Journal of Chromatography B 1073 (2018) 90–95

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Comparison of salting-out and sugaring-out liquid–liquid extraction methods for the partition of 10-hydroxy-2-decenoic acid in royal jelly and their co-extracted protein content

Xijuan Tu[a,b], Fanyi Sun[a], Siyuan Wu[a], Weiyi Liu[a], Zhaosheng Gao[a], Shaokang Huang[a,b], Wenbin Chen[a,b,*]

[a] College of Bee Science, Fujian Agriculture and Forestry University, Fuzhou, China
[b] MOE Engineering Research Center of Bee Products Processing and Application, Fujian Agriculture and Forestry University, Fuzhou, China

ARTICLE INFO

Keywords:
Sample preparation
Homogeneous liquid–liquid extraction
Salting-out
Sugaring-out
Co-extracted protein
Fatty acid

ABSTRACT

Homogeneous liquid–liquid extraction (h-LLE) has been receiving considerable attention as a sample preparation method due to its simple and fast partition of compounds with a wide range of polarities. To better understand the differences between the two h-LLE extraction approaches, salting-out assisted liquid–liquid extraction (SALLE) and sugaring-out assisted liquid–liquid extraction (SULLE), have been compared for the partition of 10-hydroxy-2-decenoic acid (10-HDA) from royal jelly, and for the co-extraction of proteins. Effects of the amount of phase partition agents and the concentration of acetonitrile (ACN) on the h-LLE were discussed. Results showed that partition efficiency of 10-HDA depends on the phase ratio in both SALLE and SULLE. Though the partition triggered by NaCl and glucose is less efficient than $MgSO_4$ in the 50% (v/v) ACN-water mixture, their extraction yields can be improved to be similar with that in $MgSO_4$ SALLE by increasing the initial concentration of ACN in the ACN-water mixture. The content of co-extracted protein was correlated with water concentration in the obtained upper phase. $MgSO_4$ showed the largest protein co-extraction at the low concentration of salt. Glucose exhibited a large protein co-extraction in the high phase ratio condition. Furthermore, NaCl with high initial ACN concentration is recommended because it produced high extraction yield for 10-HDA and the lowest amount of co-extracted protein. These observations would be valuable for the sample preparation of royal jelly.

1. Introduction

Extracting target compounds from matrices is the key procedure for the qualitative and quantitative analysis [1]. Conventional liquid–liquid extraction (LLE), which transfers target compounds from one liquid solution into another immiscible liquid phase, is the classical extraction technique in sample preparation. LLE is simple and has been commonly used. However, it has limitations because it is unable to extract a wide polarity range of compounds. Extracting solvent is usually hydrophobic in conventional LLE, which is immiscible in water, thus the hydrophilic compounds in aqueous sample are difficult to be extracted.

In recent years, homogenous liquid–liquid extraction (h-LLE) technique has been developed as an alternative method to the conventional LLE [2]. In h-LLE, water-miscible solvents are used to perform the extraction followed by adding a phase partition agent to induce a second immiscible phase. By using water-miscible solvents such as isopropyl alcohol, acetonitrile (ACN) and acetone, the extracted targets can be extended to more hydrophilic compounds which are not efficiently recovered by conventional LLE.

For example, salt induced phase partition in h-LLE, also called salting-out assisted liquid–liquid extraction (SALLE), has been widely used for the extraction of compounds with a wide polarity range [3–5]. In a typical SALLE procedure, samples are dispersed in the mixture of water and water-miscible solvent. After adding solid salt or salt solution and a gentle mixing step, the mixture is centrifuged to form a stable separated phase above the aqueous matrix. Since the separated phase is compatible with ensuing HPLC separation, it can be directly injected into chromatography system. The extract solution can also be combined with further clean-up to reduce interferences. For instance, SALLE combined with dispersive solid phase extraction has been developed as a popular method for multi-residue analysis in various matrices [6,7].

