

Qualitative Characterization of the Purity of Milled Durum Wheat Products by Multidimensional Statistical Analysis of Their Mid-Infrared Diffuse Reflectance Spectra

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ABSTRACT

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Because assessment of durum wheat semolina purity by the standard ash test has been widely criticized, we attempted to characterize products of a semolina mill by mid-infrared diffuse reflectance spectroscopy, which up to now has met with limited success. Recent technological advances may open new possibilities for this spectral range; new mathematical treatments are reported that extract the main spectral phenomena in a set of spectra. A collection of milled wheat products ranging from very pure semolinas to brans was chosen for this study. Three mathematical treatments (multidimensional statistical analyses) were applied to the spectra to

condense the data with minimal loss of information: principal component analysis, morphological analysis, and correspondence factorial analysis. By these methods, characteristic wave numbers of the main components of wheat were identified for starch (1,166, 1,102, and 1,066 cm^{-1}), cellulose (1,134 and 1,178 cm^{-1}), noncellulosic cell-wall components (1,730 and 1,594 cm^{-1}), and gluten (1,694 and 1,554 cm^{-1}). Maps showing a separation of the three kinds of milled products (semolinas, flours, and brans) and a rough classification of these products according to purity were obtained without biochemical calibration.

One of the main specifications for milled products of durum wheat, and particularly of semolinas, is what practitioners call "purity" (Irvine 1971). This criterion characterizes the degree of contamination of starchy endosperm (semolina) by the outer layers of the kernel (aleurone layer and bran). During milling, the semolina is purified, but a varying proportion of bran particles is retained in the end product. A high bran content leads to unsatisfactory technological properties of semolina (Houliaropoulos et al 1981). Assessment of purity is usually measured based on mineral content, by the ash test, because outer kernel layers have higher ash contents than the starchy endosperm. However, there are problems in evaluating semolina purity by the ash test (Godon 1977, Abecassis and Feillet 1985): different wheats have different mineral contents and ash increments when comparing bran to "pure endosperm." A technique providing rapid and reliable results for bran itself, mostly made of cell walls, would be preferable. Although many chemical methods have been developed to measure the fiber content of biological material (Van Soest 1963, Van Soest and Wine 1967, AOAC 1975), they are time-consuming and only apply to highly fibrous material.

Some physical methods have also been investigated. The major food constituents, including cell-wall components, absorb in the near-infrared region (1,100–2,500 nm), where these constituents present overtones and combination bands. Near-infrared reflectance is widely used to rapidly measure the composition of foods and food products (Norris 1978), notably cereal products (Osborne et al 1982). This technique has also been used to determine fiber in cereal foods (Baker 1983, Horvath et al 1984). The fundamental vibration-rotation bands and the skeletal vibrations fall in the mid-infrared range (400–4,600 cm^{-1}). This spectral region was recently used to study cell-wall components (Morikawa and Senda 1978, Hopkinson et al 1985). Use of the mid-infrared to characterize complex products such as foods and food products has, however, long been precluded by technical problems. The advent of Fourier transform spectrometers, with their many advantages over traditional dispersive instruments, has improved the quality of infrared data while making data collection faster and easier (Griffiths and De Haseth 1986). Also, new devices and easier techniques have been developed to study opaque powders (Fuller and Griffiths 1978). Mid-infrared spectra contain a wealth of information about material constituents. Complex

spectra make interpretation difficult, but this problem may be overcome by using multidimensional statistical analyses (Devaux et al 1986, Cowe and Mc Nicol 1985).

This preliminary study investigates the potentialities of mid-infrared diffuse reflectance spectroscopy for characterizing the quality of milled durum wheat products (including semolinas, flours, and brans) using new technical developments combined with multidimensional statistical analyses. This combination has not yet, to our knowledge, been used for cereal products, and could be interesting. Identification of characteristic absorption bands of cell-wall components was attempted.

MATERIALS AND METHODS

Samples

Nineteen milled products were produced from the durum wheat Agathe in an experimental durum mill of the Laboratoire de Technologie des Céréales, INRA (Montpellier, France). The samples included seven semolinas, eight flours, and four brans collected at different stages of milling. They were coded as follows: semolinas, SE1 to SE6 and SET (mixture of all the semolinas); flours, FB1 to FB4 and FD1 to FD4; brans, BR1 to BR4; and wheat, WHE.

