



Synthesized of poly(vinyl benzyl dithiocarbonate-dimethyl amino ethyl methacrylate) block copolymer as adsorbent for the vortex-assisted dispersive solid phase microextraction of patulin from apple products and dried fruits

Mustafa Tuzen^{a,b}, Baki Hazer^{c,d}, Adil Elik^e, Nail Altunay^{e,*}

^a Department of Chemistry, Tokat Gaziosmanpaşa University, Tokat, Turkey

^b King Fahd University of Petroleum and Minerals, Research Institute, Center for Environment and Marine Studies, Dhahran, 31261 Saudi Arabia

^c Department of Aircraft Airframe Engine Maintenance, Kapadokya University, Nevşehir, Turkey

^d Zonguldak Bülent Ecevit University, Department of Chemistry, 67100 Zonguldak, Turkey

^e Department of Chemistry, Sivas Cumhuriyet University, Sivas, Turkey

ARTICLE INFO

Keywords:

Patulin
Apple products
Dried fruits
Dispersive solid phase microextraction
UV–VIS spectrophotometer
Poly(vinyl benzyl dithiocarbonate-dimethyl amino ethyl methacrylate) block copolymer

ABSTRACT

A new and novel poly(vinyl benzyl dithiocarbonate-dimethyl amino ethyl methacrylate) block copolymer (Pvb-DMA-Xa) as adsorbent was synthesized for the vortex-assisted dispersive solid phase microextraction (VA-DSPME) of patulin from apple products and dried fruits using UV–visible spectrophotometer. The characterization of synthesized Pvb-DMA-Xa block copolymer was performed with Fourier Transform Infrared spectroscopy (FTIR-ATR) technique. Analytical characteristics such as pH, sorbent amount, adsorption time, eluent type and its volume, desorption time and adsorption capacity were optimized. Limit of detection (3Sb/m) and limit of quantitation (10Sb/m) were found 0.3 and 1.0 ng mL⁻¹. Linear dynamic range (LDR), relative standard deviation (RSD) and recovery values were found in the range of 1–30 ng mL⁻¹, 2.1–2.7 % and 93.5–97.3 %, respectively. Enhancement factor (EF) was found 193. The accuracy of the method was confirmed with standard addition method and analyzing of samples by reference method.

1. Introduction

Patulin (PAT) is a most toxic secondary metabolite produced by certain fungal species of the genera *Penicillium*, *Eupenicillium*, *Paecilomyces*, *Aspergillus* and *Byssoschlamys* (Marín, Mateo, Sanchis, Valle-Algarra, Ramos, Jiménez, 2011). *Penicillium expansum*, which is commonly found as a pollutant of apples and other fruits, is the most important of these PAT-producing molds (Fuchs, Sontag, Stidl, Ehrlich, Kundi, & Knasmüller, 2008). All patulin-producing fungi have been isolated from various vegetables and fruits such as peach, apple, pear, plum, etc. (Li, Li, Li, & Zhang, 2017). Patulin elicits a variety of chronic, acute symptoms and cause immune toxic, neuro toxic, gastro intestinal and genotoxic effects in rodents according to toxicologic studies (Wright, 2015). Therefore, it may affect the human health for long time consumption of beverages and foods contaminated with patulin. Various regulatory agencies have put strict restrictions on the content of patulin in various foods due to its adverse health effects. Maximum

recommended limit for PAT is 50 µg kg⁻¹ in fruit products and fruit juice according to Codex Alimentarius Commission (CAC, 2003). The Europe Union Commission has recommended 10 µg kg⁻¹ PAT limit for apple products to young children and infants and 25 µg kg⁻¹ for solid apple products (EC, 2006). It is recommended a tolerable daily intake of PAT as 0.4 µg kg⁻¹ body weight for long-term exposure according to WHO Food Additives Expert Committee (JECFA, 1995). Due to the toxicity and widespread presence of patulin, a sensitive, fast, green and simple analytical method is important for the control of patulin contamination in foodstuff.

Several analytical methods have been used for the determination of patulin in different samples including high performance liquid chromatography with diode array detection (HPLC-DAD) (Anene, Hosni, Chevalier, Kalfat, & Hbaieb, 2016), ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) (Beltrán, Ibáñez, Sancho, & Hernandez, 2014), gas chromatography mass spectrometry (GC-MS) (Kharandi, Babri, & Azad, 2013), micellar

* Corresponding author.

E-mail address: naltunay@cumhuriyet.edu.tr (N. Altunay).

<https://doi.org/10.1016/j.foodchem.2022.133607>

Received 30 January 2022; Received in revised form 20 June 2022; Accepted 28 June 2022

Available online 30 June 2022

0308-8146/© 2022 Elsevier Ltd. All rights reserved.

electrokinetic chromatography (MEKC) (V́ctor-Ortega, Lara, Garća-Campaña, & del Olmo-Irueala, 2013), liquid chromatography-mass spectrometry (LC-MS) (Kataoka, Itano, Ishizaki, & Saito, 2009), capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) (Moreno-González, Jáč, Riasová, & Nováková, 2021), Uv visible spectrophotometer (Taşpinar, Elik, Kaya, & Altunay, 2021). Uv visible spectrophotometer has some advantages such as simple, low cost, versatile, fast, and easy usage than above reported methods. However, direct determination of patulin by using spectrophotometer is difficult because of low concentration of analytes than instrument detection limit and matrix components of real samples. So, separation and preconcentration techniques are necessary to solve these problems and increase sensitivity and selectivity of the analytical method. Several sample preparation methods such as solid phase extraction (Moreno-González et al., 2021; Wang, Wen, & Ling, 2017), magnetic solid phase extraction (Yu, & Fan, 2017), liquid-liquid microextraction (Abu-Bakar, Makahleh, & Saad, 2014), molecularly imprinted polymer (Fang, Wang, Yang, Liu, & Wang, 2016), dispersive solid phase extraction (Sadok, Szmagara, & Staniszewska, 2018; Wu, Dang, Niu, & Hu, 2008), ionic liquid based dispersive liquid liquid microextraction (Mohammadi, Tavakoli, Kamankesh, Rashedi, Attaran, & Delavar, 2013) have been used before patulin determination in various food samples. Vortex-assisted dispersive solid phase microextraction has some advantages such simple, green, cheap, sensitive, selective and easy extraction of analytes using newly synthesized polymeric material.

The production of various types of block copolymers are a common procedure for tailoring application properties of polymers (Yıldız, Hazer, & Tauer, 2012). Comb/brush type block/graft copolymers show very useful material properties. Reversible addition fragmentation chain transfer (RAFT) polymerization is a powerful technique for macromolecular synthesis of a broad range of well-defined polymers (Ozturk, Atalar, Goktas, & Hazer, 2013; Boyer, Bulmus, Liu, Davis, Stenzel, & Barner-Kowollik, 2007). The xanthate derivatives can also be used in the controlled living free radical polymerization as reversible addition fragmentation chain transfer agent. Potassium salt of xanthate can easily be exchanged with a halogen derivative to produce a RAFT agent with xanthate group (Easterling, Xia, Zhao, Fanucci, & Sumerlin, 2019; Şanal, Oruç, Öztürk, & Hazer, 2015; Zounr, Tuzen, Hazer, & Khuhawar, 2018).

