CLASSIFICATION OF RED WINES ANALYSED BY MIDDLE INFRARED SPECTROSCOPY OF DRY EXTRACT ACCORDING TO THEIR GEOGRAPHICAL ORIGIN

ANALYSE PAR SPECTROSCOPIE INFRAROUGE D'EXTRAITS SECS DE VINS ROUGES ET CLASSIFICATION EN FONCTION DE LEUR ORIGINE GÉOGRAPHIQUE

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Summary: Mid-infrared transmission spectroscopy and classical analyses have been used to classify 165 wines of the same variety (Gamay), from three geographical origins (Gaillac, Beaujolais and Touraine). For spectroscopic analyses; the wines were dried on polyethylene membrane and infrared spectra were recorded by transmission between 800 cm^{-1} and 1800 cm^{-1}. Classical analyses included pH, total acidity, total phenol, colour, sugars and major acids. The three groups of wines were classified by applying factorial discriminant analyses with cross validation. 97 p. cent of wines were correctly classified, based on the infrared data, versus 67 p. cent based on the classical analyses.

Résumé : 165 vins de même cépage (Gamay) et issus de trois régions différentes (40 Gaillac, 53 Beaujolais et 72 Touraine) ont été analysés par des méthodes classiques physico-chimiques de caractérisation et par spectroscopie moyen infrarouge à transmission.
Les méthodes classiques de caractérisation incluent notamment des mesures de pH, d'acidité totale, de phénol totaux, de couleur, de concentrations en sucres et en acides organiques.
L'analyse spectroscopique a été réalisée sur des membranes en polyéthylène sur lesquelles le vin était déposé puis séché. Après l'étude de différents paramètres de préparation de l'échantillon (volume, temps, mode de séchage et température), 75 µl de vin sont mélangés avec le même volume d'acétone, déposés sur la membrane et séchés sous vide au dessicateur. La zone spectrale retenue était comprise entre 800 cm^{-1} et 1 800 cm^{-1}. La sélection des nombres d'ondes les plus significatifs a été réalisée par analyse discriminante pas à pas. Parmi les 34 nombres d'onde retenus, plusieurs correspondent aux zones de vibrations des fonctions alcool, C=O et des noyaux aromatiques.
L'analyse avec validation croisée sur la base des trois groupes géographiquement définis a conduit à un taux de classification correcte des vins de 67 p. cent à partir des données d'analyses classiques. Les facteurs les plus discriminants sont la concentration en acide acétique, la teneur en extrait sec et l'intensité colorante. À partir des données de spectroscopie infrarouge, 97 p. cent des vins analysés sont correctement classés dans leur zone géographique d'origine. Seul 5 vins (1 Gaillac, 3 Beaujolais, 1 Touraine) sont mal classés.

Keywords:infrared spectroscopy, origin, wines, discriminant analyses

Mots clés : spectroscopie infrarouge, origine, vins, analyses discriminantes

Knowledge of the origin, variety, type or other features of products attract considerable attention from the consumer and distributor. The concept of quality in general and authenticity are a current and essential stake for the food industries. In order to guarantee the quality and origin of products, the food industry must have reliable tools for control and rapid diagnoses if possible. Wine is produced in many countries throughout the world and is one of the products attracting much attention.

Recently, several studies have been conducted to classify wines. BAXTER *et al.* (1997), RIZZON *et al.* (1997), SUN *et al.* (1997) and THIEL *et al.* (1998) showed that the mineral composition or metals from alkaline-earth are a mean of characterising wines according to their geographical origin, i.e. country and subregion.

The amino acid composition of wine has been studied extensively and used to discriminate between Chardonnay wines according to origin (CARNEVILLIER *et al.*, 1999). Although the dis-

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tinction among European wines was good, it was more difficult for wines from other countries. The anthocyanin composition led to a 100 p. cent correct classification of 23 Spanish wines for the 6 varieties and 2 regions studied (AROZARENA *et al.*, 2000).

By using several analyses (inorganic compounds, traditional determinations, aroma compounds), MORET *et al.* (1994) correctly classified five Venetian white wines collected over 8 years. SIVERTSEN *et al.* (1999) obtained a better classification of French wines based on biochemical analyses (alcohol, acidity, organic acids, aroma compounds) versus sensorial analyses (82 and 64 p. cent correctly classified, respectively).

