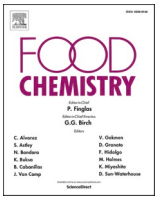
Food Chemistry 399 (2023) 133929

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Optimization of vortex-assisted switchable hydrophilicity solvent liquid phase microextraction for the selective extraction of vanillin in different matrices prior to spectrophotometric analysis

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ARTICLE INFO

Keywords:
Switchable hydrophilicity solvent
Food composition
Vanillin
Experimental design
Green chemistry
Food analysis

ABSTRACT

The main purpose of this research article is to develop a vortex-assisted switchable hydrophilicity solvent liquid phase microextraction (VA-SHS-LPME) for the selective and efficient extraction of trace vanillin in food samples. Four switchable hydrophilicity solvents (SHSs) were prepared and tested for the extraction of vanillin. The obtained extract phase after phase separation was analyzed by UV–vis spectrophotometry. The extraction parameters including pH, vortex time, NaOH volume, and SHS volume were optimized using central composite design based on response surface methodology. Under optimized conditions, the linear range (0.2–400 ng mL^{-1} with $r^2 = 0.9985$), limit of detection (0.06 ng mL^{-1}), limit of quantitation (2.0 ng mL^{-1}), extraction recovery (97 ± 4 %) and enhancement factor (220) were obtained. Also, relative standard deviations were less than 2.1 % indicating good precision. The VA-SHS-LPME procedure showed some advantages including good extraction, low consumption of chemical and low matrix effect. Finally, the VA-SHS-LPME procedure was applied for the determination of vanillin in food samples, and acceptable recoveries (91 ± 3–99 ± 3 %) were obtained.

1. Introduction

The systematic name of vanillin is 4-hydroxy-3 methoxy benzaldehyde. It is the main component of natural vanilla, one of the important flavor enhancer species (Walton et al., 2003). Since flavoring substances are found in small amounts in plants, purification is difficult and costly. Therefore, the production of artificial vanillin by extraction methods is more demanded because it is more economical (Raril and Manjunatha, 2020). Vanillin is widely used in chocolates, confectionery, butter, ready-made cakes and cakes, powder puddings (Wang et al., 2016). When this unique flavor is consumed excessively, health problems such as migraine, liver and kidney can occur (Ramesh, & Muthuraman, 2018). According to Food and Drug Administration (FDA) regulation, the vanillin concentration in food additives should not exceed 70 mg kg^{-1}.Therefore, it is important to develop selective, accessible and simple analytical methods for monitoring trace levels of vanillin in food samples.

Sample preparation procedures play an important role in trace analysis due to the matrix effect. Therefore, sample preparation procedures such as liquid-phase microextraction (LMPE) (Ji et al., 2022), solid phase extraction (Fu et al., 2019), ultrasound assisted extraction (Jadhav et al., 2009), membrane-based supercritical fluid extraction (Cabezas et al., 2020), microwaves-assisted extraction (Dong et al., 2014), vortex-assisted ionic liquid-dispersive microextraction (Altunay, 2018) and enzyme-assisted extraction (Zhang et al., 2014) have been widely used for this purpose. These procedures have disadvantages such as the use of organic solvents, time-consuming experimental steps, low enrichment factor and practical application difficulties.

The LPME has been widely studied by researchers, and many papers have been published in this field (Rutkowska et al., 2019). LPME based sample preparation procedures including ultrasonic-assisted switchable solvent liquid-phase microextraction (Durak et al., 2020), deep eutectic solvent-based air assisted liquid phase microextraction (Zounr et al., 2018), vortex-assisted liquid-phase microextraction (Altunay & Elik, 2020), hollow fiber-based liquid-phase microextraction (Hrdlička et al., 2021), ultrasound-assisted emulsification liquid phase microextraction (Li et al., 2019) and homogeneous liquid phase microextraction (Tsanaktsidou et al., 2022) have been developed. The basic step of these LPME procedures is to maximize the contact area between the extraction solvent and the sample solution. To achieve this, physical effects such as dispersive solvents, microwaves, ultrasound and vortex mixing are applied. The LPME has eliminated some disadvantages of the classical

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https://doi.org/10.1016/j.foodchem.2022.133929
Received 8 March 2022; Received in revised form 23 July 2022; Accepted 9 August 2022
Available online 11 August 2022

sample preparation techniques, such as the use of large amounts of reagents and toxic waste, long operating time, tedious procedures, and risk to the operator.