In this study, we synthesized a novel comb type block/graft copolymer (Pvb-DMA-Xa) with rich sulfur and amine groups in order to use for the vortex-assisted dispersive solid phase microextraction of patulin from apple products and dried fruits.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical purity. Patulin (4-hydroxy-4H-furo [3,2-c]pyran-2(6H)-one, PAT) was obtained from Sigma-Aldrich company (St. Louis, MO, USA) and its 1000 mg L⁻¹ stock solution was prepared by dissolving the its appropriate amount in acetonitrile/water mixture. The working solutions for the studies such as optimization and validation were freshly prepared by daily dilution of the stock solution. The solvents including acetone (Merck, Darmstadt, Germany), methanol (Merck), ethanol (Merck), THF (Sigma) and acetonitrile (Sigma) were used in the sorption and desorption steps. The pH adjustment required for the solutions in the studies was achieved with acetate, citrate, borate and phthalate buffer solutions. Vinyl benzyl chloride (vbc) and dimethyl amino ethyl methacrylate (DMA) were supplied from Sigma-Aldrich (Germany) and purified by passing through from basic Al₂O₃. Potassium-O-ethyl dithiocarbonate (Potassium xanthogonate, KXa) was supplied from Merck, Germany. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (R2) was synthesized according to the procedure in cited reference (Hazer, Eren, Senemoğlu, Modjinou, Renard, & Langlois, 2020).

2.2. Instrumentation

FTIR spectra of the polymer samples were recorded using Bruker Model, Tensor II with ATR technique. Vortex (VG3 model, IKA GmbH, Germany) was used to disperse the sorbent. Ultra-pure water was obtained by a Milli-Q water purification system (Millipore, USA). The pH of the working solutions was adjusted by means of a digital pH meter (Selecta 2001 Sartorius, North America). In the sample preparation step, ultrasonic bath (A SK5210LHC Kudos, Shanghai, China) and rotary evaporator (BUCHI R-200, Flawil, Switzerland) were used. An UV-Visible Spectrophotometer (Shimadzu UV-1800 PC model, Tokyo, Japan) was used to carry out spectral analysis equipped with quartz cuvettes with a path length of 10 mm.

2.3. Pvb-DMA-Xa block copolymer

2.3.1. Reversible addition fragmentation transfer (RAFT) polymerization of vbc

A mixture of vbc (10.08 g), R2 (0.18 g) and 2,2'-azo bis isobutyro nitrile (AIBN, 0.025 g) was polymerized at 80 °C under argon for 5 h. Pvc was recovered by precipitation from methanol. It was dried under vacuum at 40 °C for 24 h. Yield of macro RAFT initiator Pvc was 8.65 g.

2.3.2. Block copolymerization of DMA using Pvc

A mixture of Pvc (0.53 g), AIBN (0.011 g), DMA (2.0 g) and toluene (5.0 mL) as solvent was polymerized at 80 °C under argon for 5 h. The block copolymer (Pvb-DMA) was recovered by precipitation from methanol. It was dried under vacuum at 40 °C for 24 h. Yield was 1.04 g.

2.3.3. Synthesis of Xa functionalized Pvb-DMA block copolymer (Pvb-DMA-Xa)

A mixture of Pvb-DMA (0.72 g), KXa (1.47 g) and was stirred in THF (10 mL) at 40 °C for 24 h. Yield was 0.74 g.

2.4. Samples

In order to investigate the applicability of the method, all samples were collected from grocers and markets in Sivas/Turkey. In this context, nine different apple juices, six different apple-pure, nine different dried apples, three dried apricots, three dried pineapples, three dried figs, three dried prune and three dried kiwi fruit were collected. The collected samples were stored at 4 °C until sample preparation.

2.5. Sample preparation

The sample preparation steps for the collected samples are presented below (Ji, Li, Yang, Qi, Xiao, & Qian, 2017). First, solid (5 g) and liquid (5 mL) samples were added to 50 mL conical tubes containing 10 mL water and 75 µL pectinase. In the second step, the tubes were placed in an ultrasonic bath for the enzymatic reaction and then sonication was applied at 30 °C for 60 min. In the third stage, the caps of the tubes were opened and 20 mL of acetonitrile was added into them and vortex was applied for 2 min. In the fourth stage, after centrifugation at 4000 rpm for 5 min, the supernatant was transferred to a new tube and 1.5 g of *N*-propylethylenediamine and 0.5 g of MgSO₄ were added to the new tube. In the fifth step, the obtained solution was dried in a rotary evaporator at 45 °C under nitrogen flow and then was dissolved in 2.0 mL of acetonitrile-water mixture and made ready for the application of the VA-DSPME procedure.

2.6. The VA-DSPME procedure

The developed method consists of sorption and desorption steps. The sorption steps were carried out as follows. First, 10 mL of the above-described samples were added to 50 mL conical tubes containing 40 µg PAT. Subsequently, the pH of the sample solutions was adjusted to 6.5

using a phosphate buffer solution. 80 mg of the newly synthesized composite material was added to the tubes. Then, the tubes were vortexed for 3 min to ensure that the PAT in the sample solution was adsorbed on the Pvb-DMA-Xa block copolymer. Next, the tubes were centrifuged at 4000 rpm for 2 min and the sorption step was completed by separating the composite material including PAT from the aqueous phase. The desorption steps were carried out as follows. In order to provide spectrophotometric analysis of PAT, it is necessary to take the PAT adsorbed on the Pvb-DMA-Xa block copolymer into the aqueous solution. In this context, 1 mL of methanol was added to the solid sorbent and vortexed for 2 min. The tubes were centrifuged again at 4000 rpm for 2 min and then the aqueous phase was transferred to quartz cuvettes and finally spectral measurements were made at 292 nm by means of an UV-Vis spectrophotometer.

3. Results and discussion

3.1. Characterization of Pvb-PDMA-Xa block copolymer

Vinyl benzyl chloride has two different functional groups, vinyl and chloromethyl groups which improve easily modification reactions. Chloromethyl group leads to form cationic moieties obtained by the reaction with a tertiary nitrogen molecule. In our recent work, PHB-Pvbc block copolymer was reacted with trimethyl amine in order to obtain cationic polymer which was successfully used in the separation of Pb(II) and Cd(II) (Altunay, Hazer, Tuzen, & Elik, 2021).

