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Ultrasonic-Assisted Extraction of Thiols from Garlic Bulbs

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A B S T R A C T

Cysteine and glutathione (GSH) are important low molecular mass thiols or sulfhydryl compounds that play numerous important roles in metabolism. They also have myriads of applications in industry including pharmaceutical, cosmetic and foods. These compounds naturally occur in plants but less was focused on extracting them from plant sources. Garlic is of particular interest as potential source for thiol compounds since it contains high concentration of organosulfur which correlates with the presence of cysteine and GSH. In this work, water-based ultrasonic assisted extraction was used to extract thiols from garlic bulbs. The effect of garlic concentration and amplitude of sonicator on thiol concentration was investigated. The optimal garlic concentration of 10 % (w/v) was able to extract 0.170 mMthiols and 100 % amplitude of sonicator corresponds to the highest value of thiol compounds obtained.

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INTRODUCTION

Garlic (*Allium sativum)* is a hardy perennial plant that belongs to the lily family [1]. It is originated from Central Asia and has been widely used as both folk medicine and spice for thousands of years. Its characteristic white bulb is devoid of smell and flavor when intact, but when cut it is pungent in taste and smell. Garlic has complex composition with over 200 different compounds that contribute to its effects. The most important and unique features of garlic is its high contents of organosulfur. It contains at least four times more sulfur than other high sulfur vegetables like onion, broccoli, and cauliflower [1]. The organosulfur compounds are used for evaluation of garlic's quality [2].

Sulfur is an essential element for animals and plants due its structural and functional incorporation into amino acids, proteins and other biomolecules [3]. Collectively, compounds containing sulfur (sulfhydryl compounds) are called thiols. They are found in all body cells and are indispensible for life. Cysteine is a sulfur containing amino acids that is unique because of its chemically very reactive sulfhydryl group [4].Meanwhile, glutathione (GSH), a tripeptideconsisting of glutamate, cysteine and glycine that contains γ-peptide bond between glutamate and cysteine; is the most ubiquitous low molecular mass thiol is biological system. Both cysteine and GSH are considered as sulfur-containing antioxidant compounds. The sulfhydryl group (-SH) of cysteine is involved in reduction and conjugation reactions that are usually considered as the most important functions of GSH [5].

Apart from being powerful antioxidants; GSH and cysteine have a wide application in food, pharmaceutical and cosmetic industries. Thus, production of GSH and cysteine has great commercial potential.Currently, production of GSH and cysteine can be achieved through several methods including chemical synthesis, enzymatic catalysis, microbial fermentation and genetic/metabolic engineering [6,7].

These compounds naturally occur in plants but less was focused on extracting them from plants. In this study, garlic has been identified as potential source for GSH and cysteine. This is based on the pungent smell of garlic which is correlated with high sulfur content [8, 9].

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GSH and cysteine are freely soluble in water. Therefore, in this study, water-based extraction of GSH was applied. Moreover, water-based extraction is considered simple, safe and inexpensive [10]. The extraction process was assisted by sonication which is based on the sonochemical phenomenon associated with acoustic cavitation [11] that helps the disruption of cell walls and facilitate the release of contents [12]. In addition, it improves the extraction techniques by providing an efficient contact between sample and the solvent, resulting in a greater extraction. Mechanical effects of ultrasound also induce greater penetration of solvent into cellular material and improve mass transfer [12]. Moreover, the time consumed for this type of extraction is less and it is very simple, easy to operate as well as relatively cheap [11].

Consequently, ultrasonic assisted extraction was applied in this study and the effects of process conditions on the extracted thiols were investigated.

MATERIALS AND METHODS

Garlic skin was peeled off and the bulbs were sliced into small pieces prior to freeze-drying process to obtain garlic powder. Extraction of sulfhydryl compounds was performed by dissolving an amount of garlic powder in distilled water and the extraction was assisted by using sonicator with operating frequency of 30 kHz (LabsonicM, Sortorius AG, Germany). The parameters involved in this study were garlic concentration (%w/v) and amplitude of sonicator (%). The level of parameters for garlic concentration was 4, 6, 8, 10 and 12 (% w/v) while the range of amplitude of sonicator was 20, 40, 60, 80 and 100 % respectively. The temperature and extraction time were set to be constant. The water-based extraction protocol in this study was based on a previous study by Salleh et al. (2008) [10]. Quantification of thiols was done using conventional spectrophotometric method of Ellman's reagent (5, 5'-dithio-*bis*-(2-nitrobenzoic acid); DTNB) assay (www.thermoscientific.com). Absorbance against reagent blank was measured at 412 nm and cysteine (Sigma Aldrich, Germany) was used as a standard.