In order to avoid the serious harms of environmental pollution and ecological instability caused by the use of solvents, the development and use of green solvents instead of traditional organic solvents has become a social focus (Häckl, & Kunz, 2018). Therefore, the use of switchable hydrophilicity solvent (SHS) in sample preparation studies (as a new extraction solvent), is becoming widespread (Alshana et al., 2020). The SHSs are solvents that have two forms, polar and nonpolar, which differ in their physical properties. The transition of SHSs from polar form to nonpolar form or from non-polar form to polar form can be performed in a simple, fast, instantaneous reversible and controlled manner. The SHS are based on nonpolar secondary and/or tertiary amines to form protonated amine bicarbonate or alkyl carbonate salts with water in the presence of CO_2 at 1 atmosphere (Rezaeiyan et al., 2022). The basis of this reaction is the protonation of amines and is exothermic. The ammonium bicarbonate and alkyl carbonate solvents formed at the end of the reaction are polar forms of switchable solvents, that is, miscible with water (Bazel et al., 2020). If nitrogen gas (N_2) and/or air is passed through this switchable solvent medium in polar form, heated or a base such as NaOH is added, the CO_2 in the environment is removed again (Tripathy et al., 2022). When CO_2 is removed, the switchable solvent reverts to its nonpolar form. The SHSs can be to considered a form of ionic liquid but are much less expensive (Cicci et al., 2018). The simplicity and low cost of preparing these solvents are of interest to researchers in microextraction studies.

The optimization strategy is the form of analysis used to detect the effects of experimental parameters on the relevant response (Trindade et al., 2021). Statistical experiment design methods (full factorial design, central composite, Box-Behnken, Doehlert matrix..etc) experimentally describe the regression model between one or more measurable input variables (Czyrski, & Jarzębski, 2020). These methods provide great advantages in terms of optimizing the ambient conditions, increasing the efficiency, reducing the number of experiments and reducing the cost (Ferreira et al., 2018).

In this research article, vortex-assisted switchable hydrophilicity solvent liquid phase microextraction (VA-SHS-LPME) via UV–vis spectrophotometry for the selective, efficient extraction and rapid determination of trace vanillin was optimized using central composite design (CCD) based on response surface methodology. Four SHS were prepared and tested for the extraction of vanillin. In order to evaluate the accuracy of the VA-SHS-LPME procedure, the analysis of the selected food samples was also analyzed by other technique.

2. Experimental

2.1. Apparatus

An UV-1800 spectrophotometer was purchased from Shimadzu Instrument Co., ltd. (Tokyo, Japan) and employed for UV–vis analysis. VG3 model vortex stirrer was provided by IKA GmbH Company (Staufen, Germany) and used to accelerate the microextraction process. The pH of the sample solutions was measured by a Mettler Toledo FE28 pH meter (Zurich, Switzerland). Ultra-pure water was obtained by a Milli-Q water purification system (Millipore, USA). Centrifuge (Universal-320, Hettich, London, England) was used to accelerate the phases separation. In the sample preparation step, temperature and frequency control ultrasonic bath (SK5210LHC Kudos, Shanghai, China) was used.

2.2. Reagents

All reagents used were analytical grade. Stock solution (100 mg L^{-1}) of vanillin (4-Hydroxy-3-methoxybenzaldehyde were daily prepared by dissolving appropriate amounts of the solid (Sigma, St. Louis, MO. USA) in acetonitrile then stored in a refrigerator at 4 °C. The working solutions of vanillin were obtained by diluting the stock solution. NaOH solution (20 mmol/L, as a switching-off trigger) was prepared from its solid (Merck, Darmstadt, Germany) in the water. Sodium acetate buffer solution (0.2 M, pH 4.5) was prepared by dissolving appropriate amounts of sodium acetate trihydrate (Merck) and acetic acid (Sigma) solutions in the water. N,*N*-Dimethylbenzylamine (Sigma, DMBA), N,*N*-Dimethylcyclohexylamine (Sigma, DMCHA), 1-ethylpiperidine (Merck, EP) and triethylamine (Merck, TEA) were used for the preparation of SHS.

