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A quick and easy syringe-based solid-liquid extraction of lipids using a disposable pipette tip in food samples: a greener alternative to traditional methods

Extração sólido-líquido rápida e fácil de lipídios por seringa usando uma ponteira descartável de pipeta em amostras de alimentos: uma alternativa mais ecológica aos métodos tradicionais

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ABSTRACT

Lipids are essential biological molecules that influence aroma, flavor, and human health; however, excessive consumption can lead to diseases such as cardiovascular problems. Monitoring lipid content in food is crucial, and the traditional Soxhlet method is commonly used for lipid extraction. However, this method requires large amounts of petroleum-based solvents, energy, and time, making it incompatible with principles of Green Chemistry. To address this issue, a novel, environmentally friendly solid-liquid extraction method using disposable pipette tips (SLE-DT) was developed for lipid microextraction from food samples. The method was developed using a 2^2 full factorial design with salty crackers, identifying a 100 mg sample and 10 extraction cycles as the enhanced experimental condition. The SLE-DT method was tested on ten biscuit samples, showing lipid content consistent with product labels. Compared to the traditional Goldfish method, SLE-DT was significantly greener, scoring 0.56 versus 0.29, aligning better with Green Chemistry principles.

Keywords. Oil and Fat Industry, Lipids, Fats, Green Chemistry Technology.

RESUMO

Lipídios são moléculas biológicas essenciais que impactam em atributos de aroma, sabor e até na saúde humana, entretanto seu consumo excessivo pode ocasionar doenças como os problemas cardiovasculares. Monitorar o conteúdo total de lipídeos em alimentos é crucial, para isso, usualmente é empregado o método tradicional para extração de óleos e gorduras, o Soxhlet. No entanto, esse método necessita de grandes quantidades de solventes à base de petróleo, muito consumo de energia e tempo de análise, tornando-o inconsistente com os princípios da Química Verde. Nesse sentido, um novo método de extração sólido-líquido ecologicamente correto usando ponteiras descartáveis de pipeta (SLE-DT) foi desenvolvido para microextração de lipídios de amostras de alimentos. O método foi desenvolvido usando um planejamento fatorial completo de 2^2 com biscoitos salgados, identificando massa de amostra de 100 mg e 10 ciclos de extração como as melhores condições experimentais. O método SLE-DT foi testado em dez amostras de biscoitos, quantificando o conteúdo lipídico de cada amostra, sendo consistente com os rótulos dos produtos. Comparado ao método tradicional de Goldfish, o SLE-DT foi significativamente mais verde, pontuando 0,56 *versus* 0,29, alinhando-se melhor com os princípios da Química Verde.

Palavras-chave. Indústria de Óleos e Graxas, Lipídeos, Gorduras, Química Verde.

INTRODUCTION

Lipids are macronutrients present in foods containing fatty acids and glycerol in their structure, classified as triglycerides[1]. These compounds are primarily responsible for food aroma and flavor. Lipids are the body's main energy source (9 kcal/g) and are involved in transporting fat-soluble vitamins[2].

Regularly consuming specific long-chain polyunsaturated lipids, such as those with eicosapentaenoic fatty acid (EPA, 20:5n-3), can benefit human health. They act on neurological and cognitive development, preventing cancer and cardiovascular diseases[3]. However, the excessive consumption of poor-quality lipids (some with saturated fatty acids and *trans*-fatty acids) is associated with the development of cardiovascular diseases, obesity, hypertension and cancer, for instance[2].

In this sense, fast and accurate methods are necessary to determine the concentration of lipids in foods. Traditionally, lipid quantification is carried out using the Soxhlet extraction technique, which is applied to different samples, such as marine macroalgae[4], seeds[5], and fish[6]. This technique was developed in 1879 and has been used as a standard for analyzing oils, fats, and derivatives, regulated by the leading quality control agencies[7,8].

The Soxhlet extraction is based on the constant reflux of solvent through a target sample. Some characteristics rely on using a considerable amount of organic solvents (mainly petroleum ether, diethyl ether, or hexane), which are harmful to the environment and laboratory workers and are not connected with the principles of Green Chemistry. Furthermore, the procedure is laborious and time-consuming, requiring up to 8 hours for the analysis and demanding a high use of energy[8,9].

Another limitation is that the Soxhlet apparatus requires great care when handling it, as it comprises sensitive glass parts that break easily. In addition, it does not apply to small quantities of samples, limiting its application[10].

For these reasons, traditional methods do not fit with the principles of Green Chemistry developed by Anastas and Warner[11]. Some ideas of the Green Chemistry principles are reducing the amount of solvents, preventing wastes, minimizing toxicity, efficiently using energy, using safer solvents, and doing safer chemistry[12].

