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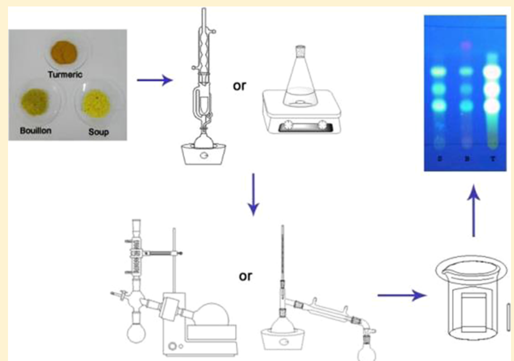
Using a Sequence of Experiments with Turmeric Pigments from Food To Teach Extraction, Distillation, and Thin-Layer Chromatography to Introductory Organic Chemistry Students

Thayssa da S. F. Fagundes,[§] Karen Danielle B. Dutra,[§,‡] Carlos Magno R. Ribeiro,[§,†] Rosângela de A. Epifanio,[§,⊥] and Alessandra L. Valverde*,[§]

[†]Programa de Pós-Graduação em Ensino de Ciências da Natureza, [‡]Programa de Pós-Graduação em Química, and [§]Departamento de Química Orgânica, Instituto de Química, Universidade Federal Fluminense, Campus Valonguinho, CEP: 24020-150 Niterói, RJ Brazil

Supporting Information



ABSTRACT: This experiment encourages students to use deductive reasoning skills to understand the correlation between different techniques used in a chemistry laboratory and to extract and analyze curcuminoids using natural products and processed food from a grocery store. Turmeric pigments were used to teach continuous or discontinuous extraction, vacuum or atmospheric pressure distillation, and thin-layer chromatography (TLC) in an introductory organic chemistry laboratory course in three laboratory sessions of 3–4 h each. Pigment extracts from the rhizome of turmeric, commercial dehydrated vegetable soup, and chicken-flavored instant bouillon (with turmeric as an ingredient) were used. The three techniques were discussed in the first laboratory session before students proposed a methodology to identify pigments in food extracts through a problem-based format. Students were assessed in a final exam where they presented a more detailed discussion of the chemistry laboratory techniques used. These results were compared to exam results from previous years. On the basis of students' and teacher's feedback, the sequence of laboratory experiments was considered interesting, and students were motivated to participate more actively during instruction.

KEYWORDS: *Second-Year Undergraduate, Organic Chemistry, Hands-On Learning/Manipulatives, Dyes/Pigments, Natural Products, Thin Layer Chromatography, Laboratory Equipment/Apparatus*

One of the most widely used natural yellow colorings in the food industry is turmeric (*Curcuma longa* L., Zingiberaceae, INS 100 (i)).[1] Tumeric root has long been used in cooking due to its preservative properties, color, and distinctive flavor. Some of the components of turmeric have been extensively studied and applied in medicine due to antioxidant, anti-inflammatory, antifungal, and anticancer properties as well as in countering Alzheimer's disease and, potentially, HIV.[2] The rhizome of turmeric has three main pigments that are responsible for its yellowish color: curcumin (**1**), desmethoxycurcumin (**2**), and bisdesmethoxycurcumin (**3**).[3] Structurally, these three substances differ only in the number of methoxy groups (Figure 1).

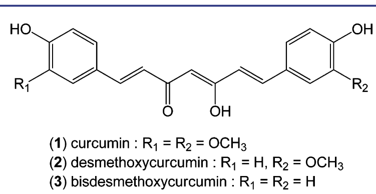


Figure 1. Main components of turmeric (**1**–**3**).

Curcuminoid **1** is described in literature as the major component of turmeric. It can be obtained from liquid–solid extraction followed by successive recrystallization or by chromatographic methods such as preparative thin-layer chromatography (TLC) and column chromatography.[4] Minor curcuminoids **2** and **3** can be obtained by the same method; however, a larger number of column chromatographic separations is needed to obtain a high level of purity. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the main method of curcuminoid analysis is TLC.[1]

Continuous and discontinuous extraction, simple distillation under atmospheric pressure, or using a rotary evaporator and TLC are important laboratory techniques commonly used to separate or analyze mixtures of organic compounds. These are generally discussed separately in textbooks.[5] For this reason, second year students, on the whole, have more difficulty in proposing how to isolate, purify, and analyze organic substances in a sequential way.[5−10] These introductory experimental

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techniques are frequently taught as part of a first- or second-year undergraduate organic chemistry laboratory curriculum in chemistry, chemistry engineering, and pharmacy programs at Brazilian universities.