2.3. Sample collection and preparation

Cream biscuit, cocoa biscuit, baby biscuit, milky chocolate, chocolate, strawberry chocolate, wafer, cake, ice cream, cookie, sugar, peanut dragee, cotton candy and milk powder were collected in commercial establishments in the city of Sivas, Turkey. These samples were prepared according to the following procedure (Altunay, 2018). Initially, the collected samples were homogenized using a laboratory blender and 0.25 g of them were carefully weighed using digital analytical balance. The weighed samples were transferred to conical tubes and 30 mL of acetonitrile was added to them. Next, the tubes were placed in an ultrasonic bath. To ensure homogenization, sonication was applied to the tubes at 50 °C for 30 min. After this step, the resulting mixture was cooled to room temperature and filtered using a membrane filter (Millipore Corp, USA). The sample blank was prepared together during the analysis of each sample. All experimental steps were performed in triplicate. Finally, the VA-SHS-LPME procedure was applied to the prepared solutions.

2.4. Preparation of switchable hydrophilicity solvent

In this study, four SHS were prepared for the extraction of vanillin. The SHS-1, SHS-2, SHS-3 and SHS-4 were composed of DMBA, DMCHA, EP and TEA mixed with water, respectively. All SHSs were prepared according to the previously described method as below (Heydari & Ramezani, 2019; Alshana et al., 2020). First, four beakers were placed on the magnetic stirrer and 200 mL of water was added to them. Then, 200 mL of DMBA, DMCHA, EP and TEA were added to separate beakers. At this stage, two separate phases were created. Then, about 20 g of dry ice was added slowly to all beakers and shaken vigorously to obtain a cloudy solution. The resulting solution was then stirred at room temperature for 2 h to ensure complete protonation of the SHSs, and 400 mL of protonated SHSs were prepared at the end of this step.

2.5. VA-SHS-LPME procedure

The VA-SHS-LPME procedure for the extraction and determination of vanillin consists of five steps. Step 1, 10 mL of sample solution was first transferred into 15 mL centrifuge tube containing 70 ng mL^{-1} vanillin. And then the pH of the sample solution was adjusted to 4.5 with sodium acetate buffer solution. Step 2, SHS-2 (545 μL, as extraction solvent), and 20 mmol L^{-1} NaOH (120 μL, as a switching-off trigger) were added to the mixture solution. Step 3, microextraction (the vanillin was extracted by SHS-2 phase) were carried out simultaneously by vortex mixing for 2.5 min. Step 4, after centrifugation at 4000 rpm for 5 min, the SHS-2 phase (upper layer) and the water phase (bottom phase) was obtained. And the aqueous phase was removed with a syringe equipped with a long needle. The final volume of the SHS-2 phases remaining in the centrifuge tube was made up to 300 μL with ethanol. Step 5, the amount of vanillin in the final phase was determined using UV–vis spectrophotometry (357 nm). UV spectra obtained for vanillin were presented in Fig. S1.

2.6. Extraction efficiency calculation

Extraction efficiency (E.E%) was an important reference indicator of the efficiency of the optimization step.

The E.E% of the overall VA-SHS-LPME procedure was is expressed by the following Eq. (1).

$$E.E(\%) = 100 \times C_{final}\, V_{final}/C_o\, V_o \quad (1)$$

Where V_{final}, V_o, C_{final} and C_o refer to the volume of the final phase, volume of the sample solution, the amount of vanillin in the final phase, and the initial amount of vanillin in the sample solution, respectively.