In this sense, alternative lipid extraction and quantification techniques are investigated, such as the use of microwave-assisted extraction of oil from *Moringa oleifera* reported by Nebolisa et al[13], and the ultrasound-assisted extraction of lipid from a salty cracker, reported by Ferreira et al[14]. These alternative techniques are based on the principles of microwave and cavitation, respectively, which significantly reduce the analysis time and the consumption of volatile organic solvents (VOS). However, their main drawback is the high equipment cost accessible in many food analysis laboratories.

Therefore, novel procedures are being used more frequently to obtain more efficient results in lipid extraction from food samples. These alternative procedures mainly seek agility in execution, waste reduction, applicability to samples in small quantities, laboratory and analyst safety, easy reproducibility, and reduction in the use of VOS.

In recent years, studies have explored alternatives to traditional procedures of extracting lipids. These studies have focused on the adaptations of different classic techniques, such as Soxhlet and Goldfish (similar to Soxhlet). This work aimed to develop a novel, efficient, and environmentally friendly method for lipid micro-extraction from food samples. The proposed method, solid-liquid extraction in disposable

pipette tips (SLE-DT), uses disposable devices and a minimum amount of VOS as a novelty, reducing the chemical waste of solvents and improving laboratory safety while maintaining the efficacy and reliability of the traditional methods.

MATERIAL AND METHODS

Samples

The sample used for the method development was obtained from the local market in Florianópolis, SC, in 2023. A salty cracker was chosen as a sample with an elemental composition of carbohydrates (60% m/m), proteins (8.6% m/m), total fat (14.3% m/m), dietary fiber (3.0% m/m), and sodium (0.4% m/m) according to the label. Ten samples of salty and sweet biscuits for analytical application were acquired in the same region in 2024. All the samples were taken to the laboratory in their original packaging. They were crushed without size adjustment in a mortar, homogenized, dried in the oven, and stored in polypropylene tubes until analysis.

Chemicals and instrumental

Ultrapure water (generated by the Elga system) was used to clean and prepare solutions. All materials used (tubes, clamps, spatulas, beaker, and glass vials) were previously cleaned with a 5% (v/v) alkaline detergent solution (Extran Supelco). Hexane (Neon) P.A. was used as a solvent for lipid extraction due to its low cost compared to other organic solvents.

Ovens (Biomatic, Brasil, and Edutec) were used for sample drying and extraction support, such as 5 mL vials. To determine humidity and set up the extraction system, the samples were weighed on an analytical balance M254Ai (Bel Engineering, Italy). A heating plate (Tecnal TE-0854) was used for hexane volatilization. To assemble the system, 10 mL polypropylene syringes (chemically resistant to VOS, such as hexane at room temperature), universal support, and 1000 µL disposable polypropylene tips were used. To verify the accuracy of the SLE-DT, the traditional lipid extraction was performed on the Goldfish equipment (Tecnal TE-044) using hexane as an extractor solvent.

Experimental procedures

Humidity determination

An aliquot (~ 5.0 g) of pulverized salty cracker sample was weighed into properly cleaned, weighed, and identified Petri dishes. Then, the sample was subjected to a temperature of approximately 105 ± 5 °C for six hours. After drying, using spatulas, the plates were placed in a desiccator to reach room temperature and then weighed again. The percentage of humidity was obtained through Equation 1.

$$\%H_2O = \frac{mi - mf}{mi} \times 100 \quad \text{Equation 1}$$

Where "%H_2O" represents the final humidity of samples, "*mi*" is the equivalent of the initial mass, and "*mf*" is the final mass. The humidity determination was performed only for the salty cracker sample used in the method's development.

Sample preparation and procedure for the SLE-DT

Figure 1 describes the procedure for preparing disposable pipette tips. Initially, the 1000 µL tips (**Figure 1A**) were identified and partially filled with degreased cotton (**Figure 1B**). The sample was weighed (according to the experimental design) and inserted into the tip (**Figure 1B**). Finally, the tip was filled with degreased cotton and placed in a conventional syringe (**Figure 1C**). The tip was adjusted to remain 1 mm above the bottom of the glass vial.

The syringe was fixed in a metallic support (**Figure 1C**) with a glass vial at the bottom to collect the solvent after elution through the sample. The glass vials were washed with alkaline detergent and dried at 105 ± 5 °C for 30 min before use.

With the system assembled, 3 mL of hexane was added to the syringe and closed. The plunger was gently pushed, pressuring the extractor solvent to elute through the sample, carrying the sample lipids into the glass vial. All the previous steps characterize one extraction "cycle", terminology used in the experimental planning. In this sense, the syringe pressuring and aspiration cycles were carried out according to the experimental planning. To guarantee the total extraction of the remaining lipid content in the cotton, at the end of each extraction cycle, another 2 mL of hexane was added to the syringe to perform a final cleaning flow.