3.1.1. Thermogravimetric analysis

Thermogravimetric Analysis (TGA) measures weight loss as a function of temperature under a nitrogen atmosphere. The dried functionalized Pvb-PDMA-Xa samples (10 mg) were heated from 20 °C to 600 °C at a rate of 10 °C/min under nitrogen atmosphere. Decomposition temperatures were measured via TGA and recorded using the first derivative (see Fig. S1). Weight loss between 216 and 382 °C is related to the xanthate groups. Polypropylene main chain starts decomposition from 382 to 450 °C. The maximum decompositions were determined as 278, 294 °C (for xanthogonate groups) and 428 °C (for polypropylene main chain).

3.1.2. SEM analysis

Topology of the Pvb-PDMA-Xa sample was determined by SEM. Pvb-PDMA-Xa morphology showed the layered structure (see Fig. S2). The sulphur content (8.36 wt%) and nitrogen content (8.33) of Pvb-PDMA-Xa were confirmed by EDX analysis (see Fig. S3).

3.1.3. BET analysis

BET specific surface areas (BETSSA) were determined on powders with a Micromeritics Gemini VII surface area and porosity under an Ar flow. The BET analysis of the Pvb-PDMA-Xa sample gave the results as: BET surface area: 0.2127 m²/g, pore volume 0.000153 cm³/g and pore size: 923.197 Å.

Here, we report a new block copolymer containing dimethyl amino ethyl methacrylate and xantogenate groups. The reaction of the chloromethyl groups of the Pvbc with tertiary amine groups of PDMA was carried out leading to cationic polymer, then chloride anions exchange with xanthogenate groups in order to obtain novel block copolymer with nitrogen and sulfide rich groups which were very effective on the chelating cationic species. This new polymer chemical formula can be designed as given in Fig. S4. The new block copolymer could not be dissolved in any solvent. It could be only characterized by FTIR-ATR technique. The characteristic signals (wavelength in cm⁻¹) of the obtained block copolymer were observed (Fig. S5): 704.79 (C–S stretch), 1724.51 (ester carbonyl), 2730–2763 (C–H stretch), 3233 (ammonium) (Nandiyanto, Oktiani, & Ragadhita, 2019).

3.2. The optimization of the VA-DSPME procedure

For the optimization of the sorption and desorption steps, respectively, pH, sorbent amount, vortex time, desorption solvent type and volume and desorption time were investigated and optimized in detail. In addition, the reusability and sorption capacity of the sorbent were also investigated. To determine the optimum value, the percent recovery was calculated according to the formula below.

$$\text{Recovery}(\%) = \left[\frac{C_{\text{found}} - C_{\text{real}}}{C_{\text{spiked}}} \right] \times 100 \quad (1)$$

Where C_{found} , C_{real} , and C_{spiked} were the amount of the PAT after spiking the standard to the sample, amount of PAT in the sample, and amount of PAT added to the sample, respectively.

3.2.1. Effect of pH

Patulin is an electrophilic molecule, and has a high effect by binding covalently to thiol groups of proteins and glutathione (Fliege, & Metzler, 2000; Morgavi, Boudra, Jouany, & Graviou, 2003). This high interaction of patulin with thiol groups, we designed a polymer with sulfur groups in order to extract patulin from apple products and dried fruits. pH of sample solution is important for vortex-assisted dispersive solid phase microextraction (VA-DSPME) of patulin in real samples for quantitative recoveries. The recovery values of analytes in VA-DSPME procedure were investigated in the pH range of between 3.5 and 9.5 by using 10 mL sample solution containing 40 µg PAT, 80 mg of Pvb-PDMA-Xa block copolymer, 3 min extraction time, 2 min centrifugation at 4000 rpm and 1 mL methanol as eluent. pH of the model solutions was investigated in the range of pH 3.5–9.5. The recovery of PAT was found quantitative at pH 6.5 (Fig. 1a).

3.2.2. Effect of Pvb-PDMA-Xa block copolymer amount

The amount of adsorbent is important for the quantitative recovery of analytes in VA-DSPME method. The amount of Pvb-PDMA-Xa block copolymer was examined in the range of 5–150 mg adsorbent in 10 mL model solution containing 40 µg PAT, 3 min extraction time, 2 min centrifugation at 4000 rpm and 1 mL methanol as eluent at pH 6.5. Quantitative recovery values of PAT were found by using 80 mg Pvb-PDMA-Xa block copolymer as adsorbent (Fig. 1b). The recovery of PAT was decreased after 80 mg adsorbent because it is necessary higher amount of eluent for the desorption of analytes. We used 1 mL methanol as eluent. High amount of eluent can cause environmental pollution.

3.2.3. Effect of sorption time

In order to accelerate the sorption of PAT in the sample solution, the solutions were shaken at a certain constant speed using a vortex mixer.

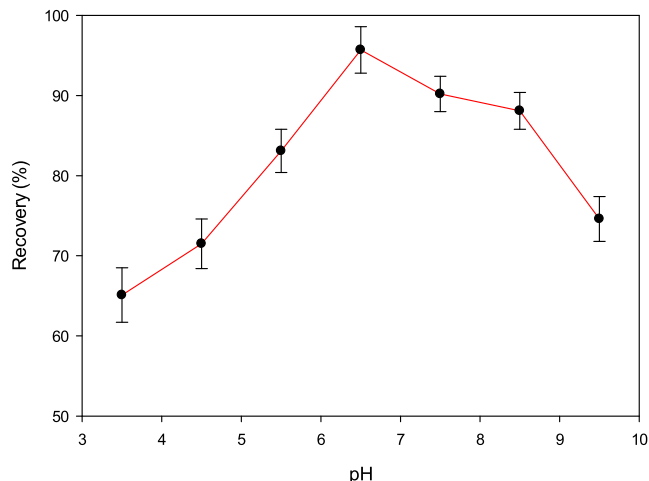


Fig. 1a. Effect of pH of sample solution on the recovery of PAT (N = 3).

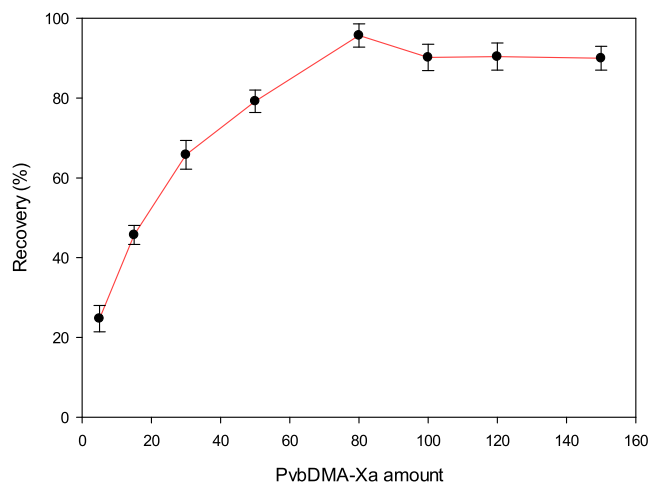


Fig. 1b. Effect of PvbDMA-Xa amount on the recovery of PAT (N = 3).

