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Front-Face Fluorescence Spectroscopy as a Rapid and Nondestructive Tool for Differentiating Various Cereal Products: A Preliminary Investigation

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The potential of intrinsic fluorescence spectroscopy was investigated for differentiating between processed grains (flours, pasta, and semolinas) of different wheat cereal products. A total of 59 samples (e.g., 20 complete Kamut, semicomplete Kamut, and soft wheat flours, 28 pasta, and 11 semolinas manufactured from complete Kamut, semicomplete Kamut, and hard wheat flours) were analyzed by front-face fluorescence spectroscopy. Tryptophan fluorescence spectra were scanned between 305 and 400 nm on samples following excitation at 290 nm. The principal component analysis (PCA) performed on flour spectra clearly differentiated complete Kamut and semicomplete Kamut samples from those produced from complete and semicomplete soft wheat flours. The PCA performed on pasta spectra discriminated samples manufactured from complete Kamut and complete hard wheat flours from those made with semicomplete Kamut and semicomplete hard wheat flours. The best discrimination was obtained from tryptophan spectra recorded on semolinas since the four groups were well discriminated. Correct classification amounting to 61.9% was obtained for pasta spectra. A better classification was obtained for flour and semolina spectra since correct classification amounted to 86.7% and 87.9%, respectively. Front-face fluorescence spectroscopy has the potential to be a rapid, low-cost, and efficient method for the authentication of cereal products.

KEYWORDS: Flour; pasta; semolina; Kamut wheat; soft wheat; hard wheat; fluorescence; tryptophan; chemometry

INTRODUCTION

In many countries, several food products owe their reputation to the traditional and specific production techniques that make such products very specific and well differentiated from the others. Nowadays, objective and authentic food information is a major concern of many consumers, and it is gaining importance. Labeling and compositional regulations, which may differ from country to country, have a fundamental place in determining which scientific tests are appropriate for a particular issue. For example, Kamut brand wheat is generally high priced and brings in a higher benefit to the producers than ordinary wheat (*1*). For consumers having an extensive choice of food commodities, authenticity is a determinant criterion for guaranteeing quality. The traditional analysis methods for major food components are slow, relatively expensive, time consuming, not easily adapted to on-line monitoring, and require highly skilled operators.

Many features of food ingredients relevant to their transformation stem from definite structural features of the macromolecules present in the system. This is true in particular for proteins, in view of the unique capacity of their high-order structures to be selectively modified upon processing. The extent of structural modification is related to the nature of the protein, the physical and chemical properties of the system, the interaction with other food components, and the nature and intensity of the treatment itself. An array of methodologies for estimating both transient and permanent structural modification of proteins has been developed, providing a wealth of information on model systems and, in a few cases, on real foods. Spectroscopic techniques have been used more and more in the agriculture and food industries in recent decades. In recent years, it has become increasingly clear that the application of spectroscopic methods to food analysis can alleviate important problems in the processing of food products. To comply with this request, a great number of noninvasive and nondestructive instrumental techniques have been developed for the determination of product composition. These new analytical techniques are relatively low cost and can be applied in both fundamental research and in the factory as on-line sensors for monitoring the cereal-based products.

Fluorescence spectroscopy offers several inherent advantages for the characterizations of food products. First, it is 100−1000

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Table 1. Investigated Flour, Pasta, and Semolina Samples[a]

samples	complete Kamut	semicomplete Kamut	complete soft wheat	semicomplete soft wheat
flours	5	7	4	4
genotype	AABB	AABB	AABBDD	AABBDD
ash (mg/100 g)	180	110	180	110

samples	complete Kamut	semicomplete Kamut	complete hard wheat	semicomplete hard wheat
semolinas	3	3	3	2
pasta	11	10	5	2
genotype	AABB	AABB	AABB	AABB
ash (mg/100 g)	180	110	180	110

[a] Kamut, *Triticum turgidum*; soft wheat, *Triticum aestivum*; hard wheat, *Triticum durum*.

times more sensitive than other spectrophotometric techniques (*2*). Second, fluorescent compounds are extremely sensitive to their environments. For example, tryptophan residues that are buried in the hydrophobic interior of a protein have different fluorescent properties than residues that are on a hydrophilic surface (*3*). This environmental sensitivity enables the characterization of conformational changes such as those attributable to the thermal, solvent, or surface denaturation of proteins, as well as to the interactions of proteins with other food components. Third, most fluorescence methods are relatively rapid.

The potential of using fluorescence in food research has increased during the past few years with the propagated application of chemometric tools and with technical and optical developments of spectrofluorimeters. It has been shown that front-face fluorescence spectroscopy can discriminate different species of cereals (e.g., rice, creso, maize, pandas) (*3*). In addition, this technique has been used to determine the relative xantophyll contents in flours obtained from four breads and five durum wheats (*4*). This technique was also utilized to study the protein−protein and protein−lipid interaction in wheat gluten (*5*, *6*) and for the measurement of riboflavin in cereal flours (*7*). Considering animal products, front-face fluorescence spectroscopy has been used to determine the geographic origin of PDO Gruyère, of L'Etivaz PDO cheeses (*8*, *9*) and of Emmental cheeses from different European geographic origins (*10*−*12*).

The aim of this work was to investigate the potential of front-face fluorescence spectroscopy coupled with chemometric tools as a rapid and low-cost technique for differentiating Kamut brand wheat from soft and hard wheat in flours, pasta, and semolinas.

MATERIALS AND METHODS

Sample Preparation. A total of 59 samples (**Table 1**), e.g., 12 complete and semicomplete Kamut (*Triticum turgidum spp. Turanicum*, tetraploid wheat (AABB)) flours, 8 complete and semicomplete soft wheat (*Triticum aestivum*, hexaploid wheat (AABBDD)) flours, and 28 pasta (spaghetti), and 11 semolinas manufactured from complete and semicomplete Kamut and from complete and semicomplete hard wheat (*Triticum durum*, tetraploid wheat (AABB)) grains. For all the cereal products produced from complete soft wheat, complete hard wheat, and complete Kamut, all the constituents of the grains were found in the flours which present an ash content of 180 mg/100 g of grain; however, a part of the envelope was removed during the milling process for all the flours produced from semicomplete soft wheat, semicomplete hard wheat, and semicomplete Kamut (ash content of 110 mg/100 g of grain).