After the SLE-DT, the lipid extract vial was immersed in a dry silica bath under a heating plate for approximately 15 min at 70 ± 10 °C. Finally, the vial was subjected to an oven (100 ± 5 °C for 60 min) for the solvent's final volatilization. Then, the vial was placed in a desiccator to reach room

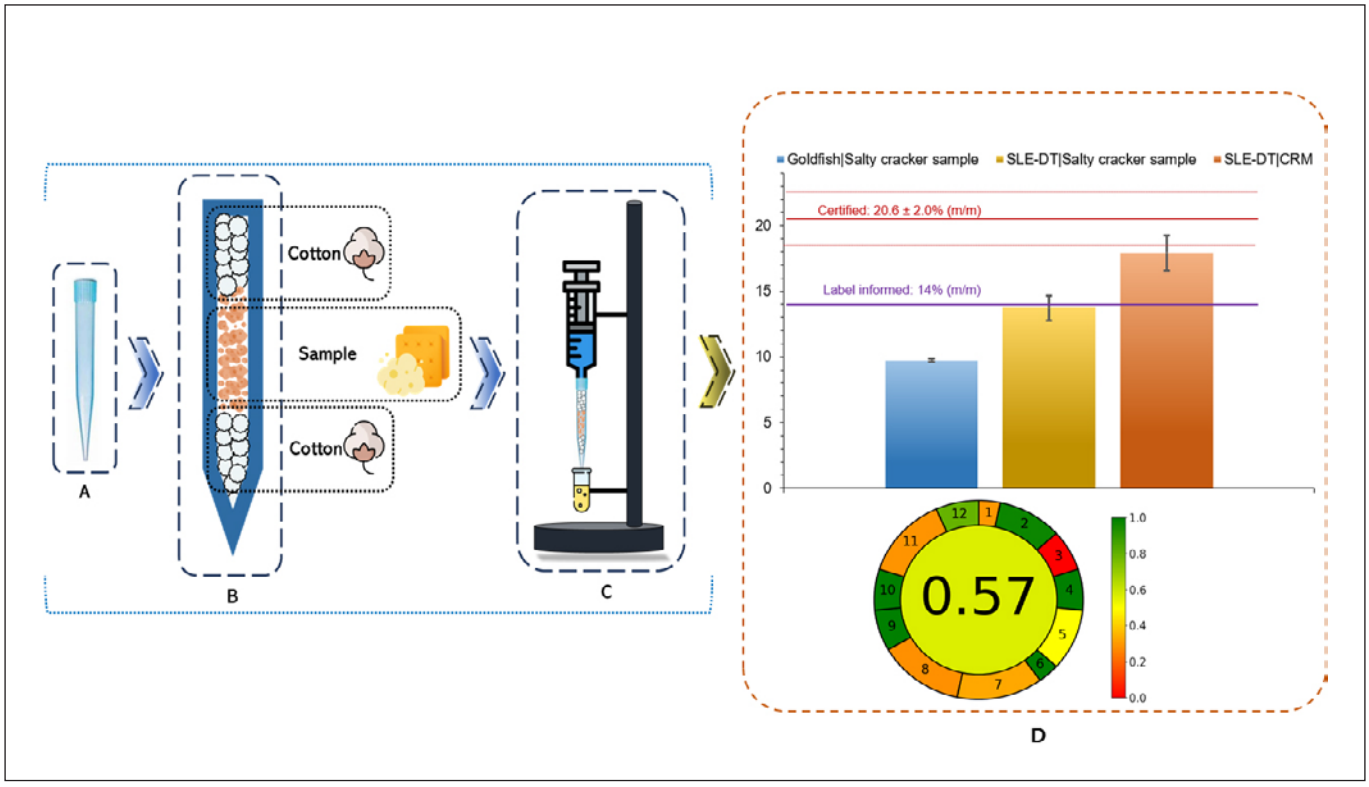

Figure 1. Scheme of tip preparation: (A) Disposable pipette tip, (B) tip as the sample holder, (C) final disposition of the apparatus and data validation with environmental impact scores of the SLE-DT method (D)

temperature, and the mass was measured again. The percentage of lipids in the sample can be obtained using Equation 2.

$$\%\ Lipids\ =\ \frac{(TE-TV)}{MA}\ x\ 100 \quad \text{Equation 2}$$

Where "TE" is the vial mass (g) containing the extracted lipids, "TV" is the initial mass (g) of the vial, and "MA" is the sample mass (g) inside the tip.

Evaluation of SLE-DT extraction parameters

A full factorial 2^2 with center points was used as a screening design to evaluate the SLE-DT. The parameters evaluated were the number of extraction cycles (10, 20, and 30 cycles) and the sample mass in the tip (100, 200, and 300 mg). The percentage of lipids in the sample was considered a response factor. The coded and real values are available in **Table 1**. After evaluating the significant extraction parameters, the compromise condition adopted for the analytical application was 100 mg of sample and 10 cycles of extraction.

