

G. NOTA*
 D. NAVIGLIO
 R. ROMANO
 V. SABIA
 G. ATTANASIO
 S. SPAGNA MUSSO

Dipartimento di Scienza degli Alimenti -
 Università degli Studi di Napoli "Federico II" -
 Via Università 100 - 80055 Portici - Napoli - Italy
 *Author to whom correspondence should be addressed

Examination of the lemon peel maceration step in the preparation of lemon liquor

SUMMARY

The kinetics of the alcoholic extraction of essential oil from lemon peels, for the preparation of lemon liquor, can be carried out either by means of spectrophotometry UV-VIS or by gas chromatography (GC). The spectrophotometric technique can determine the kinetics of extraction of the total components of the essential oils, while the gas chromatographic technique can follow the extraction of each single component. The curve representing the kinetics of extraction of the essential oils obtained spectrophotometrically has the same trend as the curves of each major component obtained through gas chromatography. At room temperature, the components found in a higher concentration reach the equilibrium after 24 hours, while those in a lower concentration are completely extracted after 3 to 4 days. Furthermore, a method is proposed to follow the kinetics of dilution of ethanol by water contained in the peel.



INTRODUCTION

Lemon liquor, commonly called "limoncello", is a typical lemon-flavoured alcoholic beverage from the Campania region of Italy. It is becoming increasingly popular, both in Italy and abroad, and is appreciated for its aroma and taste, which recall fresh lemons, as well as for its digestive properties^{1,2}. The cultivation of citrus, especially lemon, holds great importance in Southern Italy³. The production of "limoncello", obtained from lemon peels, represents a considerable source of revenue, especially in tourist areas, such as Capri, the Sorrento peninsula and the Amalfi coast. Currently, almost the entire production of lemons in these areas is used to make lemon liquor^{4,5}. Since the process used is technologically simple, production is largely carried out by small and often family-run firms. The production process of "limoncello" includes the following phases: 1) washing the fresh lemons; 2) peeling the fruit by hand; 3) steeping the lemon peel in ethyl alcohol for 7 to 10 days at room temperature; 4) filtration of the alcoholic extract; 5) diluting the extract with an appropriate amount of sugar solution; 6) bottling; 7) packing.

As shown, the most delicate phase of the process is the maceration of lemon peels in ethanol; in this phase the essential oils, together with dyes and water, are released from the peels into alcohol⁶; at the same time, the alcohol migrates into the interi-

or of the peel. The process stops when a dynamic equilibrium is reached. According to tradition, a period of time shorter than 7 days cannot guarantee the complete extraction of the essential oils from the peel and cannot assure, moreover, the production of a satisfyingly aromatic liquor. During maceration, no controls are carried out to check when the extraction of essential oils is completed. Once the equilibrium is reached, an extension of the extraction phase would be a waste of time, and thus, an increase in cost.

Aim of the paper

The aim of this study is to set up one or more procedures based on instrumental techniques to determine the kinetics of the alcoholic extraction of essential oils from lemon peels. We also propose to set up a method to follow the kinetics of the dilution of alcohol by water contained in the peels. The procedures must be simple so that they may be employed by companies that use modestly equipped chemical laboratories.

EXPERIMENTAL

Instrumentation and chemicals

UV-VIS 1601 mod. Spectrophotometer (Shimadzu, Tokyo, Japan) equipped with

1 cm optical path cuvette; Auto System XL gas chromatograph equipped with programmable split-splitless injector (PSS) and flame ionization detector (FID) (Perkin Elmer, Norwalk, CT, USA); capillary column Rtx-5, stationary phase 5% diphenyl 95% dimethyl silicone, $l=30$ m, i.d.=0.25 mm, f.t.=0.25 μ (Restek, Bellefonte, PA, USA); gas chromatograph mod. 17A equipped with split-splitless injector and interfaced with a mass spectrometer mod. QP-5000 (Shimadzu, Tokyo, Japan); capillary column SPB-5, $l=60$ m, i.d.=0.25 mm, f.t.=0.25 μ m (Supelco, Bellefonte, PA, USA); Karl Fisher water titrator mod. KF 2026 (Crisson Instruments, Baar, Switzerland).