3. Results and discussion

3.1. Preliminary studies

The extraction efficiency (E.E%) for target analytes is related to the proper choice of extraction solvent. The extraction solvent should have some characteristics, such as low solubility in the sample phase and high extraction capacity for target analytes. In this regard, SHSs have certain properties such as easy reproducibility, low solubility in water, adjustable density, low toxicity and good extraction ability for target analytes. In the study, four SHSs were designed, prepared and used as extraction solvent. As a result of the experimental studies with these SHSs prepared, the E.E % of vanillin was determined as 78.5 % for the SHS-1, 94.6 % for the SHS-2, 62.3 % for the SHS-3 and 69.4 % for the SHS-4. The SHS-2 provided the best dispersion to the sample solution among the extraction solvents studied under the same conditions. Accordingly, quantitative E.E% have been achieved since it has more interaction with vanillin. Comprehensive results were presented in Figure S2.Since the highest E.E% of vanillin was obtained in SHS-2 prepared with a mixture of water and N,*N*-Dimethylcyclohexylamine, this SHS-2 was chosen as the extraction solvent in the optimization step.

3.2. Central composite design

The adequacy of the CCD was investigated with the coefficients of determination (R^2, adjusted-R^2 and predicted- R^2), the p-value, and the lack-of-fit (LOF) test. Using the analytical data in Table S2, the ANOVA analysis was performed to evaluated the significance of the effects of optimized parameters on the VA-SHS-LPME procedure. In order for the CCD to be of high quality, its R^2, adjusted-R^2 and predicted- R^2 values should be close to 1. In the light of this explanation, when Table 1 is evaluated, it is seen that the R^2, adjusted-R^2 and predicted- R^2 are 0.9915, 0.9835, and 0.9443, respectively. These results indicate the high quality of the CCD. The contribution of the optimized parameters to the CCD is evaluated with the p-value. Here, the p-value should be$<$0.05 at the 95 % confidence level for the optimized parameters to contribute to the CCD. From the results, it is seen that the CCD (p-value:$<$ 0.0001) is significant.

Table 1
Statistical evaluation results for the CCD.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5330.73	14	380,77	124,33	$<$ 0.0001	significant
X_1	64.29	1	64.29	20.99	0.0004	
X_2	378.73	1	378.73	123.66	$<$ 0.0001	
X_3	10.09	1	10.09	3.29	0.0896	
X_4	4.06	1	4.06	1.33	0.2673	
X_1X_2	1179.92	1	1179.92	385.27	$<$ 0.0001	
X_1 X_3	0.0400	1	0.0400	0.0131	0.9105	
X_1 X_4	1584.04	1	1584.04	517.22	$<$ 0.0001	
X_2 X_3	678.60	1	678.60	221.58	$<$ 0.0001	
X_2 X_4	131.10	1	131.10	42.81	$<$ 0.0001	
X_3 X_4	108.16	1	108.16	35.32	$<$ 0.0001	
$X_1$2	14.83	1	14.83	4.84	0.0438	
$X_2$2	382.02	1	382.02	124.74	$<$ 0.0001	
$X_3$2	0.1395	1	0.1395	0.0456	0.8339	
$X_4$2	209.86	1	209.86	68.52	$<$ 0.0001	
Residual	45.94	15	3.06			
Lack of Fit	41.42	10	4.14	4.58	0.0534	not significant
Pure Error	4.52	5	0.9040			
Cor Total	5376.67	29				
Std. Dev	1.75		R^2	0.9915	Predicted R^2	0.9443
C.V%	2.45		Adjusted R^2	0.9835	Adeq Precision	42.772

In addition, the parameters that did not contribute to the CCD were SHS-2 vol (p-value: 0.0896), NaOH volume, (p-value: 0.2673), pH* SHS-2 vol (p-value: 0.9105), and (SHS-2 vol)^2 (p-value: 0.8339), respectively. F-values are evaluated to determine the parameter that contributes most to the CCD. The numerical magnitude of the F-value is directly proportional to the contribution to the CCD. It can be seen from the results that pH* NaOH volume (F-value: 517.22) is the parameter that contributes the most to the CCD. The p-value for lack of fit was calculated as 0.0534, indicating that the lack of fit was not significant compared to pure error. As a result of the ANOVA analysis, the relationship between the optimized parameters and the E.E% of vanillin can be calculated according to the equation below.

