Dewatering and Extraction of Hydrophilic Solutes and Essential Oils from Cryo-preserved Lemon Peels Using Liquefied Dimethyl Ether

Ayano NAKAMURA1, Yuki HARA1 and Tomonori KAWANO1-3*

1 Faculty and Graduate School of Environmental Engineering, The University of Kitakyushu, Kitakyushu, Japan; 2 International Photosynthesis Industrialization Research Center, The University of Kitakyushu, Kitakyushu, Japan; 3 University of Florence-LINV Kitakyushu Research Center (LINV@Kitakyushu), Kitakyushu, Japan

(Received July 21, 2016; Accepted October 10, 2016)

In general, dewatering of plant tissues (such as vegetables) and food materials is achieved by heating. In order to prevent the degradation of biologically active components in plant materials, the dewatering process should be carried out at low temperature. Therefore, in the present study, we attempted to develop a simple protocol for dewatering cryo-preserved plant tissues using liquefied dimethyl ether (DME). Prior to dewatering from frozen plant materials, we have examined the efficiency of liquefied DME for cryogenic removal of water from ice cubes. Here, lemon peel residue (consisting of flavedo and albedo) was chosen as the model plant material for dewatering and concomitant extractions of water-soluble components such as ascorbate and citric acid and hydrophobic components, chiefly, essential oils (EOs). By focusing on the exploitation of unused resources after food processing, the juice extraction residues from lemon fruits (lemon peels) were used as the starting materials. The yield of vitamin C (VC) extracted from the peel tissues derived from a single lemon fruit exceeded the amount of VC found in the manually press-extracted juice from a single lemon fruit. The major components in DME-extracted crude lemon EOs were determined and quantified with gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) to be limonene (40.4%, w/w), β-pinene (10.4%, w/w), and γ-terpinene (6.9 %, w/w).

1. Introduction

Dimethyl ether (DME), which is the simplest ether with the formula CH₃OCH₃, is well-known as a useful precursor to other organic chemicals such as liquefied petroleum gas (LPG) [1, 2] and low molecular weight hydrocarbons [3] through catalytic systems [4-6]. Today, DME is also known as an alternative fuel that can replace conventional diesel fuels [7]. In addition to the diesel engine, many types of gas turbines and petrol engines can be fuelled with DME through high-efficiency combustion with reduced emissions of NOₓ, SOₓ, and particulate matter [8]. Despite most of industrial fuels having serious issues with toxicity, production, infrastructure, or transportation, DME does not have any such problems [8].

Apart from its use as a fuel or a precursor to other organic chemicals, the liquefied form or subcritical form of DME have attracted the attentions of engineers since these forms of DME behave as solvents. Accordingly, subcritical DME acts as an effective solvent for the extraction of flavouring, pungent and medicinal agents derived from ginger, black pepper, and chili powder [9]. In addition, some organic
molecules from algal green mass can be extracted by pressure-dependent liquefied DME [10,11].

Interestingly, the use of liquefied DME for some industrial dewatering processes has been attempted as DME is highly miscible with water. Firstly, the use of liquefied DME for removal of water from sub-bituminous coal without any heating process was reported by Kanda et. al. [10, 11]. The reported efficiency for water extraction by liquefied DME was maximally 98.3%. The same group also reported that the dewatering property and capacity of DME can be applied for dry matter preparation from highly-wet (91.0% of water content) natural blue-green microalgae [10].

With liquefied DME, water from wet materials can be readily collected as the liquid mixture formed a separate phase under the oily phase. As discussed in our earlier work [12], the low boiling point (-23°C) for DME could be highly beneficial in order to facilitate the removal of the solvent (DME) from the extracted water-containing samples. We have previously demonstrated that, with liquefied DME, extraction of water-soluble proteins from water-containing plant materials such as vegetable tissues can be achieved [12]. The extraction efficiency of water-soluble proteins largely depends on the dewatering action of DME.

To obtain liquefied DME, two distinct approaches are available, namely, pressure-based liquefaction under relatively high pressure (> ca. 0.5 MPa) at ambient temperature and cryogenic liquefaction (cryo-liquefaction) at low temperature (< -23°C) at ambient pressure (0.1 MPa). We used a semi-cryogenic protocol applying moderate pressure up to 0.4 MPa while allowing liquefaction of DME in an ice-chilled metal cylinder (Figure 1).