The grains were milled with a laboratory hammer mill to obtain flour. All the samples were provided by Kamut Enterprises of Europe bvba (Gent, Belgium).

Fluorescence Spectroscopy. Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France) mounted with a variable angle front-surface accessory. The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation, and depolarization phenomena were minimized. Flour, semolina, and ground pasta samples were poured in 3 mL quartz cuvettes, and spectra were recorded at 20 °C. The emission spectra of tryptophan residues (305−400 nm) were recorded with the excitation wavelength set at 290 nm. All the spectra were corrected for instrumental distortions using a rhodamine cell as a reference channel. For each sample, three spectra were recorded on different samples.

Mathematical Analysis of Data. *Principal Component Analysis.* To reduce scattering effects and to compare the samples, fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter (*13*). Mainly, the shift of the peak maximum and the peak width changes in the spectra were considered following this normalization. The principal component analysis (PCA) was applied to the normalized spectra to investigate differences between the samples (*14*). The PCA transforms the original variables (wavelengths) into new orthogonal axes called principal components (PCs) so that the data set presented on these axes are uncorrelated with each other. Therefore, PCA expresses as much as possible the total variation in the data set in only a few PCs, and each successively derived PC expresses decreasing amounts of the variance. This statistical multivariate treatment was used earlier to observe similarities among different soft cheese samples (*14*), reducing the dimension to two or three PCs. This method is well suited to optimize the description of the spectral data collection with a minimum loss of information.

Factorial Discriminant Analysis. Factorial discriminant analysis (FDA) was performed to the first five PCs resulting from the PCA applied to the tryptophan fluorescence spectra. The aim of this technique was to predict the membership of an individual to a qualitative group defined as a preliminary (*15*). Considering flours, a group was created for each type of sample, e.g., complete Kamut, semicomplete Kamut, complete wheat, and semicomplete wheat. The method cannot be applied in a straightforward way to continuous spectra because of the high correlations occurring between the wavelengths. Advantages were found in the preliminary transformation of the data into their principal components.

FDA assesses new synthetic variables called "discriminant factors", which are linear combinations of the selected PCs, and allows a better separation of the center of gravity of the considered groups. The individual samples can be reallocated within the various groups. For each sample, the distance from the various centers of gravity of the groups is calculated. The sample is assigned to the group where its distance between the centers of gravity is the shortest. Comparison of the assigned group to the real group is an indicator of the quality of the discrimination.

As the number of samples was very low, all the samples were assigned to their original groups, and then FDA was applied. A similar approach has been applied by Karoui and Dufour (*16*) and Karoui et al. (*11*) to discriminate different soft cheeses and Gruyère and L'Etivaz cheeses (Swiss cheeses), respectively.

PCA and FDA were performed using StatBoxPro (Grimmer Logiciels, Paris, France).

RESULTS AND DISCUSSION

Fluorescence Spectra of Flour, Pasta, and Semolina Samples. It has been reported that fluorescence spectroscopy is a very sensitive technique able to measure trace substances containing one or more fluorescent chemical groups (*10*−*12*). Intrinsic protein fluorescence is due to the aromatic amino acids, mainly tryptophan, considering that phenylalanine has a very low quantum yield and emission by tyrosine in native proteins is often quenched (*6*). Fluorescence due only to tryptophan residues can be selectively measured by exciting at 290 nm, because at this wavelength there is no absorption by tyrosine.

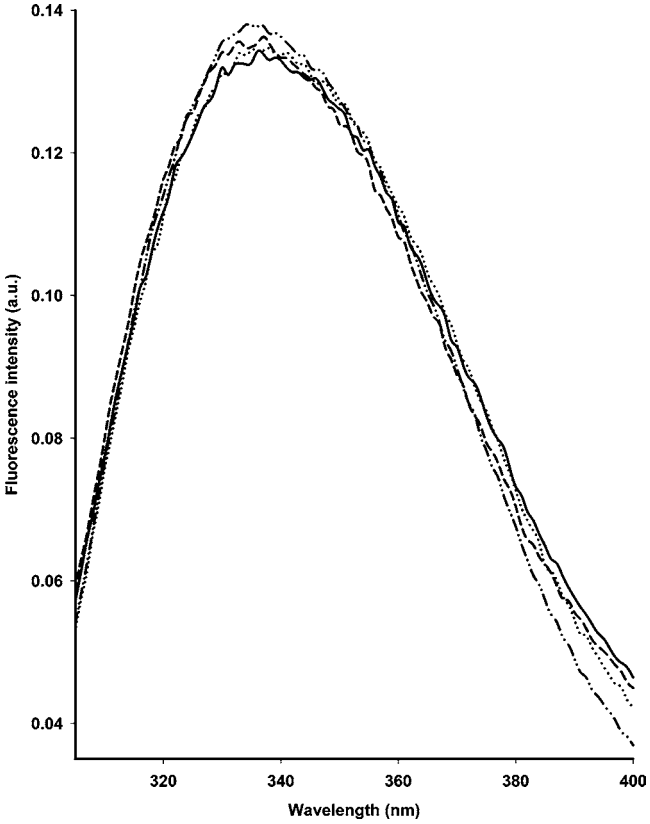

Figure 1. Normalized tryptophan fluorescence spectra of complete Kamut (—), semicomplete Kamut (• • •), complete soft wheat (– – –), and semicomplete soft wheat (– • • –) flours.

Tryptophan fluorescence is highly sensitive to the environment polarity, and shifts in its emission spectrum toward lower wavelengths (blue shift) can be seen as hydrophobicity increases. Changes in emission spectra from tryptophan can be seen in response to protein conformational transitions, subunit association, ligand binding, or denaturation, all of which can affect the local environment surrounding the indol ring (*17*).

