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Classification and analysis of citrus oils by NIR spectroscopy

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Abstract

A new NIRS method is introduced for the determination of valuable components in various citrus oils. Spectra of grapefruit, orange, mandarin, lemon and lime oils in the range from 1100 to 2500 nm have been registered. Applying principal component analysis to the spectral data a good separation of the different fruit oil types can be achieved. The application of multivariate statistics in conjunction with analytical reference data leads to good NIR calibration results. For the main components (e.g. limonene, γ-terpinene, sabinene) and general chemical–physical parameters (e.g. optical rotation value, aldehyde content) standard errors are in the range of the applied reference method. The multiple coefficients of determination (R^2) for components with an amount of more than 1.5% are generally >0.95. Furthermore reliable in-process methods for the determination of the individual nootkatone and aldehyde contents during the isolation and purification process from grapefruit and orange oil are presented. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Citrus oils; NIRS; Terpenes; Nootkatone; Aldehyde determination

1. Introduction

Usually the oils of orange [*Citrus sinensis* (L.) Osbeck], grapefruit (*Citrus*×*paradisi* MacF.), mandarin (*Citrus deliciosa* Ten.), lemon [*Citrus limon* (L.) Burm. f.] and lime [*Citrus aurantifolia* (Christm. et Panz.) Swingle] are obtained by pressure from the fruits and fruit peels. For this purpose, special machines have been developed to optimise the isolation process of the essential oil which is located in small, closed glands inside the peel. Today, in most cases, the production of juice and essential oil is carried out simultaneously; normally the obtained oil and water phases are separated by centrifugation. In addition to the pressing procedure some citrus oils such as lime oil are also produced by distillation. So-called "essence oils" are distilled from mixtures of juice and essential oil without applying any pre-separation processes. In comparison to pressed oils, distilled citrus oils do not only contain less residue in case of evaporation, but they also differ in composition. Due to the formation of artefacts resulting from exposure to acid and air, distilled oils (e.g. lime oil) may contain higher amounts especially of *p*-cymene, terpinen-4-ol and α-terpineol in comparison to the related cold pressed oil. Furthermore these citrus oils are more susceptible to peroxidative changes of the authentic terpene components (Lösing, Degener & Matheis, 1998; Schieberle & Grosch, 1988, 1989). Primarily citrus oils are used for flavouring of sweets and juices as well as applications which demand a fresh odour.

Additionally, some applications of agrumen oils are also in the perfumery section where the low volatile essential oil components play an important role as head notes (e.g. in Eaux-de-Cologne and soap perfumes).

The main component of citric peel oils is limonene with an amount of 45% in lime up to 96% in orange and grapefruit oil which is also the dominant factor for the optical rotation value, ranging from 70 to 97°. Additionally, one can find a number of terpenes such as α- and β-pinene, γ-terpinene, terpinolene and sabinene, which contents are mainly responsible for the low stability towards oxidation processes leading to various off-flavour components. Furthermore citrus oils contain different aldehydes such as octanal, decanal, citral as well as sinensal, which contribute very strongly to the whole aroma impression but also nootkatone (Fig. 1), a

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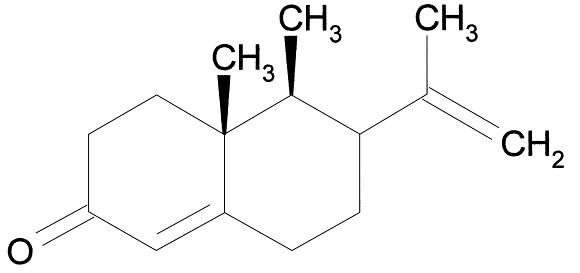

Fig. 1. Structural formula of nootkatone.

sesquiterpene ketone occurring in grapefruit oil with amounts of 0.02–0.8% which is mainly responsible for the characteristic flavour of the fruit, is commonly used for flavouring of fruit beverages (Wilson & Shaw, 1971).

In order to improve the oxidation stability and solubility in aqueous–ethanolic solutions, the mono- and sesquiterpene content is sometimes reduced by countercurrent extraction using two poorly miscible solvents of different polarity as for unpolar hydrocarbons and ethanol. Applying this procedure, a complete separation of terpene hydrocarbons such as limonene and the more polar oxygen-containing molecules such as aldehydes (e.g. citral) can be achieved. Analytical characterisation of the volatile citrus oil components is normally performed by usual GC measurements (Moshonas & Shaw, 1971; Schulz & Lösing, 1995; Ziegler, 1970). According to the more recent literature, the content of less volatile substances such as nootkatone can be successfully determined by a very fast and reliable HPLC method (Schulz, Albroscheit & Nowak, 1992). Nevertheless, the described quality analyses, mentioned in the individual International Standard Organisation (ISO) specifications of citrus oils[1] are mostly very time consuming and expensive. Therefore, the aim of this study is to present a fast and reliable NIRS method for the classification of different citrus oil types and the determination of individual valuable components. The developed innovative methods can be applied for quality control as well as for in-process measurements. As an example for in-line applications, NIRS calibration equations for nootkatone in grapefruit and total aldehyde content in orange oil were developed.