$$E.E\,(\%) + 63.14 - 1.85\,X_1 + 4.48\,X_2 - 0.7309\,X_3 - 0.4640\,X_4 + 8.59\,X_1 X_2 - 0.0500\,X_1 X_3 + 9.95\,X_1 X_4 - 6.51\,X_2 X_3 - 2.86\,X_2 X_4 + 2.60\,X_3 X_4 - 1.69\,X_1^2 + 8.59\,X_2^2 - 0.1641\,X_3^2 + 6.36\,X_4 2.$$

In Supplementary Data Figure S3, a straight line can be seen for the normal probability plot of the E.E% of vanillin. Also, this figure represents a normal distribution for experimental results and the reliability of the CCD.

The effect of binary interactions of the optimized parameters on the E.E% of vanillin was evaluated by surface response plots. The effect of the interaction between SHS-2 vol and pH on the E.E% of vanillin is given in Supplementary Data Figure S4a. In particular, quantitative E.E % of vanillin have been achieved in the SHS-2 range of 160–350 μL at pH below 5.5. The decrease in E.E% in basic regions may be due to the fact that excess OH^- ions in the sample solution cause a decrease in the effectiveness of SHS-2.

The effect of SHS-2 vol and vortex time on the E.E% of vanillin is presented in Supplementary Data Figure S4b. Here, vortex is the most important parameter for effective dispersion of SHS-2 into the sample solution. In this context, the results show that approximately 3 min of vortexing is sufficient to disperse the SHS-2 into the sample solution. No significant change in E.E% was observed at higher vortex times.

The effect of vortex time and NaOH volume on the E.E% of vanillin is presented in Supplementary Data Figure S4c. The addition of NaOH to the water-miscible system is an important step in deprotonating the exchangeable solvent to obtain an analyte-rich SHS phase. Addition of a small amount of NaOH to the sample solution causes poor deprotonation, while excessive addition of NaOH can cause dilution of the analyte-rich SHS phase. Based on the explanations made, it can be seen from Figure S4c that quantitative E.E is obtained at low vortex times ($\leq$3 min) and NaOH volumes (150–360 μL).

The effect of NaOH volume and SHS-2 vol on the E.E% of vanillin is presented in Supplementary Data Figure S4d. Quantitative recoveries were obtained when the NaOH volume was above 320 μL and the SHS-2 vol was in the 160–350 μL range. It is seen that 320 μL of NaOH is sufficient to ensure protonation of SHS-2. The reason for non-quantitative E.E% at low NaOH volumes may be due to insufficient protomization of SHS-2.

The criterion in the selection of optimum conditions is to provide quantitative phase separation and to achieve the highest E.E% of vanillin. In this context, based on the findings from the relevant CCD program and experimental studies, optimum values for sonication time, pH, SHS-2 vol, NaOH volume and vortex time were selected as 4.5, 545 μL, 120 μL and 2.5 min, respectively. The experimental E.E% obtained

using these optimum values showed a statistically high agreement with the value estimated by the CCD. A value below 0.85 % represents a strong correlation between the experimental and predicted values. Therefore, these analytical data were chosen as optimum for the studied parameters.

3.3. Analytical performance

The analytical performance of the VA-SHS-LPME procedure was investigated under optimized microextraction conditions. Regression equation, coefficient of determination (r^2), linear range, detection limit (LOD), and quantification limit (LOQ), enhancement factor (EF), relative standard deviation (RSD) and extraction recovery were calculated. The linear range were constructed to determine vanillin at a concentration range of 0.2 to 400 ng mL^{-1}. The regression equation was A = 0.065[*vanillin amount, ng* mL^{-1}]-0.007 with r^2 = 09985. LOD and LOQ were calculated as 0.06 ng mL^{-1} and 0.2 ng mL^{-1} with the help of formulas $3S_{blank}/m$ and $10S_{blank}/m$, respectively. Where S_{blank} is the standard deviation obtained from the ten-replication analysis of blank samples and m is the slope of the calibration graph. The RSD for 5 replicate measurements of 10 ng mL^{-1} vanillin was 2.1 %. EF was calculated as 220 from the ratio of the angular coefficient of the analytical curve obtained before and after the VA-SHS-LPME procedure. All result were given in Table 2a.