Figure 1 presents the spectra recorded following excitation at 290 nm of complete Kamut, semicomplete Kamut, complete soft wheat, and semicomplete soft wheat flours. The emission maximum of tryptophan residues was observed at about 335 nm and varied slightly between flours manufactured from Kamut and those manufactured from soft wheat. Slight differences were also observed between flours manufactured from complete Kamut and semicomplete Kamut, as well as between those manufactured from complete soft wheat and semicomplete soft wheat.

The tryptophan fluorescence spectra recorded on semolinas manufactured from Kamut and hard wheat exhibited a maximum at 338 nm, varying slightly between the four samples (**Figure 2**). Considering tryptophan fluorescence spectra recorded on pasta (spaghetti) (**Figure 3**), the emission spectra exhibited a maximum at 334 for pasta made with semicomplete Kamut and semicomplete hard wheat, whereas pasta manufactured with complete Kamut and complete hard wheat showed a maximum at 338 nm. In addition, the shapes of the spectra of the latter were broader than those of semicomplete Kamut and semicomplete hard pasta. The presence of high amounts of bran in pasta produced from complete hard durum and Kamut compared to those found in pasta produced with semicomplete Kamut and semicomplete hard durum could explain the difference observed in the tryptophan fluorescence spectra. From these results, it can be concluded that the environment of the tryptophan residues was more hydrophobic and/or denatured for complete Kamut and complete hard wheat pasta, in agreement with Möller and Denicola (*17*) who reported that a shift of tryptophan emission toward lower wavelengths (blue shift) can be seen as hydrophobicity increases.

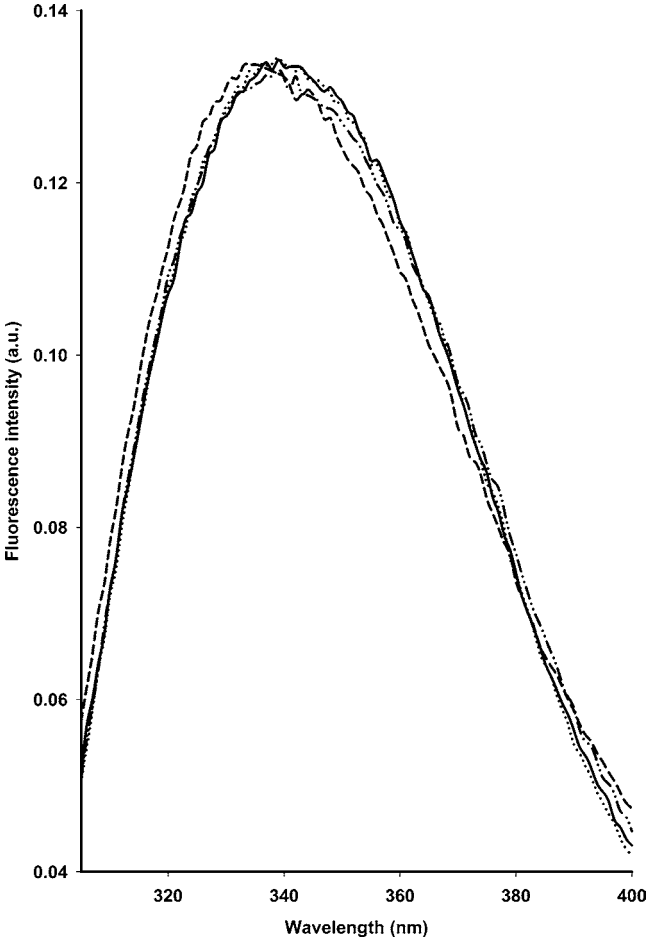

Figure 2. Normalized tryptophan fluorescence spectra of complete Kamut (—), semicomplete Kamut (• • •), complete hard wheat (– – –), and semicomplete hard wheat (– • • –) semolinas.

As the fluorescence spectral data of the different investigated samples exhibited slight differences (especially for the flours), univariate analysis of fluorescence data, e.g., changes of fluorescence intensity at a given excitation/emission wavelength pair, was not appropriate. The univariate analysis focuses on the variance of this single wavelength and did not take advantage of the information content of a complete spectrum. Statistical methods such as PCA and FDA make it possible to extract information from spectral databases.

Multivariate Statistical Analysis of the Investigated Samples. *Discrimination of Flour Samples from Their Tryptophan Fluorescence Spectra.* To compare the set of tryptophan fluorescence spectra and to emphasize the similarities and differences underlined above, PCA was carried out on the 60 spectra recorded on the 20 flour samples produced from Kamut and soft wheat grains. The score scatter plots of the PC1 versus the PC2 represented 93.7% of the total variance with a predominance of the PC1 accounting for 76.1% of the variance and separated samples according to their cereal products (**Figure 4a**). Indeed, soft wheat flours had mostly all positive score values, while those manufactured from Kamut grains had mostly all negative score values according to PC1. The PC2 discriminate complete soft wheat flours which were located on the positive side from semicomplete soft wheat flours located mostly

