Food Chemistry 216 (2017) 27–36

Contents lists available at ScienceDirect

Food Chemistry

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

Enhanced extraction of oleoresin from ginger (*Zingiber officinale*) rhizome powder using enzyme-assisted three phase partitioning

Sadineni Varakumar *, Kannamangalam Vijayan Umesh, Rekha S. Singhal

Food Engineering and Technology Department, Institute of Chemical Technology, Matunga, Mumbai 400 019, India

ARTICLE INFO

Article history:
Received 3 April 2016
Received in revised form 7 July 2016
Accepted 29 July 2016
Available online 30 July 2016

Keywords:
Zingiber officinale Roscoe
Ginger oleoresin
Three phase partitioning
Enzyme assisted three phase partitioning
Ultrasound assisted three phase partitioning

ABSTRACT

Ginger (*Zingiber officinale* R.) is a popular spice used worldwide. The oleoresin consists of gingerols, shogaols and other non-volatiles as chief bioactive constituents. Three phase partitioning (TPP), a bioseparation technique, based on partitioning of polar constituents, proteins, and hydrophobic constituents in three phases comprising of water, ammonium sulphate and *t*-butanol, was explored for extraction of oleoresin and gingerols from dry powder. Parameters optimized for maximum recovery of gingerols and [6]-shogaol were ammonium sulphate concentration, ratio of *t*-butanol to slurry, solid loading and pH. Ultrasound and enzymatic pretreatments increased the yield of oleoresin and its phytoconstituents. Ultrasound pretreatment showed separation of starch in the bottom aqueous phase but is an additional step in extraction. Enzymatic pretreatment using accellerase increased the yield of [6]-, [8]-, [10]-gingerols and [6]-shogaol by 64.10, 87.8, 62.78 and 32.0% within 4 h and is recommended. The efficacy of the enzymatic pretreatment was confirmed by SEM and FTIR.

1. Introduction

Ginger (*Zingiber officinale* Roscoe) is a monocotyledonous plant belonging to the family *Zingiberacea* and is one of the world's best known spices, cultivated in several countries and has been used since antiquity for its health benefits. It is generally consumed as fresh paste, dried powder, slices preserved in syrup, candy (crystallized ginger) for flavouring tea. In many countries, especially in India and China, fresh ginger is used as flavouring agent in beverages and many other food preparations (Shukla & Singh, 2007). However the major use of ground dried ginger is for domestic culinary purposes as flavouring agent, bakery products and desserts (Young, Chiang, Huang, Pan, & Chen, 2002). Ginger consists of two main fractions, the non-volatile resins and the volatile essential oil, both of which reside in the same cell but are extracted in different manner with different solvents (Azian, Kamal, & Azlina, 2004). The main non-volatile pungent constituents of oleoresin are polyphenolic compounds namely, [6]-, [8]-, and [10]-gingerols, as well as [6]-, [8]-, and [10]-shogaols (Schwertner, Rios, & Pascoe, 2006), which are responsible for its various pharmacological effects (Dugasani et al., 2010). Among other potential mechanisms, ginger has antioxidant and anti-inflammatory, analgesic, gastro-protective, antihepatotoxic, antifungal, antiemetic, antischistosomal effects (Said, Arya, Pradhan, Singh, & Rai, 2015), DNA damage and cell migration (Jayakumar & Kanthimathi, 2012).

Fresh ginger contains about 80% moisture which makes it vulnerable to microbial spoilage. Hence it is dehydrated to moisture content 10% or below and ground to a powder, and from which value added products like essential oil and oleoresins are prepared industrially. These products retain the characteristic ginger flavor and pungency. In fresh ginger, the gingerols are identified as the major active components. Shogaols are gingerol analogues with a 4, 5 double bond, resulting from elimination of the 5-hydroxy group in alkyl side chain. The shogaol series of compounds are more pungent than the gingerols (Cheng, Liu, Peng, Qi, & Li, 2011) and are virtually absent in fresh ginger, and is derived from the corresponding gingerols during thermal processing or long-term storage (Zhang, Iwaoka, Huang, Nakamoto, & Wong, 1994). Generally, the dehydration reaction of gingerol to shogaol takes place either because of the acidic environment or as a result of the increase in temperature. Gingerol is reportedly stable in the pH range 1–7 at 37 °C; however, it starts degrading at 60 °C and above in aqueous solutions (Bhattarai, Tran, & Duke, 2001).