Variation of purity (bran content) of milled products was evident: bran particles were visible in some flours and semolinas, notably in SE6. Purity was estimated by an ash test. Actually, although ash content cannot be used for milled products coming from different wheat cultivars, it does express their purity when applied to the products of a single wheat, as was the case in our experiment. Semolinas had the lowest ash contents (0.63–0.96% dry matter), followed by flours (1.76–2.64% dry matter). Brans had the highest ash contents (3.36–5.81% dry matter). Semolinas SE3 and SE1 were the purest, SE2 and SE4 were intermediary, and SE5 and SE6 were the most contaminated by outer layer particles.

Except for the flours, which were finely powdered, all milled products and wheat were reground using a Cyclotec grinder with a 0.5-mm mesh.

Purified wheat starch (Rofec 61-927), microcrystalline cellulose (Avicell PH 101 Mercks), wheat gluten extracted according to Godon et al (1983), and cell-wall fractions were also available. The cell walls were isolated from the milled products by the procedure of Brillouet and Carre (1983).

Mid-Infrared Diffuse Reflectance Spectra

Mid-infrared spectra were collected on a Nicolet 10-MX Fourier transform spectrometer. Diffuse reflectance spectra were obtained with a Barnes diffuse reflection cell (Messerschmidt 1985).

Finely ground homogeneous material was placed in the sample

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cup (13 mm diameter by 2 mm deep). Excess material was removed by passing a straight edge across the surface. The sample cup was then placed in the diffuse reflection cell, continually purged with dry air. The sample was purged 5 min before recording the spectra. Finely powdered dry potassium chloride was used to obtain a background against which spectra of all other samples were compared.

The data were recorded from 1,850 to 1,000 cm^{-1} in 4-cm increments as $\log(1/R)$, where R is the ratio of the reflected intensity for the background to that of the sample. Three hundred scans were averaged for each spectrum. The intensity of the spectra was low: the highest peaks had $\log(1/R)$ values lower than 0.1 (0.035–0.058 on baseline-corrected spectra).

Baseline adjustment was carried out manually by application of software supplied with the spectrophotometer.

Mathematical Treatments

Multidimensional statistical analyses were performed on an IBM PC microcomputer. Software based on the programs of Foucart (1982) was developed at the Laboratoire de Technologie des Aliments des Animaux, INRA (Nantes, France).

Multidimensional statistical analyses, such as principal component analysis (PCA) and correspondence factorial analysis, describe variation in multidimensional data by a few synthetic variables. These synthetic variables are linear combinations of all the original variables, and have the advantage of having no correlation with each other. Simpler descriptions of data sets are thus obtained with minimal loss of information. These treatments were used for morphological analysis (Le Nouvel 1981) of spectra and to graphically represent similarity of spectra.

PCA

A spectrum including n spectral data can be seen as a point in an n -dimensional space. Similarly, a set of spectra forms a multidimensional volume in that space. Each dimension corresponds to an axis or wave number at which the sample is measured. The description of the multidimensional volume using the original data may not, however, be the simplest mathematical way to characterize a collection of spectra (Bertrand et al 1984). This is notably true with mid-infrared spectra: in the mid-infrared range, data may be highly correlated. The information has a large redundancy, which can be eliminated by creating a more suitable system of axes.

The first improvement is to put the origin of the axes at the center of the multidimensional volume by centering the data, i.e., subtracting the mean spectrum from each observation. A rotation of axes is then performed to put the first axis (first principal component) in the largest dimension of the volume. The second component is chosen to be orthogonal to the first one, taking width into account. Subsequent components are calculated in the same way, each at right angles to all the preceding components, and adjusted to the largest remaining dimension. In practice, components are determined to give the largest sum of the square (variance) of the projection of the observations.

Mathematically, the directions of the new axes are defined by the eigenvectors of $X'X$, where X is the centered data table (rows = observations, columns = variables), and X' is the transposed matrix. Variances along each axis are the eigenvalues of $X'X$. Percentages of total variance, which are the ratio (in percent) of the corresponding eigenvalues to the total variance, are also commonly used.

PCA can also be performed with spectra as variables and wave numbers as observations. This mathematical treatment applied to digitized continuous curves is called morphological analysis. The principal components obtained are linear combinations of spectra; the spectra can also be described, from the opposite point of view, as linear combinations of the principal components. Morphological analysis thus splits spectra into linear combinations of independent "spectral patterns." The spectral pattern corresponding to a principal component can be drawn by associating each wave number to its coordinate on that principal component. Spectral patterns are abstract features that represent the spectral variation

in the set of spectra. It is interesting to study the shapes of spectral patterns, which often resemble spectra of known constituents, or known absorption bands (Robert and Bertrand 1985a, Cowe et al 1985).