3.4. Matrix effect

Since the optimization strategy is carried out using model solutions, the matrix effect should be investigated before the analysis of real samples. Therefore, the possibility of matrix effect caused by matrix ions in the preconcentration and extraction was investigated using 50 ng mL^{-1} vanillin solutions. Each matrix ion was added to the model solutions including vanillin, and the analytical signal was compared with those corresponding to the sample solution signal containing only vanillin. A matrix ion was considered interfering when the analytical signal in its presence varies ± 5 % about the analytical signal of the vanillin. In addition, recovery% and RSD% were calculated for each matrix ion. The results in Table 2b indicated that high tolerable limits were achieved for the studied components. The tolerable limit, recovery, and RSD were in the range of 50–1500, 94.7–99.5 %, and 2.1–4.4 %, respectively. It was observed that the method was not affected by the presence of the species in the tested quantities.

3.5. Robustness test

The robustness of the VA-SHS-LPME procedure was investigated by making small changes in optimum conditions. In this context, minor modifications of four different microextraction parameters such as pH, vortex time, SHS-2 vol and NaOH volume were made to investigate the robustness of the method. In experimental studies, while the optimization parameter was changed, other parameters were kept constant at their optimum levels. The RSD% of the E.E% of vanillin were calculated for each parameter. After three repetitive experiments, the RSD% of the E.E% of vanillin for pH, vortex time, SHS-2 vol and NaOH volume were calculated as 2.1 %, 1.9 %, 2.8 %, and 2.4 %, respectively. These RSD values obtained were showed the robustness of the VA-SHS-LPME procedure.

Table 2a
Analytical figures of merit of the optimized VA-SHS-LPME procedure.

Analytical figures	Optimal value
Regression equation	A = 0.065[vanillin amount, ng mL^{-1}]-0.007
Coefficient of determination (r^2)	0.9985
Linear range (ng mL^{-1})	0.2–400
LOD ($3S_{blank}/m$, ng mL^{-1})	0.06
LOQ ($10S_{blank}/m$ ng mL^{-1})	0.2
EF	220
RSD % (for 10 ng mL^{-1} of vanillin, N = 5)	2.1
Extraction recovery ± standard deviation	97 ± 4

LOD: limit of detection; LOQ: limit of quantification, EF: Enhancement factor, RSD: Relative standard deviation.

Table 2b
Matrix effect research results of the optimized VA-SHS-LPME procedure.

Matrix ions	Recovery (%)	RSD (%)	Tolerable limit
SO_4^{2-}	99.5	2.7	1500
F^-	99.1	2.1	1500
Cl^-	98.8	2.6	1500
Zn^{2+}	98.7	2.2	1500
Mg^{2+}	98.1	2.8	1000
Cu^{2+}	98.3	2.8	1000
Tartaric acid	98.5	3.3	1000
Eugenol	98.6	3.1	750
Glucose	97.7	3.4	500
Coumarin	97.1	3.6	500
Maltol	97.6	3.3	250
Xanthine	95.0	3.9	250
Lactose	95.4	3.7	100
Ethyl vanillin	96.2	4.4	50
Methyl vanillin	94.7	4.2	50

3.6. Intra-day and inter-day precision

The precision of the VA-SHS-LPME procedure was investigated by intraday and interday studies. These studies were carried out as follows. In both studies, 10 ng mL^{-1}, 50 ng mL^{-1} and 200 ng mL^{-1} of vanillin solution was added to the samples. In the intraday study, each sample was analyzed four times a day, while in the interday study, each sample was analyzed four times in four consecutive days. At the end of intra-day study, the RSD and recovery values ranged from 1.9 to 3.2 % and 94–98 %, respectively. As a result of the inter-day study, the RSD and recovery ranged between 2.4 and 3.6 % and 92–96 %, respectively. In addition, a high agreement was observed between the added and found values. The comprehensive analytical data were given in Table 2c.