Table 1. Real and coded parameters of full factorial design 2^2 with central points, evaluating sample mass and number of extraction cycles

Experiment	Mass (mg)		Cycles		Lipids (% m/m)
1	100	(-1)	10	(-1)	13.87
2	300	(1)	10	(-1)	11.88
3	100	(-1)	30	(1)	13.89
4	300	(1)	30	(1)	11.88
5	200	(0)	20	(0)	12.45
6	200	(0)	20	(0)	12.77
7	200	(0)	20	(0)	12.32

Mean values in Lipids % column. N = 3

Accuracy verification and analytical application

To verify the accuracy, lipid extraction was performed using the traditional continuous solvent extraction Goldfish method[15], with modifications, using the same sample (salty cracker). The technique was carried out in triplicate, with approximately 5.00 g of sample per unit inserted into degreased cotton cartridges and fixed to extraction tubes that were already adequately cleaned, dried, and weighed. With the system assembled, the sample was subjected to solvent reflux (Hexane P.A.) for approximately 5 hours at a temperature of roughly 98 ± 5 °C. Finally, the extraction tubes were subjected to an oven (100 ± 5 °C) for approximately 1 hour. The accuracy was also verified throughout the analysis of a certified reference material (Total Diet CRM 1548), with a certified content of lipids (20.6 ± 2.0%, m/m), following the same procedure described for the cracker samples using SLE-DT.

Ten samples of salty and sweet biscuits were used for the analytical application. 100 mg of each sample was packed inside the disposable pipette tip, and the lipids were extracted using 10 cycles of SLE-DT.

Evaluation of the SLE-DT method's environmental impacts

The environmental impacts of the proposed SLE-DT method were compared with the traditional Goldfish methodology. To perform the comparison, a metric approach based on the 12 principles of Green Analytical Chemistry was used to aid the evaluation of the proposed method's greenness. This approach is feasible using the Analytical GREEnness calculator software[16].

Statistical analysis and parameters of merit

Extractions were performed in real triplicate, and the results were expressed as mean ± standard deviation. The comparison assessment was performed using the paired t-test with a 95% confidence interval to verify statistical differences between the proposed SLE-DT and traditional Goldfish methods. Statistical analysis and data for the full factorial 2^2 design were performed at TIBCO Statistica v.13.5 (TIBCO Statistica Ltd, USA). As the SLE-DT method is based on gravimetric analysis, the limit of detection was defined as the minimum mass range that the analytical balance was built to quantify precisely (Resolution: 0.1 mg; Linearity: ± 0.0003 g; Repeatibility 0.1 mg; Minimum load: 10 mg).

RESULTS AND DISCUSSION

Due to the differences in the polarity of water and lipid content, the humidity of salty cracker samples was determined to be 1.37%. Because of this, the dry sample was used for further investigations and method development.

Screening of extraction parameters

The results for lipid extraction by the experimental design of the proposed SLE-DT by full factorial design 2^2 with central points are shown in **Table 1**. The obtained lipid concentrations varied from 11.88 to 13.89% m/m, where the highest extraction efficiency was obtained in experiment 3 (100 mg of sample, and 30 cycles of extraction). On the other hand, the lowest values were observed in experiments 2 (300 mg of mass, and 10 cycles) and 4 (300 mg of mass, and 30 cycles), suggesting that a "high" amount of sample mass does not positively impact the extraction of lipids by the SLE-DT.

The lipid extraction process using the proposed method presented a satisfactory result, with an extraction percentage close to what was expected compared to the value described in the sample's label (14%).

The significance of the proposed SLE-DT and its confidence interval were evaluated through a full 2^2 factorial design, and the results are available in **Figure 2D**. The SLE-DT did not show a lack of fit (p-value > 0.05), and the coefficient of determination R^2 was 0.9221, suggesting an excellent mathematical fit to the experimental data.

The Pareto chart (**Figure 2A**) shows the parameters evaluated in the full factorial design, highlighting the model's significant and non-significant parameters, as observed in **Figure 2D**.