A standard PCA (with spectra as observations) was also performed. This mathematical treatment is used to draw similarity maps and analyze resemblances and differences among spectra.

Because our program could not handle more than 80 variables, a selection was made among available wave numbers, based on results of the morphological analysis: parts of the spectral range corresponding to the highest coordinates of spectral patterns were retained. Data in each column of the data table were divided by the standard deviation of that column to give the same weight to each wave number in the creation of principal components. Contributions of wave numbers to creation of principal components were used to help to interpret the spectral significance of principal components. Contributions can be evaluated from the formula:

$$C_{i\bar{\nu}} = \frac{(x_i^{\bar{\nu}})^2 \times 1,000}{P_i} \quad (1)$$

where $C_{i\bar{\nu}}$ = the contribution of the wave number $\bar{\nu}$ to the creation of the principal component i , $x_i^{\bar{\nu}}$ = the coordinate of the wave number $\bar{\nu}$ on the principal component i , and P_i = the variance along the principal component i .

Correspondence Factorial Analysis (CFA)

CFA (Benzecri and Benzecri 1980) is a statistical technique that gives the same role to rows and columns of a data table, allowing representation of observations and variables on the same graph.

The principle of CFA is very similar to that of PCA. The reciprocity of the analysis on the rows and the columns and the mixed representations require data transformation before a standard PCA. Good descriptions of CFA and justification of the transformation are given in Lebart et al (1977) and Lefebvre (1983). The synthetic variables calculated by CFA are called principal factors.

One advantage of CFA is that it can be performed using either spectra or wave numbers as observations. The most convenient line of action, which, for practical reasons, is the one where variables are less numerous, can be chosen. Wave numbers were thus considered to be observations, and spectra were treated as variables.

RESULTS

Direct Observation of Spectra

Spectra of starch, gluten, cellulose, and purified cell walls are given in Figure 1. Some spectral distortion was observed, because of the physical state of the samples (undiluted powders) and the specular component of the reflected light. Particle size and sample preparation, together with the diffuse reflectance technique, caused this disturbance. Gluten showed an absorption band at 1,694 cm^{-1} , an unexpectedly high frequency for an amide I band; this shift was ascribed to the chemical and physical nature of gluten proteins and to measurement conditions. Absorption bands of cellulose were found in the spectrum of the cell walls (fibrous material made of cellulose, hemicelluloses, and lignin).

Spectra of wheat (WHE), of bran (BR1), and of semolina (SE3) (Fig. 2) show some similarities between the spectrum of semolina and that of starch, and between the spectrum of bran and that of cell walls. The spectrum of wheat showed characteristic absorption bands of starch and of cell walls. Comparison with earlier works (Lin and Pomeranz 1965) showed a good overall resemblance with other wheat spectra (particularly with durum wheat): broad bands at 1,660 cm^{-1} and 1,400–1,300 cm^{-1} , peaks at about 1,450, 1,160, and 1,060 cm^{-1} .

