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Optimisation of Ultrasonic-Assisted Extraction (UAE) of Allicin from Garlic (Allium sativum L.)

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Garlic (Allium sativum L.) is one of the most widely used traditional medicines. Allicin is the main bioactive components of garlic which is responsible for many of its benefits, such as antimicrobial, anti-inflammatory and antioxidant agent. This study investigated the ultrasound-assisted extraction (UAE) of allicin from garlic. The extraction conditions associated with allicin yield, including particle size, extraction temperature and extraction time, are discussed. HPLC has been used for the quantification of allicin. The optimal condition for the extraction of allicin from garlic was found with sliced garlic, at temperature of 25 °C and 90 min of extraction for the maximum yield (112 µg/mL). Overall, the results obtained indicate that UAE method provided a good alternative for the extraction of allicin from garlic.

1. Introduction

Garlic (Allium sativum L.) and garlic extracts are not only used as food ingredients, but also as phytopharmaceuticals for the prevention and treatment of various illnesses and as plant protector in agriculture. The biologically active component of fresh garlic extract is responsible for many of its benefits, which have been attributed to the thiosulfinates, the single most abundant class of organosulfur compounds found in freshly chopped or crushed garlic (Upadhyay, 2012). Allicin (2-propene-1-sulfinothioic acid S-2-propenyl ester) is the most abundant thiosulfinate, typically accounting for 70 % (w/w) of the total thiosulfinates (approximately 0.4 % by fresh mass) found in fresh garlic (Chyau et al., 1999). It is only released when the garlic cloves are crushed, cut, or chewed where the pressure ruptures the cell membranes. The S(+)- allyl-L-cysteine sulfoxide (alliin) inside the cells quickly degrades into allicin and other sulphur containing compounds with the catalytic action from the enzyme allinase (Han et al., 1995). Pharmacologically, allicin is active against many bacteria and viruses, besides acting as antioxidant agent. To take benefit of all the valuable advantages of garlic-derived allicin, efficient technique is required to extract, quantify and identify them from their host plant. In that case, a few reports have described the studies on allicin in this area where Farías-Campomanes et al. (2014) performed studies on garlic extraction for allicin by using Pressurised Liquid Extraction technique technique with ethanol as the solvent. Chong et al. (2015) have investigated on the use of reversed-phase high performance liquid chromatography method to analyse allicin in garlic extracts whereas Arzanlou et al. (2015) developed a semi-preparative high performance liquid chromatography (HPLC) method for the purification of allicin from garlic extract.
The common extraction technique of garlic extracts is by using aqueous solution, maceration (Rodríguez-Jimenes, 2014) and solvent extraction (Ratti et al., 2007). Conventional extraction techniques have several drawbacks including the degradation of sensitive compounds due to the use of high temperatures, the consumption of large amounts of solvent, high toxicity of some solvents, the long processing times and the low selectivity, among others (Ribeiro et al., 2001). According to Samsudin et al. (2014), using high temperatures extraction on fresh garlic resulted in a loss of activity or destruction of the alliinase, thus preventing allicin production. In contrast, over drying garlic at low temperature (< 333 K) has little effect on the yield of the allicin and other thiosulfinates. Additionally, garlic products in the form of oils do not generate allicin, but rather, allicin-derived compounds. As a means to overcome this sort of drawbacks, an advance and improved method

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such as pressurised liquid extraction (PLE) (Farías-Campomanes et al., 2014), supercritical fluid extraction (SFE) (de Valle et al., 2012), and ultrasound-assisted extraction (UAE) (Bose et al., 2014) have been applied to shorten the extraction time, improve the extraction yield and reduce the operational costs.
Recently, UAE procedures for natural product extraction have become attractive for use in laboratories and industry due to its fast energy transfer; need no heating and as an environmental friendly extraction technique. The application of ultrasound disrupts the cell wall structure and accelerates diffusion through membranes; thus, the cell lyses and facilitates the release of cell contents (Falleh et al., 2012). Ilić et al., (2012) has used ultrasonic as the extraction tool for garlic extracts. They found that ultrasound has accelerated the extraction process by 98.5 % while He and Ma (2006) found an allicin yield of 334.9 mg/ 100 g fresh garlic using the same extraction method. The composition of allicin compounds in garlic products depends on the processing conditions (Bhagyalakshmi et al., 2005). To the best of this author's knowledge, the optimisation study of the UAE parameters in the extraction of allicin from garlic and simultaneous identification using HPLC has not been reported yet.
This study aimed to optimise the UAE of allicin from garlic. The effect of operational parameters such as extraction time, extraction temperature and particle size were evaluated to identify its optimum condition for extraction and this applicability was appreciated by using the result of subsequent HPLC analysis on allicin content.