Ethyl alcohol 95% (v/v) (Fluka, Dublin, Ireland), Hydranal Titrant 5 and Hydranal Solvent (Riedel-de Haen, Seelze, Germany), n-dodecane, limonene, β -pinene, γ -terpinene, α -pinene, sabinene, geranial were all analytical grade (Fluka, Dublin, Ireland).

Gas chromatographic conditions

The gas chromatographic conditions for the instrument Perkin Elmer Auto System XL were the following: the carrier gas (helium) flow-rate was 2.0 mL/min; the split ratio 1:60; the oven was programmed at 75°C for 8 min, raised at 8°C/min increment to 240°C and held for 5 min at 240°C; the injector was programmed from 60°C for 12 sec, raised at 900°C/min increment to 250°C and held for 3 min; detector temperature was 260°C; capillary column was Rtx-5.

The gas chromatographic conditions for the instrument Shimadzu GC-17A were the following: carrier gas (helium) flow rate was 1.5 mL/min; split ratio 1:80; temperature program was the same as the GC above. Capillary column was SPB-5. Operating conditions: scan mode (TIC) from 19 to 250 amu at 2.9 scan/s, ionization by EI at 70 eV by autotuning; ion source temperature 250°C.

Procedure

Extraction of essential oils from lemon peel through ethyl alcohol

1. Wash and dry the lemons.
2. Peel the lemons, separating the albedo from the flavedo as much as possible.
3. In a glass container, place the appropriate amount of lemon peel (30 g is sufficient) and 100 mL of ethanol 95% v/v.
4. Cover the solution and keep it at a constant temperature of 20°C.
5. Let it macerate, stirring it from time to time.

Determination of the kinetics of extraction of essential oils from peel by spectrophotometry

At pre-determined intervals of extraction time shake the container, draw a sample of the extract and measure the absorbance at 400 nm without dilution; draw another part and measure the absorbance at 275 nm after the appropriate dilution with ethanol 95% v/v.

For each wavelength plot the absorbance values against the extraction time.

Determination of the kinetics of extraction of essential oils from peel by gas chromatography (GC)

At the pre-determined intervals of extraction time, after shaking, draw 1.00 mL of the extract and add 1.00 mL of ethanol solution of normal dodecane 500 mg/L as an internal standard (IS). Analyze the solution obtained by GC.

Report the ratio between the peak areas of the major compounds such as limonene,

β -pinene, γ -terpinene and the peak area of the internal standard against extraction time.

Report the ratio between the peak areas of the minor compounds such as α -pinene, sabinene, geranial and the peak area of the internal standard against extraction time.

Determination of the kinetics of dilution of ethanol by water contained in the peel during the essential oil extraction step After shaking, transfer 100 μ L of the alcoholic extract in the Karl Fisher titrator and determine the water content.

Carry out the measurement of the water content of the extract every 15 min for the first two hours, then every hour. Plot extract water content against the extraction time.

RESULTS AND DISCUSSION

The kinetic of extraction of the essential oils from lemon peel by spectrophotometry

The kinetics of extraction of essential oils through ethanol can be carried out using instrumental measurements. The absorption spectrum of alcoholic extract between 200 and 500 nm shows that the analyzed solution significantly absorbs in the whole range of UV wavelengths. The absorption spectra between 200 and 500 nm, obtained from the extracts drawn at differ-