Numerous techniques for extraction of ginger oleoresin are reported in the scientific literature. These include the conventional Soxhlet extraction and cold percolation which employ various organic solvents and high temperatures. The major disadvantages of these techniques are longer extraction time (>10 h), enormous requirement of organic solvents and exposure of the extract to a

* Corresponding author.
E-mail address: svarakumar@gmail.com (S. Varakumar).

http://dx.doi.org/10.1016/j.foodchem.2016.07.180
0308-8146/

severe risk of degradation or modification of some of the constituents. In the last few decades, there has been an increasing demand for new extraction techniques with concise extraction times with minimum usage of organic solvents to avoid potential environmental pollution and to reduce the cost burden. Recently, extraction methods such as microwave-assisted extraction (Rahath Kubra, Kumar, & Rao, 2013), concurrent application of ultrasound during supercritical extraction (Balachandran, Kentish, Mawson, & Ashokkumar, 2006; Supardan, Fuadi, Alam, & Arpi, 2011) and supercritical fluid extraction (Said et al., 2015) have been reported for ginger constituents. However these techniques require equipment with high investment and maintenance costs. In the light of these aspects, there is a continued effort globally to investigate inexpensive, rapid and greener techniques of extraction.

Three phase partitioning (TPP) is simple, scalable, rapid and relatively recent bioseparation technique involving mixing of *t*-butanol with aqueous antichaotropic salt, ammonium sulphate forming two phases, the aqueous and organic layers at room temperature. It has been used for extraction of wide variety of biomolecules mainly proteins. Generally *t*-butanol is completely miscible with water but on the addition of ammonium sulphate at adequate concentration, it separates into lower aqueous phase and an upper *t*-butanol phase. This upon mixing with either microbial, plant or animal cell homogenates forms three layers, separating the proteinaceous layer at the interphase of the organic and the aqueous layer (Chougle, Singhal, & Baik, 2014). It is believed that ammonium sulphate at high concentration imparts a high dielectric property to water due to which the *t*-butanol layer becomes hydrophobic in nature and it gets excluded from water enabling extraction of lipophilic constituents while some polar molecules get concentrated in the lower aqueous phase (Harde & Singhal, 2012). Although TPP has been extensively evaluated for simultaneous separation and purification of proteins and enzymes from crude suspensions, it has been widely employed for extraction of polar and non-polar constituents as well. Sharma, Khare, and Gupta (2002) employed TPP for extraction of oil from soybean. Later, TPP has been employed for extraction of oil from almond, apricot, and rice bran (Sharma & Gupta, 2004), edible oils (Gaur, Sharma, Khare, & Gupta, 2007), oleoresin from turmeric (Kurmudle, Bankar, Bajaj, Bule, & Singhal, 2011), forskolin (Harde & Singhal, 2012), cocoa butter (Vidhate & Singhal, 2013), astaxanthin (Chougle et al., 2014), trichosanthin from *Trichosanthes kirilowii* roots (Mondal, 2014) and lipids from *Chlorella* (Mulchandani, Kar, & Singhal, 2015). Although TPP has been employed successfully for extraction of wide range of molecules, scale up studies are scarce and more studies are also warranted as a future direction towards its economic feasibility at larger scale operations.

In this context, the present study aimed at evaluating the possible application of TPP as an efficient technique for the extraction of oleoresin from dried ginger rhizome powder. Further, the effect of various pretreatments such as enzyme and ultrasound were explored to further improve the TPP process. These processes were termed as enzyme-assisted three phase portioning (EATPP) and ultrasound-assisted three phase partitioning (UATPP).