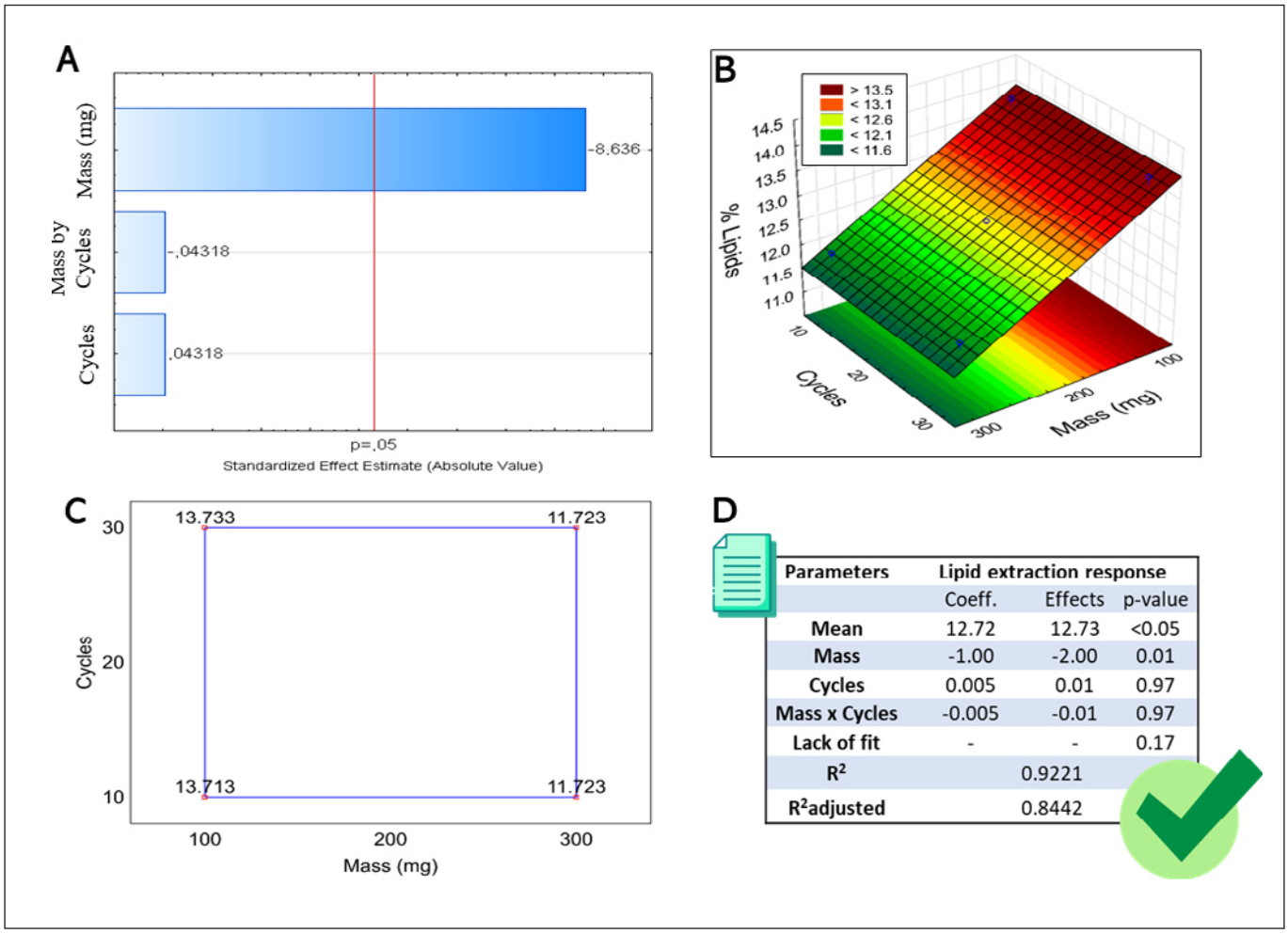

Figure 2. Pareto's chart (A), response surface (B), predicted values (C), and SLE-DT design parameters for % lipids response (D)

As observed in the chart, the mass amount is statistically significant and negative for the extraction of lipids using the SLE-DT. According to the data presented in **Table 1**, when the parameters were changed from level -1 to +1, with the mass ratio of the sample changed from 100 mg to 300 mg, it resulted in a lower extraction of lipids using the proposed method. Therefore, in experiments 1 and 3, which showed the highest lipid extraction, the sample mass was 100 mg. On the other hand, the number of cycles proved not significant for the application of the SLE-DT. This suggests that 10 cycles are already sufficient for the efficient extraction of lipids by the proposed procedure. Furthermore, it is worth mentioning that, considering the range of the parameters studied, the interaction between mass and number of cycles was not significant.

According to the response surface (**Figure 2B**), the percentage of obtained lipids is unaffected by varying the number of cycles between 10 and 30. However, when the mass parameter is increasingly varied, the rate of extracted lipids decreases, revealing the importance of evaluating the mass-independent parameter for lipid extraction.

Based on the evaluated parameters, the most effective conditions for achieving greater lipid extraction involve using the smallest mass amount and the fewest number of cycles. This results in a fast

and efficient extraction process that requires the preparation of a minimal amount of sample and involves a short handling time. Additionally, this approach enhances analyst safety and reduces waste generation.

