

Laboratory Production of Lemon Liqueur (Limoncello) by Conventional Maceration and a Two-Syringe System To Illustrate Rapid Solid–Liquid Dynamic Extraction

Daniele Naviglio,[†] Domenico Montesano,[‡] and Monica Gallo^{*,§}

[†]Department of Chemical Sciences, University of Naples Federico II, via Cintia 4, 80126 Naples, Italy

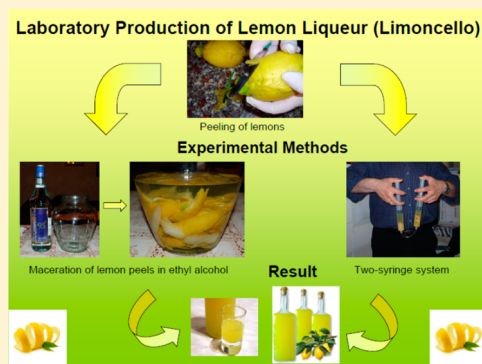
[‡]Department of Pharmacy, University of Naples Federico II, via Domenico Montesano, 49 80131 Naples, Italy

[§]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, via Pansini, 5, 80131 Naples, Italy

S Supporting Information

ABSTRACT: Two experimental techniques of solid–liquid extraction are compared relating to the lab-scale production of lemon liqueur, most commonly named “limoncello”; the first is the official method of maceration for the solid–liquid extraction of analytes and is widely used to extract active ingredients from a great variety of natural products; the second is a two-syringe system based on a Naviglio extractor, which represents a new solid–liquid extractive technology called rapid solid–liquid dynamic extraction (RSLDE) that changes the philosophy of the process of solid–liquid extraction. Maceration is based on the principle of diffusion (Fick’s law) that depends mainly on temperature; in fact, to increase the speed of maceration, heating of the system is required. In the case of RSLDE, the principle is based on the generation, in the presence of an appropriate solvent, of a negative pressure gradient between the outside and the inside of the solid matrix, followed by a sudden restoration of the initial conditions of equilibrium, which induces the forced extraction of compounds not chemically bonded to the matrix; consequently, the extraction can be performed at room temperature. In the experiment described, students evaluate the efficiency of extraction by the two methods. This laboratory experiment can be used in a food science laboratory course for secondary schools and for university students.

KEYWORDS: Graduate Education/Research, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Alcohols, Chemical Technicians, Gas Chromatography, Solutions/Solvents, High School/Introductory Chemistry



INTRODUCTION

In many industrial processes, the initial phase of the preparation of a product requires the application of a technique of solid–liquid extraction to isolate the extractable material content in the most varied type of vegetable matrices.¹ The most important example is the field of medicinal plants, from which are derived active ingredients with pharmacological properties for the treatment of some human diseases; related fields are herbal, cosmetic and perfumery plants, from which are derived the main ingredients of their preparations by solid–liquid extraction of plant parts such as flowers, leaves, or roots.² Also, in other industrial sectors, such as the beverage industry, solid–liquid extraction is employed to obtain alcoholic extracts of citrus peels, flowers, and leaves.^{3,4} Conventional extraction methods, such as maceration and Soxhlet extraction, have shown low efficiency and potential environmental pollution due to large volumes of organic solvents used and long extraction times required, periods ranging from a few hours up to several days.^{5–7} Among the techniques of extraction, maceration is the simplest and the cheapest, and widespread.⁸ Unfortunately, maceration is not always applicable because it requires a long contact time between the solid and the liquid; for example,

plants cannot be soaked in water due to the phenomenon of putrefaction. Therefore, solid–liquid extraction techniques that are currently used are not universally applicable, but limited. In addition, the extraction principle on which solid–liquid extraction techniques are based is essentially linked to the phenomena of diffusion and osmosis of substances in the solid, which tend to occupy the entire volume of extracting liquid after being extracted. To increase the efficiency of these extraction systems, a temperature increase is used to increase diffusion in order to reduce extraction times and increase yields. This expedient does not apply to vegetable matrices because they contain substances that degrade from heat. In particular, waste citrus peels can be used as source of several bioactive compounds. Among these, the limonene is the major constituent in lemon essential oil and is of great interest in several fields. Citrus essential oils can be extracted from citrus peels by traditional methods such as cold pressing, distillation through the exposure to boiling water or steam⁹ and Soxhlet system.¹⁰ These conventional methods have some disadvantages, mainly related to high energy costs and long extraction