2. Materials and method

2.1 Plant Samples

Garlic cloves were purchased from local market which was originated from China. During the project, all the samples were maintained in lab at appropriate conditions (dark, 25 °C).

2.2 Reagents

Methanol used was HPLC grade (Purity > 99.9 %) purchased from Sigma Aldrich (US) and deionised water used was purified by Milli-Q purification system (Millipore) (Massachusetts, USA).

2.3 Pretreatments

10 g of garlic cloves were blended with 100 mL deionised water for 1 min in a commercial blender for blended samples. For sliced sample, 10 g of garlic cloves were sliced into small pieces with equal size distribution and mixed with 100 mL deionised water.

2.4 Ultrasonic-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) was performed in an ultrasound cleaning bath (Sonorex, DT1028/H, 500 mm × 300 mm × 200 mm interior dimensions) by the mode of the indirect sonication, at the fixed-frequency of 35 kHz using as the working liquid. The 100 mL sample flask was charged with the pre-treated sample mixture and placed into the ultrasonic cavity for extraction process. Parameters optimised were extraction time (30 min, 60 min, 90 min, 120 min and 150 min), extraction temperature (25 °C, 30 °C, 35 °C) and particle size (Blended and sliced garlic bulb). After sonication the solution was then separated from impurities by centrifugation at 3,000 g for 2 min. Then the solution is filtered to remove the undissolved garlic and stored at 4 °C.

2.5 Quantification of Allicin

Allicin in the garlic extracts was quantified by a HPLC method which involved using a Zorbax Eclipse XDB-C18 column with a size of 4.6 mm × 150 mm, A G1312A bin pump, and a G1315A UV detector operating at the temperature of 25 °C with 10 uL injection. Above parts were included in the Agilent-1100 HPLC system (Agilent Technologies, Santa Clara, California, USA). Allicin or allicin-containing garlic extracts were applied onto the column and eluted by the isocratic solvent of water/methanol (50 / 50) at a flow rate of 0.75 mL/min. The total running time was 20 min. The absorbance of allicin or garlic extract was monitored at 254 nm. Quantification of allicin in garlic extract was made by comparing its peak area with the calibration curve of the standard allicin with known concentrations (Chong et al., 2015).

3. Results and Discussion

Quality and quantity of extracts mainly depends on the extraction procedures. The optimising extraction procedure is considered as a vital process. In current research, the following parameters were studied to identify the optimum operating condition of UAE in extraction of allicin from Garlic: Particle Size, Sliced and

Blended, Extraction Temperature, 25 °C, 30 °C, 35 °C; Extraction Time, 30 min, 60 min, 90 min, 120 min and 150 min

3.1 Effect of particle size

Figure 1 shows the allicin content of garlic extracts obtained from sliced and grinded garlic bulbs under 25 °C. Regardless of the sonication duration, extraction of allicin from grinded sample was significantly richer in allicin content (150 µg/mL) than sliced samples (122 µg/mL). Increase in the duration of sonication from 30 min to 150 min enhanced the allicin contents of garlic extracts from sliced bulb but not in the case of grinded bulb. Same findings were reported by Farías-Campomanes et al. (2014) where allicin was found to be more stable in sliced garlic extracts compared to grinded. This is due to the caking phenomenon which is more favourably occurred in sticky lumps.

