

# Edible oils and fats authentication by Fourier transform Raman spectrometry

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The European project FAIR-CT96-5053 concerned the application of the FT-Raman and infrared spectroscopy in food chemistry and quality control. Our research mainly concerned the study of the potential of Raman spectroscopy and the comparison with the results achieved in infrared spectroscopy. The discrimination of virgin olive oil from other edible oils, and the detection and quantification of virgin olive oil adulteration have been experimented with this new technique of fast and non-destructive analysis.

**Keywords.** FT-Raman spectroscopy, virgin olive oil, stepwise linear discriminant analysis, adulteration, authentication.

**Authentification des huiles et graisses végétales par spectroscopie FT-Raman.** L'objectif du projet européen FAIR-CT96-5053 était l'application des spectroscopies Raman et infrarouge dans le domaine de la chimie alimentaire et le contrôle de qualité. Notre travail a principalement concerné l'étude du potentiel de la spectroscopie Raman et la comparaison des résultats de cette technique aux résultats de la spectroscopie infrarouge. La différenciation de l'huile d'olive vierge des autres huiles végétales, ainsi que la détection et la quantification de l'adulteration de l'huile d'olive vierge ont été expérimentées avec cette nouvelle technique d'analyse rapide et non-destructive.

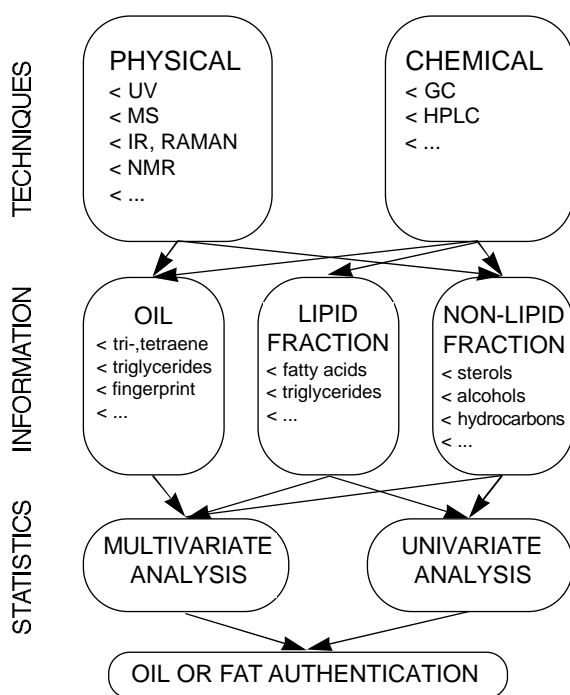
**Mots-clés.** Spectroscopie FT-Raman, huile d'olive vierge, stepwise linear discriminant analysis, adulteration, authentification.

## 1. INTRODUCTION

Authentication is of paramount importance in the food industry where incoming batches of raw materials and finished products must be tested for compliance with regulatory and health specifications. The recent dioxin crisis highlighted the importance of checking raw materials for their compliance with these specifications. In the past, other incidents such as the adulteration of olive oils with non-edible oils have shown the potentially serious consequences of such practices. With the emergence of international markets, however, the authentication of food products is receiving increased attention. One of the consequences of trade agreements, such as EU trade agreements and the GATT ratification accord, is the creation of a complex network of excise duties and agricultural subsidies which, in turn, require the establishment of official mechanisms to ensure that a food product is what it is claimed to be (Lees, 1999). There has also been an increase in the number of the products carrying Denomination of

Origin (DO) information and/or labels. This trend is a result of efforts by regional authorities and producers to protect and support local production. An additional important aspect in the authentication of fat products is the social and cultural impact of adulteration.

Several techniques for assessing the authenticity of food products have been proposed. The authentication methods applied to oils and fats can be classified as chemical (= separative) or physical (= non-separative) (**Figure 1**). Separative techniques, such as gas chromatography, focus on the existence or absence of certain chemical compounds in the adulterated sample. Physical techniques, such as infrared or Raman spectrometry, are based on a combination of measurements (e.g., light absorbance at different frequencies or the whole spectrum). The measurements are carried out directly on the samples or after dilution in a suitable solvent. The most widely used and accepted physical technique for oil and fat authentication is ultraviolet (UV) spectrometry. Other promising physical techniques which have been investigated for oil and fat authen-



**Figure 1.** Presentation of some of the methods used to identify oils and fats (Aparicio, Baeten, 1998) — *Diagramme présentant une partie des méthodes utilisées pour l'authentification des huiles et graisses.*

tication include mass spectrometry, pyrolysis mass spectrometry, GC-electron ionisation mass spectrometry, nuclear magnetic resonance and infrared spectrometry. Several papers have discussed the potential of near- and mid-infrared spectrometry in the authentication of food products; some of these papers are listed in **table 1**.