It was observed that students can be greatly motivated by the use of everyday products such as foods, dyes and pigments, pharmaceuticals, and beauty products. Furthermore, experiments must make sense in relation to their course specificities. For example, for a pharmaceutical course student, they should be related to obtaining products concerning pharmacological activity; for a chemical engineering student, related to industrial products; and for a chemistry student, the experiments should involve discussions on properties and methods of obtaining substances.

This problematization was considered as the pedagogical approach of this study and aimed to use curcuminoids **1**−**3** present in processed or raw foods as the motivating theme for the development of a sequence of experiments using extraction, distillation, and TLC. Besides, this methodology sought to demonstrate to students that these techniques are important in the extraction, isolation, and analysis of organic compounds and that the experiments should be taught sequentially. Moreover, the knowledge of laboratory techniques also aimed to facilitate discussion of theoretical concepts about molecular interactions such as hydrogen bonding.

The food products used in this sequence of experiments were dehydrated vegetable soup, chicken-flavored instant bouillon (with turmeric as an ingredient), and turmeric rhizome. All products were purchased at a relatively low cost and are available in local markets. The experimental procedure was implemented in three laboratory sessions over 3−4 h during an undergraduate organic chemistry course.

■ LITERATURE OVERVIEW

Foods are commonly used as motivational tools for teaching laboratory techniques and theoretical concepts. Chocolate, yogurts, and essential oils are some examples of this type of educational approach to teaching TLC, high-performance liquid chromatography (HPLC), and NMR spectroscopy, respectively.[11−13] Curcumin (**1**), for example, is used to teach the principles of NMR spectroscopy and demonstrate the properties of nanoparticles in a lab-on-paper device for pH measurement as a natural acid−base indicator as well as in chromatography and fluorescence studies.[14−18] Previous studies have demonstrated that learning and understanding of laboratory techniques are improved if applied as a sequence of experiments, for example, the use of lemon essential oils for analysis of organic compounds through extraction and evaporation followed by TLC,[19] the fermentation of sugar cane juice followed by simple distillation and titration,[20] and the use of freeze-dried berries to teach solid−liquid extraction, paper chromatography, UV−visible spectroscopic characterization, and detection and evaluation of radical scavenging properties.[21]

■ EXPERIMENTAL PROCEDURE

Sequence of Experiments (Three Sessions of 3 or 4 h)

Approximately 40 g of turmeric powder, dehydrated vegetable soup, or chicken flavored instant bouillon was subjected separately to Soxhlet extraction for a period of 2 h using approximately 170 mL of ethanol as the solvent.

In the second session, the solutions of these extractions were distilled under reduced pressure using a rotary evaporator or at atmospheric pressure using simple distillation apparatus.

In the third session, the ethanolic extracts obtained after distillation were analyzed by TLC plates using a mixture of 5% methanol in dichloromethane (as the eluent). Chromatographic plates were observed by eye (white light), under a UV lamp (at 365 and 254 nm), under a black light lamp (400−320 nm), and 20% H_2SO_4 solution in ethanol.

Experimental details and additional experiments are in the Supporting Information.

■ HAZARDS

The curcuminoids are natural products used as spice or food pigments and are only present in small amounts, so they are not deemed dangerous. Since the methanol and ethanol used in these experiments are volatile and flammable organic solvents, all work with these solvents was conducted in a fume hood. Methanol can cause damage to vision. These solvents are harmful if ingested, inhaled, or come into contact with skin. Dichloromethane is an eye and skin irritant and potentially carcinogenic; therefore, gloves should be worn while handling. Sulfuric acid is very hazardous to the skin, if in contact with the eyes, or if ingested, and it should not be inhaled. UV lamps were used with goggles due to the potential to cause eye damage and skin cancer. Throughout all procedures, a lab coat, gloves, and goggles were used.

■ RESULTS AND DISCUSSION

The three techniques were presented in the first session: extraction, distillation, and analyses by TLC of organic compounds (1 h), and the students understood all the techniques. The time of extraction was enough to extract curcuminoids from all used samples. After the solvents were removed by simple distillation or rotator evaporator, students' yields were about 6.85% (turmeric), 4.45% (dehydrated soup), and 4.68% (chicken bouillon). Curcuminoids **1**−**3** were easily detected by TLC, but the best result was observed using the black light lamp, which was an excellent detector, as it is an inexpensive lamp and can be purchased commercially. Pigments were detected with various developers, and the comparisons of their features were highlighted.

Application of the Sequence of Experiments

The sequence of experiments was carried out by six different groups of introductory organic chemistry students containing 2−12 students/class (total of 48 students) from chemistry, pharmacy, and chemical engineering programs at the Universidade Federal Fluminense (UFF) in 2014 and was executed by groups of one or two students.