Morphological Analysis

Results of the morphological analysis carried out on the original

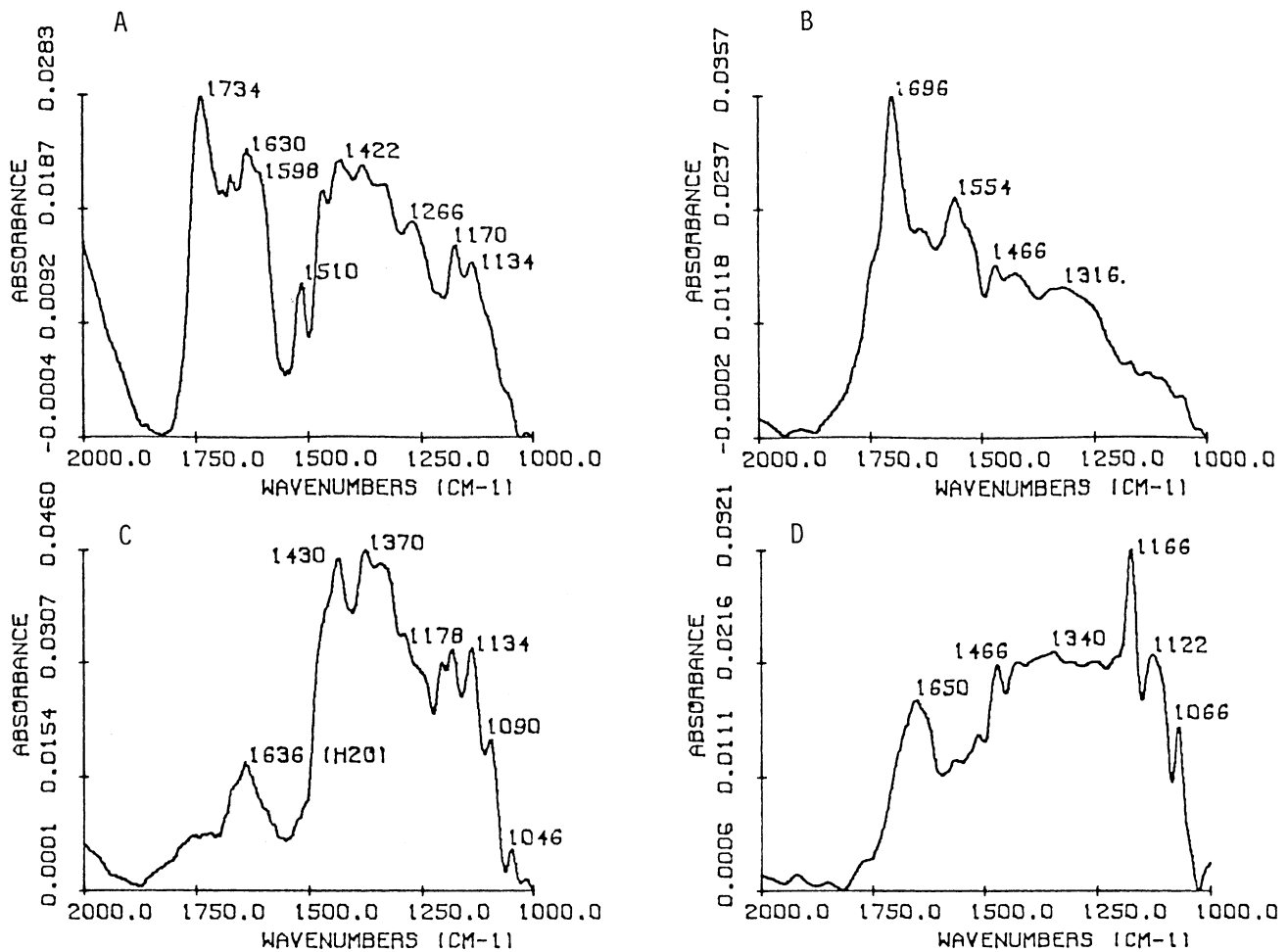


Fig. 1. Diffuse reflectance spectra of A, cell walls extracted from BRI by the procedure of Brillouet and Carre (1983); B, wheat gluten; C, microcrystalline cellulose, and D, wheat starch from 2,000 to 1,000 cm^{-1} . The samples were run as undiluted powders.

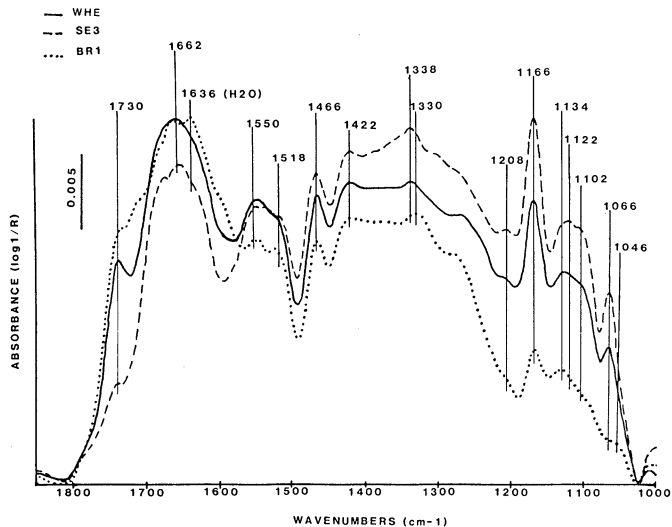


Fig. 2. Diffuse reflectance spectra of wheat (—), semolina (---), and bran (····) from 1,850 to 1,000 cm^{-1} . The samples were ground in a Cyclotec grinder (0.5-mm mesh) and run as undiluted powders.

spectral data ($\log 1/R$) are given in Figure 3. The first four principal components accounted for 99.6% of the total variance.