Another branch of vibrational spectrometry is Raman spectrometry which, like infrared spectrometry, provides information about the vibrations of the molecules. This information is contained within the wavenumber or frequency (or Raman shift) spectrum of scattered intensity. From a chemical point of view, both Raman and infrared spectrometry are based on the vibrational transitions occurring in the ground electronic state of the molecules. Raman scattering arises from the changes in the polarisability or shape of the electron distribution in the molecule as it vibrates; in contrast, infrared absorption requires a change of the intrinsic dipole moment with the molecular vibration.

For many years Raman spectrometry was considered to be of very limited use in food science. Several drawbacks were noted: the fluorescence, the photodecomposition and the wavenumber calibration problems, and the difficulty of obtaining high resolution spectra. Only recently, with the introduction of instrumental advances such as the interferometer technology and the near-infrared source, has Raman spectrometry become more widely used. **Table 2** summarises the

analytical, spectroscopic and instrumental advantages of FT-Raman spectrometry. Almost all spectroscopic techniques have these features, but some are specific to FT-Raman spectrometry and are indicated.

The European project FAIR-CT96-5053 (Aparicio, Baeten, 1998) was carried out at the Instituto de la Grasa to evaluate the potential of FT-Raman spectrometry for the authentication of oils (with virgin olive oil as the model).

## 2. MATERIALS AND METHODS

### 2.1. Sampling

A set of 138 commercial samples of the most representative oils and fats used in the food industry was analysed. The samples were purchased from Belgian and Spanish producers, retailers and laboratories.

### 2.2. Raman analysis

All the FT-Raman spectra were acquired on a Nicolet 910 FT-Raman spectrometer (Nicolet Analytical Instrument, Madison, WI). FT-Raman spectra were obtained by placing each sample in front of the laser and focusing the Nd: YAG laser beam onto the sample. Spectra were produced over the Raman shift 3250–0  $\text{cm}^{-1}$ . Typically, 200 interferograms were co-added at 4  $\text{cm}^{-1}$  resolution with a sampling time of 4 min.

### 2.3. Near-infrared (NIR) and FT-mid-infrared (FT-MIR)

NIR spectra of fat and oil samples were recorded with a Pacific Scientific Model 6250 spectrometer (PSCO, NIRSystems, Silver Spring, MD, USA) working in the single beam mode. The FT-MIR instrument used for this work was the collegian model spectrometer of Midac (Costa Mesa, CA, USA). The NIR and part of the FT-MIR spectra were recorded at the Unité de Biochimie de la Nutrition at the Université Catholique de Louvain (Louvain-la-Neuve, Belgium).

## 3. RESULTS

### 3.1. The Raman spectrum of an edible oil

**Figure 2** presents the FT-MIR (inverse scale, top) and FT-Raman (bottom) spectra of the same virgin olive oil. Both techniques present a spectrum with well-resolved bands showing various scattering intensities (Raman spectrum) or absorbances (FT-MIR spectrum). The polar groups (e.g., C=O, O-H) have strong mid-infrared absorption bands, whereas the non-polar groups (e.g., C=C) show intense Raman scattered bands.