Abbreviations: LLE, liquid liquid extraction; h-LLE, homogenous liquid–liquid extraction; SALLE, salting-out assisted liquid–liquid extraction; SULLE, sugaring-out assisted liquid–liquid extraction; 10-HDA, 10-hydroxy-2-decenoic acid; ACN, acetonitrile

* Corresponding author at: College of Bee Science, Fujian Agriculture and Forestry University, Fuzhou, China.
E-mail address: wbchen@fafu.edu.cn (W. Chen).

https://doi.org/10.1016/j.jchromb.2017.12.020
Received 8 June 2017; Received in revised form 3 December 2017; Accepted 10 December 2017
Available online 12 December 2017
1570-0232/ © 2017 Elsevier B.V. All rights reserved.

Recently, micro-extraction techniques based on SALLE have been reported for analyzing biological samples [8,9].

Beside salts, sugars have been reported to be used as another phase partition agent to construct the sugaring-out assisted liquid–liquid extraction (SULLE) method [10]. Compared with SALLE, SULLE shows advantages of rapid phase separation and a more friendly environment to the biomolecules. Recently, SULLE has been applied for the recovery of protein [11], partition of vanillin [12] and metal ions [13], and for the analysis of drugs in honey [14] or human plasma [15].

Royal jelly is a viscous substance secreted by the hypopharyngeal glands and mandibular glands of young worker bees to feed the larvae and queen honeybee. Royal jelly is the dietary cause of the caste differentiation in honeybees [16]. Queen larvae are fed with royal jelly throughout its larval development, while worker larvae are fed with worker jelly for the first three days. This diet difference induces queen honeybees which are distinct from worker honeybees in morphology, behavior and physiology [17]. The nutrition value and the biological activities of royal jelly have been intensively studied, enabling it to be widely used in functional foods and cosmetics products [18]. Royal jelly contains high protein content which constitutes nearly 27–41% of the dry matter. Another important component is lipids (8–19% of dry matter) [18]. In particular, 10-hydroxy-2-decenoic acid (10-HDA), a unique fatty acid specifically found in royal jelly, forms nearly 32% of the fatty acid fraction [18]. 10-HDA has been reported to have multiple biological functions such as enhancing insulin sensitivity [19], immunomodulatory effect [20,21], and antidepressant activity [22].

Despite of the fact that SALLE and SULLE have been attracting growing interests in bioanalysis, little is known about the distribution of fatty acid and protein in these sample preparation methods. In addition, though HPLC methods have been developed for the determination of 10-HDA [23–29], there are few reports regarding the application of h-LLE in royal jelly. To better understand the extraction differences in h-LLE methods and explore the possible applications of h-LLE in royal jelly matrix, the partition of 10-HDA from royal jelly by using SALLE and SULLE was investigated, and the protein residue in these two h-LLE methods were evaluated.

2. Materials and methods

2.1. Materials

ACN and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany), 10-HDA standard (99.2%) was purchased from National Institutes for Food and Drug Control (Beijing, China), other reagents including anhydrous magnesium sulfate, sodium chloride, glucose, anhydrous ethanol, hydrochloric acid, Coomassie brilliant blue G250, and bovine serum albumin (BSA) were all of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ultrapure water (18.2 MΩ) was produced by Wortel WP-UPLH20 ultrapure water system (Chengdu, China). Royal jelly was produced from *Apis mellifera* colonies at College of Bee Science, Fujian Agriculture and Forestry University (Fuzhou, China).

2.2. h-LLE

Royal jelly sample (0.25 g) was mixed with 2 mL ACN aqueous solution at the ratio of 40% to 70% (v/v) with a 10% (v/v) increment. After vortexing for 1 min, different phase partition agents (NaCl, $MgSO_4$, and glucose) were added into the mixture. After another 30 s vortexing, the mixed solution was centrifuged at 5000 rpm for 5 min to make a clear phase separation. The upper phase was collected and then diluted to 25 mL with anhydrous ethanol. This extract solution was analyzed by HPLC. The phase ratio was defined as $r = V_{upper}/V_{lower}$, where V_{upper} and V_{lower} was the volume of upper phase and lower phase, respectively. All experiments were triplicates.