The spectral pattern associated with principal component 1 (96.3% of total variance) (Fig. 3A) described the general outline of the spectra. Multidimensional analyses split data in a sum of independent phenomena. With spectral data, the main phenomenon (which corresponds to the first principal component) is a difference in general intensity: in first approximation, spectra are varying proportions of the average spectrum. The spectral

patterns in Fig. 3B–D corresponded to principal components 2 (1.9% of the total variance), 3 (1% of the total variance), and 4 (0.4% of the total variance). They exhibited positive peaks at 1,730 or 1,714 cm^{-1} and 1,594 cm^{-1} related to cell walls. The spectral patterns associated with principal components 2 and 3 also featured negative peaks assigned to starch (1,066, 1,102, 1,122, and 1,166 cm^{-1}). These peaks in opposite directions corresponded to a comparison between those parts of spectra related to a statistical opposition in concentrations of starch and of cell walls in milled products. In addition to the peaks at 1,730 and 1,594 cm^{-1} , the spectral pattern corresponding to principal component 4 showed absorption bands that were characteristic of cellulose (1,178 and 1,134 cm^{-1}).

PCA

Three portions of the spectral range (1,750–1,654 cm^{-1} , 1,602–1,550 cm^{-1} , and 1,194–1,050 cm^{-1}), containing a total of 76 data points, were selected for PCA. The main absorption bands of starch and cell walls extracted by morphological analysis were within these limits.

The first four principal components accounted for 99.5% of the total variance. Previous experiments (Robert and Bertrand 1985b) showed the first principal component (91.2% of the cumulated variance) to be representative of the general intensity of the spectra (which variation is the main quantitative phenomenon). Principal components 2 and 3 (5.7 and 1.8%, respectively, of the total variance) defined the similarity map in Figure 4. A discrimination of spectra according to the nature of samples was observed: brans, flours, and semolinas were clearly separated. Semolina SE6, which was known to have a high bran content, is near the brans. The contributions of the wave numbers to the creation of the principal components, calculated according to formula 1, are given in Table

I; the contributions of wave numbers with a negative coordinate were differentiated by a minus sign. Principal component 2 contrasted wave numbers 1,166, 1,102, and 1,066 cm^{-1} (starch) to wave numbers 1,730, 1,714, and 1,594 cm^{-1} (cell walls). Principal component 3 set absorption bands of cell wall (1,730 and 1,594 cm^{-1}) over against absorption bands of gluten (1,694 and 1,554 cm^{-1}) and one absorption band of starch (1,166 cm^{-1}). Only spectral data were used in creating principal components; thus PCA extracted potentially useful information based solely on spectra.

CFA

CFA was performed on all spectral data. The first four principal factors accounted for 96.3% of the total variance. Principal factor 1 (62.3% of the total variance) represented the variations of the general intensity.

On graphical representations featuring both spectra and wave

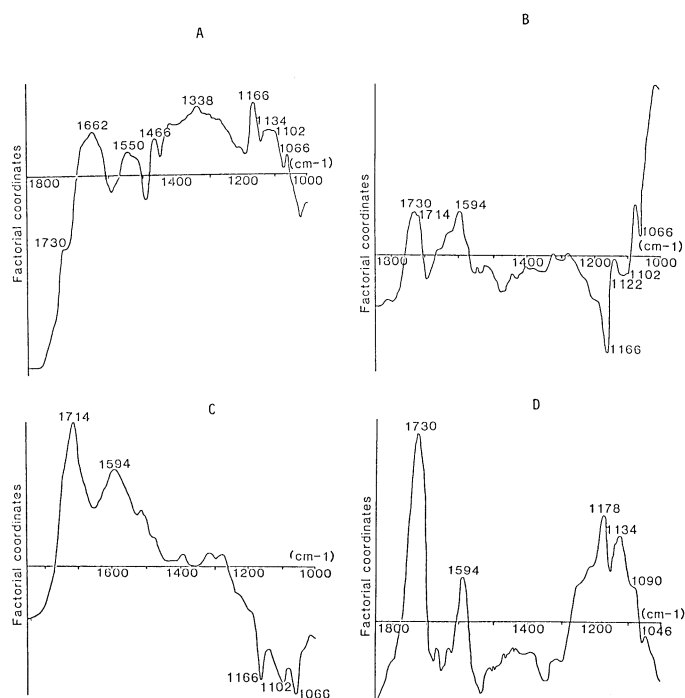


Fig. 3. Morphological analysis: spectral patterns of **A**, principal component 1 (96.3% of total variance); **B**, principal component 2 (1.9% of total variance); **C**, principal component 3 (1% of total variance); **D**, principal component 4 (0.4% of total variance). X axes are wave numbers (from 1,850 to 1,000 cm^{-1}), and Y axes are factorial coordinates for the represented principal component (arbitrary intensity units).