More sophisticated methods have also been used. The isotopic ratio of hydrogen, oxygen and carbon from ethanol or water extracted from wine and measured by nuclear magnetic resonance spectroscopy led to the distinction of the geographical origin of products from different countries or subregions (BIGWOOD *et al.*, 1998; MARTIN *et al.*, 1999).

In comparison to these techniques, reviewed by ARVANITOYANNIS (1999), infrared spectroscopy has been less used to differentiate among products in the field of winemaking. Several studies have been performed in order to classify vegetable oils (DAHLBERG *et al.*, 1997; LAI *et al.*, 1994), coffees (BRIANDET *et al.*, 1996; KEMSLEY *et al.*, 1995; DUPUY *et al.*, 1995) and raspberry purees or jam (KEMSLEY *et al.*, 1996; DEFERNEZ *et al.*, 1995), showing the interest of this technology.

The purpose of this work was to assess a dry extract technique using microporous polyethylene film and the potential of Fourier Transform middle infrared spectroscopy (FTIR) by transmission in order to characterise red wines. The application concerned the classification of red wines (Gamay) from three different geographical regions (Gaillac, Beaujolais, Gamay). The results based on FTIR analyses were compared to those based on chemical analyses.

MATERIAL AND METHODS

I - WINES

165 red wines from vintages 1998 and 1999 of the same variety (Gamay) were collected by the Institut Technique de la Vigne et du Vin in three French production areas i.e. Gaillac, Beaujolais and Touraine (table I).

II - CHEMICAL ANALYSES

Several standardised analyses, used in the quality control of wines, were carried out. The experimental protocols were described by RIBÉREAU-GAYON *et al.* (1972). They involved the measurement of pH, total acidity by the titrimetric method, total phenol content by measurement of absorption at 280 nm, content of anthocyanins after discoloration in acid medium, colour intensity and colour hue calculated as the sum and the ratio between the absorbances at 420 nm and 520 nm, respectively, and the extract at 100°C. Additionally, the concentration of sugars (glucose, fructose), glycerol and some organic acids (acetic, tartaric, malic and lactic acids) were measured by high pressure liquid chromatography (HPLC). These compounds were separated by isocratic chromatography (H_2SO_4 0,01 M, 0,6 ml/min) on a Biorad Aminex HPX87H column. Sugars and glycerol were detected by refractometry, the organic acids by the absorption at 210 nm.

III - SAMPLE PREPARATION FOR SPECTROSCOPIC ANALYSES

Infrared analyses were conducted with 3M type 61 disposable cards (Spectra Tech Inc., USA). The sample was deposited on a microporous polyethylene membrane, usable in the spectral range of 4000 cm^{-1} to 400 cm^{-1}, except between 2918 cm^{-1} and 2849 cm^{-1}, the zone of stretching of aliphatic C-H groups present in the membrane. The spectrum of the card was recorded before sample application and was used as blank for the acquisition of the sample spectrum.

The samples were dried to eliminate water, ethanol and volatile components. Several application and drying methods were tested. Drying in a desiccator at 30°C, in a desiccator under vacuum at 30°C, in a ventilated oven (50°C or 70°C) or under an infrared lamp at 70°C.was evaluated. Tests with different sample volumes (10 µl, 25 µl, 50 µl, 75 µl) mixed with the same volume of acetone were also run.

IV - EQUIPMENT AND SPECTRAL ACQUISITION

A Nicolet Magna 750 purge spectrophotometer equipped with a DTGS detector was used. Thirty-two interferograms were collected at an optical resolution of 4 cm^{-1}. The single beam spectra of wines were transformed in absorbance units using the background spectra of the membrane. Baseline correction was conducted between the two extreme points of the zone chosen.

Table I - List of wines

Liste des vins

Origin	Vintage 1998	Vintage 1999
Gaillac	20	20
Beaujolais	19	34
Touraine	20	52
Total	59	106

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The spectra were normalised on the integrated spectral area. The repeatability of spectra has been assessed by the measurement of the same sample nine times in the fingerprint zone, between 800 cm^{-1} and 1800 cm^{-1}. Three transmission cards were used by wine and the average spectrum was introduced in the data set.