2. Materials and methods

The oils were obtained from the commercial company Miritz Citrus Ingredients GmbH (MCI), Kirchgandern (Germany).

2.1. Reference analysis

The reference values have been obtained by GC/FID of the citrus oils [diluted in pentane/diethyl ether (63:37)] using a Shimadzu GC 14A/GC 14B chromatograph. The instrument was fitted with a J&W DBWax 30 m×0.32 mm column (film thickness: 0.5 mm). Injector and detector temperatures were set at 220 and 240°C, the oven temperature was programmed from 60° — 5 min with 2°C/min to 240°C (split); carrier gas was hydrogen at a constant flow of 4 ml/min. The percentage composition was computed from the GC peak areas by the 100% method. Aldehyde contents were determined by a titration method according to the FCC (1996). Optical rotation values were measured using a Zeiss polarimeter 32-G580c with a circular scale.

2.2. NIR-spectroscopy

All citrus oil samples were scanned with a near-infrared spectrometer NIRSystems 5000 (Foss Analytical GmbH, Hamburg) in the range from 1100 to 2500 nm with a resolution of 2 nm. The measurements were performed in the transflection mode using quartz cuvettes equipped with a gold reflector (path length 0.4 mm). Data processing and development of appropriate chemometric methods were carried out with the commercial analysis programme NIRS2, Vers. 4.0 (Infrasoft International Inc., Port Mathilda, USA).

Principal component analysis (PCA) was performed for the spectral data only. For the calibration development a modified partial least squares (PLS) algorithm was used with an optimum number of PLS factors. The calibration accuracy is described by the multiple coefficient of determination (R^2) and the overall error between modelled and reference values [standard error of cross-validation (SECV)].

For the development of calibration equations for nootkatone and aldehyde contents, synthetic samples have been produced by diluting a nootkatone enriched (54%) grapefruit oil (mixtures with 11 different grapefruit oils in the range from 0 to 54%, 5% increments) and a pure decanal standard with different orange oil terpenes (20 samples range from 0 to 100%, 5% increments). Thus by this procedure a nearly authentic variation was simulated in order to avoid linear dependencies in the mixtures.

3. Results and discussion

3.1. NIRS discrimination of different citrus oil qualities

All NIRS spectra obtained are in accordance with the results of similar measurements reported earlier (Schulz & Lösing, 1995). Generally, no spectral outliers

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[1] ISO 3140-1976, orange oil, sweet; ISO 3809-1976, lime oil, cold pressed; ISO 3519-1976, lime oil, distilled; ISO/R-855-1968, lemon oil, Italian; ISO 3053-1975, grapefruit oil.1. ISO 3140-1976, orange oil, sweet; ISO 3809-1976, lime oil, cold pressed; ISO 3519-1976, lime oil, distilled; ISO/R-855-1968, lemon oil, Italian; ISO 3053-1975, grapefruit oil.

(Mahalanobis distance > 3) have been found. As shown in Fig. 2, the different citrus oil types regarded in this study can be discriminated on the basis of their individual spectral data applying principal component analysis by the first three PCA factors (98.3% of the variation explained). It can be seen, that the highest influence is found for factor 1 in accordance to high (orange, grapefruit) or low (lime) limonene contents. Three mandarin samples are found very close to the orange and grapefruit groups which is caused by the enrichment of limonene up to 93 and 95%, respectively. As demonstrated in Fig. 2 for both lime oil types (distilled and cold pressed quality), even a discrimination between products received from different refining processes is possible. In Table 1, the mean values for the detected oil components with amounts higher than 0.5% are presented. It is shown that each fruit type presents a special chemical fingerprint of a distinctive terpenoid composition.

3.2. NIRS calibration

For the NIR calibration procedure, the most valuable components as well as the optical rotation value and aldehyde content have been taken into account. All chemometric results are presented in Table 2. Since the optical rotation is strongly correlated with the limonene content in citrus oils also this physical parameter can be predicted very precisely and leads to similar loading factors. Obviously, there is a good correspondence between the first limonene loading resulting from the calibration procedure and the recorded spectrum of the pure substance (Fig. 3). The loading factor 1 contains predominantly overtones and combination bands of C–H– stretching and deformation vibrations in the areas of 1634–1766 and 2250–2350 nm. The negative calculated peaks refer to C–H absorptions of other oil substances listed in Table 1.