**Table 1.** Review of the papers on the authentication of food products by infrared (NIR and FT-MIR) and Raman spectroscopy — *Articles traitant d'authentification de denrées alimentaires par spectroscopie infrarouge (NIR et FT-MIR) et Raman.*

Food product		Issue	Analytical method	References
Cereals	Rice grains	Geographic origin	NIR	Osborne <i>et al.</i> , 1993 Krzanowski, 1995
Coffee	Whole dried or/and ground coffee beans	Species discrimination	NIR	Downey <i>et al.</i> , 1994 Downey, Boussion, 1996
			FT-MIR	Dupuy <i>et al.</i> , 1995a Kemsley <i>et al.</i> , 1995 Briandet <i>et al.</i> , 1996a
	Adulteration	NIR	Downey, Spengler, 1996	
		FT-MIR	Briandet <i>et al.</i> , 1996a Briandet <i>et al.</i> , 1996b	
Dairy products	Milks	Adulteration	NIR & FT-MIR	Collomb, Spahni, 1991
	Butters		FT-MIR	Sato <i>et al.</i> , 1990
	Skim milk powder		NIR	Downey <i>et al.</i> , 1990; 1992
Fruit-based products	Fruit juices	Geographical origin and species discrimination	NIR	Scotter, Legrand, 1994 Twomey <i>et al.</i> , 1995 Li <i>et al.</i> , 1996
			FT-MIR	Kemsley <i>et al.</i> , 1996
	Fruit purées	Fruit concentration	FT-MIR	Wilson <i>et al.</i> , 1993 Defernez <i>et al.</i> , 1995
	Jams	Fruit type	FT-MIR	Defernez, Wilson, 1995
Meat	Meats	Process discrimination	NIR	Downey, Beauchêne, 1997a Downey, Beauchêne, 1997b Thyholt, Isakson, 1997
		Species discrimination	NIR	Thyholt <i>et al.</i> , 1997
		Adulteration	NIR	Thyholt <i>et al.</i> , 1998
Oils and fats	Edible oils and fats	Species discrimination	NIR	Bewig <i>et al.</i> , 1994 Safar <i>et al.</i> , 1994 Sato, 1994
			FT-MIR	Dupuy <i>et al.</i> , 1995b Dupuy <i>et al.</i> , 1996 Lai <i>et al.</i> , 1994 Marigheto <i>et al.</i> , 1998
			Raman	Baeten <i>et al.</i> , 1998a Baeten <i>et al.</i> , 1998b Marigheto <i>et al.</i> , 1998
	Virgin olive oil	Adulteration	NIR	Wesley <i>et al.</i> , 1995 Wesley <i>et al.</i> , 1996
			FT-MIR	Lai <i>et al.</i> , 1995
			Raman	Baeten <i>et al.</i> , 1996
				Aparicio, Baeten, 1998

### 3.2. Classification of edible oils and fats: “How to spin spectral data into chemical information”

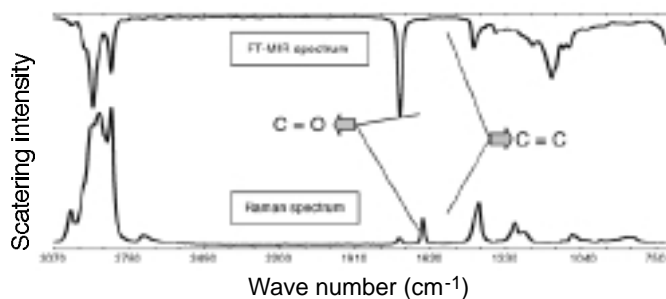
The main analytical operation in spectroscopy is to extract the information in such a way that it can be used in quantitative or qualitative analysis. The Raman spectrum is a rich source of multivariate data (about 1000 data for one spectrum [4000 to 0 cm<sup>-1</sup>] collected with a resolution of 4 cm<sup>-1</sup>). The challenge facing the analyst is how to distinguish the spectral data set and

isolate the variables that can be correlated with the information of interest (in our case, the authentication issue). A wide range of statistical and data analysis software packages is available. The trick in the multivariate data analysis is not the computation, but the definition of the objective and the subsequent choice of the appropriate statistical analysis or chemometrics.

The various chemometric approaches can be classified according to whether they are supervised (e.g., discriminant analysis) or unsupervised (e.g.,

**Table 2.** Analytical, spectroscopic and instrumental advantages of FT-Raman spectroscopy (Aparicio, Baeten, 1998) — *Avantages analytiques, spectroscopiques et instrumentaux de la spectroscopie FT-Raman.*

