for ATR-FTIR-PCA coupled analysis. Cases (samples) were evaluated by the flour type (wheat and rice), processing (unprocessed or processed), and egg addition. In the Statistica 7.1 (PC&CA module, StatSoft), FTIR data were centered. On the other hand, the cross-validation method have been considered in the ATR-FTIR-PCA analysis.

3. Results and discussion

3.1. ATR-FTIR of wheat and rice pasta

Pasta mainly contain carbohydrates (70.0%), proteins (12.3%) and fats (2.9%) if these products are obtained with eggs. Moreover, the dietary fiber and minerals have concentration of about 3.4% and 1.0%, respectively [1, 2, 12-20]. Consequently, the bonds corresponding to α-D-glucopyranose units from carbohydrates, amide groups and amino acid moieties from proteins as well as ester groups and fatty acid moieties from triglycerides in lipids are the main elements that can be evaluated by FTIR [21-26].

The superimposed FTIR spectra and band assignments for bond stretching and bending in unprocessed or processed wheat and rice pasta, without or with egg yolk addition are presented in Figures 1, 2, and Tables 1,2, respectively.

In the region of 3200-3400 cm^{-1} the OH stretching appear. It can be due even to OH groups from water molecules and other OH groups such as alcoholic of phenolic ones. This large band appears in all above-mentioned constituent compound classes. The values of 1738-1748 cm^{-1} corresponds to the ester C=O stretching vibrations in lipids form both cereal flour and egg yolk. Generally, these values are lower in the unprocessed samples. NH bending vibration (amide-II band) appears at 1538-1541 cm^{-1} and is specific to proteins. No important variations for the wavelength values were observed. CH deformation from ring vibration in polysaccharides was identified at 1338-1342 cm^{-1}, while the C-O-C ring vibration of carbohydrates was identified at 928-932, 854-858, and 757-761 cm^{-1} (Tables 1 and 2).

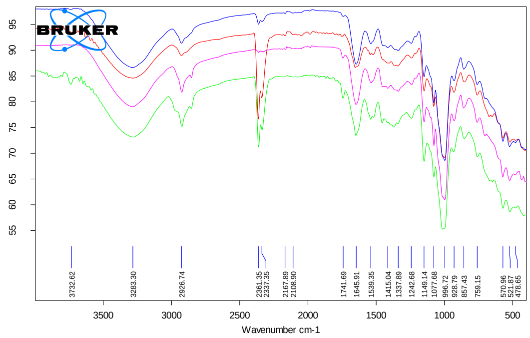


Figure 1. Superimposed ATR-FTIR spectra for wheat pasta without egg (unprocessed and processed – blue and red) and with egg (unprocessed and processed – pink and light green)

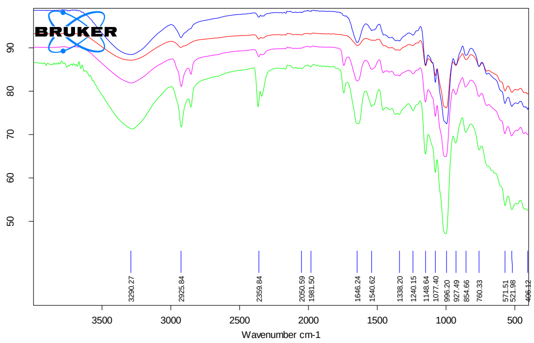


Figure 2. Superimposed ATR-FTIR spectra for rice pasta without egg (unprocessed and processed – blue and red) and with egg (unprocessed and processed – pink and light green)

Table 1. ATR-FTIR results for wheat pasta (*n* = 3, excepting *)