Also, thanks to this agitation step, the Pvb-DMA-Xa block copolymer was distributed evenly in the sample solution. In this context, the effect of sorption time on recovery of PAT ions was tested in the range of 0.5–10 min (Fig. 1c). The results show that 3 min vortex time is sufficient for effective sorption of PAT. Also, recovery of PAT at periods greater than 3 min followed a flat plateau. Therefore, 3 min of sorption time was chosen as optimum for further studies.

3.2.4. Effect of desorption eluent type

In solid phase extraction studies, one of the important variables in obtaining quantitative and acceptable results is the type of desorption solvent and its volume. Appropriate desorption solvent should be selected to take the analyte adsorbed on the Pvb-DMA-Xa block copolymer back into the aqueous solution. If the appropriate solvent is not selected, full desorption step cannot be achieved and therefore the desired analytical results cannot be obtained. Therefore, acetone, methanol, ethanol, THF and acetonitrile were tested as desorption solvents in this study. The results in Fig. 1d indicated that the most suitable desorption solvent for this study was methanol. Therefore, methanol was used as the desorption solvent for further studies.

3.2.5. Effect of desorption eluent volume

The second most important variable in achieving full desorption is the volume of desorption solvent. Sufficient solvent must be added to the Pvb-DMA-Xa block copolymer so that all PAT can be taken into the

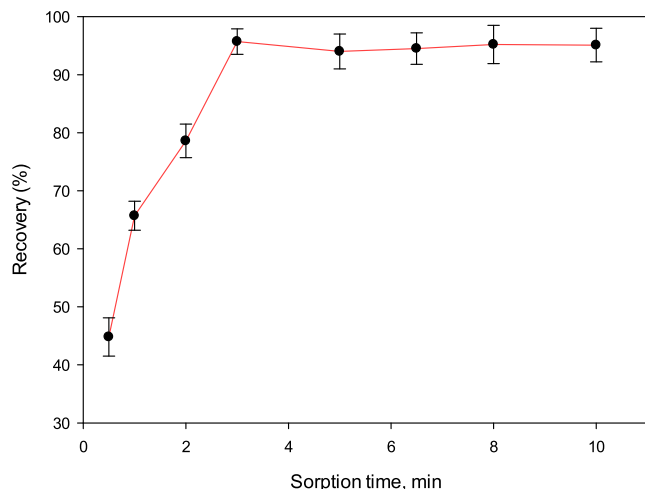


Fig. 1c. Effect of sorption time on the recovery of PAT (N = 3).

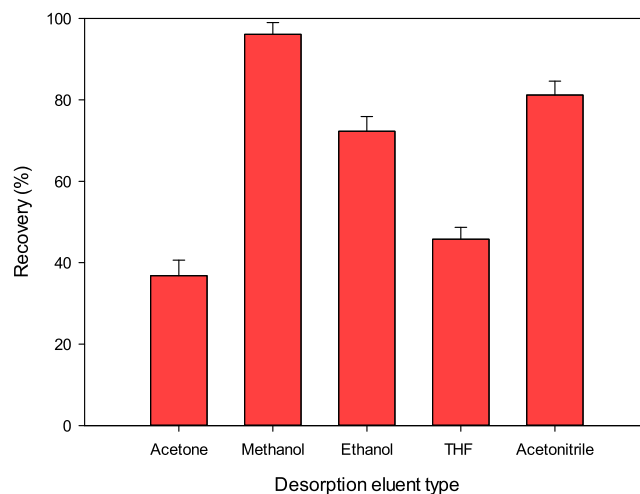


Fig. 1d. Effect of desorption eluent type on the recovery of PAT (N = 3).

aqueous solution. Therefore, the effect of methanol volume on the recovery of PAT was investigated in the volume range from 0.5 mL to 3 mL. The results in Fig. 1e showed that 1 mL of methanol was sufficient to absorb acetone into the aqueous solution. In particular, the reduction in recovery of PAT at volumes above 2 mL is due to the increase in the final volume. Therefore, 1.0 mL of methanol was used as the desorption solvent volume for further studies.

3.2.6. Effect of desorption time

After the deposition solvent (methanol) is added to the Pvb-DMA-Xa block copolymer, it must interact well with the Pvb-DMA-Xa block copolymer to ensure complete desorption. If complete desorption is not achieved, some amount of PAT remains on the adsorbent. Therefore, both the recovery is reduced and less PAT is detected than expected. For these reasons, after adding methanol on the Pvb-DMA-Xa block copolymer, vortex was applied to this mixture to ensure effective interaction. The change in the recovery of PAT with the applied vortex time is presented in Fig. 1f. It can be seen in the Fig. that a 2 min vortex is sufficient for quantitative recovery of PAT. Therefore, the 2 min vortex time chosen as the optimum desorption time for further studies.

3.2.7. Reusability

The high number of reusability of the Pvb-DMA-Xa block copolymer reduces the cost of the solid phase method. Therefore, in order to test its reusability, the Pvb-DMA-Xa block copolymer was washed three times

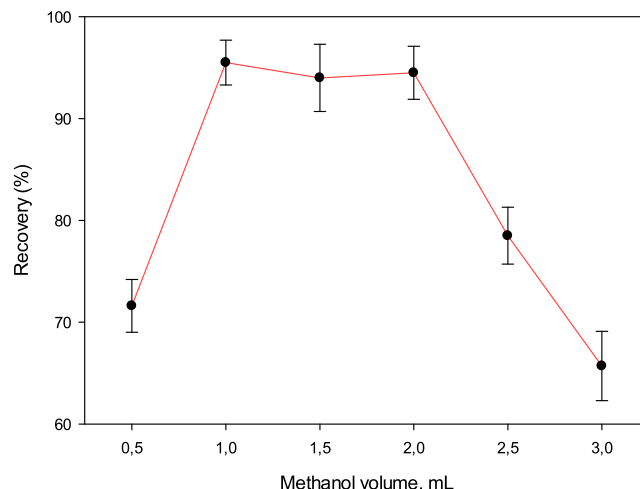


Fig. 1e. Effect of pH of methanol volume on the recovery of PAT (N = 3).