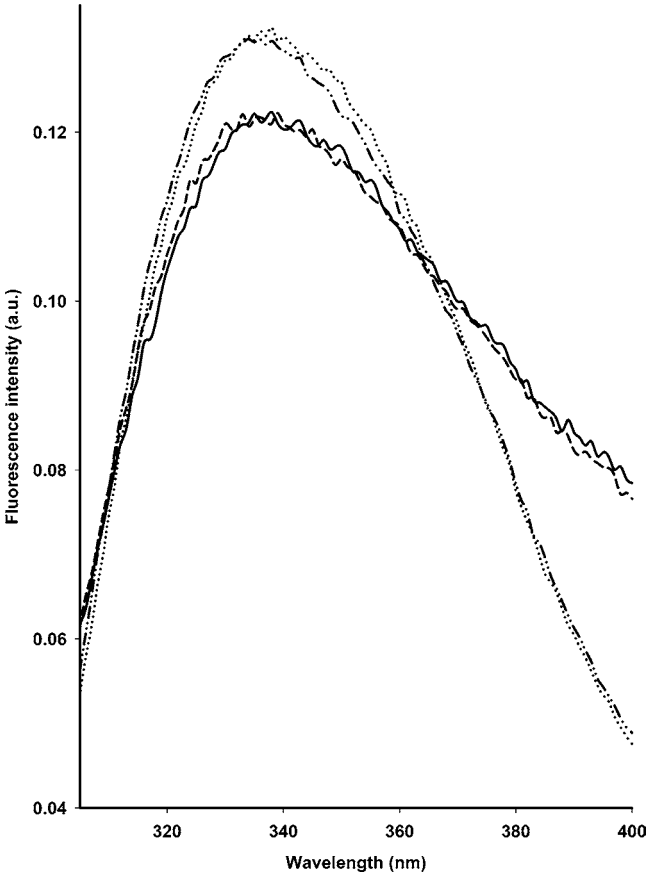

Figure 3. Normalized tryptophan fluorescence spectra of complete Kamut (—), semicomplete Kamut (· · ·), complete hard wheat (– – –), and semicomplete hard wheat (– · · –) pasta.

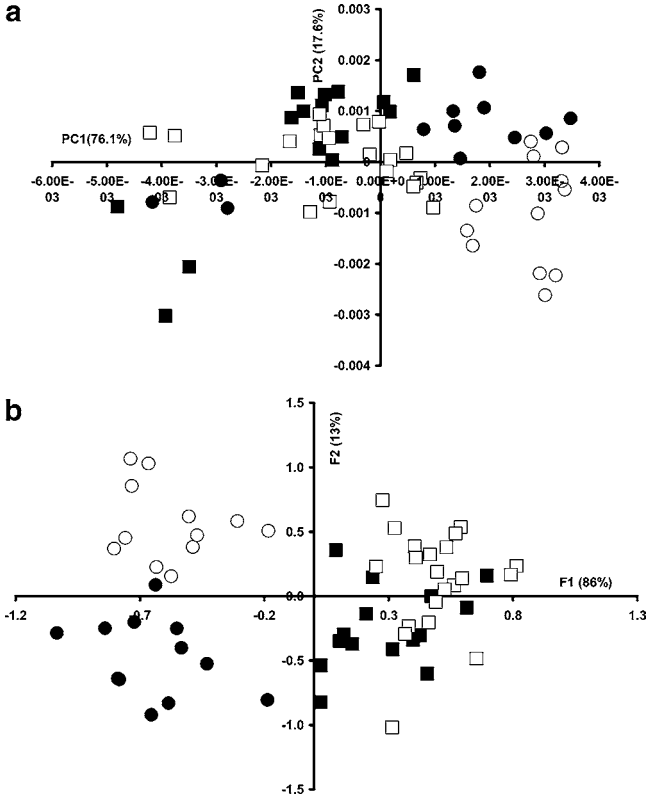

Figure 4. (**a**) Principal component analysis similarity map determined by principal components 1 and 2 of the principal component analysis (PCA) performed on tryptophan fluorescence spectra for complete Kamut (■), semicomplete Kamut (□), complete soft wheat (●), and semicomplete soft wheat (○) flours. (**b**) Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on tryptophan fluorescence spectra for complete Kamut (■), semicomplete Kamut (□), complete soft wheat (●), and semicomplete soft wheat (○) flours.

on the negative side. No discrimination was observed between complete and semicomplete Kamut flours.

In a second step, the ability of tryptophan fluorescence spectra to differentiate between the four groups of flours was investigated by applying FDA on the first five PCs of the PCA performed on the tryptophan fluorescence spectra. Four groups were created for the investigated samples (complete Kamut, semicomplete Kamut, complete soft wheat, and semicomplete soft wheat flours). Again, a good discrimination of soft wheat flours from Kamut flours was observed according to discriminant factor 1 which accounted for 86% of the total variance (**Figure 4b**). Indeed, considering the discriminant factor 1, Kamut flours were observed on the right, whereas soft wheat flours were located on the left. The discriminant factor 2 which took into account 13% of the total variance essentially discriminated complete soft wheat flours from semicomplete soft wheat flours, while only a trend to a good discrimination between complete and semicomplete Kamut flours was also observed according to the same discriminant factor.

The percentage of samples correctly classified into four groups was 86.7% (**Table 2**). **Table 2** shows that any misclassification was observed between Kamut and soft wheat flours. Moreover, 100% correct classification was observed for semicomplete soft wheat flours. Considering complete soft wheat flours, only one spectrum was misclassified as belonging to the semicomplete soft wheat group, while some misclassification occurred between semicomplete and complete Kamut flours.

Discrimination of Semolina Samples from Their Tryptophan Fluorescence Spectra. A number of 33 tryptophan fluorescence spectra were collected from the 11 semolina samples, e.g., 3 complete Kamut, 3 semicomplete Kamut, 3 complete hard wheat, and 2 semicomplete hard wheat semolinas. The map defined by the PC1 and PC2 of the PCA applied to this data set showed some discrimination between the investigated samples (data not shown).