FTIR band assignment	Wheat pasta without egg		Wheat pasta with egg	
	Unproc.	*Process.**	*Unproc.*	*Process.**
ν_{OH}	3286±2.3	3285	3286±1.0	3281
ν^{as}_{CH2}	2926±0.3	2927	2924±0.3	2924
ν^{s}_{CH2}	2857±0.6	2857	2855±0.7	2857
$\nu_{C=O}$	1742±1.0	1745	1741±4.3	1742
δ_{OH}/ ν_{A-I}/sk$_{Ar}$	1647±0.7	1648	1646±0.8	1646
δ_{A-II}	1540±1.3	1541	1538±0.3	1539
$\nu_{bCH2/3}$	1455±1.2	1454	1453±0.7	1455
sc$_{CH2}$/ $\delta_{OH/CCAr}$	1416±1.0	1417	1416±0.6	1416
δ^{s}_{CH}/ν_{CO}	1362±1.0	1366	1364±0.2	1369
δ_{CH}/δ_{CO}	1338±0.4	1342	1339±1.1	1340
$\delta_{CH2/CO}$/ sk$_{Py}$	1242±0.9	1241	1240±0.4	1242
ν_{COC}	1149±0.4	1149	1149±0.1	1149
ν_{CO}/ν_{COC}	1077±0.2	1078	1077±0.3	1077
ν_{CO}/ δ_{CHAr}	1012±0.4	1011	1013±0.8	1013
$\nu_{CO\&CC}$	996±0.2	999	999±1.7	1000
ν^{as}_{Rg}	928±1.2	929	929±1.5	932
δ_{EqC1H}	858±1.3	854	858±2.6	854
δ_{COC}	760±0.1	759	759±1.2	757
sk$_{PyG1}$	572±0.3	570	572±0.3	571
sk$_{PyG2}$	522±0.3	520	522±1.3	520

Table 2. ATR-FTIR results for rice pasta (*n* = 3, excepting *)

FTIR band assignment	Rice pasta without egg		Rice pasta with egg	
	Unproc.	*Process.**	*Unproc.**	*Process.**
ν_{OH}	3292±2.9	3292	3283	3280
ν^{as}_{CH2}	2928±2.1	2927	2924	2924
ν^{s}_{CH2}	2857±2.5	2856	2854	2855
$\nu_{C=O}$	1738±5.1	1748	1742	1743
δ_{OH}/ ν_{A-I}/sk$_{Ar}$	1646±1.2	1647	1645	1647
δ_{A-II}	1540±1.2	1541	1539	1541
$\nu_{bCH2/3}$	1456±0.8	1456	1456	1457
sc$_{CH2}$/ $\delta_{OH/CCAr}$	1415±1.4	1417	1414	1410
δ^{s}_{CH}/ν_{CO}	1362±0.7	1363	1363	1368
δ_{CH}/δ_{CO}	1338±0.4	1338	1338	1340
$\delta_{CH2/CO}$/ sk$_{Py}$	1241±0.9	1242	1239	1239
ν_{COC}	1149±0.2	1149	1149	1150
ν_{CO}/ν_{COC}	1077±0.1	1077	1078	1078
ν_{CO}/ δ_{CHAr}	1011±0.4	1013	1012	1010
$\nu_{CO\&CC}$	997±1.2	997	1003	1002
ν^{as}_{Rg}	928±0.3	929	928	930
δ_{EqC1H}	858±2.3	858	858	857
δ_{COC}	761±0.6	759	761	759
sk$_{PyG1}$	572±0.5	572	572	571
sk$_{PyG2}$	523±0.7	522	523	522

3.2. ATR-FTIR-PCA coupled analysis of wheat and rice pasta

The evaluation and discrimination of pasta samples based on FTIR spectra is very difficult. The wavelength and intensity values corresponding to stretching and bending of different bonds/groups in the components from pasta samples can vary according to cereal flour types, unprocessed and processed conditions as well as the addition of egg yolk. Some changes are more important, but many of these have small changes. A useful combined technique to discriminate between pasta samples and to identify the importance of FTIR parameters to such discrimination is ATR-FTIR-PCA. Wavelength and intensity values for all FTIR bands or only one type of parameters have been used as input parameters in PCA analysis. The samples were coded as unprocessed and processed (boiled and dried) wheat and rice-based pasta (WU/WP and RU/RP). On the other hand, these samples were classified according to the addition of chicken egg yolk (E – with egg, N – without egg).

PCA analysis using all FTIR data provide a relatively well discrimination between wheat and rice samples. Rice pasta were grouped in the upper side of the *PC_2 vs. PC_1* scores plot (excepting one sample), while wheat pasta are located in the center of this plot. The most important parameters are ν_{COC} in the positive part and most of intensities in the negative part for PC_1 and parameters such as δ_{COC} for the positive part and δ^s_{CH}/ν_{CO} in the negative region for PC_2 (Figures 3a-3d). The explained variance for PC_1 was 50.57%, for PC_2 17.41% and for PC_3 9.75% (Figure 3e). It seems that the first three PCs explain 77.73% from the variance of the data, instead of twenty input parameters.