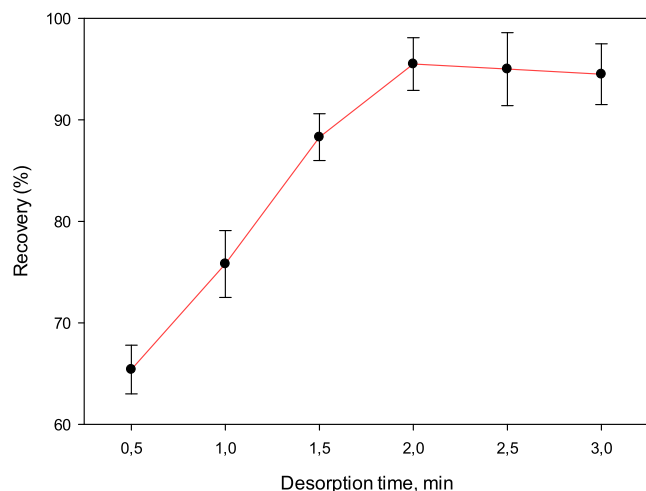


Fig. 1f. Effect of desorption time on the recovery of PAT (N = 3).

with methanol and water after the desorption step and then dried for reuse. No significant change in recovery of PAT ions was observed until twenty repetitions of the reuse of the Pvb-DMA-Xa block copolymer. The recoveries obtained were in the range of 95.4–99.7% for PAT. The analytical result showed that the Pvb-DMA-Xa block copolymer was stable in the VA-DSPME procedure and had excellent reusability. The high number of reusability of the Pvb-DMA-Xa block copolymer caused a relative decrease in the cost of the method.

3.2.8. Sorption capacity

Sorption capacity is one of the important parameters to test the recovery of PAT. The sorption capacity of the Pvb-DMA-Xa block copolymer for PAT was determined according to the batch adsorption procedure. The related study includes the following experimental steps. A 50 mL of test solution containing 50 mg of PAT was added to 100 mL beaker containing 80 mg of the Pvb-DMA-Xa block copolymer. Next, the mixture was vortexed for about 1 h. After separation of the Pvb-DMA-Xa block copolymer from the aqueous solution, the possible amounts of PAT in the remaining aqueous solution were determined by UV-VIS spectrophotometer. Using the formula in the batch adsorption procedure (Duran, Ozdes, Gundogdu, Imamoglu, & Senturk, 2011), we calculated the adsorption capacity as 185.6 mg g⁻¹ for PAT.

Table 1
Analytical characteristics of the VA-DSPME method.

Analytical characteristics	With VA-DSPME	Without VA-DSPME
Regression equation (C in ng mL ⁻¹)	$A = 82.9 \times 10^{-3}C + 1.4 \times 10^{-3}$	$A = 4.3 \times 10^{-4}C - 9.5 \times 10^{-4}$
Linear dynamic range (ng mL ⁻¹)	1–300	150–2000
Correlation coefficient, r ²	0.9995	0.9941
Limit of detection (3Sb/m, ng mL ⁻¹)	0.3	45.5
Limit of quantitation (10Sb/m, ng mL ⁻¹)	1.0	150
RSD (%), N = 10	2.7% (at 5 ng mL ⁻¹) 2.1% (at 100 ng mL ⁻¹)	6.2% (at 200 ng mL ⁻¹) 4.8% (at 800 ng mL ⁻¹)
Recovery (%), N = 10	93.5% (at 5 ng mL ⁻¹) 97.3% (at 100 ng mL ⁻¹)	88.4% (at 200 ng mL ⁻¹) 94.6% (at 800 ng mL ⁻¹)
*Enrichment factor	193	–

*Enhancement factor was calculated from the ratio of the slopes of the calibration graphs after and before the VA-DSPME method.

3.3. Analytical performance

The analytical parameters of the VA-DSPME method were presented in Table 1 in comparison with the data obtained without the VA-DSPME method. The regression equation of the VA-DSPME method was $A = 0.0827C + 0.0139$ with a coefficient of determination of 0.9995. The proposed method exhibited a linear dynamic range from 1 to 300 ng mL⁻¹. Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated as 0.3 ng mL⁻¹ and 1.0 ng mL⁻¹ from the formulas $3Sb/m$ and $10Sb/m$, respectively. Where m and Sb were the slope of the calibration equation and the standard deviation of the blank samples (N = 10), respectively. As a result of the application of the VA-DSPME method for the two concentrations (5 and 100 ng mL⁻¹) of PAT in the linear dynamic range, the percent relative standard deviation (RSD%) and percent recovery were found in the range of 2.1–2.7% and 93.5–97.3%, respectively. Enhancement factor (EF) was calculated as 193 from the ratio of the slopes of the calibration graphs after and before the VA-DSPME method. Validation test, concentrations 20-fold higher than the LOQ concentration of PAT were studied with the VA-DSPME method and a calibration curve was created according to the analytical signals obtained. The satisfactory r² value obtained (see Fig. S6) was supported the validation of the VA-DSPME method.

3.4. Selectivity

One of the most important quality parameters in extraction studies is the selectivity of the method for the analyte. If the developed method is not selective for the analyte studied, the reliability of the analytical results obtained will decrease. In this context, the proposed method was applied by adding the chemical species specified in Table 2 to the model solutions including 25 ng mL⁻¹ PAT. Then, tolerance limit, RSD and percent recovery values were calculated for each chemical species. The results obtained showed that the recovery (200–3000) was quantitative and the RSD values (1.3–2.2%) were lower than the desired limit (5%). Also, the tolerable limit for the studied chemical species was quite high. These results indicate that especially the synthesized Pvb-DMA-Xa block copolymer has a high selectivity for PAT.

3.5. Precision

The precision of the VA-DSPME method was checked by intraday/interday studies. In this context, four different PAT concentrations were added to the control samples (apple juice), and then the VA-DSPME method for intraday and interday study was applied to them five times a day and five times in five consecutive days, respectively. Then,

Table 2
Tolerant limits of matrix ions for the determination and extraction of PAT (N = 3, 25 ng mL⁻¹).

Matrix ions	Tolerant limit*	Recovery (%)	RSD (%)
CO ₃ ²⁻	3000	99 ± 2	1.6
SO ₄ ²⁻	3000	99 ± 1	1.5
K ⁺	3000	98 ± 3	1.9
Ca ²⁺	3000	98 ± 3	1.3
Fe ³⁺	1000	97 ± 4	1.8
Na ⁺	1000	96 ± 2	2.0
Flumethrin	1000	97 ± 3	1.9
Cyfluthrin	1000	95 ± 5	1.8
Cypermethrin	750	98 ± 2	2.2
Ascorbic acid	750	97 ± 4	1.9
Tartaric acid	500	97 ± 3	1.7
5-Methylfurfural	500	95 ± 3	1.5
Beauvericin	500	94 ± 5	1.7
Ochratoxin	500	92 ± 4	2.2
Citrinin	200	93 ± 3	2.0
5-HMF	200	91 ± 3	2.1

* [matrix ion concentration] / [PAT concentration].

using the obtained results, RSD and percent recovery were calculated for each study. The RSD and percent recovery in the intraday study were in the range of 2.6–3.4% and 93 ± 5 – $98 \pm 3\%$, respectively, while the RSD and percent recovery in the interday study were in the range of 3.0–4.2% and 90 ± 6 – $97 \pm 3\%$, respectively (Table 3a). These results indicate the high precision of the VA-DSPME method. In addition, the obtained quantitative recoveries also showed that the proposed method exhibits good accuracy in intraday and interday operation.