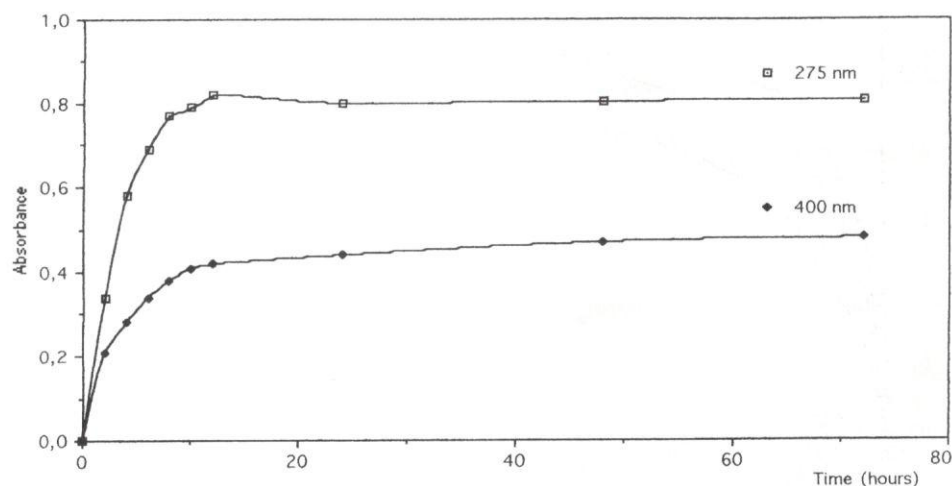


Fig. 1 - Absorbance values of alcoholic extract against extraction time at two wavelengths ($\lambda=275$ and $\lambda=400$ nm).

ent intervals during maceration, have the same appearance. This behaviour of absorbance shows that the principal compounds of the essential oil, are extracted at the same ratio during the entire extraction time. In **fig. 1** the absorbance, at 275 and at 400 nm, against extraction time is reported. The absorbance at 400 nm follows the kinetics of extraction of natural colorants and can be carried out on the extract exactly alike, up to 30 min, after the beginning of the extraction process; as for the absorbance at 275 nm,

it is necessary to dilute the extract with ethanol. As it can be seen by both wavelengths, during the maceration phase, the absorbance first increases and then becomes constant. The trends of both curves indicate that the absorbance becomes constant at the same extraction time. By overlapping the two curves, it can be noted that the absorbance in both cases reaches the constant value at the same time; thus, we can say that it is possible to follow the extraction of essential oils from peels either by using the measure-

ment of the colour intensity at 400 nm (yellow) or the variation in absorbance at 275 nm.

In **fig. 2a** and in **fig. 2b** are reported the kinetics of extraction measured at 275 and 400 nm for three alcoholic extracts prepared by macerating 30 g lemon peels per 100 mL ethyl alcohol; peels came from the same lemon tree. As you can see the curves cannot be superimposed; the deviation of absorbance values compared at the same extraction time from their average value can get 20%. This happens if the values of the zone of curves which reach a plateau are confronted. This fact is simply justified by the irregular distribution of essential oils and dyes in peels of different fruits, even if picked from the same plant. Moreover, the three curves have the same trend and the absorbance values get constant after the maceration process has undergone the same extraction time.