Similar classifications were obtained if only FTIR wavelengths or intensities were used as input parameters. In the first case, wheat samples were grouped especially in the center and right side of the PC_2 vs. PC_1 scores plot, or in the right side in the PC_3 vs. PC_1 scores plot. The main parameters involved in these classifications were δ^s_{CH}/ν_{CO}, ν_{COC}, ν_{CO}/δ_{CHAr} and $\delta_{A\text{-}II}$. It must be emphasized the importance of the wavelength corresponding to amide-II bending for the discrimination along the PC_3 axis.

The explained variance for the first three PCs is 70.6% (Figures 4a-4e). In Figures 5a-5e are presented the scores and loadings plots, as well as eigenvalues of the correlation matrix corresponding to ATR-FTIR-PCA analysis using the intensities of FTIR bands. Wheat samples are especially located in the center of PC_2 vs. PC_1 scores plot, while for PC_3 vs. PC_1 these samples were grouped in the upper side.

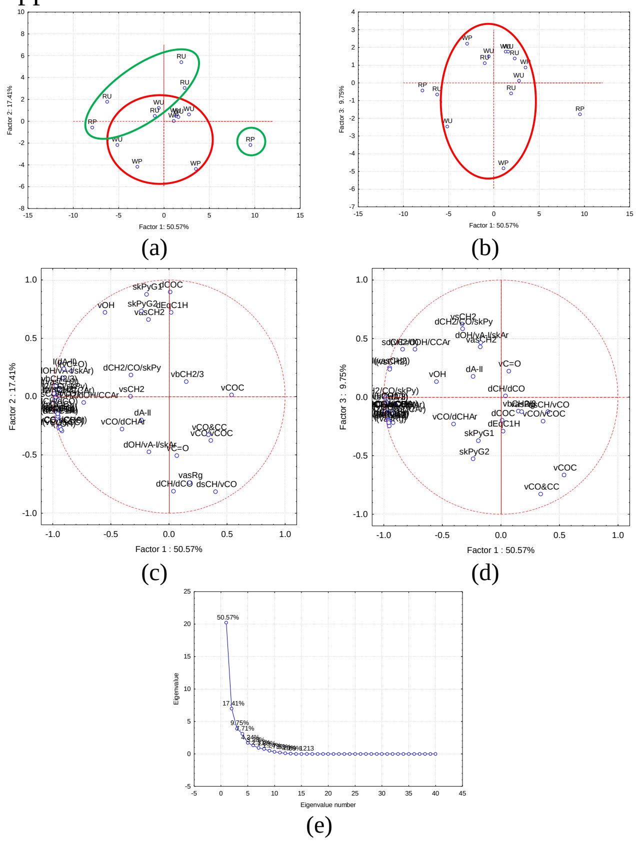
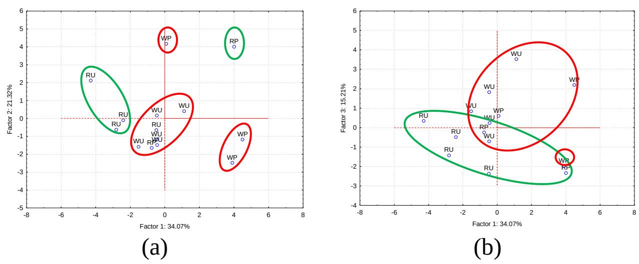


Figure 3. ATR-FTIR-PCA results for unprocessed and processed wheat and rice pasta (WU / WP and RU / RP, respectively) – wavelength and intensity of FTIR bands as input variables: (a) *PC2 vs. PC1* scores plot; (b) *PC3 vs. PC1* scores plot; (c) *PC2 vs PC1* loadings plot; (d) *PC3 vs. PC1* loadings plot; (e) eigenvalues of the correlation matrix