Furthermore, the determination of minor components such as α-pinene, sabinene, myrcene and γ-terpinene, which are only present in lemon, mandarin and lime oils is reliably performed even when the mean contents are

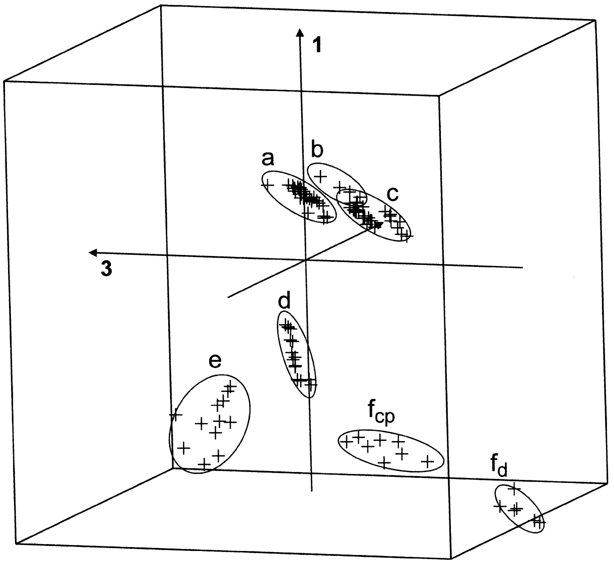

Fig. 2. Principal component analysis of seven commercial citrus oil types and qualities under studies: a, orange, b, bitter orange, c, grapefruit, d, lemon, e, mandarin, f_{cp}, lime (cold pressed), f_d, lime (distilled).

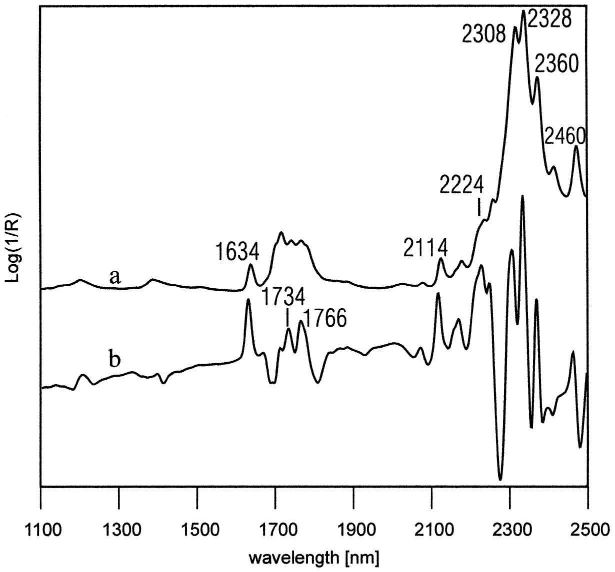

Fig. 3. NIR spectra of (a) pure limonene and (b) the 1st PLS loading.

Table 1
GC composition (mean values for each component in %) and sum parameters of the investigated citrus oils

	Mean concentration									
	Limonene	Myrcene	α-Pinene	β-Pinene	Sabinene	γ-Terpinene	Terpinolene	Optical rotation (α_D^{20})	Aldehyde content	Number of samples
Grapefruit	94.8	1.8	–	–	–	–	–	94.9	1.3	26
Orange	95.1	1.8	0.6	–	–	–	–	97.1	1.2	39
Mandarin	77.3	1.7	1.8	1.1		14.2	0.6	70.7	0.8	13
Lemon	68.6	1.5	1.9	12.1	1.7	8.7	–	63.9	2.6	14
Bitter orange	95.5	1.8	0.5	–	–	–	–	97.5	1.2	7
Lime (distilled and pressed)	49.9	1.3	1.4	4.2	0.5	12.3	6.2	39.9	1.7	14

Table 2
Range, means and NIRS calibration statistics for the individual citrus oils components as well as sum parameters[a]

	Range	Mean	S.D.	S.E.C.	R^2	S.E.C.V.	N
Limonene	45–96	85.5	14.7	0.58	0.99	0.65	91
Myrcene	1.38–1.95	1.74	0.14	0.05	0.87	0.06	81
α-Pinene	0.27–2.40	0.95	0.67	0.09	0.98	0.10	88
β-Pinene	8.9–14.4	12.1	1.7	0.03	1.0	0.28	15
Sabinene	0.07–2.30	0.57	0.59	0.13	0.95	0.15	79
γ-Terpinene	1.35–20.60	11.51	4.9	0.15	0.99	0.25	26
Optical rotation	34.6–99.0	84.9	17.8	1.09	0.99	1.18	91
Aldehyde	0.3–3.2	1.42	0.65	0.30	0.79	0.33	83

[a] S.D. standard deviation; S.E.C. standard error of calibration; N, number of samples used for calibration.