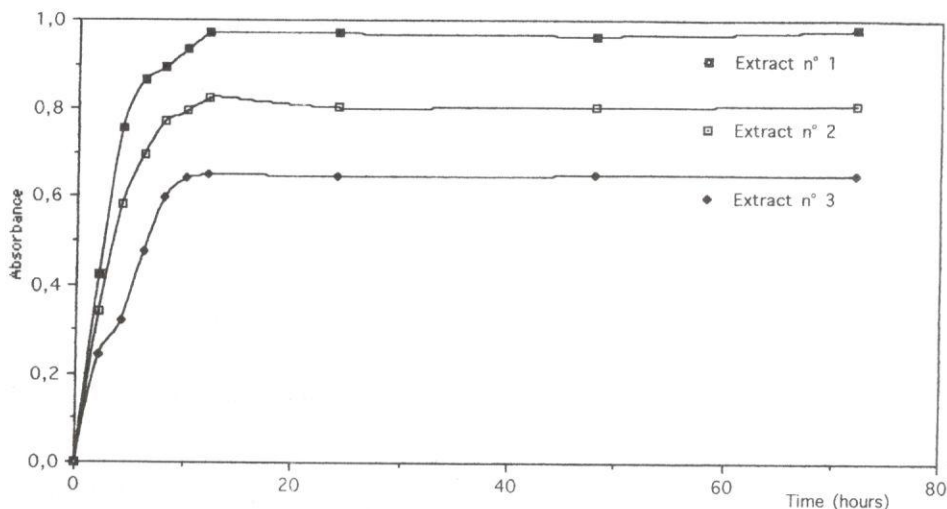


Fig. 2a - Absorbance values ($\lambda=275$ nm) of three alcoholic extracts against extraction time. Lemons were picked from the same plant.

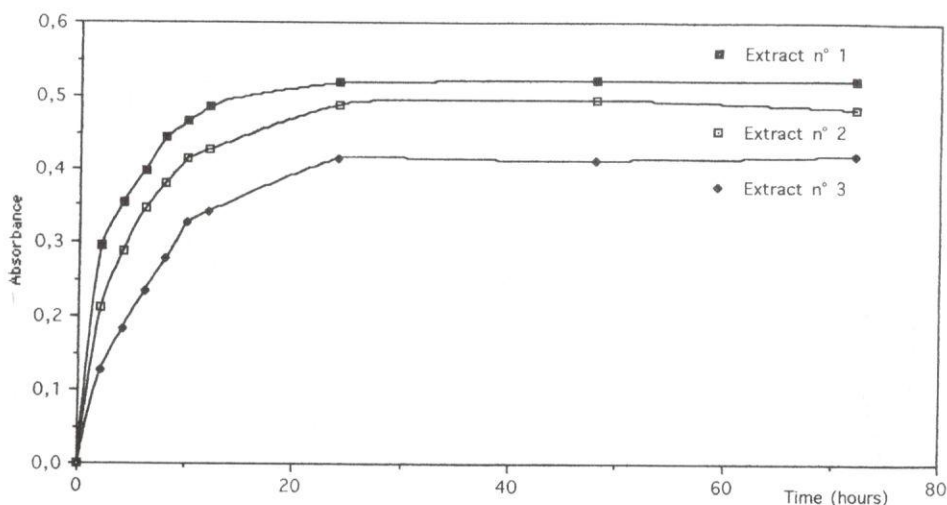


Fig. 2b - Absorbance values ($\lambda=400$ nm) of three alcoholic extract against extraction time. Lemons were picked from the same plant.

The kinetic of extraction of the essential oils from lemon peel by gas chromatography

In **fig. 3** a typical gas chromatogram of an alcoholic extract, obtained by means of GC/MS technique, is reported; the alcoholic extract was analyzed after one day of lemon peel maceration in ethyl alcohol. The identification of the peaks was done by GC/MS library search and by retention time of authentic standards. In **table 1** peaks identification and their percentual composition are reported. Extract samples were withdrawn at different times during the maceration process and then were analyzed by means of gas chromatographic technique. In **fig. 4** the ratios between areas of limonene, β -pinene, γ -terpinene, which are contained in a greater amount (major components) of essential oils of lemon and the internal standard n-dodecane are reported against the extraction time; in **fig. 5** the ratios between areas of α -pinene, sabinene, geranial, which are contained in a smaller amount (minor components) of the essential oils of lemon and the internal standard n-dodecane are reported against the extraction time. As can be seen, the con-

Table 1 - Compounds of alcoholic extract identified by GC/MS and their percentage composition. Compound numbers corresponds to peak numbers in fig. 3.