times. In order to have fast, efficient and cost-effective extraction with enhanced quantity of essential oil, several new methods have been studied to extract essential oils from citrus peels or plants, such as supercritical fluid extraction (SFE),^{11,12} ultrasound extraction (UAE),¹³ controlled pressure drop process,¹⁴ subcritical water extraction,¹⁵ and microwave-assisted extraction (MAE).^{16–19} On the other hand, in recent years, there is an increasing demand for new extraction techniques with shortened extraction time, reduced organic solvent consumption, and increased pollution prevention (“green” methods). Novel extraction methods including (UAE),¹³ microwave-assisted extraction (MAE),^{16–19} supercritical fluid extraction (SFE)^{11,12} and accelerated solvent extraction (ASE)^{20–22} are fast and efficient for extracting chemicals from solid plant matrixes. These techniques have the possibility of working at elevated temperatures and/or pressures, greatly decreasing the time of extraction. In the field of innovative techniques, a new technology called rapid solid–liquid dynamic extraction (RSLDE) was developed as a valid alternative to maceration. It is an inexpensive technique and even if not properly “green” has many advantages; it requires a minimum energy expenditure, as compared to the extraction with SFE or ASE. In addition, it is possible to perform an aqueous extraction due to reduced extraction time, which is impossible to achieve for maceration. It completely changes the philosophy of solid–liquid extraction thanks to the discovery of a new principle of extraction, Naviglio’s Principle.^{23,24} The extraction is carried out using a Naviglio extractor for the generation of a negative pressure gradient from the inside to the outside of the solid matrix, so solid–liquid extraction can be conducted at room temperature (see Supporting Information). Recently, RSLDE was successfully used for the extraction of lycopene from tomato-waste using water as extracting liquid²⁵ and for rapid rehydration of cannellini beans.²⁶ In addition, while there have been many advances in green chemistry in the industrial and research fields, integration of these concepts into the teaching environment is still uncommon. In the teaching laboratory, introduction of greener experiments improves safety, allows for the routine use of macroscale techniques, and provides an ideal context for the discussion of chemical safety. This low diffusion may be due to the limited availability of educational materials that illustrate the methods, techniques, and principles of green chemistry. Consequently, in recent years, a new green organic laboratory curriculum has been developed to teach fundamental chemical concepts and techniques along with the tools and strategies of green chemistry.²⁷ Similarly, based on the Naviglio extractor, a system of extraction has been devised that can be used for student laboratory experiments to illustrate RSLDE. This extractor constitutes a two-syringe system connected at the bases by a tube (Figure 1) (Supporting Information). In the experiment described, students compare two extraction techniques for the preparation of a lemon liqueur (limoncello), maceration and the two-syringe system. The extracts are subjected to gas chromatography, spectrophotometric analyses, and gravimetric analysis. Finally, an appropriate volume of ethanol extract derived from the two extractions is mixed with a sucrose solution to prepare lemon liqueurs; liqueurs produced are tasted by students (respecting the legal limits for consumption of alcohol) to compare the liqueurs by the different extraction processes.

The pedagogic goals for this experiment are to illustrate to students the process of alcoholic maceration, i.e., a simple lab procedure for the production of alcoholic beverages, such as

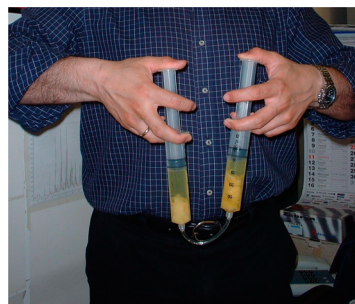


Figure 1. Two-syringe system for extraction.

lemon liquor, that can be performed at home without risk and is based only on diffusion regulated by Fick’s law. (In Italy, it is the traditional way to make many types of alcoholic beverages, e.g., orange, mint, etc., and it has been used for more than a century.) In addition, this slow process of solid–liquid extraction is compared to a new two-syringes system for obtaining the same alcoholic extract in a reduced time. The basis for this new RLSDE is Naviglio’s Principle. At the end of this experiment, students will know a new procedure to obtain more rapidly and in a more efficient manner any vegetable alcoholic extract, avoiding the long times required for maceration.