V - STATISTICAL ANALYSES

The spectral analyses provided 250 wavenumbers for each sample.The full data set contained 41 000 variables (164 x 250). The discriminant analyses could not be applied whilst the number of spectral data exceeded the number of samples (wines analysed). A selection of a subset of variables ($p< 0.10$) was performed by stepwise analyses (stepdisc procedure, stepwise method, SAS, 1988a) in order to retain just the more significant wavenumbers.

This subset was used in a factorial discriminant analyses to determine the origin of the wines (discrim procedure/crosssvalid method, SAS, 1988b). The cross validation diagnostic allowed the evaluation of the method's performance. It classified each sample as if it was a validation sample. This was accomplished by sequentially removing each sample from the data set, discriminant analyses on n-1 samples and prediction of the class of the removing sample. The same methods were used for the discrimination among wines according to the results of chemical analyses.

RESULTS AND DISCUSSION

I - METHODOLOGICAL CONSIDERATIONS AND SPECTRAL ANALYSES

Prior mixing of wine and acetone enabled good wetting of the card and a uniform spreading of the sample as assessed visually. After 30 min in a desiccator under vacuum, the sample could be analysed and the spectrum obtained did not change over time. In the case of higher temperatures (oven or infrared lamp), the first measurement could be conducted after 15 min. Subsequent measurements, however, revealed spectral changes characterized by a continuous reduction in absorbance. As an example, absorbance at 1040 cm^{-1} decreased by 0.05 units per hour.

Reducing the sample volume applied to the card reduced drying time. It was thus 25 min for 50 µl, 15 min for 25 µl and only 5 min for 5 µl of wine. At volumes lower than 25 µl, measured absorbance values were low and there was a loss of data in the zones of 400 cm^{-1}-950 cm^{-1} and 1200 cm^{-1}-1450 cm^{-1} (figure 1). The quantity, applied to the membrane, depends on the concentration of the different molecules that vibrate in the spectral domain examined. In the rest of the study, 75 µL of wine mixed with the same volume of acetone were deposited on the card and dried in a desiccator.

For measurements, carried out with the same wine on nine polyethylene cards, the coefficient of variation was about 7,5 p. cent in the spectral zone studied, except between 1450 cm^{-1} and 1500 cm^{-1}. This zone was not used for the data analyses.

Figure 2 shows the spectra of all 59 wines of the 1998 vintage, representing the composition of the dry matter of the wine. Sharp absorption bands and shoulders at different frequencies could be attributed to functional groups. The peak at 1710 cm^{-1} is characteristic of stretching of the C=O double bond, present in aldehydes, ketones, esters and acids. C-O stretching for acids, esters, ethers and alcohols occurs at 1300 cm^{-1} to 1000 cm^{-1}. Absorptions attributed to the antisymmetric and symmetric stretching of the carboxylic acid group ($RCOO^-$) was at 1600 cm^{-1} and 1450 cm^{-1}, respectively. The zone between 1000 cm^{-1} and 1150 cm^{-1} corresponds to the antisymmetric stretching of primary and secondary alcohols. The same move-

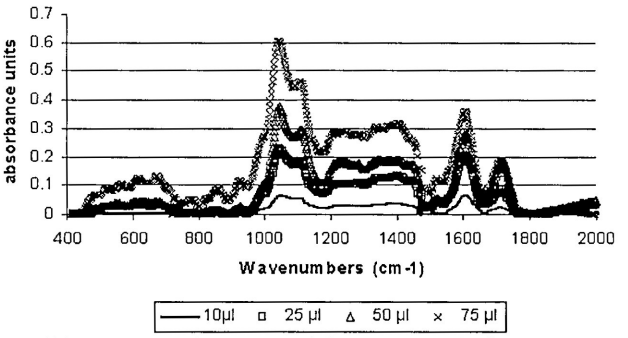


Fig. 1 - Typical spectra of dry extract of wine as a function of sample volume deposited on the membrane

Spectres caractéristiques d'un extrait sec de vin en fonction du volume déposé sur la membrane