2.3. Analytical methods

10-HDA content was determined based on the reversed phase HPLC method [23,24]. The HPLC system (Shimadzu) was composed of LC-20AT pumps, SIL-20AC autosampler, CTO-10AS column oven, and SPD-20A UV detector. An InertSustain (Shimadzu) C18 column (5 μm, 4.6 × 150 mm) was used for the separation. The mobile phase consists of 55% methanol and 45% water, and the pH was adjusted to 2.5 with hydrochloric acid.[1] Flow rate was 1 mL/min, the column temperature was 35 °C, and the detection wavelength was set at 210 nm. The concentration of 10-HDA was calculated by using the external calibration, which was prepared by plotting the peak area of HDA versus the corresponding concentration. The partition efficiency was compared by 10-HDA extraction yields (EYs, %) = (amount of 10-HDA in the upper phase/amount of 10-HDA in the royal jelly sample) × 100.

Bradford assay [30] was used to compare the co-extracted protein content in the final extract solution. The upper phase was collected and evaporated to dryness under nitrogen. The residue was reconstituted by 1 mL H_2O, then transferred and diluted to 10 mL by H_2O for the protein assay. Absorbance intensity at 595 nm was recorded by a UV–vis spectrophotometer (YOUKE-UV759CRT, Shanghai, China), and BSA was used to construct the standard curve for the quantification of protein content.

3. Results and discussion

3.1. Partition of 10-HDA

ACN, ethanol, isopropyl alcohol and acetone have been used as solvents in SALLE. ACN is the most preferred solvent because of its smaller volume to produce phase separation, better partition of both hydrophilic and hydrophobic compounds, and the compatibility with HPLC instruments. In SULLE, ACN also is the most widely used organic solvent to perform the extraction. Therefore, ACN was used in this study to investigate the h-LLE methods in royal jelly sample.

NaCl and $MgSO_4$ are the two typical salts for SALLE [31], and effects of NaCl and $MgSO_4$ amounts on the partition of 10-HDA in ACN aqueous solution (50%, v/v) are shown in Fig. 1a and b, respectively. The EYs of 10-HDA were increased from 62.6% to 69.3% with the addition of NaCl from 0.1 to 0.4 g. The partition triggered by $MgSO_4$ showed higher EYs than NaCl, and the trend under the increase of salt concentration was different with NaCl. Specifically, EYs reached the maximum value of 93.2% with the addition of 0.2 g $MgSO_4$, and then decreased to 82.1% with salt amount up to 0.4 g.

Glucose has been demonstrated to be the better phase partition agent than other sugars in SULLE [10], and effects of added glucose amount on the partition of 10-HDA were studied in the range of 0.2–0.6 g. It is valuable to note that glucose is a component in RJ with concentration of 4–8% (w/w) [18]. The sample amount of royal jelly in this work was 0.25 g, thus the content of intrinsic glucose in the extraction solution was about 10–20 mg. Though this content is much lower than the concentration required to trigger phase separation, the data presented in the figures were plotted against the added amount of glucose with consideration of this factor. As shown in Fig. 1c, EY was linearly increased from 32.6% to 59.3% as the amount of sugar increased from 0.2 g to 0.4 g, and reached the plateau over 0.4 g.

These results indicated that the partition induced by $MgSO_4$ was more efficient than NaCl and glucose. The EYs of 32.6% and 66.0% were obtained with 0.2 g glucose and 0.2 g NaCl, respectively, while much higher EY of 93.2% could be found in $MgSO_4$ under the same amount of salt. In these experiments, the volume of the upper phase induced by $MgSO_4$ was observed to be larger than both NaCl and

[1] Use of phosphoric acid is recommended to sustain the chromatographic column life time.

Download English Version:

https://daneshyari.com/en/article/7615504

Download Persian Version:

https://daneshyari.com/article/7615504

Daneshyari.com