	Compound	tr., min	%
1	α -thuyene	13.493	0.40
2	α -pinene	13.883	1.81
3	sabinene	15.597	1.85
4	β -pinene	15.883	13.25
5	myrcen	16.323	1.43
6	α -phellandrene	16.798	0.35
7	α -terpinene	18.038	0.19
8	limonene	18.351	65.29
9	trans-ocimene	18.401	0.06
10	γ -terpinene	19.612	9.81
11	trans-sabinene hydrate	19.994	0.24
12	cis-sabinene hydrate	21.280	0.31
13	linalol	21.413	0.33
14	citronellale	22.333	0.15
15	4-nonanol	23.225	0.18
16	terpinen-4-olo	23.555	0.14
17	α -terpineol	25.326	0.35
18	neral	27.170	0.87
19	geranial	28.262	1.47
20	neryl acetate	31.587	0.44
21	geranyl acetate	32.329	0.46
22	β -caryophyllene	34.118	0.28
23	α -bergamotene	34.390	0.43
24	β -bisabolene	36.687	0.61

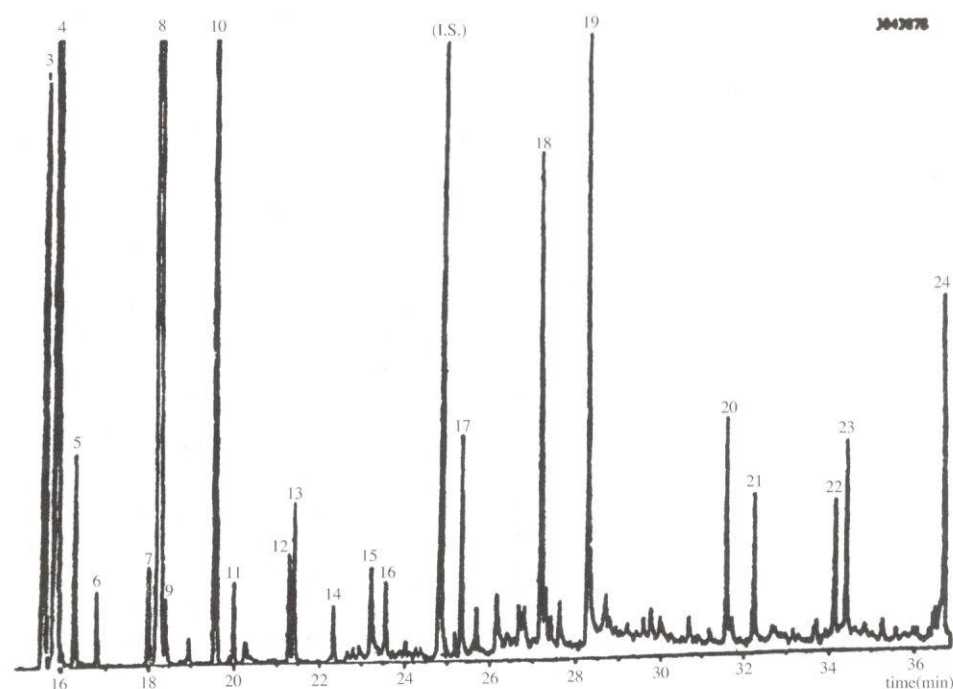


Fig. 3 - GC/MS chromatogram of alcoholic extract of lemon peel with internal standard (I.S.) n-dodecane. Peak numbering corresponds to compounds listed in table 1.

centrations of major components in the alcoholic extract reach a constant value after the same time; after 24 hours these compounds are completely extracted. The curves relating to the minor components take a different trend, indicating that they are extracted more slowly. This behaviour was to be expected, since these compounds are extracted into the solution more slowly because of their lower concentration gradient. To reach the complete extraction of these compounds, a period of about three days is necessary.

Comparison of spectrophotometric curves and gas chromatographic ones for major components demonstrates that these curves reach the same value after the same time. So, the progression of extraction regarding the major components can be seen either by spectrophotometry UV-VIS or by GC.