3.6. Recovery

For a successful microextraction process, both the matrix effect and the accuracy of the method should be tested by performing a recovery study in the real sample application. In this context, three-level PAT concentrations were added to the real samples. Then, the percent recovery was calculated for each concentration by applying the VA-DSPME method. The percent recovery range was also calculated for each sample analyzed. All the results obtained are presented in Table 3b. The percent recovery ranged from $91 \pm 3\%$ to $99 \pm 1\%$. The results indicated that quantitative recoveries were achieved. This indicates that the method has good accuracy. In addition, the VA-DSPME method can be safely applied in real samples with low matrix effect.

3.7. Applications

The applicability of the VA-DSPME method was tested on apple products and dried fruits. In addition, the same samples were also analyzed with the reference method (Ji, Li, Yang, Qi, Xiao, & Qian, 2017) for the reliability of the analytical results obtained with the VA-DSPME method. The application results were presented in Table 4. It was seen that there was no significant difference between the results obtained with the VA-DSPME method and the reference method. Also, the experimental *t*-values were lower at the critical *t*-value for six degrees of freedom at the 95% confidence level, which indicates that the method does not contain significant errors. These results confirm that the VA-DSPME method is suitable for the measurement of PAT content in the apple products and dried fruits. As listed in Table 4, no the PAT was found in apple juice-2, dried apple-1, dried pineapple, dried fig, and dried kiwi fruit, probably because their complex manufacturing process eliminated the PAT. But, the concentration of PAT was detected in $6.95 \pm 0.46 \mu\text{g g}^{-1}$ in apple Juice-1, $2.35 \pm 0.08 \mu\text{g g}^{-1}$ in apple Juice-3, $16.18 \pm 0.94 \mu\text{g g}^{-1}$ in apple puree-1, $8.16 \pm 0.75 \mu\text{g g}^{-1}$ in apple puree-2, $3.51 \pm 0.06 \mu\text{g g}^{-1}$ in dried apple-2, $4.85 \pm 0.09 \mu\text{g g}^{-1}$ in dried apple-3, $1.09 \pm 0.02 \mu\text{g g}^{-1}$ in dried apricot and $1.63 \pm 0.03 \mu\text{g g}^{-1}$ in dried prune. The PAT level in analyzed samples were within the permitted limit established by European regulation ($50 \mu\text{g kg}^{-1}$). The data obtained with both methods showed that the selected sample matrices did not have a significant effect on the results and that the VA-DSPME method could be applied efficiently and reliably for the determination of PAT content in the apple products and dried fruits.

3.8. Comparison with other methods

In order to better understand the effectiveness of the VA-DSPME method, the analytical data of the VA-DSPME method were compared

Table 3a
Values determined of mean recovery, accuracy and precision of the VA-DSPME.

Spiked amount (ng mL ⁻¹)	Intra-day precision, % (N = 5)	Intra-day accuracy, % (N = 5)	Inter-day precision % (N = 5 × 5)	Inter-day accuracy % (N = 5 × 5)
1	3.4	93 ± 5	4.2	90 ± 6
5	3.1	95 ± 4	3.8	92 ± 6
150	2.8	97 ± 3	3.5	95 ± 4
200	2.6	98 ± 3	3.0	97 ± 3

Table 3b

Investigation of the metric effect of the VA-DSPME method in real samples.

Samples	Spiked PAT concentrations			
	1 ng mL ⁻¹	10 ng mL ⁻¹	50 ng mL ⁻¹	100 ng mL ⁻¹
Apple Juice-1	92 ± 2	94 ± 3	96 ± 4	97 ± 2
Apple Juice-2	94 ± 4	96 ± 4	97 ± 3	98 ± 1
Apple Juice-3	93 ± 3	95 ± 4	98 ± 3	98 ± 2
Apple puree-1	91 ± 3	94 ± 4	96 ± 5	98 ± 3
Apple puree-2	95 ± 2	97 ± 3	98 ± 4	99 ± 3
Dried apple-1	96 ± 4	97 ± 2	98 ± 4	99 ± 2
Dried apple-2	92 ± 3	95 ± 4	97 ± 3	98 ± 2
Dried apple-3	93 ± 5	96 ± 4	97 ± 3	99 ± 1
Dried Apricot	94 ± 4	95 ± 5	98 ± 4	99 ± 3
Dried pineapple	95 ± 2	96 ± 3	97 ± 3	98 ± 2
Dried fig	94 ± 3	96 ± 4	98 ± 2	98 ± 1
Dried prune	96 ± 4	97 ± 3	98 ± 2	99 ± 1
Dried kiwi fruit	92 ± 5	94 ± 3	96 ± 4	97 ± 3

Table 4

Application results of the VA-DSPME to selected dried fruit and apple products (N = 4P = 0.95; tk = 2.78).

Samples	VA-DSPME method Found	Reference method Found	** <i>t</i> -value
Apple Juice-1	$6.95 \pm 0.46^*$	6.59 ± 0.44	0.98
Apple Juice-2	<LOD	<LOD	–
Apple Juice-3	2.35 ± 0.08	2.41 ± 0.09	0.87
Apple puree-1	16.18 ± 0.94	16.83 ± 0.97	0.84
Apple puree-2	8.16 ± 0.75	8.64 ± 0.72	0.80
Dried apple-1	<LOD	<LOD	–
Dried apple-2	3.51 ± 0.06	3.44 ± 0.06	1.43
Dried apple-3	4.85 ± 0.09	4.76 ± 0.08	1.29
Dried Apricot	1.09 ± 0.02	1.12 ± 0.03	1.47
Dried pineapple	<LOD	<LOD	–
Dried fig	<LOD	<LOD	–
Dried prune	1.63 ± 0.03	1.59 ± 0.04	1.40
Dried kiwi fruit	<LOD	<LOD	–

Juice sample (ng mL⁻¹); dried fruit samples ($\mu\text{g kg}^{-1}$). *Mean \pm SD, **The criterion *t*-value established by two paired ANOVA analysis for 6-degree of freedom at 95% confidence limit where $t_{\text{exp}} = (m_a - m_b) / S_{\text{pooled}} \times [(n_1 + n_2) / n_1 \times n_2]^{1/2}$ and $S_{\text{pooled}} = [(n_1 - 1) S_{m,1}^2 + (n_2 - 1) S_{m,2}^2 / (n_1 + n_2 - 2)]^{1/2}$.

with the methods previously reported in the literature. The analytical data were given in Table 5. It is seen that all analytical comparison parameters (LDR, LOD, RSD, recovery, and EF) of the VA-DSPME method are better than similar determination techniques. When compared with expert users and expensive techniques such as LC-MS/MS and HPLC-DAD, the working range and LOD values obtained with the VA-DSPME method were comparable. Also, the RSD values of our method are lower than those of the method. In addition to all these, higher EF was obtained with the VA-DSPME method compared to similar microextraction procedures. These results demonstrate that the VA-DSPME method can be powerfully applied to a wide range of applications.