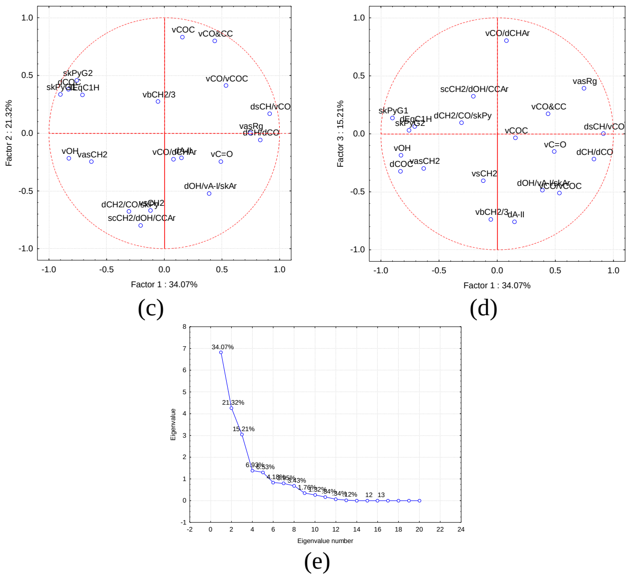


Figure 4. ATR-FTIR-PCA results for unprocessed and processed wheat and rice pasta (WU / WP and RU / RP, respectively) – wavelength of FTIR bands as input variables: (a) *PC2 vs. PC1* scores plot; (b) *PC3 vs. PC1* scores plot; (c) *PC2 vs PC1* loadings plot; (d) *PC3 vs. PC1* loadings plot; (e) eigenvalues of the correlation matrix

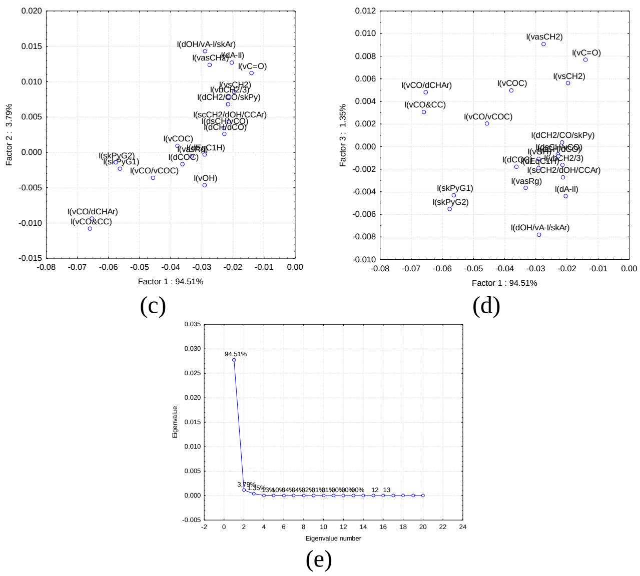


Figure 5. ATR-FTIR-PCA results for unprocessed and processed wheat and rice pasta (WU / WP and RU / RP, respectively) – intensity of FTIR bands as input variables: (a) *PC2 vs. PC1* scores plot; (b) *PC3 vs. PC1* scores plot; (c) *PC2 vs PC1* loadings plot; (d) *PC3 vs. PC1* loadings plot; (e) eigenvalues of the correlation matrix

The same coupled analysis was applied for unprocessed and processed samples (U and P). The unprocessed samples were grouped especially in the center of scores plots, even the FTIR wavelengths and intensities were used together or separately (Figures 6a-6f). However, the best ATR-FTIR-PCA discriminations were obtained if the FTIR intensities were used as input parameters for discriminations between wheat and rice pasta without or with chicken egg yolk (E – pasta with egg, N – pasta without egg, Figures 7a-7f). It can be seen that pasta without eggs are located in the upper side of the PC_2 vs. PC_1 scores plot, while the pasta with eggs are clearly grouped in the bottom part. The first two PCs are sufficient to discriminate between these samples, the sum of the explained variance being 98.3% (this statistical parameter is 99.65% if PC_1 to PC_3 is considered, but the first two PCs almost completely explain this classification).