Advantages	
Analytical	Rapid analysis
	Direct, non-invasive and non-destructive
	☞ <i>In situ</i> analysis
	Qualitative and quantitative analysis
	Economic method (e.g. labour saving)
Spectroscopic	No use of pollutant solvents
	☞ Scattering process
	Intensity proportional to concentration
	Well-resolved spectra
	High-resolution spectra
	Precise spectral frequency measurement
	☞ Entire vibrational spectra
	☞ Non-polar group information
	More significant information
	☞ Trace element determination
☞ Fluorescence-free spectra	
☞ Spectra insensitive to temperature	
Instrumental	Push button instrumentation
	None or reduced sample preparation
	☞ Works well with aqueous samples
	☞ Suitable for use at-, on- or in-line process
	Compatible with suitable fibre optics



**Figure 2.** FT-mid-infrared (inverse scale) and FT-Raman spectra of a virgin olive oil — *Spectre FT-moyen-infrarouge (échelle inverse) et FT-Raman d'une huile d'olive vierge.*

cluster analysis). The supervised (Principal Component Analysis, PCA) and unsupervised (Stepwise Linear Discriminant Analysis, SLDA) methods were used in the project (Baeten *et al.*, 1998a, b; Baeten *et al.*, 1999).

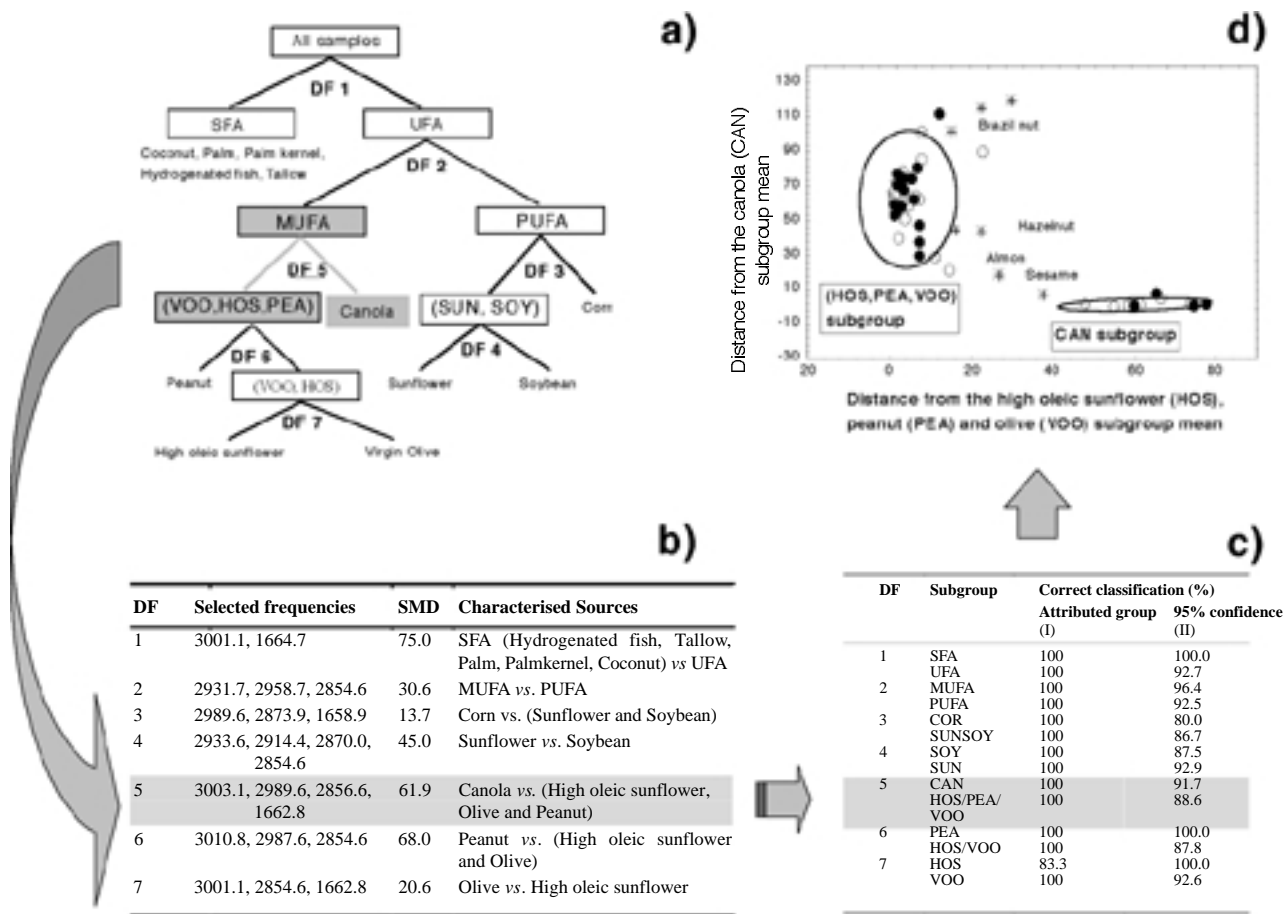
**Principal component analysis.** In the first stage, the PCA method was applied to the mean values of the fatty acids content (determined by GC) of the all