4. Conclusions

In this research paper, a green, rapid, accurate, and precise vortex-assisted dispersive solid phase microextraction (VA-DSPME) procedure was presented for the extraction and determination of PAT in apple products and dried fruits. A new and novel poly(vinyl benzyl dithiocarbonate-dimethyl amino ethyl methacrylate) block copolymer (Pvb-DMA-Xa) was synthesized and applied as reusable adsorbent with superior adsorption capacity in VA-DSPME procedure. The sorption and desorption stages were investigated in detail and optimized. The optimum values obtained were pH 6.5, mass of 80 mg Pvb-DMA-Xa block copolymer, sorption time of 3 min, 1 mL of methanol, and desorption time of 2 min, respectively, to ensure quantitative recoveries. After the necessary verification work, the VA-DSPME method successfully applied to the selected samples. The successful effects of the VA-DSPME method

Table 5

Comparison of VA-DSPME procedure with other reported methods for PAT extraction and determination.

Extraction procedure	Determination method	LDR(ng mL ⁻¹)	LOD(ng mL ⁻¹)	RSD (%)	Recovery (%)	EF	References
VA-DSPME	UV-VIS spectrophotometry	1–300	0.3	≤2.7	93.5–97.3	193	Current method
SD-LLLME	LC-MS	2–2000	0.5	≤3.6	83.6–96.3	3.1	(Li et al., 2018)
MISPE	CE-MS/MS	1–100	0.3	≤14.9	94–98	93	(Moreno-González et al., 2021)
SPE	LC-MS/MS	1–400	0.2	≤3.99	97.2–100.2	–	(Li et al., 2021)
SPE	LC-MS/MS	1–100	0.05	≤4.5	81.3–106.3	–	(Zhao et al., 2019)
UA-ELPME-DES	UV-VIS spectrophotometry	7.5–420	2.2	≤4.2	90.2–106.9	88	(Altunay et al., 2019)
AA-NADES-SH-LPME	UV-VIS spectrophotometry	10–750	3.5	≤3.5	94–104	150	(Taşpınar et al., 2021)
VALLME	HPLC-DAD	0.5–100	0.4	≤7.6	90–112	–	(Abu-Bakar et al., 2014)

LDR: Linear dynamic range, EF: Enhancement factor; SD-LLLME: Single-drop liquid-liquid-liquid microextraction; LC-MS: Liquid chromatography-mass spectrometry; CE-MS/MS: Electrophoresis coupled with tandem mass spectrometry; MISPE: In-line molecularly imprinted polymer solid phase extraction; SPE: Solid phase extraction; UA-ELPME-DES: Ultrasound-assisted emulsification liquid phase microextraction based on alcohol-based deep eutectic solvent; AA-NADES-SH-LPME: Air-assisted natural deep eutectic solvent-based solidified homogeneous liquid phase microextraction; VALLME: Vortex-assisted liquid-liquid microextraction; HPLC-DAD: high performance liquid chromatography coupled with diode array detector.

in the determination of PAT in the real matrix lead to further improvements in application character and feasibility for column treatment in separation and pre-concentration. All these results indicate that the synthesized block copolymer material exhibits good selectivity for PAT.

CRedit authorship contribution statement

Mustafa Tuzen: Supervision, Conceptualization, Writing – original draft. **Baki Hazer:** Conceptualization, Investigation, Writing – original draft. **Adil Elik:** Investigation, Supervision. **Naïl Altunay:** Investigation, Validation, Writing – original draft, Writing – review & editing.

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

Patuline

Acknowledgments

This work was supported by the Kapadokya University Research Funds (#KÜN.2020-BAGP-001). Dr. M. Tuzen thanks to Turkish Academy of Sciences (TUBA) for partial supports.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2022.133607>.