In a second step, FDA was applied to the first five PCs of the PCA applied to the tryptophan fluorescence spectra scanned on semolina samples. The approach described in the above section was also used in this case, e.g., four groups were created (complete Kamut, semicomplete Kamut, complete hard wheat, and semicomplete hard wheat pasta). The map defined by the discriminant factors 1 and 2 is shown in **Figure 5**. A fairly good separation of complete Kamut and complete hard wheat semolinas from semicomplete Kamut and semicomplete hard wheat semolinas was observed according to the discriminant factor 1, which accounted for 73% of the total variance. The discriminant factor 2 discriminated between complete Kamut and semicomplete Kamut semolinas from complete hard wheat and semicomplete hard wheat semolinas. In fact, it appeared that (1) complete hard wheat semolinas exhibited negative scores according to discriminant factor 1 and mostly positive score values according to discriminant factor 2, (2) semicomplete hard wheat semolinas showed positive score values according to discriminant factors 1 and 2, (3) semicomplete Kamut semolinas exhibited positive values according to discriminant factor 1 and negative score values according to discriminant factor 2, and (4) complete Kamut semolinas were characterized by almost negative score values according to discriminant factors 1 and 2. These results suggest that the tryptophan fluorescence probe

Table 2. Classification Table of the Tryptophan Fluorescence Spectra

	observed[a]				
predicted[b]	complete Kamut	semicomplete Kamut	complete soft wheat	semicomplete soft wheat	% correct classification
			Flours		
complete Kamut	**12**	3			**80**
semicomplete Kamut	4	**17**			**80.3**
complete soft wheat			**11**	1	**91.7**
semicomplete soft wheat				**12**	**100**
total					**86.7**

	observed[a]				
predicted[b]	complete Kamut	semicomplete Kamut	complete hard wheat	semicomplete hard wheat	% correct classification
			Semolinas		
complete Kamut	**5**	2	2		**55.6**
semicomplete Kamut		**9**			**100**
complete hard wheat			**9**		**100**
semicomplete hard wheat				**6**	**100**
total					**87.9**
			Pasta		
complete Kamut	**20**	5	8		**60.6**
semicomplete Kamut	4	**15**		11	**50**
complete hard wheat	4		**11**		**73.3**
semicomplete hard wheat				**6**	**100**
total					**61.9**

[a] The number of observed samples. [b] The number of predicted samples.

may be a potential technique for recognizing the type of wheat used for manufacturing semolina. This could be due to the different arrangement of protein tryptophan in semolina produced from the different grains.

Correct classification was observed for 87.9% (**Table 2**). As shown in **Table 2**, 100% correct classification was observed for complete hard wheat, semicomplete hard wheat, and semicomplete Kamut semolinas. Considering complete Kamut semolinas, four spectra were misclassified: two were assigned as complete hard wheat semolinas, and two others were assigned to the semicomplete Kamut group. It was concluded that tryptophan fluorescence spectra are fingerprints allowing a good discrimination of cereal products used for producing semolinas.

Discrimination of Pasta Sample from Their Tryptophan Fluorescence Spectra. Pasta samples were made from 11 complete Kamut, 10 semicomplete Kamut, 5 complete hard wheat semolinas, and 2 semicomplete hard wheat semolinas. PCA was applied to the 28 samples in order to discriminate

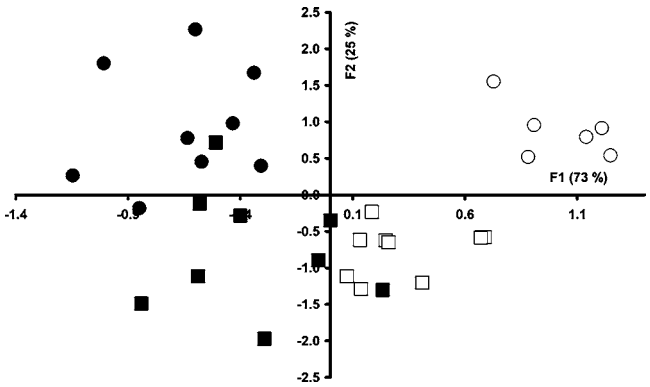

Figure 5. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on tryptophan fluorescence spectra for complete Kamut (■), semicomplete Kamut (□), complete hard wheat (●), and semicomplete hard wheat (○) semolinas.

between the four groups used for manufacturing pasta. The map defined by the PC1 and the PC2 accounted for 98.4% of the total variance (**Figure 6a**). The results obtained from the PCA were quite similar to those obtained with semolina samples. Indeed, The PC1 accounted for 90.8% and discriminated between complete Kamut and complete hard wheat pasta which were located mostly on the negative side from semicomplete Kamut and semicomplete hard wheat pasta located mostly on the positive side.

Spectral patterns associated with the PCs provide the characteristic wavelengths that may be used to discriminate between spectra. Spectral patterns are similar to spectra and were used to derive structural information at the molecular level (*18*). The spectral pattern 1 associated with the PC1 (**Figure 6b**) presented a positive peak at 334 nm. The spectral pattern 1 indicated that the fluorescence spectra of complete Kamut and complete hard wheat pasta exhibited fluorescence in the 380−400 nm region. Such fluorescence is observed in neither the fluorescence spectra of pasta made from semicomplete Kamut and hard wheat semolinas, nor those of flour (**Figure 1**) and semolina (**Figure 2**) samples. As explained here above, the presence of high amounts of bran in complete semolina, complete Kamut, and complete hard wheat compared to those of the other cereal products made with semicomplete grains could explained these difference.

It was concluded that the manufacturing steps (mixing, extruding, and drying phases) of pasta from complete semolinas induced the formation of compounds fluorescing in the 380−400 nm region. Despite the nature of these compounds which are to date unknown, products of the Maillard reactions may explain fluorescence in this region of the spectra (*19*). The differences in fluorescence properties between pasta made from complete Kamut and hard wheat grains and those manufactured with semicomplete Kamut and hard wheat grains may also be related to a higher level of moisture for pasta samples made from complete Kamut and hard wheat grains. In addition, the semicomplete Kamut and semicomplete hard wheat pasta are