sample sources. Three clusters of samples appeared: those rich in saturated fatty acids (SFA), those rich in monounsaturated fatty acids (MUFA) and those rich in polyunsaturated fatty acids (PUFA). In the second stage, PCA was applied to the FT-Raman spectral data. The oils and fats clustered into the same three groups described for the first stage. The results of the unsupervised method show clearly that FT-Raman spectroscopy can classify samples according to their degree of unsaturation.

**Stepwise linear discriminant analysis.** The third stage of the study was to design an arborescent structure for the classification of edible oils and fats. The objective was to establish a series of discriminant functions (DFs) (i.e., mathematical equations) able to separate unknown oil and fat samples from different sources and to study the Raman data used to do that. A flow diagram describing the procedure used to build the arborescent structure was established and was applied to the sample set in order to determine the different DFs. The first step of the flow diagram consisted of assigning an arbitrary number to each sample according to its source. The SLDA procedure was then applied to discriminate the sample groups and to construct the pair of DFs (one for each group).

The next stage focused on the validation of the DFs. The squared Mahalanobis distance from the sample group mean observations (i.e., centroid) was calculated for each sample. Each oil or fat was assigned to the nearest group centroid. If the conclusions of the internal and external validation (i.e., samples used and not used to build the equations) were positive, the established DFs were validated DFs for the two studied sample groups. In the case of one or both groups including samples from the same source, the discrimination was considered partially or fully achieved, respectively. When the sample group included samples from two or more sources, the flow diagram was again applied until each group contained only oils from a single source.

**Figure 3a** presents the results of the construction of the arborescent structure with the established pair of DFs and the defined groups. First, the calibration samples were separated into two groups (DF 1) on the basis of total amount of unsaturated fatty acids; thus, one group contained samples with a high content of saturated fatty acids (SFA), while the other contained samples rich in unsaturated fatty acids (UFA). The UFA group was then divided into two sub-groups (DF 2) on the basis of type of unsaturation; thus, one sub-group contained oils with a high amount of monounsaturated fatty acids (MUFA), while the samples in the other sub-group contained a predominant fraction of polyunsaturated fatty acids (PUFA). In the PUFA branch, the next stage (DF 3) was the discrimination of the corn oils (COR) —richer in MUFA— from the



**Figure 3.** Summary of the vegetable oils classification by SLDA procedure. **a)** Arborescent structure constructed. **b)** Characteristics of the pair discriminant functions (DF) included at each node of the arborescent structure. **c)** Percentage of the samples correctly classified according to the nearest group centroid (classification procedure I) and the samples included in the 95% confidence region (classification procedure II). **d)** Results of the DF 5 as plot of the squared Mahalanobis distance from each group centroid (Aparicio, Baeten, 1998) — *Résumé de la classification des huiles végétales par la procédure SLDA. a) Structure arborescente construite. b) Caractéristiques des paires de fonctions discriminantes (DF) présentes à chaque nœud de la structure arborescente. c) Pourcentage des échantillons correctement classés suivant le plus proche centroïde (procédure de classification I) et les échantillons compris dans l'intervalle de confiance 95 % (procédure de classification II). d) Résultats de l'étape DF5, carré de la distance de Mahalanobis de chaque centroïde.*

group including the sunflower (SUN) and soybean (SOY) oils —richer in PUFA. The SUN group was discriminated in the last step (DF 4) involving the PUFA branch. Turning to the MUFA branch, canola (CAN, DF 5) and peanut (PEA, DF 6) oils —richer in PUFA— were successively discriminated from the group which included the high oleic sunflower (HOS) and the olive (VOO) oils. The DF 7 stage involved discriminating between the HOS and VOO samples.