Considering the mass as the only significant factor, mathematical modeling was performed. **Figure 2C** shows the predicted values of the lipid extraction response by the SLE-DT within the experimental range evaluated, varying between levels -1 and +1 of mass amount and cycles.

As observed in **Figure 2C**, using the minor levels (-1) of each parameter of mass and cycles, the lipid amount obtained in the SLE-DT method is 13.71% m/m. This value is similar to using -1 of mass and +1 of cycles, obtaining an extraction response of 13.73% m/m. This data suggests that 100 mg of sample mass and 10 extraction cycles can get a straightforward and quick lipid extraction.

Different lipid extraction methods were applied to anise, numeg, and white mustard seed samples by Kozłowska et al[17]. The Soxhlet methodology used by the authors required an extraction time of 8 hours, while Folch's method required an extraction time of 2 hours. Therefore, the traditional lipid extraction procedures are time-consuming. However, the proposed SLE-DT has a shorter analysis time (approximately 15 min) than those carried out by Kozłowska et al[17].

Ribeiro et al[18] utilized the Soxhlet lipid extraction method on seed samples of *Cnidoscolus quercifolius*, which required an analysis time of 8 hours. Their research also performed lipid extraction using the cold pressing process, aiming for faster and solvent-free extraction. However, this approach yielded a lower extraction rate than the Soxhlet procedure.

Ferreira et al[14] evaluated the lipid extraction method using bath-ultrasound as an extracting agent. The authors reported that the technique uses 1 g of sample (the same type used in this paper) for one extraction cycle and two more washes with hexane as a solvent to ensure complete lipid extraction. In this regard, the proposed SLE-DT used a smaller sample size, ranging from approximately 3.3 to 10 times less compared to the paper of Ferreira et al[14]. This indicates that the SLE-DT procedure can quantify lipids using low amounts of samples.

Forfang et al[10] monitored different lipid extraction processes, such as Bligh and Lewis, Folch, and Soxhlet extraction, revealing that the amount of mass required in this procedure is a limiting factor. Soxhlet extraction does not apply to samples with low masses, while the Bligh and Lewis procedures do not apply for industrial use on a large scale. Furthermore, they also evaluated the time required for analysis, defining Soxhlet as the slowest and most laborious among the procedures evaluated. Thus, fast, simple, safe, and efficient alternative methods for lipid extraction, with minimum waste of VOS, have been researched for years in the literature.

Since the 1970s, researchers have proposed a lipid extraction procedure using hexane and isopropanol solvents, with steps like those described by the Bligh-Dyer. The aim is to reduce waste generation in lipid extractions[19]. Waste reduction and laboratory safety have been key considerations, with efforts to minimize the use of solvents commonly used in lipid extractions. The proposed SLE-DT stands out by using only 5 mL of hexane, 100 mg of sample, and 10 cycles of extraction for efficient lipid extraction.

Accuracy check

The accuracy of the proposed procedure using SLE-DT was evaluated by comparing the results with those obtained after the Goldfish extraction procedure and by analyzing a CRM with a certificate concentration of lipids.

The Goldfish method was used because the solvent chosen for extraction directly influences the quantity and type of lipids extracted, as Ramanathan et al[20] described. Thus, the Goldfish procedure, like the proposed SLE-DT, uses hexane as an extractor solvent.

The lipid extracted values varied from 9.62% to 9.84%, with a mean value of 9.74 ± 0.11%. The relative standard deviation of 1.14% indicates a good accuracy of the results. The data indicate that although Goldfish is a widespread and official methodology, the results were lower than those reported by the SLE-DT method and the sample label used for the investigation. Moreover, considering the analytical frequency, the Goldfish method is unsuitable.

Evaluating analytical methods is crucial to ensuring the reliability of developing new analytical techniques. It aims for reproducibility, accuracy, and precision. Additionally, these techniques demonstrate that the method is suitable for its intended use[21].

Aiming to validate the analytical method, a certified sample (Total Diet CRM 1548) was evaluated using the SLE-DT. The results of the lipid extraction are shown in **Figure 1D** and compared to those obtained using the traditional Goldfish and the SLE-DT method proposed for the salty cracker sample.

Observing **Figure 1D**, it can be seen that the traditional Goldfish method, applied to the salty cracker sample, presented a percentage of extracted lipids lower than the expected amount described in the sample's nutritional table (14% lipids), as demonstrated by the purple line (**Figure 1D**), even considering the standard deviation of each average bar. According to the t-test (95% significance), the *p*-value of comparing Goldfish to the proposed SLE-DT method was 0.0018, suggesting a statistically significant difference between the methods.

On the other hand, using the SLE-DT method (using the same sample), the amount of lipids obtained was higher than that of the traditional Goldfish method. Compared with the purple line, which represents the lipid information on the salty cracker label, the SLE-DT method followed the nutritional table (14% m/m of lipids), considering its standard deviation.