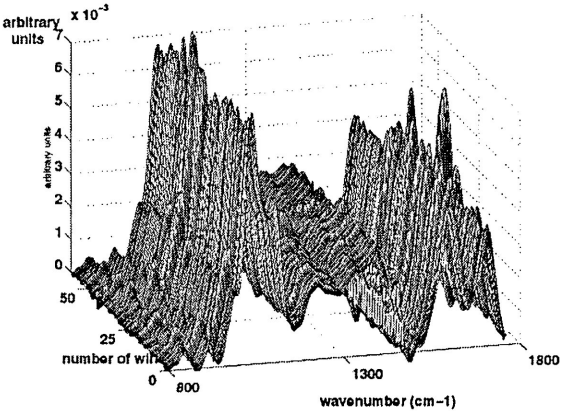


Fig. 2 - Infrared spectra of dry extract of wines from vintage 1998

Spectres infra rouge de l'extrait sec des vins de 1998

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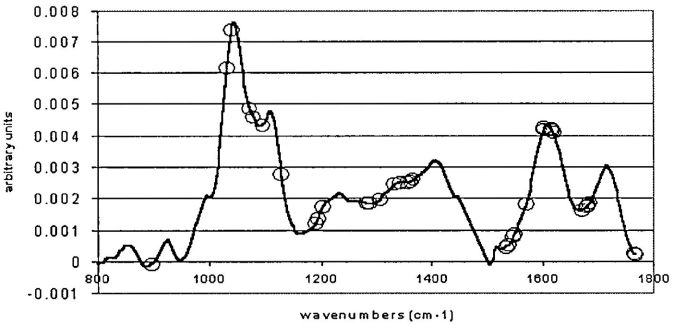


Fig. 3 - Wavenumbers selected by the stepwise procedure

Nombres d'ondes sélectionnés par la procédure « stepwise »

Table II - Crossvalidation results of discriminant analyses of wines analysed by infrared spectroscopy.

Classification par analyse discriminante avec validation croisée des vins analysés par spectroscopie infra rouge

	Gaillac	Beaujolais	Touraine
Gaillac	39	0	1
Beaujolais	0	50	0
Touraine	1	3	71
% Classified	97	94	99

Table III - Crossvalidation results of discriminant analyses of wines analysed by classical methods

Classification obtenue par analyse discriminante avec validation croisée des vins analysés par les méthodes classiques

	Gaillac	Beaujolais	Touraine
Gaillac	30	10	17
Beaujolais	4	33	8
Touraine	6	10	47
% classified	75	64	65

ment is found around 930 cm^{-1} but in phase. The distinct peak at 1040 cm^{-1} reflects the presence of the functional group CH_2-O. There were aromatic ring vibrations between 650 cm^{-1} and 920 cm^{-1} and the C=C ring stretching of aromatic rings occurs at 1600 cm^{-1} and 1450 cm^{-1}.

II - DISCRIMINANT ANALYSES OF WINES ANALYSED BY SPECTROSCOPY

The stepwise analyses method performed on the data set led to the selection of 34 wavenumbers (figure 3), that are distributed throughout the spectrum. Some corresponded to zones of vibrations defined earlier. For example, six wavenumbers were selected between 1000 cm^{-1} and 1150 cm^{-1}, the zone of alcohol stretching and five around 1600 cm^{-1}, the zone of aromatic ring vibrations. Around 1300 cm^{-1}, the zone of C-O stretching, several wavenumbers were selected.

Cross validation led to a mean percentage of well classified wines of 97 p. cent; i.e. 97 p. cent, 94 p. cent and 99 p. cent for wines from Gaillac, Beaujolais and Touraine, respectively. The distribution of the various wines is listed in table II. Only five wines were misclassified, one Gaillac and three Beaujolais into Touraine, and one Touraine into Gaillac. This distribution of the wines is quite visible on the factorial plane formed by the two discriminant axes, accounting for 55 p. cent and 45 p. cent of total variability (figure 4). Axis 1 separated the wines of Gaillac from the other two. On axis 2, the discrimination between the Touraine and Beaujolais is clearly seen.

III - DISCRIMINANT ANALYSES OF WINES ANALYSED BY CHEMICAL METHODS

Discriminant analyses of the chemical data made it possible to correctly classify 67 p. cent of the wines analysed, i.e. 75 p. cent, 64 p. cent and 65 p. cent for Gaillac, Beaujolais and Touraine, respectively.