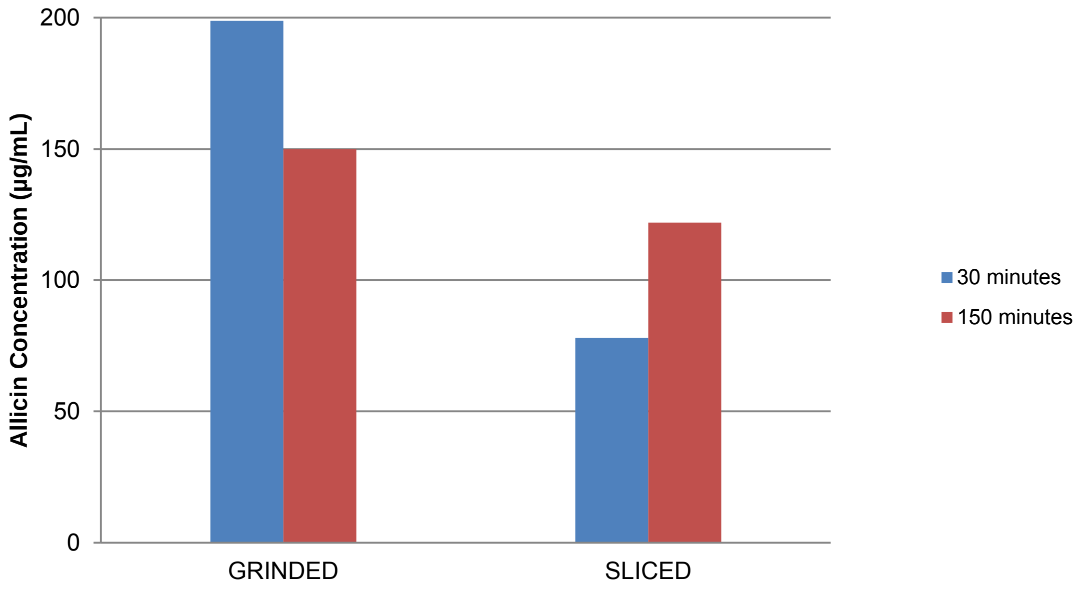

Figure 1: Effect of particle size in extraction of Allicin from Garlic (Allium sativum L.) at different extraction time under 25 °C.

The caking phenomena is commonly observed in vegetal matrixes such as garlic which have high water content (65 - 70 %) (Prati et al., 2014). Allicin is a very labile compound that readily decays, thus crushing the allicin –containing materials with substantial mechanical force may result in a significant reduction in the stability of allicin (de Valle et al., 2012). Sliced garlic sample shows more stable value of allicin by time compared to grinded. A sound analysis on the interaction between storage and stability analysis need to be done in order to choose the best pre-treatment method.

3.2 Effect of extraction time

Figure 2 shows the influence of sonication time on the extraction yields of allicin over the range 30 - 150 min under a fix temperature of 25 °C from sliced garlic bulbs. From the graph, the amount of yield does not change significantly after 90 min where the yield obtained up to this period was 112 µg/mL. According to Bose et al., (2014) who performed conventional maceration method for the extraction of allicin from garlic, they only reported 33.3 µg/mL of yield in 3 h of maceration. This momentous extraction rate by UAE is due to the cavitation effects induced by ultrasound on the cell material, causing cell disruption and facilitate the release of the intracellular matter (Falleh et al., 2012). These create an instant and high rate mass transfer platform. All these results indicated a substantial saving in time, energy and its cost. As being depicted in Figure 2, the rate of extraction was high at the beginning of the extraction but get slower gradually over time. Bose et al. (2014) have studied the effect of extraction time on extraction of Garlic and he found almost the same configuration as illustrated in Figure 2. These results confirmed the Fick's second law of diffusion which stated about the final equilibrium achieved by the solute concentrations in plant matrix and in the solvent after a certain time. This cause into no significant improvement in oil yield when prolonging the extraction time.

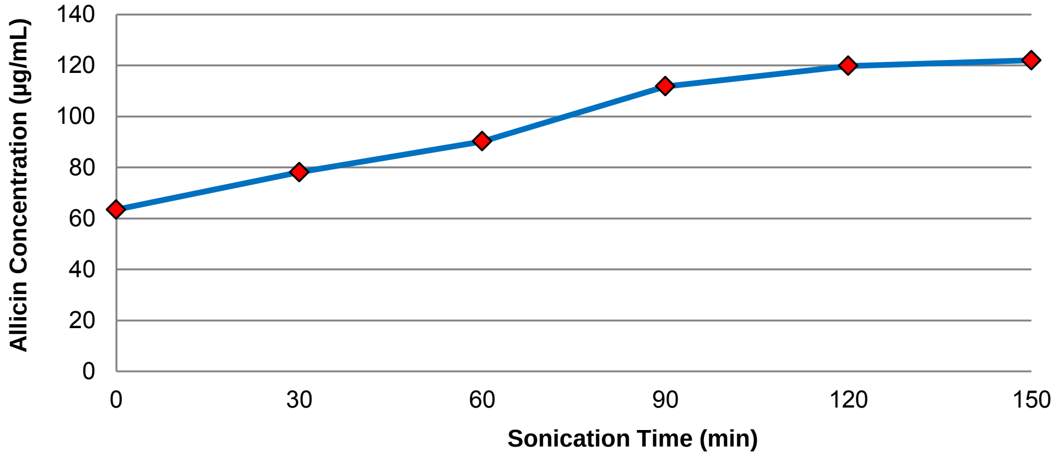

Figure 2: Effect of sonication time in extraction of Allicin from sliced Garlic (Allium sativum L.) under 25 oC