Notably, cryo-liquefied DME can be used as a low-temperature extraction solvent for a variety of laboratory procedures for extraction of neutral fats from food wastes such as rice brans [13] and pigments such as chlorophylls and carotenoids from plant tissues [14].

In general, dewatering of plant tissues (such as vegetables) and food materials are usually achieved by the application of heat [15]. However, in order to prevent the degradation of biologically active components in plant materials, the dewatering process should be carried out at low temperature as discussed elsewhere [12]. Therefore, in the present study, we attempted to develop a simple protocol for effectively achieving dewatering and extractions of essential oils (EOs), citric acid (CA), and ascorbate (vitamin C, VC) at the same time, from frozen lemon peel tissues, used as model plant tissues, by incubating the frozen tissues in the liquefied DME under cryogenic conditions (at -30°C).

EOs can be basically obtained from specific plant organs/tissues such as flower petals and fruit peels in specific plant species such as roses and citruses. Bulky plant tissues are often required for extraction of, even a single drop of, an EO. Use of an organic solvent such as hexane is one of the commonly employed protocols for extraction of EOs. In this case, heat- and vacuum-enhanced separation of EOs from the recovered solvent-oil mixture is required. However, some EOs are highly sensitive to heat and/or one highly volatile. Thus it is appropriate to complete all extraction procedures under controlled low-temperature conditions. In the last decade, the use of DME as a novel organic solvent substitute, which can be liquefied from its gaseous form under low temperature or high pressure has been proposed. Since the liquefied DME behaves similarly to most organic solvents, we expected that it could be used for the extraction of EOs from plant tissues under freezing temperatures.

Citrus fruits form a group of major pomological products cultivated in Japan [16]. Often than, direct
consumption, citrus fruits are used primarily in juice production [17]. As a result, a huge amount of citrus residues chiefly peels are discarded although citrus peels still contain useful components such as EOs and VC [18]. Accordingly, disposal of citrus peels requires the removal of a large amount of water contained in the peels [19]. Generally, EOs in citrus peels can be obtained by the cold-press or steam distillation method. However, there are problems in both procedures. The yield of EOs in the cold-press method is low because many EOs remain in the citrus peels [20]. From the steam distillation method, the components of EOs are readily modified by heat because the method is often carried out at high temperature between 130 and 150°C. On the other hand, during the extraction of oils using liquefied DME, the solvent can be evaporated without heating [10, 11]. Therefore, the use of liquefied DME is one of the most attractive approaches for extraction of EOs.

As previously demonstrated [12], liquefied DME can be used as a dewatering solvent actively removing water from plant tissues, therefore, in the present study, we further demonstrated that dewatering and extraction of industrially important components can be achieved in one step under cryogenic conditions.

2. Materials and methods

Handling of DME was basically achieved according to Hara et al. [13]. With some modifications, a batch-type extraction system was employed. The system consisted of the semi-cryogenic DME liquefaction unit allowing liquefaction of DME in an ice-chilled metal cylinder under partially elevated pressure (0.4 MPa; Figure 1) and the dewatering and extraction unit kept under cryogenic conditions in a freezer (Figure 2). The semi-cryogenic DME liquefaction unit consisted of a DME gas cylinder obtained from a local gas vendor.
(Air-Gases Kitakyushu Inc.) and cooling units (liquid collection cylinders incubated with ice). Supply of gaseous DME was made through aluminum capillaries. Then the resultant liquefied DME was supplied to the pressure-proof chambers made of PFA resin (Tetrafluoroethylene-perfluoroalkyl vinyl ether copolymer) (see Figure 4B) used as the dewatering/extraction units kept at -30°C (Figure 2). In order to enhance the dewatering and extraction processes, magnetic stirring bars were used for continuous mixing of the cold solvent and the frozen samples inside the dewatering/extraction chambers.