3.7. Recovery studies

The accuracy of the VA-SHS-LPME procedure was investigated by the standard addition method. Three different concentrations (10, 150 and 300 ng mL^{-1}) of vanillin were added to all prepared samples. Then, the VA-SHS-LPME procedure was applied to these samples and the recovery value was calculated for each added vanillin. Recovery values for 10, 150 and 300 ng mL^{-1} vanillin were calculated in the range of 91–96 %, 94–98 % and 96–99 %, respectively. From the results (see Table 2d), it was seen that quantitative recoveries were achieved even at low concentrations. This shows both the high accuracy and low matric effect of the VA-SHS-LPME procedure.

Table 2c
Results of intra-day/inter-day precision studies.

Spiked (ng mL^{-1})	Intra-day studies (N = 4)			Inter-day studies (N = 4 × 4)		
	Found (ng mL^{-1})	RSD (%)	Recovery (%)	Found (ng mL^{-1})	RSD (%)	Recovery (%)
10	9.4	1.9	94 ± 4	9.2	2.4	92 ± 4
50	48.5	2.5	97 ± 2	47	2.9	94 ± 5
200	196	3.2	98 ± 3	192	3.6	96 ± 2

Table 2d
Results of the recovery study for the selected samples using the optimized VA-SHS-LPME procedure.

Different matrixes	Spiked vanillin concentration		
	Low (10 ng mL^{-1})	Middle (150 ng mL^{-1})	High (300 ng mL^{-1})
Cream biscuit	93 ± 2*	95 ± 3	97 ± 4
Cocoa biscuit	95 ± 3	96 ± 3	98 ± 4
Baby biscuit	96 ± 3	97 ± 2	97 ± 5
Milky Chocolate	96 ± 2	98 ± 3	99 ± 3
Chocolate	94 ± 5	96 ± 3	97 ± 3
Strawberry chocolate	93 ± 3	95 ± 4	97 ± 4
Wafer	94 ± 4	96 ± 5	98 ± 3
Cake	96 ± 3	98 ± 3	99 ± 3
Ice cream	91 ± 3	94 ± 3	96 ± 4
Cookie	95 ± 2	96 ± 2	98 ± 4
Sugar	94 ± 3	95 ± 2	97 ± 2
Peanut dragee	96 ± 5	97 ± 5	98 ± 3
Cotton candy	94 ± 4	95 ± 3	96 ± 5
Milk powder-1	93 ± 2	94 ± 3	96 ± 5
Milk powder-2	95 ± 3	97 ± 4	98 ± 4
Milk powder-3	95 ± 3	96 ± 4	98 ± 3

* Mean relative recovery ± standard deviation (N = 5).

Table 3
Determination of vanillin in different matrixes.

Different matrixes	VA-SHS-LPME procedure	HPLC method	*t*-value	F-value
	Found (μg kg^{-1})	Found (μg kg^{-1})		
Cream biscuit	84.7 ± 2.5*	84.1 ± 2.3*	1.2	2.7
Cocoa biscuit	15.8 ± 1.3	15.4 ± 1.2	0.7	3.2
Baby biscuit	74.9 ± 2.2	75.6 ± 2.0	0.8	2.2
Milky Chocolate	95.3 ± 2.3	95.8 ± 2.1	1.0	3.4
Chocolate	26.7 ± 1.9	26.9 ± 1.7	1.1	3.9
Strawberry chocolate	66.1 ± 3.5	66.7 ± 3.3	0.9	4.2
Wafer	142.6 ± 3.7	141.3 ± 3.4	0.6	4.8
Cake	85.3 ± 3.8	84.6 ± 3.4	0.7	2.6
Ice cream	122.8 ± 2.9	123.4 ± 2.7	1.1	2.3
Cookie	66.4 ± 1.7	67.2 ± 1.9	1.0	3.4
Sugar	21.6 ± 1.5	22.2 ± 1.4	0.7	3.9
Peanut dragee	63.5 ± 3.1	63.9 ± 3.2	0.5	5.2
Cotton candy	95.9 ± 2.3	96.7 ± 2.4	0.8	5.7
Milk powder-1	162.1 ± 4.9	161.7 ± 4.5	1.2	3.6
Milk powder-2	146.7 ± 5.4	146.2 ± 5.6	0.9	3.1
Milk powder-3	133.8 ± 2.2	132.5 ± 2.3	0.7	4.9

*Mean amount ± standard deviation (N = 5).