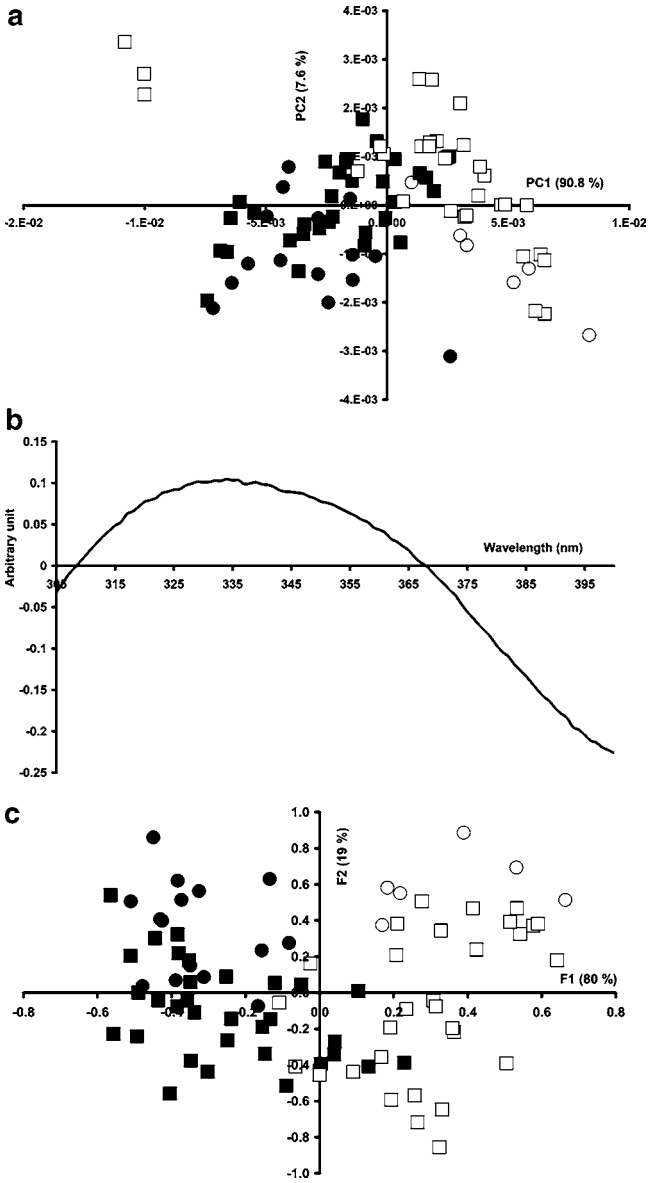

Figure 6. (**a**) Principal component analysis similarity map determined by principal components 1 and 2 of the principal component analysis (PCA) performed on tryptophan fluorescence spectra for complete Kamut (■), semicomplete Kamut (□), complete hard wheat (●), and semicomplete hard wheat (○) pasta. (**b**) Spectral pattern corresponding to the principal component 1 of tryptophan data for complete Kamut, semicomplete Kamut, complete hard wheat, and semicomplete hard wheat pasta. **(c**) Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on tryptophan fluorescence spectra for complete Kamut (■), semicomplete Kamut (□), complete hard wheat (●), and semicomplete hard wheat (○) pasta.

comparatively richer in starch and less rich in all other elements since part of the envelope was removed by sifting after grinding inducing changes in the physicochemical composition of these products. For pasta produced from complete Kamut and complete hard wheat, the grinding is done without sorting. Thus, all the parts of the grain are found in the flour, i.e., endosperm with the white flour and germ rich in protein and oil.

The ability of tryptophan fluorescence spectra to discriminate pasta was investigated by applying the FDA to the first five PCs of the PCA applied to the pasta. The approach described in the above section was also used in this case, i.e., four groups were created (complete Kamut, semicomplete Kamut, complete hard wheat, and semicomplete hard wheat pasta). The map

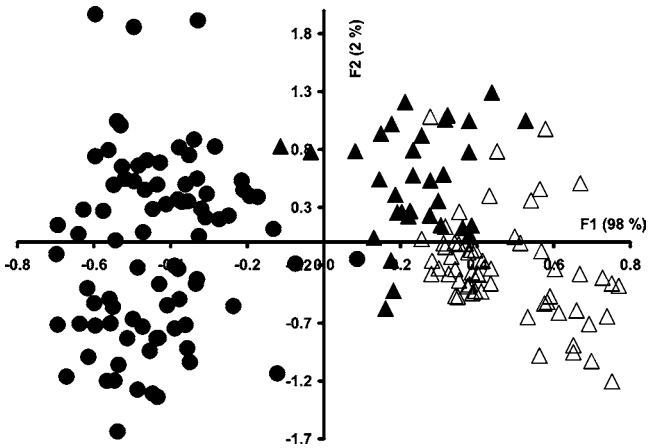

Figure 7. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on tryptophan fluorescence spectra for flour (●), pasta (△), and semolina (▲) samples.

defined by discriminant factors 1 and 2 represented 99% of the total variance with discriminant factor 1 accounting for 80% (**Figure 6c**). Considering the discriminant factor 1, semicomplete Kamut and semicomplete hard wheat pasta had mostly positive score values, whereas complete Kamut and complete hard wheat pasta presented mostly negative scores. In addition, a fairly good separation of semicomplete hard wheat pasta from complete hard wheat pasta was observed according to discriminant factor 1, similar to what was found for complete and semicomplete Kamut.

Correct classification was observed for 61.9% of the samples (**Table 2**). This table illustrate that 100% correct classification was observed for spectra of pasta manufactured with semicomplete hard wheat semolinas, while some misclassification occurred for the other pasta spectra. Considering pasta made with complete hard wheat, four spectra were classified as belonging to the complete Kamut group. For complete Kamut pasta, 13 spectra were misclassified: five spectra were with the semicomplete Kamut group and eight with the complete hard wheat group. The worst classification was obtained with semicomplete Kamut pasta since only 50% of them were correctly classified. This could be due to the pasta processing which can also produce changes in carbohydrate content. Indeed, during mixing, extruding, and drying phases, starch can suffer damage, releasing free maltose (*20*). Indeed, pasta is made up of a high content of carbohydrates (70.0 g/100 g of dried pasta), principally starch (63.0 g/100 g of dried pasta) and free carbohydrates. Proteins are also present in minor proportion (11.5 g/100 g of dried pasta) (*21*). Thus, the release of free maltose could indicate a different arrangement of tryptophan protein residues and for consequent different shapes of the tryptophan spectra.