Moreover, **Figure 1D** describes the lipid amount obtained in the certified reference material using the SLE-DT method (17.92 ± 1.35%). Considering the standard deviation range, the SLE-DT method reached the values concerning the certified lipid content of 20.6 ± 2.0%, as shown in **Figure 1D**, considering the red lines. Also, according to the t-test (t-value of -2.32 and t-critical 4.30), no significant difference exists between the SLE-DT applied to the CRM sample and the Total Diet CRM 1548 certified value.

Therefore, the SLE-DT method offers the benefits of low organic solvent consumption, minimal instrumental cost, and practicality. Using a low sample consumption (100 mg) with few extraction cycles (10 cycles), it was possible to develop a straightforward, effective, cheap, and fast method that contributes to the analytical frequency in laboratories and for the principles of Green Chemistry.

Analytical application

Ten samples of salty and sweet biscuits were used to apply the developed SLE-DT method and verify its applicability. **Table 2** shows the % of lipids (m/m) obtained using the proposed method. As indicated by the *p*-value column, which means the comparison between the amount of lipids obtained by SLE-DT and the amount of lipids reported in the label, only four samples (2, 4, 8, and 9) had not their results statistically similar between the two groups compared to each other.

Table 2. Analytical application of the SLE-DT method in biscuit samples

Sample	Type	% Lipids (mean ± SD)	95% Confidence interval	% Lipids (label informed)	*p*-value
1	Sweet	16.65 ± 1.37	13.24 – 20.05	19	0.09
2	Sweet	13.73 ± 0.20	13.23 – 14.23	13	< 0.05
3	Sweet	10.92 ± 1.02	8.38 – 13.45	13	0.07
4	Sweet	10.03 ± 0.04	9.93 – 10.14	13	< 0.05
5	Sweet	16.37 ± 1.32	13.09 – 19.65	17	0.49
6	Salty	14.33 ± 0.51	13.07 – 15.60	14	0.37
7	Salty	11.04 ± 0.34	10.21 – 11.88	11	0.84
8	Sweet	17.46 ± 0.24	16.87 – 18.05	15	< 0.05
9	Salty	17.80 ± 0.62	16.25 – 19.35	15	< 0.05
10	Salty	9.93 ± 0.41	8.91 – 10.96	10	0.80

The % of lipids (m/m) from sample 2 (sweet type) was not statistically similar to the label-informed value due to the slightly superior value of 13.73, suggesting a 95% confidence interval varying from 13.23% to 14.23%, also superior to the 13% available in the biscuit's label. Sample 4 (sweet type) had an amount of lipids of 10.03%, approximately 22% below the label-informed value. On the other hand, salty and sweet samples, respectively, 8 (17.46%) and 9 (17.80%) had values of 16.4% and 18.6%, superior to the 15% indicated in the labels of both samples.

Ferreira et al[14] proposed a method using ultrasound-assisted extraction of lipids in salty crackers. Two samples were used and analyzed by groups of regular undergraduate students, and 66% of the groups that analyzed the first sample agreed with the stipulated concentration of lipids. In contrast, for the second sample, all groups agreed with the specified value of lipids.

In our results, 60% of samples were in accordance with the label value in the nutritional table. Considering that food samples are complex matrices and macronutrients, such as lipids, could not be entirely uniformly distributed in the biscuits, the data obtained using the proposed method are suitable, and minor differences regarding the obtained values and the label-informed values were observed.

In gravimetric analysis, assessing the error profile is essential to ensure the accuracy and reliability of results. Errors can be systematic (e.g., contamination, incomplete precipitation, co-precipitation, or loss of precipitate) or random (e.g., fluctuations in weighing or handling). A step-by-step review of the procedure helps identify where such errors might occur. Replicate analyses were performed to evaluate precision and detect random errors, allowing standard deviation and confidence intervals to be calculated. Accuracy was assessed by analyzing the certified reference materials, which helped identify systematic errors.

In this sense, the SLE-DT method is an alternative to traditional methods such as Soxhlet and Goldfish. The proposed method emphasizes simplicity and affordability as its main attributes. The total cost, including the tip, syringe, and cotton, essential disposable materials in the development process, is notably lower than the expenses associated with general analytical methods. Furthermore, these materials are readily available in numerous laboratory settings, making them easily accessible for researchers and scientists.

Assessment of the SLE-DT method's environmental impacts

The environmental impact calculation was compared with the SLE-DT with the Goldfish method and was assessed using the Analytical GREEnness calculator[16]. **Table 3** summarizes the results based on the 12 principles of Green Chemistry.