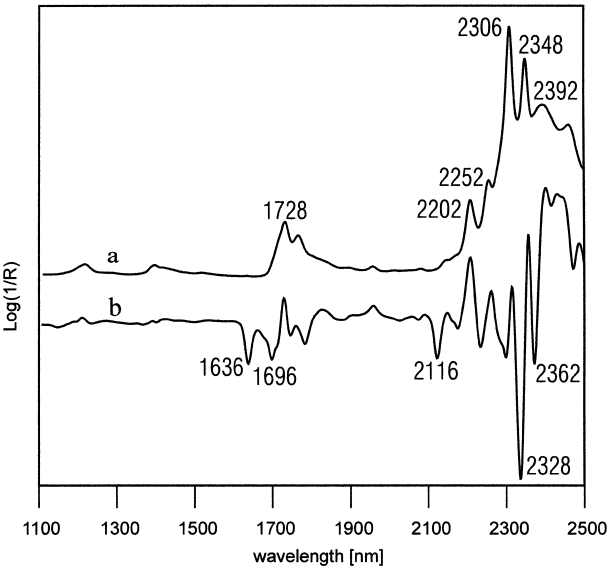

Fig. 4. NIR spectra of (a) pure decanal and (b) the 1st PLS loading for its dilution in orange oil terpenes (loading with no mathematical treatment).

only approx. 1%. The lower accuracy of titrimetric aldehyde determinations leads to a lower NIRS prediction quality. With the intention of getting also reliable results for the aldehyde content during enrichment processes a calibration on synthetic mixtures of decanal and orange oil terpenes was performed. A very good correlation between predicted NIRS and reference decanal data is achieved. The overall error SECV using eight cross-validation groups is 0.2% with a R^2 value of 1.0. Furthermore, 11 dilutions of different amounts of nootkatone in grapefruit oils ranging from 1 to 50% have been calibrated. With a SECV value of 0.25% and a multiple coefficient of determination of 0.99 the NIRS method can also be applied for in-line measuring purposes.

The 1st PLS loading factor resulting from the decanal/nootkatone calibration procedure shows the expected high influence of the C–H bending–stretching interactions in the range from 2240 to 2360 nm (Figs. 4,5). A high weight is observed at 2202 nm for decanal caused by the combination of the C=O and C–H stretching frequencies of the aldehyde group. Negative bands in the 1st loadings are observed according to the influence of limonene in the dilution mixtures with decanal and nootkatone (2220, 2328, 2364 and 2464 nm).

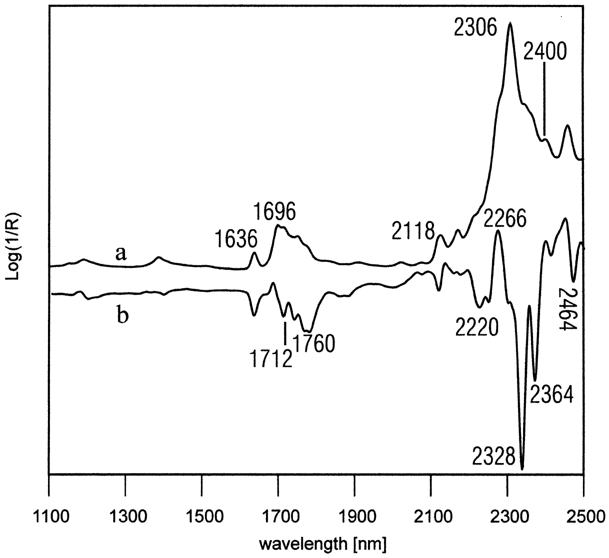

Fig. 5. NIR spectra of (a) pure nootkatone and (b) the 1st PLS loading for its dilution in different grapefruit oils (loading with no mathematical treatment).

In conclusion, near-infra-red spectroscopy can be used to predict the content of valuable components such as aldehydes, nootkatone, limonene as well as other terpene hydrocarbons rapidly and accurately. Visual assessment of the calculated spectral loadings suggests, that analytically useful absorbance is dominated by effects related to C–H and C=O groups caused by combination and overtones of the individual analyt molecules. All relevant chemical–physical parameters, which are usually mentioned in international specifications of citrus oils can be determined simultaneously in a very short time. Therefore, the described NIRS technique has potential to replace the existing quality control standard methods which are mostly very time consuming. Furthermore, NIRS measurements using optical fibers with the advantage of on-line control during distillation, extraction or other industrial processes are principally possible. In this context a new generation of powerful and low-priced diode array NIRS systems will get increasing importance in the near future.

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