A discussion on continuous and discontinuous extractions and distillation under vacuum and at atmospheric pressure as well as TLC analysis was initially conducted before students started the experiments. After this, students were informed that the three curcuminoids (**1**−**3**) were present in turmeric. However, the students had to participate in an experimental theoretical discussion to decide how best to analyze the presence of these compounds in raw and processed food using methods of extraction, distillation, and TLC. Finally, the sequence was performed in three sessions of 3 or 4 h each, as described in the Experimental Procedure. Depending on the number of groups, each one used a different source of turmeric (rhizome, soup, or bouillon) to obtain the organic extract, but

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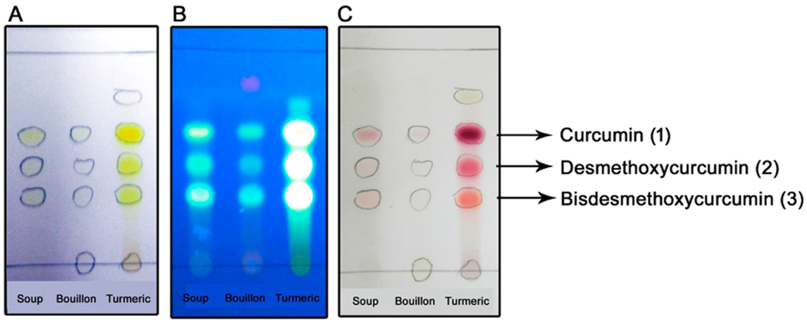

Figure 2. TLC of ethanolic extracts of commercial food and purified **1** (c). Eluent: 5% methanol in dichloromethane. Detectors: (A) white light and UV 254 nm (circles); (B) black light lamp; (C) H_2SO_4 in EtOH (20%).

all groups used all extracts to analyze the presence of curcuminoids by TLC. Small groups used rhizome and one processed food to develop this sequence of experiments.

The results of proposed experimental procedures were reproducible in all classrooms. Students were able to correlate the structure of the curcuminoids with its R_f in TLC because of its intramolecular interactions such as hydrogen bonding. It was important to consider that the main focus of this sequence was to use extraction and distillation to obtain extracts for analysis by TLC; isolating the curcuminoids, however, is also a possibility to be explored by a professor. Figure 2 shows an example of a students ́ TLC results.

The professor of each class formed their questions to involve prior knowledge of the theory, techniques, and apparatus used in this sequence. By comparing this to the results of previous years, an improvement of students' understanding was observed in their responses in the final exams in 2014. A better description and discussion of these techniques were provided. A questionnaire was applied after completing the laboratory classes to gauge students' opinions about the sequence of experiments. From the 48 students' opinions, they found: (a) it interesting and felt more motivated to actively participate during instruction (70%); (b) the time for execution was adequate (100%); and (c) they were helped to better understand the laboratory techniques (56%). The same questions were asked to six teachers who applied this sequence of experiments in their instruction; all teachers had the same opinions/answers as the students.

■ SUMMARY

Laboratory techniques should be explored sequentially as pedagogic goals for students to understand that the techniques can be applied in sequence to isolate and analyze extracts of organic compounds.

This research was proposed to develop a student's reflective and critical investigation using the problematization (use of questioning in problem solving) to learn laboratory techniques for investigative research work, as previously discussed in the literature.[22]

To this end, and as mentioned before, the sequence of experiments was initiated with a general discussion about the techniques used to obtain and analyze organic extracts of natural products. Then students were asked to suggest a way to obtain extracts from food, soup, or bouillon, and then the presence of the curcuminoids pigments in these products was analyzed. In general, after reflexive and critical discussions about the techniques commonly used in organic chemistry laboratories, students' groups proposed a sequence to solve the problem given to them. Generally, the students' sequence involved: (a) use of turmeric as a standard method to analyze the presence of curcuminoids in these processed food; (b) obtaining the extracts using continuous extraction by Soxhlet apparatus; and (c) the use of TLC to analyze the extracts. As well as this sequence of experiments, use of turmeric could alternatively be used to help provide valuable learning outcomes in organic chemistry where students could develop understanding of theoretical aspects such as intra- and intermolecular hydrogen bonds, tautomerism, resonance, solubility, and polarity (Supporting Information).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00138.

Experimental detail; additional experiments; theoretical aspects; apparatus and chromatograms (PDF, DOC)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: alessandravalverde@id.uff.br.

Author Contributions

⊥Rosângela de A. Epifanio, in memoriam.

Notes

The authors declare no competing financial interest.

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