Table 3. Summary of the 12 principles, criteria, and their responses and weights for the SLE-DT and Goldfish method

Criteria		SLE-DT	Goldfish	Weight
1	Direct analytical techniques should be applied to avoid sample treatment	External sample pre- and treatment and batch analysis (reduced number of steps)	External sample pre- and treatment and batch analysis (reduced number of steps)	1
2	Minimal sample size and minimal number of samples are goals	0.1 g	5 g	3
3	If possible, measurements should be performed *in situ*	Off-line	Off-line	2
4	Integration of analytical processes and operations saves energy and reduces the use of reagents	3 or fewer	3 or fewer	2
5	Automated and miniaturized methods should be selected	Degree of automation: manual / Sample preparation: miniaturized	Degree of automation: manual / Sample preparation: not miniaturized	3
6	Derivatization should be avoided	Not applicable	Not applicable	1
7	Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided	15 g (eg. sample, pipette tips, solvent, and syringe)	140 g (eg. sample, and solvents)	4
8	Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time	Number of analytes determined in a single run: 1 / Sample throughput (samples analysed per hour): 4	Number of analytes determined in a single run: 1 / Sample throughput (samples analysed per hour): 0.5	4

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Criteria		SLE-DT	Goldfish	Weight
9	The use of energy should be minimized	Hot plate solvent evaporation (10-150 min)	Soxhlet extraction	2
10	Reagents obtained from renewable sources should be preferred	No reagents	No reagents	2
11	Toxic reagents should be eliminated or replaced	Yes, 5 mL	Yes, 200 mL	4
12	Operator's safety should be increased	Highly flammable	Highly flammable	2

As demonstrated, the 12 input variables were evaluated for each methodology to verify the score on a scale of 0 to 1. Moreover, the weight factor in each criterion was modified for those highly relevant to the methodologies' characteristics. In this regard, the calculator generates **Figure 1D**, which represents the distribution of the 12 principles and the final score of the methods.

The score of the SLE-DT method in Figure 1D is 0.57, with the lowest score (in red) attributed to principle number 3, which is related to the in situ measurements of the target analytes[16]. In this case, the weight of principle number three was two (**Table 3**) due to its low applicability to measuring *in situ* lipid content in food samples.

The score of the Goldfish method was 0.28. The main negative scores (in orange and red) are related to criteria 1, 3, 5, 7, 8, 9, and 11. Principle 1 is associated with the direct analysis of samples, avoiding steps of sample treatment[16], which, in the case of goldfish, the sample needs to be prepared first (powdered, dried, packed in the cotton sample holder, etc.).

Criterion 5 is linked to automated and miniaturized methods[16]; the Goldfish method required about 5.00 g of sample for the analysis, whereas SLE-DT only uses 100 mg of sample. Also, the degree of automation of the proposed method is manual due to its simplicity and low cost. Parameter 7 is linked to waste generation; the Goldfish method produces about 140 g of waste due to the sample and solvents used.

Parameter 8 deals with the analytical frequency; the analytical frequency of the Goldfish method is low because of the time required to perform the lipid extraction. Parameter 9 is related to energy use, which concerns the long period of analysis of the Goldfish apparatus versus its potency, which makes systems that consume > 1.5 kWh get a low score[16].

According to principle 11, toxic reagents should be eliminated or replaced. In this case, Goldfish's extraction of lipids for each sample requires approximately 200 mL of petroleum-based solvent, which negatively impacts the environment.

The SLE-DT proposed in this paper is twice as environmentally friendly as the traditional Goldfish method. This suggests that the SLE-DT is well-suited for applications in Green Chemistry, particularly in developing more sustainable methodologies and processes. It indicates the potential of the SLE-DT to contribute to environmentally conscious practices within the field.

CONCLUSION

The SLE-DT method was effective for extracting lipids, demonstrating high precision and accuracy. It is quick to execute and does not require specific equipment such as an ultrasound, Soxhlet, or a Goldfish apparatus. This method could be a feasible alternative to traditional extractions that use large volumes of solvents, which are often toxic and harmful to both the analyst and the environment, and lead to a significant amount of waste. In line with the principles of Green Chemistry, the SLE-DT method was found to be more environmentally friendly than the traditional Goldfish method, primarily due to its lower energy and solvent usage, and requiring less sample and time. This method can be adjusted to work with other less toxic solvents, ensuring efficient lipid extraction while being gentler and less aggressive. Additionally, SLE-DT is simple and could be considered an official extraction method for lipid quantification.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR'S CONTRIBUTIONS

Bernardo Moura Zapelini: Methodology, formal analysis, investigation, writing – original draft. Caroline Gonçalves: Formal analysis, writing – original draft, writing – review & editing. Eduardo Sidinei Chaves: Conceptualization, writing – review & editing, resources. Bruno Luís Ferreira: Conceptualization, formal analysis, writing – original draft, writing – review & editing, resources.

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