Finally, the tryptophan fluorescence spectra recorded on flours, pasta, and semolinas were pooled into one matrix and analyzed by PCA. Considering the similarity map defined by the PC1 and PC3, a good discrimination between flours and pasta or semolinas was observed according to the PC1 (data not shown). The FDA was then applied to the first five PCs of the PCA. The map defined by the discriminant factors 1 and 2 of the FDA performed on the data sets is shown in **Figure 7**. Considering discriminant factor 1 accounting for 98% of the total variance, flour samples were observed on the left, while pasta and semolina samples were observed on the right. In addition, a good discrimination between pasta and semolina samples was observed.

Table 3. Classification Table of the Tryptophan Fluorescence Spectra

	observed[a]			
predicted[b]	flours	pasta	semolinas	% correct classification
flours	**82**		2	**97.6**
pasta		**51**	9	**85**
semolinas		4	**29**	**87.9**
total				**91.6**

[a] The number of observed samples. [b] The number of predicted samples.

Correct classification was observed for 91.6% (**Table 3**). This table shows that most of flour spectra were correctly classified, since only two out of 84 spectra were classified as belonging to semolina group. Some misclassification occurred between semolina and pasta spectra, but none of them was misclassified with flour spectra. It appeared that front-face fluorescence spectroscopy could be used for recognizing flour products from pasta and semolina products.

The identities of the different samples in each class were also investigated. Good discrimination between cereal products produced from Kamut grains was observed (**Figure 8a**). Considering the PC1 which accounted for 93% of the total variance, pasta samples had mostly positive scores, whereas semolina and flour samples exhibited negative scores values. Although semolina and flour samples were overlapped on the map, a trend to a good discrimination between these samples was observed according to the PC2. Similar results were obtained from cereal products produced from hard wheat grains (**Figure 8b**). Indeed, pasta samples were observed mostly on the right according to the PC1, whereas semolina samples were located on the left. In addition, this map differentiates between samples made from hard wheat grains from those produced with soft wheat grains.

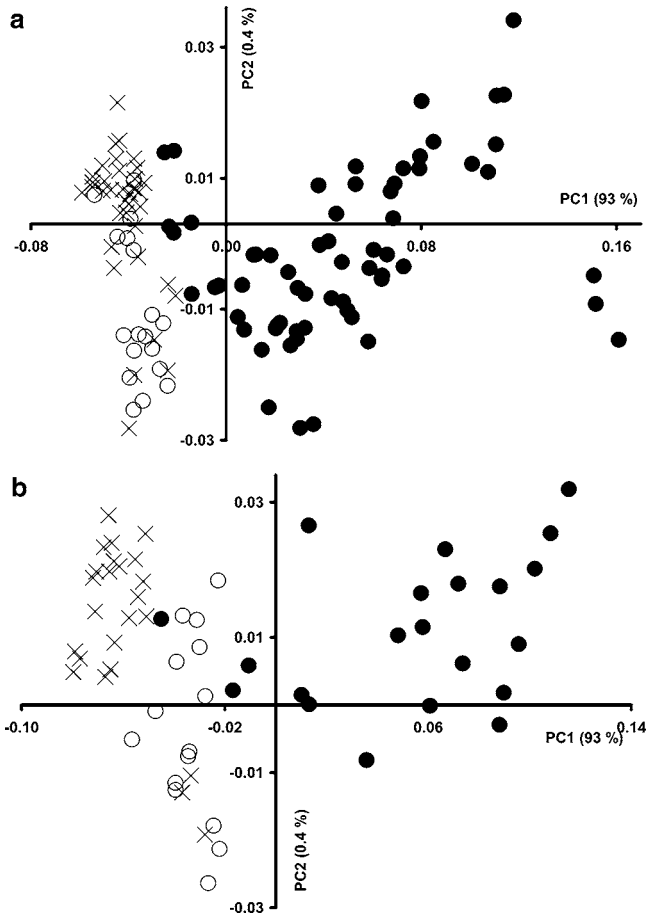

Figure 8. (**a**) Principal component analysis similarity map determined by principal components 1 and 2 of the principal component analysis (PCA) performed on tryptophan fluorescence spectra for flours (×), pasta (●), and semolinas (○) produced from Kamut grains. (**b**) Principal component analysis similarity map determined by principal components 1 and 2 of the principal component analysis (PCA) performed on tryptophan fluorescence spectra for flours (×) produced with soft wheat grains, pasta (●), and semolinas (○) made with hard wheat grains.

From the **Figure 8**, parts **a** and **b**, it appeared that (i) flours made from Kamut and soft wheat grains exhibited negative scores according to PC1 and mostly positive scores according to PC2; (ii) semolina samples made from Kamut and hard wheat grains showed mostly negative values according to PC1 and PC2; and (iii) pasta samples produced with Kamut and hard wheat grains had mostly positive score values according to PC1. It was concluded that tryptophan fluorescence spectra are fingerprints allowing the identification of the cereal products according to their manufacturing processes. These spectroscopic differences result from different protein−protein interactions and different network structures. They are the consequences of the processes used to manufacture these products. This result was in agreement with previous findings reporting that the shape of tryptophan fluorescence spectra change according to the cheese-making procedures (*14*).

The current results showed that tryptophan fluorescence spectra could be used for differentiating between Kamut flours and soft wheat flours. In addition, the same intrinsic probe show a good discrimination of pasta and semolinas made from complete hard wheat and complete Kamut pasta from those produced from semicomplete hard wheat and semicomplete Kamut pasta. The best results were obtained on tryptophan fluorescence spectra recorded on semolinas since the four groups were clearly discriminated.