■ EXPERIMENT

Students work in groups of three. Lemons of “Ovale di Sorrento” species, ethyl alcohol 96% (v/v) commonly used for the preparation of alcoholic beverages, sugar purchased from a local market, drinkable water with a low mineral content (dry residue <200 mg/L), glass containers hermetically closable (macerator), and a potato peeler are required. If taste tests are used, it is mandatory to use laboratory equipment that is new or previously used only for food. Detailed procedures are in the Supporting Information; extraction using a Naviglio extractor is described in the Supporting Information.

Preparation of Lemon Extract by Maceration

Clean, dry lemons are peeled to obtain the outer peel (flavedo); the white part of the peel (which is very bitter) should be avoided. The peels are cut into small strips, transferred to a glass container hermetically closable (macerator), and covered with ethanol (peels/ethanol ratio: 30 g/100 mL (w/v)). The mixture is allowed to macerate for 7 days with occasional stirring. At the end of this time, the alcoholic extract is recovered and filtered to remove naturally occurring solid impurities. Lemon peels used are steam-distilled for gravimetric measurement of the residue of ethyl alcohol using a hydrostatic balance.^{8–10}

Preparation of Lemon Extract by the Two-Syringe System (60 mL Each Syringe)

For the preparation of lemon extract, a two syringe system is used (Figure 1). This system is constituted by two 60 mL syringes connected by a tube in the lower part of syringes. Then, 15 g of lemon peels is added to each syringe for a total of 30 g. Finally, 50 mL of ethyl alcohol (96% (v/v)) is added to each syringe for a total of 100 mL and the system is closed with two pistons. In this way, the same solid–liquid ratio of maceration was achieved. The extractive process starts by moving the pistons alternately (dynamic phase) for 1 min (10 s for piston up and 10 s for piston down); then the system is put under pressure for 30 s by pressing on the liquid with the two

Table 1. Results of the Experiments Obtained by Students of Analytical Chemistry in the Academic Year 2011–2012^a

Sample	Maceration Technique	Two-Syringe System Technique	RSLDE Technique
Kinetics parameters	$k = 15 (\pm 1) \text{ h}$; $S = 3.5 (\pm 0.5) \text{ g/L}$	$k = 0.14 (\pm 0.01) \text{ h}$; $S = 3.3 (\pm 0.3) \text{ g/L}$	$k = 0.15 (\pm 0.01) \text{ h}$; $S = 3.5 (\pm 0.3) \text{ g/L}$
Alcoholic extract	Limonene (GC): 45% (± 3) (w/w)	Limonene (GC): 47% (± 3) (w/w)	Limonene (GC): 48% (± 3) (w/w)
Alcoholic grade of the final extract	Ethyl alcohol: 88% (± 1) (v/v)	Ethyl alcohol: 90% (± 1) (v/v)	Ethyl alcohol: 90% (± 1) (v/v)
Dry material	Residue: 3.3 (± 0.5) g/L	Residue: 3.2 (± 0.3) g/L	Residue: 3.2 (± 0.3) g/L
Lemon liqueur (Limoncello)	Taste test: good	Taste test: very good	Taste test: very good

^aResults from RSLDE are shown for comparison to the two-syringe system.

pistons (static phase). The cycle is repeated 20 times (30 min; 10 min for each student), and then the tube is removed to collect the ethyl alcohol extract in a beaker. An aliquot of extract is used for characterization.

Kinetic Study of the Extraction Process

With the use of five two-syringe systems, five students start the extraction process at the same time with the same ratio of lemon peels/alcohol as described above. After 5, 10, 15, 20, and 25 min, the extraction process is stopped and the ethanol extract is recovered. The alcohol content in the ethanol extracts, the quantity of limonene in the ethanol extracts, and the quantity of dry material in each ethanol extract are determined at each time point as described below. The alcohol content (% ethanol), quantity of limonene (area of a GC trace that can be converted to mg of limonene if a calibration curve is used), and the quantity of dry material (in mg/L) are plotted as a function of time to obtain a rate constant for the extraction process. A mathematical program of interpolation is used to fit the data to the equation $y(t) = S_{t=\infty} \cdot e^{-(k/t)}$ to obtain kinetic constants for the different processes, where $S_{t=\infty}$ is the value for the measured quantity at infinite time.