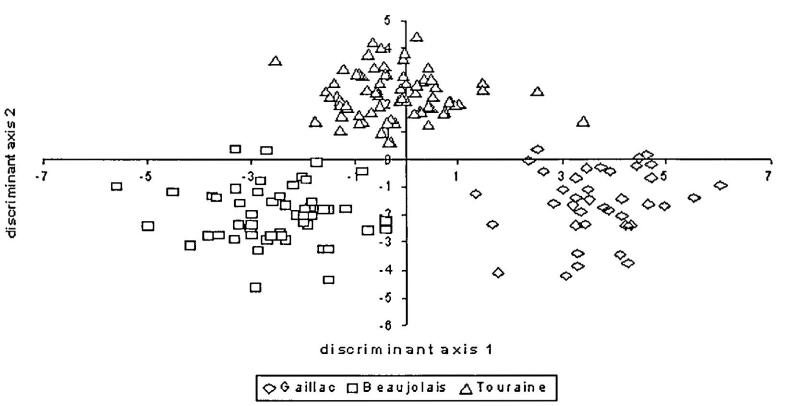


Fig. 4 - Discriminant map of the wines from the infrared spectroscopy data

Carte factorielle de l'analyse discriminante réalisée sur les spectres des extraits secs de vins

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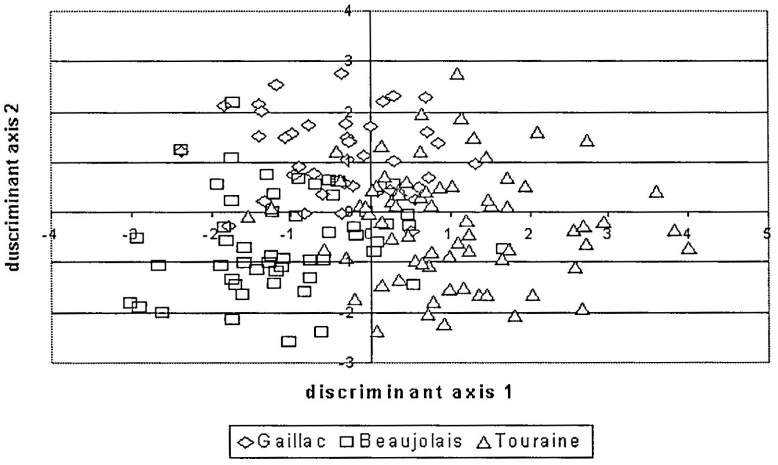


Fig. 5 - Discriminant map of the wines from the chemical data

Carte factorielle de l'analyse discriminante réalisée sur les données d'analyses classiques des vins

59 samples were misclassified. The distribution of the wines is listed in the table III. There was a high degree of overlapping between regions.

The position of the wines in the plane formed by the two discriminant axis confirmed these result (figure 5). On axis 1, accounting 60 p. cent of total variability, the discrimination between Touraine and the other two regions appeared. This axis is correlated with acetic acid concentration, the dry extract and to a lesser extent with colour hue. Axis 2 separated Gaillac from Beaujolais and Touraine. This axis is correlated with colour intensity. Nevertheless, separation between the three groups was poor.

CONCLUSIONS

The aim of this work was to assess a method of sample preparation and the mid-infrared transmission spectroscopy for the classification of wines from three different geographical regions.

The dry extract technique using microporous polyethylene membrane was easy to implement and solved the problem of water and ethanol interference. The concentration of the non volatile components by drying allowed to obtain a intense signal from 25 µl of sample.

Analyses of the dry extract of wines by infrared spectroscopy made it possible to differentiate between wines from 3 French production areas, i.e. Touraine, Beaujolais and Gaillac, respectively. Among the 165 red wines analysed, only 5 (2.4 p. cent) were misclassified by the cross validation method.

Distinction was also obtained from the traditional chemical and physical analyses. The level of discrimination according to geographic origin, however, was lower in the case of traditional analyses than in the case of infrared spectroscopy. The misclassified wine rate in classical analyses was 33 p. cent.

For discrimination, the infrared spectroscopy of the of dry extract could advantageously replace the various chemical analyses employed. The potential of middle infrared spectroscopy was shown for the classification of wines of the same variety from three different geographic regions. It would be interesting to apply this procedure to wines of different varieties and/or from other countries. The effects of winemaking technologies and of ageing could be also studied by IRTF.

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