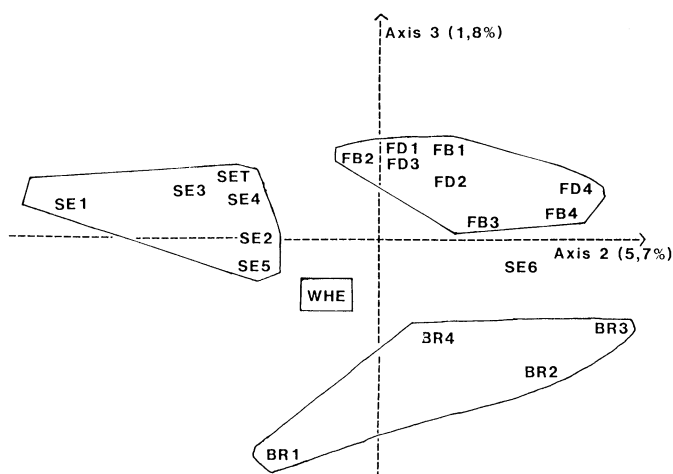


Fig. 4. Principal component analyses: similarity map defined by principal components 2 and 3 (5.7 and 1.8% of total variance, respectively).

numbers, a striking point was that consecutive wave numbers followed one another in an ordered manner. For better legibility, we linked the wave numbers in increasing order. The line thus obtained is called the "parametric curve." Le Nouvel (1981) has shown that, in multidimensional analyses of digitized continuous curves, replacing the individual data points by such parametric curves is possible and meaningful. Changes in direction of parametric curves usually indicate significant wave numbers. An interpretation of the proximity between a given wave number and the spectrum of a sample is possible if that sample has characteristic composition, i.e., is very rich in one chemical component. These proximities were used to confirm assignments of the most significant absorption bands.

The graphical representation defined by principal factors 2 (16.7% of the total variance) and 3 (11.5% of the total variance) is shown in Figure 5. The layout of spectra was similar to that obtained by PCA (Fig. 4). Clear differentiation of brans, semolinas, and flours was observed. Semolina SE6, rich in cell wall components, was located near the brans.

The parametric curve featured changes in direction (in the area of semolinas) at 1,166, 1,102, and 1,066 cm^{-1} (attributed to starch) and 1,134 cm^{-1} . A local opposition was observed between wave numbers of starch and the wave number at 1,134 cm^{-1} , characteristic of cellulose. Brans are in the same quadrant as characteristic absorption bands of cell wall components (1,730–1,714 cm^{-1} , 1,594 cm^{-1}).

The similarity map given in Figure 6 is defined by principal factors 2 and 4 (5.8% of the cumulated variance). The main features of the parametric curve were changes in direction at 1,730 and 1,594 cm^{-1} , set against wave numbers at 1,166 and 1,066 cm^{-1} . This information can indicate purity of milled products of durum wheat (line 1). Moreover, results are in agreement with those reported by

TABLE I
Contributions to the Creation of the Axes
of Principal Component Analysis ($\times 100$)

Wave Number	Principal Component		
	1	2	3
1,730	12.3	22.5	-21.4
1,714	12.1	30.3	-11.8
1,694	13.1	9.3	29.4
1,594	13	14	-15.3
1,554	13.5	5.3	19.1
1,178	13.3	-7	20.3
1,166	12.7	-15.8	32.8
1,134	13.7	-10.3	0.3
1,102	13.3	-18.1	0.3
1,090	13.4	-15.9	-3.3
1,066	12.8	-22.1	-4.4