**Figure 3b** shows the scattering intensities from Raman shifts selected, the source samples included in the two defined groups and the squared Mahalanobis mean distance (SMD) calculated between the two groups. To study the power of the DFs, different factors were considered.

- The squared Mahalanobis distance between the group centroids (i.e., the point defined by the means of the Mahalanobis distance for all variables in the group) was calculated.
- The classification matrix displayed by the SLDA module of the STATISTICA package allowed the number and percentage of samples to be correctly classified for each group involved; a sample is classified in the group to which it is nearest.
- The ellipses of the 95% confidence region were calculated during the calibration step, using the Mahalanobis distance from the group mean. Their centres were calculated from the means of the group coordinates, and their axes represent the values of the confidence regions. These ellipses allow an

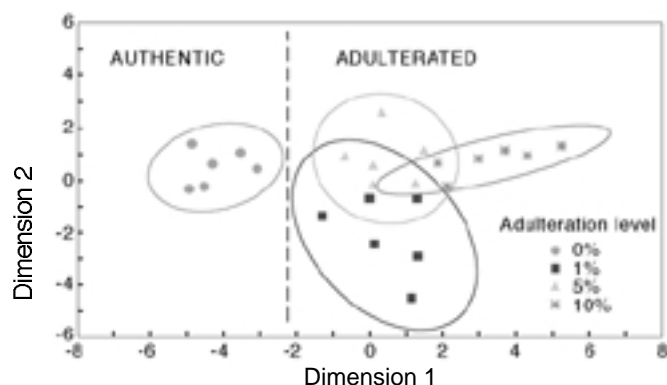
interpretation beyond the simple location of the sample and the calculation of the percentage of samples correctly classified.

For each pair of DFs and each subsequent group, **figure 3c** regroups the percentage of samples correctly classified according to the nearest group (classification procedure I) and the samples included in the 95% confidence region (classification procedure II). **Figure 3d** shows the results of DF 5.

### 3.3. Detection of virgin olive oil adulteration: “Which samples are authentic?”

The potential of Raman spectroscopy for detecting the adulteration of virgin olive oil was also assessed. The FT-Raman spectrometer could be calibrated for the quantification of some triglycerides (e.g., LLL) in edible oil. LLL is present in virgin olive oil at trace levels; a high content of these triglycerides therefore indicates that the virgin olive oil has been adulterated with other kinds of vegetable oil.

The potential of Raman spectrometry was demonstrated for adulterant oils rich in LLL (soybean, corn) as well as for oils poor in LLL (pomace). For this experiment, genuine Virgin olive oils were spiked at the range of 1, 5 and 10% with different adulterant types. The Raman data used in the classification of fats and oils were useful in the authentication of virgin olive oils. Using the information contained in the Raman spectra it is possible to discriminate clearly between genuine and 1% spiked samples (**Figure 4**). This observation attests to the potential of FT-Raman spectroscopy in the detection of low levels of adulteration. The results of discrimination between the three groups (1, 5, 10%) of adulterated samples indicate that the technique could also be useful in quantifying the adulteration (Baeten *et al.*, 1996).



**Figure 4.** Discrimination between authentic and adulterated virgin olive oils — *Différentiation entre les huiles d'olive vierges authentiques et adultérées.*

## 4. CONCLUSION

Advances in knowledge and technology have undoubtedly led to the increased success in the battle against fraud. Unfortunately, however, the same advances have been used by dishonest people to invalidate the usefulness of official standards. Consequently, it is necessary to update and improve food authentication methods continuously, to develop new analytical techniques, to keep pace with changing practices in the food industry and to keep ahead of those indulging in unscrupulous practices.

The results of the European project FAIR-CT96-5053 (Aparicio, Baeten, 1998) have demonstrated that FT-Raman spectrometry could be useful for classifying samples of oils and fats according to their sources and for detecting and quantifying virgin olive oil adulteration. A combination of spectroscopy and chromatography (where the latter could be used to quantify chemical compounds revealing adulterations, and the results could be used in the multivariate calibration of the former) might be an easier, faster and more accurate way of determining virgin olive oil adulteration than currently available methods.

The methodology developed and tested in the project could be applied to the authentication of other edible oils such as peanut, cottonseed or sesame oil, as well as fat products such as butter and chocolate.

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