Lemon fruits were purchased at a local market in Kitakyushu city. Here, lemon fruits and their peels were used as model plant materials. An anatomical cross-section of a lemon fruit is shown in Figure 3. The tissues (pulp) rich in lemon juice sacs (rich in juice) are surrounded by the peel layer. The lemon peel layer can be clearly dissected into flavedo (or exocarp) rich in oil glands and spongy tissues known as albedo (or mesocarp). EOs are specifically located in the oil glands but not in any other portion of the fruits while aqueous solvents such as VC and CA are widespread in the fruits.

Extraction of EOs from the lemon peels, especially from flavedo (yellow colored outer peels) was carried out using liquefied DME. 20 g fresh weight (fw) of manually sliced flavedo tissues were frozen in liquid nitrogen and sealed inside the pressure-resistant extraction chamber kept at -30°C, with ca. 50 g of liquefied DME, for 2 to 24 hrs. Since at least 2 hrs were required to liquefy DME for a single extraction, a 2 hr-interval was set as the minimal length between solvent renewals.

Analysis of EOs using gas chromatography-mass spectrometry (GC-MS) and a gas chromatography-flame ionization detector (GC-FID) was outsourced to the analytical laboratory at Mie Prefecture Environmental Conservation Agency.

Assuming that the residues of lemon tissue after juice extraction are typical examples of potential unused waste resources from the food processing industry, the condition after juice extraction was reproduced by subjecting the cut fruits to a manual press-extraction procedure prior to DME-dependent extraction of VC and CA (two typical industrially important water-soluble components from lemons). Then, the resultant lemon residues were manually sliced and immediately frozen in liquid nitrogen and further used for cryogenic dewatering and extraction with liquefied DME as illustrated in Figure 2. In this way, extraction and separation of an oily phase containing EOs and a water phase containing solutes (such as minerals, vitamins, soluble proteins, etc.) could be achieved.

Determinations of VC and CA were performed by using an analytical VC determination kit provided by COSMO BIO (Tokyo, Japan) and an Acidity Meter (for fruit citric acid, FS-101N, Atago Co. Ltd., Tokyo, Japan), respectively.
3. Results and Discussion

3.1 Preliminary cryogenic dewatering from ice cubes

Prior to dewatering from frozen lemon tissues, we have examined the efficiency of the liquefied DME for removal of water in the frozen state, namely, from ice cubes. To demonstrate the action of liquefied DME for dewatering frozen samples, extraction of water from ice cubes was performed. Extraction of water from the model ice cubes was successfully carried out under cryo-preservative conditions at -30°C (Figure 4).

By allowing enhanced contact with ice cubes and solvent DME by stirring, the efficiency for removal of water from the ice cubes was largely enhanced (Figure 4C). In the end, by addition of 15-20 g of liquefied DME to 1 g of ice, water from the ice could be completely removed under cryogenic stirring conditions. This quantification helps us estimate the amount of liquefied DME required for removal of water from cryo-preserved samples.

3.2 DME-based dewatering and extraction of EOs from frozen lemon tissues

From the lemon peels, slices of flavedo tissues were prepared for both dewatering and extraction of hydrophobic components, In particular, EOs stored in the oil glands in the flavedo. By incubating the frozen

Figure 4. Extraction of water from model ice cubes at -30°C. (A) Ice cubes used. (B) A pressure-proof PFA chamber. (C) Effect of stirring on the enhancement of water removal from the ice cubes.

Figure 5. Extraction of EOs from lemon peels (flavedo) at -30°C. Excised lemon peels prior to extraction (A). Lemon peels after single (B), double (C) and quintuple (D) extractions show gradual loss of pigment colors. (E) Layer of EOs (oily phase) formed above the aqueous layer after extraction with cryo-liquefied DME.
lemon flavedo slices in liquefied DME at -30°C, removal of water by repeatedly adding liquefied DME was performed under static condition or with stirring. Here, the efficiency of the two distinct extraction procedures, namely, the short-term extraction exercise where solvent DME replacement was performed at 2 hrs intervals.
and the long-term extraction exercise where solvent renewal was performed at daily intervals, were compared. As shown in Figure 5 (A-D), excised lemon peel flavedo subjected to single, double and quintuple extractions with daily renewed liquefied DME showed a gradual loss of pigment colours reflecting the behaviour of carotenoids. After recovery of the extraction mixture and passive evaporation of DME, a hydrophobic phase rich in EOs tended to form a layer over the water phase (Figure 5E).