3.3 Effect of temperature

Temperature is one of the important factors in the extraction of heat sensitive compounds. Along with the increase of temperature, the solvent diffusion rate and the mass transfer amplification result in the dissolution of objective components. Meanwhile, the dissolution of impurities can also increase, and some thermal labile components such as allicin may degrade (Zou et al., 2011). In the current study, three temperature levels were analysed in the extraction of allicin using UAE and their results were shown in Figure 3 (sliced garlic bulbs and 90 min of extraction time). The extraction was deteriorated by raising the extraction temperature from 25 °C to 35 °C with a resulting allicin content of 112 µg/mL, 67 µg/mL and 44 µg/mL. The obtained result is in accordance with the findings from Mansor et al. (2016) on the thermal stability of Allicin, where their reported data illustrated increasing in temperature shows gradual and even immediate decomposition of allicin. As an organic compound, allicin is susceptible to decompose at higher temperature. A disulfide bond (S-S), typically has a bond dissociation energy of 250 kJ/mol when a higher temperature is maintained, as more heat energy is supplied into the system which allows the bond's dissociation energy to be overcome easily. As the disulfide bond present in allicin is 40 % weaker than C – C and C – H bonds, the disulfide bond is the weakest link in the allicin structure which is most susceptible to cleavage when higher heat is supplied (Kim et al., 2001). Therefore, ambient temperature was found to be more favourable for the extraction of allicin.

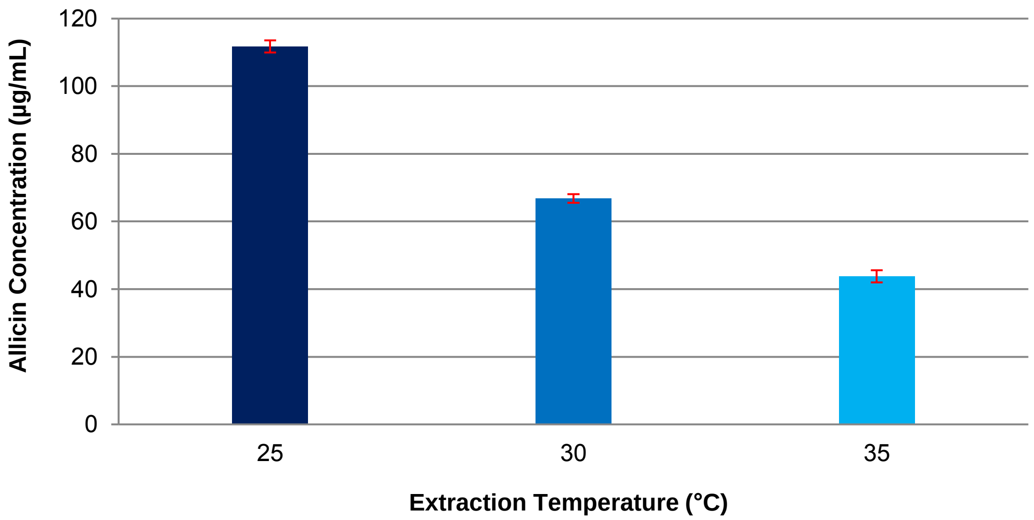

Figure 3: Effect of temperature in extraction of Allicin from sliced Garlic (Allium sativum L.) at 90 min of extraction

An optimisation study was carried out by de Valle et al., (2012) in the extraction of allicin from garlic by Supercritical Fluid Extraction (SFE) which is another recent and environmental friendly extraction method. The optimum allicin yield obtained through SFE (75 μg/mL) was lower than the one obtained by UAE (112 μg/mL). The low allicin concentration in the SFE extracts could be due to the instability of allicin in garlic oil, which forms the SFE extract. Another advantage of UAE in this case is its ability of working at ambient temperature for the extraction of active compounds from plants. Thus, this avoids the thermal overexposure especially in the instance of heat sensitive materials.

4. Conclusion

An UAE method has been developed for the extraction of allicin from Garlic (Allium sativum L.). Ultrasonic wave is a powerful tool, which can efficiently improve the extracting performance of allicin. In this paper, UAE method was studied for optimisation based on the evaluation of several experimental parameters, namely particle size, extraction time and extraction temperature. The best combination of response function was sliced garlic, at a temperature of 25 °C and 90 min of extraction time with ultrasonic irradiation. Under the optimal conditions, the yield of allicin reached 112 μg/mL. The results obtained are helpful for the full utilisation of Garlic (Allium sativum L.), which also indicated that the UAE as a powerful tool for the extraction of important phytochemicals from plant materials and bring volume of worth. It is recommended to carry out the interaction study between the experimental parameter and storage time to recognise the stability of allicin which is essential for industrial production.

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