Characterization of the Lemon Extract

The lemon extracts are characterized by the following analyses, depending on time and instrumentation available: (1) determination of the alcohol content in the ethanol extracts using a densitometer and/or hydrostatic balance; (2) determination of the alcohol content in the water extracts using a densitometer and/or hydrostatic balance (gravimetric analysis); (3) GC of essential oils; (4) GC/MS of essential oils; (5) UV–vis absorption spectrum of the ethanol extracts; (6) determination of the dry material in the ethanol extracts. Only the determination of the dry material of the ethanol extracts takes more than 5 h because of the evaporation process in an oven, and so the final weight can be obtained on another day.

Students use a gas chromatograph equipped with an HP-1 dimethylpolysiloxane capillary column ($l = 25 \text{ m}$; i.d. = 0.2 mm; f.t. = 0.2 μm), flame ionization detector (FID) coupled with an electronic integrator to analyze each ethanol extract (1 μL sample size). GC/MS analyses are carried out on the same chromatograph equipped with an MS computerized system.

Students calibrate a spectrophotometer in the range of 200–600 nm with ethyl alcohol and record the spectra for ethanol extracts (1 mL) in a quartz cuvette.

To determine the quantity of dry material in the ethanol extracts, students evaporate an aliquot of the ethanol extract (10 mL) in an oven at 70 °C, and the weight obtained on an analytical balance. (Fresh ethyl alcohol (96% (v/v)) (10 mL) may be added back to the ethanol extract to maintain the initial volume of the extract.)

Preparation of Lemon Liqueur

Maceration extract: A sugar solution is prepared by dissolving sucrose in water (sugar/water ratio: 50/50 (w/v)). This solution is added to the alcoholic extract (2/1 (v/v)) and the mixture is stirred gently for 2 min to obtain complete homogenization. (If allowed by law and local regulations, students taste the liqueur.)

Two-syringe system extract: Drinkable water is added to the two syringes containing the peels and only a static phase is performed for one min; the water is collected in a beaker, the peels are washed again in the same manner, and the two washings are combined. Sugar is added to the collected water (50/50 (w/v)) and dissolved. The ethyl alcohol extract and sugar solution are mixed (1/2 (v/v)) for 1 min to obtain a homogeneous solution. (If allowed by law and local regulations, students taste the liqueur.)

HAZARDS

The experimental part does not present any particular risk for the chemicals used. Students follow the main rules of laboratory safety, wear lab coats, and use the hood.

RESULTS AND DISCUSSION

Analyses were carried out in the sequence reported in order to minimize the analysis time and to have the minimum disturbance of the extraction system. For the construction of kinetics curves, samples were taken every day from a maceration batch and every 10 min from a batch RLSDE.

Determination of Alcohol Content

An aliquot of the alcohol extract (10 mL) was used to measure the ethanol content using a densitometer or hydrostatic balance. The density was converted into % ethyl alcohol according to conversion tables, and showed a content of 88 and 90% (v/v) (Table 1).

Determination of Essential Oils by GC and GC/MS

Identification of the components was based on GC retention times, computer matching with an NBS library, comparison of the fragmentation patterns with those reported in the literature^{17,18} and, whenever possible, coinjections with authentic samples (data and chromatogram not shown). The GC data (Table 1) showed limonene was present in 45–48% (w/w). The limonene is chosen as reference for the construction of kinetics curve, because it is the major compound of lemon essential oil. Other minor terpene compounds found in the extract are α -pinene, β -pinene, γ -terpinene, myrcene, geranial, sabinene.^{28–30}

Determination of the Absorption Spectrum

With the aim to follow the kinetics of extraction in a very simple way, before performing other analyses, the alcoholic extract undergoes UV/vis analysis that is not destructive technique. UV/vis spectra showed a maximum absorbance at

Table 2. Sensory Evaluation Form Compiled by Students for the Taste Test

Sensory Evaluation Form					
Recipe Name:			Category:		
Directions: Circle one rating in the yellow boxes for each of the following: Appearance, Taste/Flavor, Texture/Consistency, Aroma/Smell, and Overall					
Appearance	Extremely Attractive	Moderately Attractive	Attractive/matches photo	Unappetizing	Unattractive
Taste/Flavor	Tasted great	Flavorful	Acceptable	Off flavor	Flavor did not appeal to me
Texture Rating	Wonderful texture	Good texture	Acceptable texture	Off texture	Inappropriate texture/flat/runny
Aroma/Smell Rating	Wonderful aroma	Appealing aroma	Acceptable aroma	Aroma is not appealing	Unappetizing aroma
Overall Acceptability	Extremely Acceptable	Moderately Acceptable	Acceptable	Moderately Unacceptable	Unacceptable
School Use Only					
Panelist Code:			Date:		