However, with only 20 independent samples for flours, 28 for pasta, and 11 for semolinas, the current model was not very robust, and further research is needed. The spectral database should include the spectra of additional intrinsic probes such as riboflavin (*7*). The reported work is only a feasibility study, and further studies using considerably more samples (with known genotype and botanical origin) are required before its value may be validated and adopted in routine analysis. The effect of different botanical origins needs to be investigated in order to provide a robust model to discriminate between wheat, pasta, and semolina using front-face fluorescence spectroscopy.

The simplicity of the method offers rich opportunities for efficient characterization of cereal-based products and could assist in solving problems linked with the authentication of food manufactured with high-priced wheat grains from the others. Front-face fluorescence spectroscopy is easy to perform, rapid, and nondestructive. It neither needs any particular sample preparation nor special qualification of the personnel. This technique could be easily extended to other complex solid substrates, including nonalimentary ones, providing the sample retained fluorescent molecules.

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LITERATURE CITED

(1) Stallknecht, G. F.; Gilbertson, K. M.; Ramey, J. E. Alternative wheat cereals as food grains: Einkorn, emmer, spelt, kamut, and triticale. In *Progress in New Crops*; Janick, J., Ed.; ASHS Press: Alexandria, VA, 1996; pp 156−170.

(2) Strasburg, G. M.; Ludescher, R. D. Theory and application of fluorescence spectroscopy in food research. *Trends Food Sci. Technol.* **1995**, *6*, 69−75.
(3) Lakowicz, J. R. Fluorophores. In *Principles of Fluorescence Spectroscopy*; Pelenum Press: New York, 1983; pp 63−93.
(4) Zandomeneghi, M.; Festa, C.; Carbonaro, L. Front-surface absorbance spectra of wheat flour: determination of Carotenoids. *J. Agric. Food Chem.* **2000**, *48*, 2216−2221.
(5) Genot, C.; Tonetti, F.; Montenay-Garestier, T.; Marion, D.; Drapon, R. Front-face fluorescence applied to structural studies of proteins and lipid−protein interactions of viscoelastic food products. 2. Application to wheat gluten. *Sci. Aliments* **1992**, *12*, 687−704.
(6) Yeboah, N. A.; Freedman, R. B.; Popineau, Y.; Shewry, P. R.; Tatham, A. S. Fluorescence studies of two γ-gliadin fractions from bread wheat. *J. Cereal Sci.* **1994**, *19*, 141−148.
(7) Zandomeneghi, M.; Carbonaro, L.; Calucci, L.; Pinzino, C.; Galleschi, L.; Ghiringhelli, S. Direct fluorometric determination of fluorescent substances in powders: the case of riboflavin in cereal flours. *J. Agric. Food Chem.* **2003**, *51*, 2888−2895.
(8) Dufour, É.; Karoui, R.; Bosset, J. O. Utilisation de la fluorescence frontale intrinsèque de fromages de type L'Etivaz AOC et Gruyère AOC pour reconnaître leur origine géographique. *Mitt. Geb. Lebensmittelunters. Hyg.* **2003**, *94*, 379−393.
(9) Karoui, R.; Dufour, É.; Pillonel, L.; Picque, D.; Cattenoz, T.; Bosset, J. O. Fluorescence and infrared spectroscopies: a tool for the determination of the geographic origin of Emmental cheeses manufactured during summer. *Lait* **2004**, *84*, 359−374.
(10) Karoui, R.; Dufour, É.; Pillonel, L.; Picque, D.; Cattenoz, T.; Bosset, J. O. Determining the geographic origin of Emmental cheeses produced during winter and summer using a technique based on the concatenation of MIR and fluorescence spectroscopic data. *Eur. Food Res. Technol.* **2004**, *219*, 184−189.
(11) Karoui, R.; Bosset, J. O.; Mazerolles, G.; Kulmyrzaev, A.; Dufour, É. Monitoring the geographic origin of both experimental French Jura hard cheeses and Swiss Gruyère and l'Etivaz PDO cheeses using mid-infrared and fluorescence spectroscopies. *Int. Dairy J.* **2005**, *15*, 275−286.
(12) Karoui, R.; Dufour, É.; Pillonel, L.; Schaller, E.; Picque, D.; Cattenoz, T.; Bosset, J. O. Determination of the geographic origin of Emmental cheeses by combining infrared and fluorescence spectroscopies. *Int. Dairy J.* **2005**, *15*, 287−298.
(13) Bertrand, D.; Scotter, C. N. G. Application of multivariate analyses to NIR spectra of gelatinized starch. *Appl. Spectrosc.* **1992**, *46*, 1420−1425.
(14) Herbert, S.; Mouhous Riou, N.; Devaux, M. F.; Riaublanc, A.; Bouchet, B.; Gallant, J. D.; Dufour, É. Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. *Lait* **2000**, *80*, 621−634.
(15) Safar, M.; Bertrand, P. R.; Devaux, M. F.; Genot, C. Characterization of edible oils, butters, and margarines by Fourier transform infrared spectroscopy with attenuated total reflectance. *J. Am. Oil Chem. Soc.* **1994**, *71*, 371−377.
(16) Karoui, R.; Dufour, E. Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. *Int. Dairy J.* **2003**, *13*, 973−985.
(17) Möller, M.; Denicola, A. Protein tryptophan accessibility studied by fluorescence quenching. *Biochem. Mol. Biol. Educ.* **2002**, *30*, 175−178.
(18) Dufour, É.; Subirade, M.; Loupil, F.; Riaublanc, A. Whey proteins modify the phase transition of milk fat−globule phospholipids. *Lait* **1999**, *79*, 217−228.
(19) Kulmyrzaev, A.; Dufour, É. Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. *Lait* **2002**, *82*, 725−735.
(20) García-Baños, J. L.; Corzo, N.; Olano, A. Maltulose and furosine as indicators of quality of pasta products. *Food Chem.* **2004**, *88*, 35−38.
(21) Souci, S. W.; Fatchmann, W.; Kraut, H. *Food Composition and Nutrition Tables*; Wissenshaftliche Verlagsgesellshaft mbH: Stuttgart, Germany, 1986; pp 560−564.

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