2. Materials and methods

2.1. Plant material and reagents

Fresh ginger rhizome roots were procured from local market, Mumbai, India. Rhizomes were cut into small pieces and dried in lab dryer at 40 ± 5 °C overnight, ground in lab mill and passed through 40 mesh sieve to get particle size of 0.420 mm and stored in an air tight container at 4 °C until completion of analysis (moisture content of rhizome powder was 10%). The portion retained on the sieve was ~10% w/w and discarded. Gingerol standards, [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol were procured from Natural Remedies, Bangalore, India. Acetonitrile and methanol HPLC grade were procured from Merck, Mumbai, *t*-butanol and ammonium sulphate (analytical grade) were procured from S. D. Fine Chemicals, Mumbai, India. Stargen® 002 (with activity of 570 GAU/g) and Accellerase® 1500 (endoglucanase activity of 2200–2800 CMC U/g; β-Glucosidase activity of 450–775 pNPG U/g) were gifted by Genencor International, Mumbai, India. Ultrasonication was carried out in Branson Sonifier S 450A, Danbury, USA (maximum power output is 400 W) with a ½″ diameter tapped biohorn (frequency 20 kHz). The height of the probe from the base of the container was kept constant at 1 cm and the samples were protected in an ice bath to avoid overheating.

2.2. Conventional extraction

2.2.1. Soxhlet method

The ginger rhizome powder was extracted in a Soxhlet apparatus using methanol for 8 h above 65 °C. The extract was cooled and then concentrated by evaporating in rotary vacuum evaporator under reduced pressure at 50 °C (IKA RV 10 digital, Germany). The oleoresin was collected in amber coloured bottles and stored in a refrigerator and analysed for gingerols and shogaol. The yield of these constituents was expressed as percent w/w of dry rhizome powder.

2.2.2. Cold percolation method

The ginger rhizome powder was percolated using methanol as solvent for 8 h at room temperature, protecting from bright light. Methanol was filtered using 4-layered cloth and concentrated by evaporating in rotary vacuum evaporator under reduced pressure at 50 °C (IKA RV 10 digital, Germany). The oleoresin was collected in amber coloured bottles and stored in refrigerator and yield of gingerol was expressed as percent w/w of dry rhizome powder.

2.3. Three phase partitioning (TPP)

TPP was optimized by varying the concentration of ammonium sulphate, *t*-butanol to slurry ratio, solid loading in the slurry, and pH of the medium appropriate for maximum extraction of gingerols. Initially for optimization of TPP, 5% (w/v) slurry was prepared by dispersing 1 g ginger rhizome powder in 20 mL distilled water and mixed well. Weighed amount of ammonium sulphate was added to the slurry prepared and vortexed gently, followed by addition of measured amount of *t*-butanol. The extraction was carried out for 1 h by gentle stirring with magnetic stirrer covering the medium with aluminium foil, protecting from the bright light. The mixture was allowed to stand for 1 h in dark for the formation of three phases. The three phases so formed were separated by centrifugation at 5000*g* for 20 min. The upper organic (*t*-butanol) layer was collected and the solvent was evaporated under reduced pressure at 50 °C for 5 min (IKA RV 10 digital, Germany). The extract so obtained was re-dissolved in known volume of methanol and quantified for gingerol content by HPLC. Ammonium sulphate loading was varied from 10 to 50% w/v of slurry while ratio of *t*-butanol to slurry was varied from 0.5:1 to 1.5:1 with all the other extraction conditions being unchanged. To check the effect of pH on extraction of gingerols, pH of the medium was adjusted to pH 3, 4, 5, 6, 7 and 8 by the addition of 1 N HCl and 1 N NaOH after addition of ammonium sulphate to the slurry. Later to find out the effect of solid loading in the slurry, solid loading of 5, 10, 15, 20 and 25% (w/v) were tested for maximum yield of gingerols. The optimized concentrations of ammonium sulphate, *t*-butanol,