DME-based dewatering and EO extraction with and without stirring during short-term and long-term exercises were compared (Figure 6A-C). Removal of water by repeatedly added liquefied DME was shown to be enhanced by stirring of the cold solvent and frozen tissue during the long-term extraction exercise (Figure 6A). The ratio of solvent required for cryogenic dewatering from frozen peel flavedo tissues was shown to be within the range between 20 and 25 (over water) as predicted from the preliminary experiments with model ice cubes. A maximum of ca. 0.8 g of EOs was extracted and recovered from 20 gfw of frozen peel flavedo tissues (Figure 6C), suggesting that the extraction of EOs from the lemon peels was successfully performed under cryo-preservative conditions at -30°C.

Notably, extraction and recovery of hydrophobic and volatile components, chiefly, EOs such as limonene were effectively achieved. We performed the extraction of EOs from flavedo (yellow colored outer peels) of lemon using liquefied DME. The flavedo slices (20 gfw) were sealed inside the pressure-resistant extraction chamber kept at -30°C, with ca. 50 g of liquefied DME, for 2 to 24 hrs. Analysis of EO components using gas chromatography-mass spectrometry (GC-MS) and a gas chromatography-flame ionization detector (GC-FID) revealed that the major components in DME-extracted crude EOs from lemon peel were limonene (40.4%, w/w), β-pinene (10.4%, w/w), and γ-terpinene (6.9 %, w/w) as shown in Figure 6 (D-F).

It has been shown that ordinary lemon fruits available in the European market show 6-17 ml of extractable EOs per kgfw of peel (both flavedo and albedo) [21]. Our demonstration showed that the yields of total EOs greatly exceeds that level suggesting that our protocol increased the extractability of EOs from lemon peels.

Figure 7. Extraction of water soluble components from lemon peel residues at -30°C. (A) Lemon residues after manual juice extraction. These materials were used for DME-based dewatering and extraction of solutes. (B) Comparisons per fruit of DME extracted VC from lemon peel residues and VC found in manually extracted juice. (C) Comparison per fruit of DME extracted CA from lemon peel residues and CA found in the juice.
3.3 DME-based extraction of VC and CA from lemon peel residues after manual extraction of juice

In addition to the simple dewatering exercise and extraction of hydrophobic components such as EOs, we attempted to extract water-soluble components from lemon fruits. We have chosen the residues of lemon fruits left after manual juice extraction as a model plant/food material for performing dewatering at frozen temperatures accompanying the water-soluble components. The water-soluble components examined here include VC and CA (Figure 7). As shown here, VC and CA could be successfully extracted and recovered through the dewatering action of liquefied DME.

By manually compressing a single fruit (ca. 130 g), juice containing ca. 4 mg of VC and 1 g of CA was extracted (Figure 7). The concentrations of VC and CA in the juice were 43.0 mg and 10.75 g per 100 g juice, respectively. Since the VC concentration in fresh lemon juice reportedly ranges from 2.4 to 43 mg/100 g juice depending on the duration and conditions of post-harvest fruit preservation [22], it is likely that the fruit samples used here were in a relatively favorable condition for preventing the post-harvest loss of VC.

Note that upon addition of 200 g or more of liquefied DME, the yield of VC extracted from the frozen peel tissues (lemon juice extraction residues containing both flavedo and albedo) derived from a single lemon fruit exceeded the amount of VC found in the juice from a single lemon fruit, thus, the total yield of VC from a single fruit could be more than double compared to the case where simple juice extraction only was performed.

4. Conclusion

In conclusion, extraction of EOs from cryo-preserved lemon peels accompanying the dewatering process using liquefied DME was successfully carried out. Simultaneously, extractions of VC and CA from frozen samples were effectively achieved under cryogenic condition.

Acknowledgement

This work was supported by a grant of Regional Innovation Strategy Support Program implemented by Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan; and also by a grant from Tonen General Sekiyu Research/Development Encouragement & Scholarship Foundation.

References