On the contrary, the proceeding of the extraction of minor components can only be followed by means of gas chromatography.

Effect of the dilution of alcohol during the maceration of the peels

Fresh lemon peel contains a noteworthy amount of water. We assumed that during maceration, there was a diffusion of alcohol into the peel and of water from the peel into alcohol. According to this hypothesis, during the extractive phase, the alcohol becomes diluted and diffuses into the peels, becoming unrecoverable. Since the content of water in the peel is about 70% w/w, and since we usually macerate 30 g of peel per every 100 mL of ethyl alcohol 95% v/v, when the equilibrium is reached, the concentration of the alcohol should lower to about 80% v/v and about 20 mL of solution should remain in the pores of the peel. Experimental measurements carried out on the alcoholic extract in equilibrium, indicate that the concentration of alcohol changes from 95% (v/v) to 78% v/v, which is in accordance with the assumed values. In **fig. 6** the experimental curve for the kinetics of the dilution of the alcohol during maceration is reported. As one can note, it takes about four hours to reach a water-alcohol equilibrium. It thus seems advisable to limit the maceration time to one day.

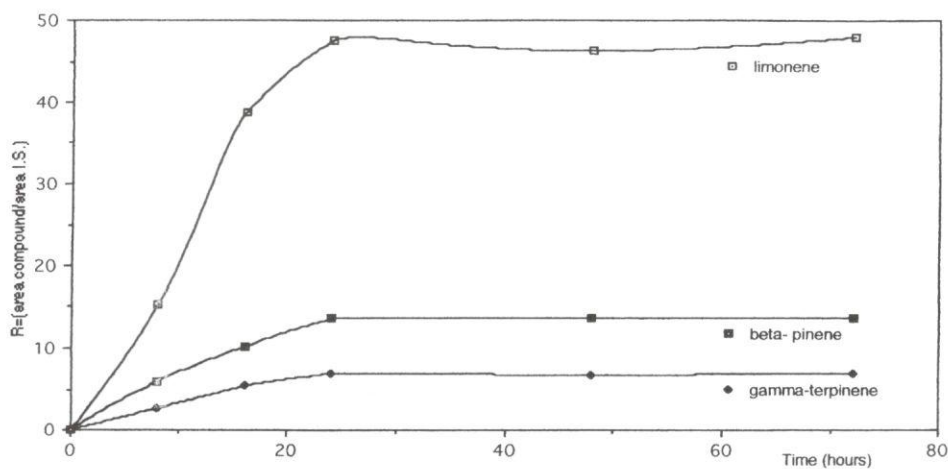


Fig. 4 - The kinetics of extraction of the compounds showing a greater presence in the lemon peel. The ratios between the peak areas of the substances and peak area of the internal standard are reported over extraction time.

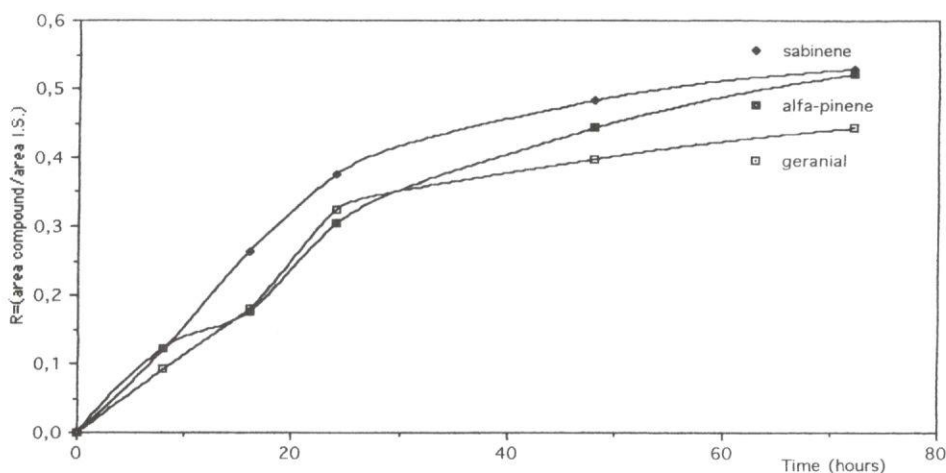


Fig. 5 - The kinetics of extraction of the compounds showing a lesser presence in the lemon peel. The ratios between the peak areas of the substances and peak area of the internal standard are reported over extraction time.