3.8. Application to real samples

The VA-SHS-LPME procedure was applied for the extraction and determination of vanillin in cream biscuit, cocoa biscuit, baby biscuit, milky chocolate, chocolate, strawberry chocolate, wafer, cake, ice cream, cookie, sugar, peanut dragee, cotton candy and milk powder. The preparation of these samples was described in Section 2.3. All samples were analyzed in five replicates and results are given as mean amount ± standard deviation. Among the analyzed samples, the highest vanillin content (162.1 ± 4.9 μg kg^{-1}) was determined in milk powder-1, while the lowest vanillin content (15.8 ± 1.3 μg kg^{-1}) was determined in cocoa biscuit. The vanillin contents of the analyzed samples were presented in Table 3. For the food samples, the allowable daily intake (ADI) of vanillin is 0 ~ 10 mg kg^{-1} per kilogram of body weight. In this context, the amount of vanillin in all analyzed samples was below the daily tolerable limits. In order to evaluate the accuracy of the VA-SHS-LPME procedure, the analysis of the selected food samples was also analyzed by HPLC technique. The reliability of the data obtained as a result of the application of both methods was evaluated using the *t*-test and F-test. As a result of the triplicate, all calculated t and F values were smaller than the critical t- (2.77) and F-(19) values at the 95 % confidence level. All results were given in Table 3. As a result, the VA-SHS-LPME procedure has been successfully applied to different matrices.

3.9. Comparison with other reported methods

The results of the comparison between this VA-SHS-LPME procedure and other reported methods for the determination of vanillin were given in Supplementary Data Table S3. This procedure was used in combination with UV–vis spectrophotometry without expensive HPLC-MS/MS, which makes it easier to promote, especially in remote or economically underdeveloped areas. The LOD and RSD of the VA-SHS-LPME procedure were comparable to or lower than those of other reported methods. The EF and linear range of the procedure was higher than those of other methods. In addition, a switchable hydrophilicity solvent was used as the extraction solvent instead of an organic solvent in this article. Therefore, the VA-SHS-LPME procedure was a simple, rapid, sensitive, low-loss and green method that can be used for the separation and extraction of trace vanillin in real samples.

4. Conclusion

The VA-SHS-LPME procedure presented a simple, low-cost, and fast approach for the extraction and determination of the trace vanillin in food samples. Herein, SHSs were prepared and first tested as a suitable extraction solvent for the extraction and preconcentration of vanillin. The optimization strategy of the experimental steps was carried out using the CCD. This VA-SHS-LPME procedure showed some advantages including short microextraction time (only 2.5 min), less consumption of SHS-2 (545 μL), low LOD (0.06 ng mL^{-1}) and high EF (2 2 0). The good recovery and precision of the VA-SHS-LPME procedure were demonstrated. Further, the VA-SHS-LPME procedure appears to be a good alternative extraction method for determining vanillin in food samples because it is simple, low-cost, effective, and green. The VA-SHS-LPME procedure is based on the principles of green chemistry, as it does not employ toxic or persistent solvents. Especially, despite the rapid extraction process and the low quantity of extraction solvent used, the LOQ was lower than the permissible level of vanillin in selected samples. Finally, the VA-SHS-LPME procedure was applied to simultaneously extract and determine vanillin in food samples and the results showed that the proposed method for determining its concentrations in aqueous samples is reliable.

CRediT authorship contribution statement

Adil Elik: Supervision, Writing – review & editing. **Nail Altunay:** Investigation, Validation, Writing – original draft.

Declaration of Competing 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.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133929.

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