References

- Abu-Bakar, N. B., Makahleh, A., & Saad, B. (2014). Vortex-assisted liquid liquid microextraction coupled with high performance liquid chromatography for the determination of furfurals and patulin in fruit juices. *Talanta*, *120*, 47–54.
- Altunay, N., Elik, A., & Gürkan, R. (2019). A novel, green and safe ultrasound-assisted emulsification liquid phase microextraction based on alcohol-based deep eutectic solvent for determination of patulin in fruit juices by spectrophotometry. *Journal of Food Composition and Analysis*, *82*, Article 103256.
- Altunay, N., Hazer, B., Tuzen, M., & Elik, A. (2021). A new analytical approach for preconcentration, separation and determination of Pb(II) and Cd(II) in real samples using a new adsorbent: Synthesis, characterization and application. *Food Chemistry*, *359*, Article 129923.
- Anene, A., Hosni, K., Chevalier, Y., Kalfat, R., & Hbaieb, S. (2016). Molecularly imprinted polymer for extraction of patulin in apple juice samples. *Food Control*, *70*, 90–95.
- Beltrán, E., Ibáñez, M., Sancho, J. V., & Hernandez, F. (2014). Determination of patulin in apple and derived products by UHPLC-MS/MS. Study of matrix effects with atmospheric pressure ionisation sources. *Food Chemistry*, *142*, 400–407.
- Boyer, C., Bulmus, V., Liu, J., Davis, T. P., Stenzel, M. H., & Barner-Kowollik, C. (2007). Well-defined protein-polymer conjugates via in situ RAFT polymerization. *J. Am. Chem. Soc.*, *129*, 7145–7154.
- Cac. (2003). *Code of practice for the prevention and reduction of patulin contamination in apple juice and apple juice ingredients in other beverages* (pp. 1–6). Geneva: Food and Agriculture Organization.
- Duran, C., Ozdes, D., Gundogdu, A., Imamoglu, M., & Senturk, H. B. (2011). Tea-industry waste activated carbon, as a novel adsorbent, for separation, preconcentration and speciation of chromium. *Analytica Chimica Acta*, *688*(1), 75–83.
- Easterling, C. P., Xia, Y., Zhao, J., Fanucci, G. E., & Sumerlin, B. S. (2019). Block copolymer sequence inversion through photoiniferter polymerization. *ACS Macro Letters*, *8*, 1461–1466.
- EC. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff. Official Journal of the European Communities, *L364*, 5–24.
- Fang, G., Wang, H., Yang, Y., Liu, G., & Wang, S. (2016). Development and application of a quartz crystal microbalance sensor based on molecularly imprinted sol-gel polymer for rapid detection of patulin in foods. *Sensors and Actuators B: Chemical*, *237*, 239–246.
- Fliege, R., & Metzler, M. (2000). Electrophilic properties of patulin. adduct structures and reaction pathways with 4-bromothiophenol and other model nucleophiles. *Chemical Research in Toxicology*, *13*, 363–372.
- Fuchs, S., Sonntag, G., Stidl, R., Ehrlich, V., Kundi, M., & Knasmüller, S. (2008). Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. *Food and Chemical Toxicology*, *46*, 1398–1407.
- JECFA. (1995). Evaluation of certain food additives and contaminants: forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series No. 859, 9241208597, Geneva.
- Hazer, B., Eren, M., Senemoğlu, Y., Modjinou, T., Renard, E., & Langlois, V. (2020). Novel poly(3-hydroxy butyrate) macro raft agent. Synthesis and characterization of thermoresponsive block copolymers. *Journal of Polymer Research*, *27*, 147. <https://doi.org/10.1007/s10965-020-02133-1>
- Ji, X., Li, R., Yang, H., Qi, P., Xiao, Y., & Qian, M. (2017). Occurrence of patulin in various fruit products and dietary exposure assessment for consumers in China. *Food Control*, *78*, 100–107.
- Kataoka, H., Itano, M., Ishizaki, A., & Saito, K. (2009). Determination of patulin in fruit juice and dried fruit samples by in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry. *Journal of Chromatography A*, *1216*, 3746–3750.
- Kharandi, N., Babri, M., & Azad, J. (2013). A novel method for determination of patulin in apple juices by GC-MS. *Food Chemistry*, *141*, 1619–1623.
- Li, X., Li, H., Li, X., & Zhang, Q. (2017). Determination of trace patulin in apple-based food matrices. *Food Chemistry*, *233*, 290–301.
- Li, X., Li, H., Ma, W., Guo, Z., Li, X., Li, X., & Zhang, Q. (2018). Determination of patulin in apple juice by single-drop liquid-liquid-liquid microextraction coupled with liquid chromatography-mass spectrometry. *Food Chemistry*, *257*, 1–6.
- Li, X., Ma, W., Zhang, Q., Li, H., & Liu, H. (2021). Determination of patulin in apple juice by amine-functionalized solid-phase extraction coupled with isotope dilution liquid chromatography tandem mass spectrometry. *Journal of the Science of Food and Agriculture*, *101*(5), 1767–1771.
- Marín, S., Mateo, E. M., Sanchis, V., Valle-Algarra, F. M., Ramos, A. J., & Jiménez, M. (2011). Patulin contamination in fruit derivatives, including baby food, from the Spanish market. *Food Chemistry*, *124*, 563–568.
- Mohammadi, A., Tavakoli, R., Kamankesh, M., Rashedi, H., Attaran, A., & Delavar, M. (2013). Enzyme-assisted extraction and ionic liquid-based dispersive liquid-liquid microextraction followed by high-performance liquid chromatography for determination of patulin in apple juice and method optimization using central composite design. *Analytica Chimica Acta*, *804*, 104–110.
- Moreno-González, D., Jác, P., Riasová, P., & Nováková, L. (2021). In-line molecularly imprinted polymer solid phase extraction-capillary electrophoresis coupled with tandem mass spectrometry for the determination of patulin in apple-based food. *Food Chemistry*, *334*, Article 127607.

- Morgavi, D. P., Boudra, H., Jouany, J. P., & Graviou, D. (2003). Prevention of Patulin Toxicity on Rumen Microbial Fermentation by SH-Containing Reducing Agents. *Journal of Agricultural and Food Chemistry*, *51*, 6906–6910.
- Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret FTIR spectroscopy of organic material. *Journal of Science and Technology*, *4*(1), 97–118.
- Ozturk, T., Atalar, M. N., Goktas, M., & Hazer, B. (2013). One-step synthesis of block-graft copolymers via simultaneous reversible-addition fragmentation chain transfer and ring-opening polymerization using a novel macroinitiator. *Journal of Polymer Science Part A: Polymer Chemistry*, *51*, 2651–2659.
- Sadok, I., Szmagara, A., & Staniszewska, M. M. (2018). The validated and sensitive HPLC-DAD method for determination of patulin in strawberries. *Food Chemistry*, *245*, 364–370.
- Şanal, T., Oruç, O., Öztürk, T., & Hazer, H. (2015). Synthesis of pH- and thermo-responsive poly(ϵ -caprolactone-*b*-4-vinyl benzyl-*g*-dimethyl amino ethyl methacrylate) brush graft copolymers via RAFT polymerization. *Journal of Polymer Research*, *22*(3), 1–12.
- Taşpınar, H., Elik, A., Kaya, S., & Altunay, N. (2021). Optimization of green and rapid analytical procedure for the extraction of patulin in fruit juice and dried fruit samples by air-assisted natural deep eutectic solvent-based solidified homogeneous liquid phase microextraction using experimental design and computational chemistry approach. *Food Chemistry*, *358*, Article 129817.
- Víctor-Ortega, M. D., Lara, F. J., García-Campana, A. M., & del Olmo-Iruela, M. (2013). Evaluation of dispersive liquid-liquid microextraction for the determination of patulin in apple juices using micellar electrokinetic capillary chromatography. *Food Control*, *31*, 353–358.
- Wang, Y., Wen, Y., & Ling, Y. C. (2017). Graphene oxide-based magnetic solid phase extraction combined with high performance liquid chromatography for determination of patulin in apple juice. *Food Analytical Methods*, *10*(1), 210–218.
- Wright, S. A. I. (2015). Patulin in food. *Current Opinion in Food Science*, *5*, 105–109.
- Wu, R. N., Dang, Y. L., Niu, L., & Hu, H. (2008). Application of matrix solid-phase dispersion-HPLC method to determine patulin in apple and apple juice concentrate. *Journal of Food Composition and Analysis*, *21*(7), 582–586.
- Yıldız, U., Hazer, B., & Tauer, K. (2012). Tailoring polymer architectures with macromonomer azoinitiators. *Polymer Chemistry*, *3*, 1107–1118.
- Yu, Y., & Fan, Z. (2017). Determination of patulin in apple juice using magnetic solid phase extraction coupled with high-performance liquid chromatography. *Food Additives & Contaminants: Part A*, *34*(2), 273–281.
- Zhao, M., Shao, H., He, Y., Li, H., Yan, M., Jiang, Z., ... She, Y. (2019). The determination of patulin from food samples using dual-dummy molecularly imprinted solid-phase extraction coupled with LC-MS/MS. *Journal of Chromatography B*, *1125*, Article 121714.
- Zounr, R. A., Tuzen, M., Hazer, B., & Khuhawar, M. Y. (2018). Solid-phase microextraction and determination of tin species in beverages and food samples by using poly(ϵ -caprolactone-*b*-4-vinyl benzyl-*g*-dimethyl amino ethyl methacrylate) polymer in syringe system: A Multivariate study. *Food Analytical Methods*, *11*, 2538–2546.