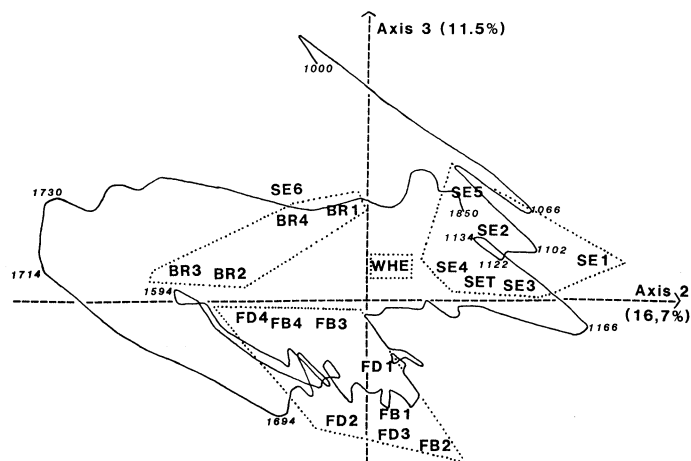


Fig. 5. Correspondence factorial analyses: similarity map defined by principal factors 2 and 3 (16.7 and 11.5%, respectively, of total variance). The continuous line is the parametric curve, drawn by linking the points corresponding to wave numbers in increasing order. The italicized numbers are wave numbers where changes in direction occur.

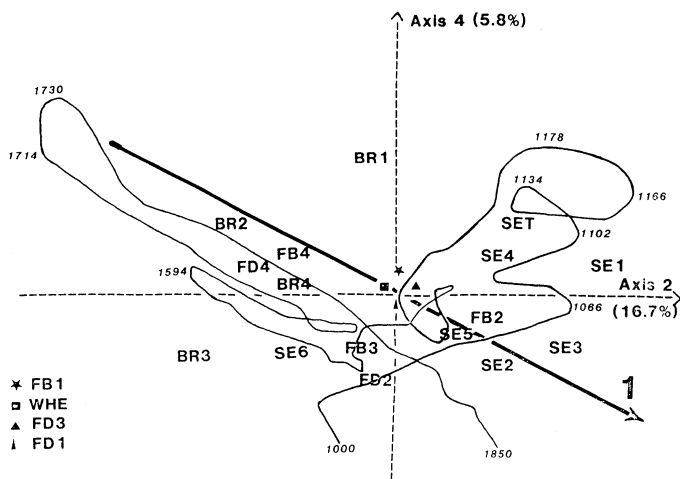


Fig. 6. Correspondence factorial analyses: similarity map defined by principal factors 2 and 4 (16.7 and 5.8%, respectively, of total variance). Line 1 corresponds to a purity axis.

Houliaropoulos et al (1981) concerning the histological origin of the various mill streams.

CONCLUSION

Two main conclusions were reached: 1) Diffuse reflectance infrared Fourier transform spectroscopy has potential to characterize milled products of durum wheat. Components characteristic of cell walls were identified by absorption bands at 1,178 and 1,134 cm^{-1} (cellulose) and 1,730 and 1,594 cm^{-1} (non-cellulosic cell wall material). Significant wave numbers were also extracted for starch (1,166, 1,102, and 1,066 cm^{-1}). Development of a test to detect and measure the presence of peripheral layers in semolina seems to be possible. However it would be necessary to confirm these results by using various wheat cultivars from different locations.

2) Multidimensional statistical analyses allow a clear description of spectral data. Morphological analysis gives access to the patterns of spectral variation: the portions of spectra that have high factorial coordinates in morphological analysis match important absorption bands of components that have varying concentrations between the samples. Resemblances between spectra are visualized on the similarity maps of PCA. An assignment of the wave numbers can be realized using CFA.

LITERATURE CITED

- ABECASSIS, J., and FEILLET, P. 1985. Pureté des semoules de blé dur, taux de cendres et réglementation. *Ind. Céréales* 36:13-18.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Official Methods of Analysis, 12th ed. The Association: Washington, DC.
- BAKER, D. 1983. The determination of fiber in processed cereal foods by near-infrared spectroscopy. *Cereal Chem.* 60:217-219.
- BENZECRI, J. P., and BENZECRI, F. 1980. *Pratique de l'Analyse des Données*. Dunod: Paris.
- BERTRAND, D., ROBERT, P., and TRAN, V. 1984. Traitements mathématiques de spectres NIR de mélanges. Page 11 in: Proc. 11th ICC Mtg., Vienna.
- BRILLOUET, J. M., and CARRE, B. 1983. Composition of cell-walls from cotyledons of *Pisum sativum*, *Vicia faba*, and *Glycine max*. *Phytochemistry* 22:841-847.
- COWE, I. A., and MC NICOL, J. W. 1985. The use of principal components in the analysis of near-infrared spectra. *Appl. Spectrosc.*