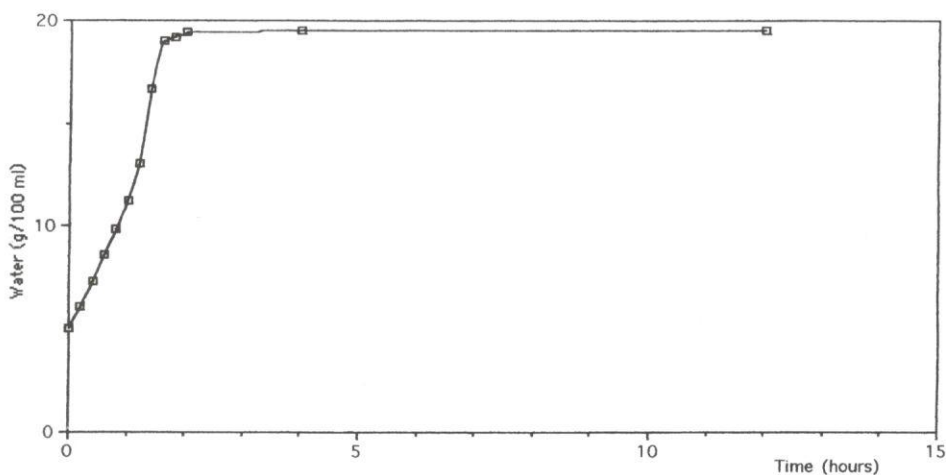


Fig. 6 - The kinetics of extraction of water contained in the lemon peel. The quantity of water (g) contained in 100 mL of alcoholic extract is reported against extraction time.

Since, as shown, a 24 hour maceration time is necessary to completely extract major components of essential oils contained in lemon peels, it was not possible to avoid the dilution of ethyl alcohol in the final extract.

CONCLUSIONS

The alcoholic extraction of the major components of the essential oils from lemon peel using ethyl alcohol can be carried out using visible and/or UV spectrophotometric measurements. Using GC, we can follow the kinetics of the major components as well as the minor ones. The major components are extracted completely after one day, whereas for the minor components, about three days of infusion of lemon peels into the ethyl alcohol are required. When the equilibrium is reached during the maceration phase, the concentration of alcohol lowers from 95 to 80% v/v, due to the dilution of alcohol by water naturally present in the peel. The kinetics of the dilution of alcohol is faster than the kinetics of the extraction of the major components of the essential oils; so, it is not possible to extract the latter compounds without lowering the alcoholic grade of ethanol used for the extraction.

BIBLIOGRAPHY

1. Kimball D. Citrus processing - Quality control and technology. Van Nostrand Reinhold. New York. (1991).
2. Different Authors. Prodotti agro-alimentari tipici della Campania. Dip. Econ. e Pol. Agraria, Facoltà di Agraria, Università degli Studi di Napoli "Federico II" (1995).
3. Spina P. Trattato di agrumicoltura. Edagricole (1985).
4. Di Giacomo A., Rapisarda P., Safina G., L'industria dei derivati agrumari. Problematiche generali del settore. Riv. Ess. e Der. Agrum., 31, 209-226, (1991).
5. Di Giacomo A., Mincione B., Gli oli essenziali agrumari in Italia; RAISA-CNR sottoprogetto 4, Laruffa Editore (1994).
6. Rodriguez-Amaya D. Stability of carotenoids during the storage of food. Departamento de Ciencia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Brazil (1993).