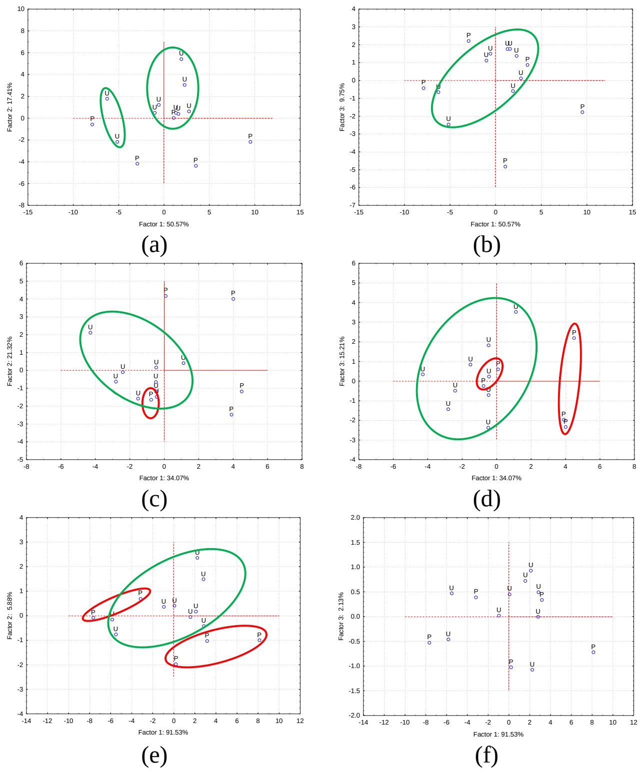
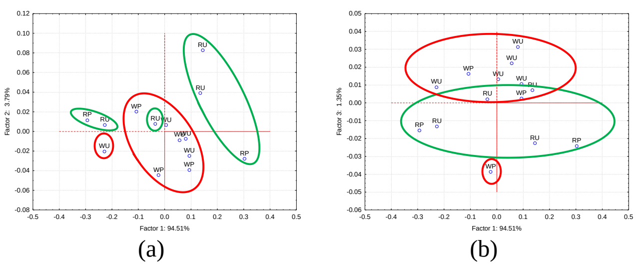


Figure 6. ATR-FTIR-PCA *PC2 vs. PC1* (left) and *PC3 vs. PC1* (right) scores plots for unprocessed and processed pasta (U and P, respectively) – both wavelength and intensity (a and b), only wavelength (c and d) or only intensity (e and f) FTIR bands as input variables



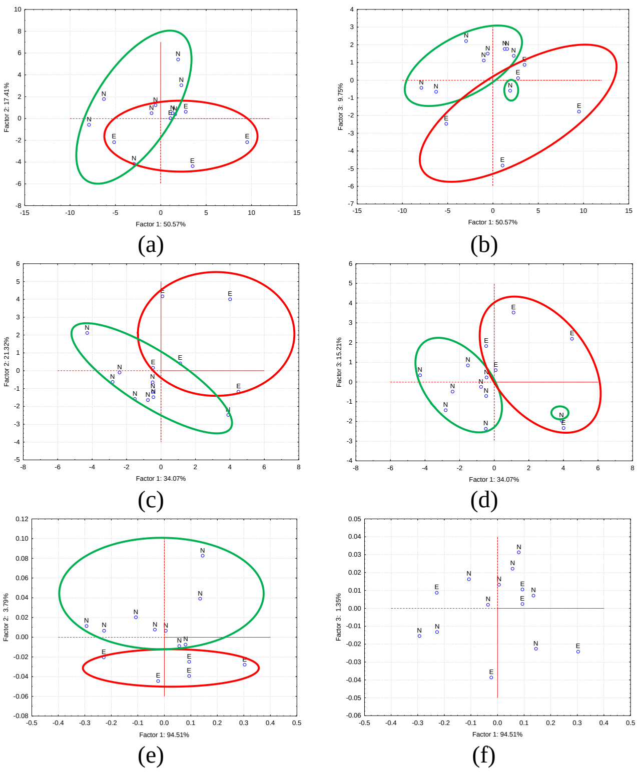


Figure 7. ATR-FTIR-PCA *PC2 vs. PC1* (left) and *PC3 vs. PC1* (right) scores plots for pasta without or with egg yolk (N and E, respectively) – both wavelength and intensity (a and b), only wavelength (c and d) or only intensity (e and f) FTIR bands as input variables

4. Conclusion

In this study, the discrimination between wheat and rice pasta products have been performed using the ATR-FTIR-PCA combined technique. Good classifications were obtained both for cereal flour types, level of processing (boiling and drying) and the egg yolk addition. The FTIR intensities corresponding to bending vibrations of the amide groups in proteins were "key" parameters for such discriminations. This coupled technique can be useful for the detection and evaluation of the quality of wheat and rice pasta, as well as for possible adulterations of such food products. However, more studies are needed in order to increase the statistical significance in this regard.

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