- 39(2):257-266.
- COWE, I. A., MC NICOL, J. W., and CUTHBERTSON, D. C. 1985. A designed experiment for the examination of techniques used in the analysis of NIR spectra, I: Analysis of spectral structure. *Analyst* 110:1227-1232.
- DEVAUX, M. F., BERTRAND, D., MARTIN, G. 1986. Discrimination of bread-baking quality of wheats according to their variety by near-infrared reflectance spectroscopy. *Cereal Chem.* 63:151-154.
- FOUCART, T. 1982. *Analyse Factorielle. Programmation sur Microordinateur*. Masson: Paris.
- FULLER, M. P., and GRIFFITHS, P. R. 1978. Diffuse reflectance measurements by infrared Fourier transform spectrometry. *Anal. Chem.* 50(13):1906-1910.
- GODON, B. 1977. *Matières minérales du grain de blé et de la farine*. Bulletin Anc. Eleves Ec. Meun. ENSMIC 50:74-87.
- GODON, B., LEBLANC, M. P., and POPINEAU, Y. 1983. A small device for wheat gluten separation. *Qual. Plant Foods Hum. Nutr.* 33:161-168.
- GRIFFITHS, P. R., and DE HASETH, J. A. 1986. *Fourier transform infrared spectrometry*. John Wiley & Sons: New York.
- HOPKINSON, J., MOUSTOU, C., CHARLWOOD, K. A., NEWBERY, J. E., and CHARLWOOD, B. V. 1985. Investigation of the enzymatic digestion of plant cell walls using reflectance Fourier transform infrared spectroscopy. *Plant Cell Rep.* 4:321-324.
- HORVATH, L., NORRIS, K. H., HORVATH-MOSONYI, M., RIGO, J., and HEGEDUS-VOLGYESI, E. 1984. Study into determining dietary fiber of wheat by NIR technique. *Acta Aliment. Acad. Sci. Hung.* 13:355-372.
- HOULIAROPOULOS, E., ABECASSIS, J., AUTRAN, J. C. 1981. Produits de mouture du blé dur: Coloration et caractéristiques culinaires. *Ind. Céréales* No. 12, 3-13.
- IRVINE, G. N. 1971. Durum wheat and pasta products. Pages 777-798 in: *Wheat Chemistry and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- LEBART, L., MORINEAU, A., and TABARD, N. 1977. *Techniques de la description statistique. Méthodes et logiciels pour l'analyse des grands tableaux*. Dunod: Paris.
- LEFEBVRE, J. 1983. *Introduction aux analyses statistiques multidimensionnelles*, 3rd ed. Masson: Paris.
- LE NOUVEL, J. 1981. *Etude d'une famille de courbes par méthodes d'analyse des données. Application à l'analyse morphologique de courbes provenant de données médicales*. Thèse de 3ème cycle, Université Rennes I, France.
- LIN, F. M., and POMERANZ, Y. 1965. Characterization of wheat components by infrared spectroscopy: I. Infrared spectra of major wheat flour components. *J. Assoc. Off. Anal. Chem.* 48(5):885-891.
- MESSERSCHMIDT, R. G. 1985. Complete elimination of specular reflectance in infrared diffuse reflectance measurements. *Applied Spectrosc.* 39(4):737-739.
- MORIKAWA, A., and SENDA, M. 1978. Infrared analysis of oat coleoptiles cell walls and oriented structure for matrice polysaccharides in the walls. *Plant Cell Physiol.* 19(2):327-336.
- NORRIS, K. H. 1978. Near-infrared reflectance spectroscopy. Pages 245-254 in: *Cereals '78: Better Nutrition for the World's Millions*. 6th Int. Cereal Congr. Am. Assoc. Cereal Chem.: St. Paul, MN.
- OSBORNE, B. G., DOUGLAS, S., and FEARN, T. 1982. The application of near infrared reflectance analysis to rapid flour testing. *J. Food Technol.* 17:355-363.
- ROBERT, P., and BERTRAND, D. 1985a. Application of multidimensional statistical analysis to the prediction of forage digestibility by NIR spectroscopy. EEC Workshop on Methodology of Analysis of Feedstuffs for Ruminants: Prediction of In Vitro and In Vivo Digestibility by NIRS. Brussels.
- ROBERT, P., and BERTRAND, D. 1985b. Réduction de l'influence de la granulométrie en spectroscopie proche infrarouge par des traitements mathématiques. *Sci. Aliment.* 5:501-517.
- VAN SOEST, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II: A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Anal. Chem.* 46:829-835.
- VAN SOEST, P. J., and WINE, R. H. 1967. Use of detergents in the analysis of fibrous feeds. IV: Determination of plant cell wall constituents. *J. Assoc. Off. Anal. Chem.* 50:50-55.

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