Table 3. Comparison between Two Different Solid–Liquid Extraction Techniques: Maceration and Two-Syringe System^a

Extractive Technique	Granulometry	Solvent	Performance	Time	Quality Extract	Stability Extract
Maceration	important	fundamental	exhaustive	long	good	good
Two-syringe system	not important	indifferent	exhaustive	minimum	good	good
RSLDE	not important	indifferent	exhaustive	minimum	good	good

^aResults from RSLDE are shown for comparison to the two-syringe system.

458 nm, that is the index of the yellow color of solution, given mainly by carotenoids.

Determination of Dry Matter

The determination of dry matter is performed at the end of each extractive process (maceration, two syringe system, RSLDE). A sample of 10 mL of ethyl extract is collected and evaporated in the oven at 70 °C. The dry matter present in the ethanol extracts (maceration, two syringe system, RSLDE) showed a concentration between 3.2 (± 0.3) and 3.3 (± 0.5) g/mL (Table 1).

Construction of Kinetic Curves and Calculation of Kinetic Parameters " $S_{t=\infty}$ " and " k "

The extraction process by the three methods (maceration, two-syringes, and RSLDE) can be described by an equation of the type: $y(t) = S_{t=\infty} \cdot e^{-(k/t)}$, where $y(t)$ is the concentration at time t , the parameter $S_{t=\infty}$ is the concentration at saturation, i.e., the maximum value of the concentration that can be obtained (at time t infinity), and k is the kinetic constant. This equation is very common for many processes (i.d. solid–liquid extraction, hydration of legumes etc.) and it is easily understood. Only for $t = 0$ the equation assumes the value of infinite and this point is not representative for the process, while the half-life for the process is $t_{1/2} = k/(\ln 2)$. The above-mentioned equation can be written as $C(t) = S_{t=\infty} \cdot e^{-(k/t)}$. The interpolation of experimental data for the three processes gave very different values for kinetic constants for the quantity of dry material versus time. As shown in Table 1, kinetics constant k was very different than that obtained for the two-syringe system and RSLDE, which are nearly identical. Similarly, data obtained for limonene (gas chromatographic data) and % ethanol (gravimetric analyses) can be used for the construction of kinetic curves. These data were interpolated by the same equation above-mentioned.

However, for RSLDE, the saturation value ($S_{t=\infty}$) was gained before with respect to the maceration due to the lower value of kinetic constant (k) (figure not shown).

Taste Test

Twenty-one students, 12 males and 9 females from 20 to 22 years old, carried out the organoleptic test to compare the two liqueurs. The test required each taster to be blindfolded, and to

smell and taste two lemon liqueurs, one produced following the traditional recipe (maceration) and one made with the two-syringe system. Students completed a Sensory Evaluation Form to give their opinion on: Appearance, Taste/Flavor, Texture rating, Aroma/Smell rating, and Overall acceptability of the products (Table 2).³¹ The subjects were advised to use water to rinse their mouths between samples. The samples were presented coded by three-digit numbers and in random order. They were evaluated at room temperature following the indications as reported in literature.¹⁹ A t test was used to determine significant differences between the products at $p < 0.05$ level, using XLSTAT-PRO 7.5.2 (Microsoft) for statistical analysis of data (Table 1).

CONCLUSION

The laboratory experiment allowed students to compare two extraction methods and also to verify the two-syringe system as illustrative of the Naviglio extractor. The results showed that the preparation of lemon liquor (limoncello) by RSLDE was superior compared to the traditional method of maceration (Table 3). Furthermore, the development of the two-syringe system for simple manual operations reduced risks of the procedure. The low cost of the material required proved to be suitable for laboratory exercises for teaching purposes, even with numerous groups of students.

ASSOCIATED CONTENT

Supporting Information

Student handout; instructors' notes; some experimental conditions not present in the text of the paper. This material is available via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mongallo@unina.it.

Notes

The authors declare no competing financial interest.

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