RESULT AND DISCUSSION

The mechanical effects of ultrasound induce the greater penetration of solvent into cellular material and improve mass transfer [12]. The extraction by sonication is further enhanced by the propagation of ultrasound pressure waves resulting in cavitation phenomena [13] which helps the disruption of biological cell walls and facilitate the release of contents [12]. Mechanical effects of ultrasound also induce greater penetration of solvent into cellular and improve mass transfer [12].

Table 1 shows the effects of garlic concentration and amplitude of sonicator towards the extracted thiols. Details of the individual parameter affecting the extracted thiols will be elaborated later in this section.

Table 1: Effect of parameters on extracted thiols from garlic.

Garlic concenteration (%w/v)	4	6	8	10	12
Concentration of Extracted thiols (mM)	0.132	0.134	0.147	0.170	0.159
Amplitude of sonicator (%)	20	40	60	80	100
Concentration of Extracted thiols (mM)	0.081	0.084	0.090	0.094	0.102

Figure 1 shows the effect of garlic concentration on extraction of thiols by using sonicator. The concentrations of extracted thiols were increased as the amount or garlic concentrations increased (4% w/v (0.132 mM) to 10 %w/v (0.170 mM)). However, at garlic concentration of 12 % (w/v), the concentration of thiols decreased to 0.159 mM. This phenomenon occurred because the solvent volume was too low to extract the compounds efficiently [11]. Thus, the constant ratio of sample to solvent should be kept. In this study, the optimum garlic concentration was found to be 10% (w/v) by using sonicator to produce maximum concentration of thiols.

Meanwhile, Figure 2shows the effect of amplitude of sonicator on the extraction of thiols. The trend shows increment of thiols from the lowest to the highest amplitude of sonicator. The maximum was obtained at sonicator amplitude of 100%. However, this maximum level does not necessarily be the optimal amplitude. As the amplitude increased, the intensity and cavitation effect also increased which enhance the extraction process [14]. As the sample used is viscous, it is necessary to increase the amplitude to reach the optimal since higher amplitude vibration could lead to an increase in the intensity and cavitation effects [15]. In addition, at high value of amplitude, the shear stress to garlic cell wall would be higher, hence cell walls would easily be disrupted and release more thiols[10].

As mentioned earlier, the optimal amplitude for extracting thiols cannot be achieved due to the limitation of the sonicator. The sonicator used only has the fixed frequency of 30 kHz. In order to achieve cavitation, frequency should be increased so that the intensity of the amplitude also increases. An increment in amplitude will lead to the sonochemical effects. Furthermore, the viscous condition of garlic supernatant resists the movement of ultrasonic probe. Therefore, high amplitude is granted to obtain necessary vibration that will promote more cavitation [15].

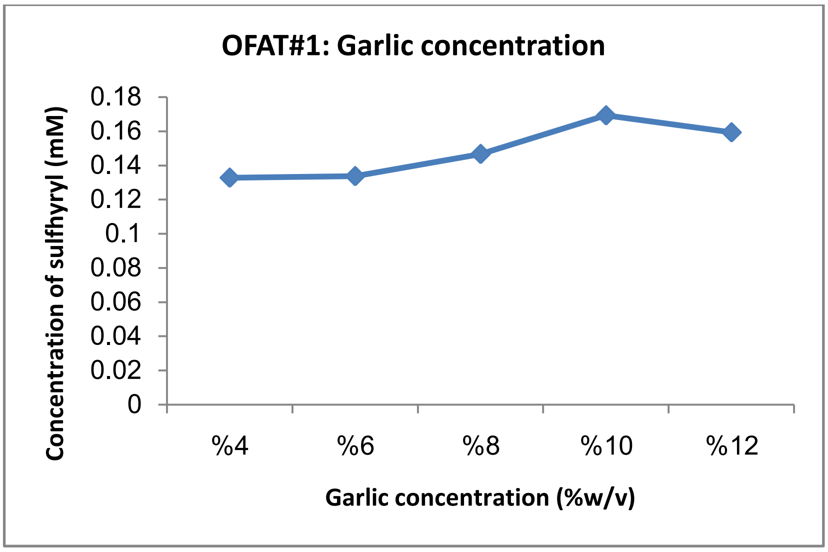

Fig. 1: Effect of garlic concentration on thiols. The extraction was carried out at various concentrations. The temperature was kept constant at room temperature and extraction time was 15 seconds with amplitude of 100%.

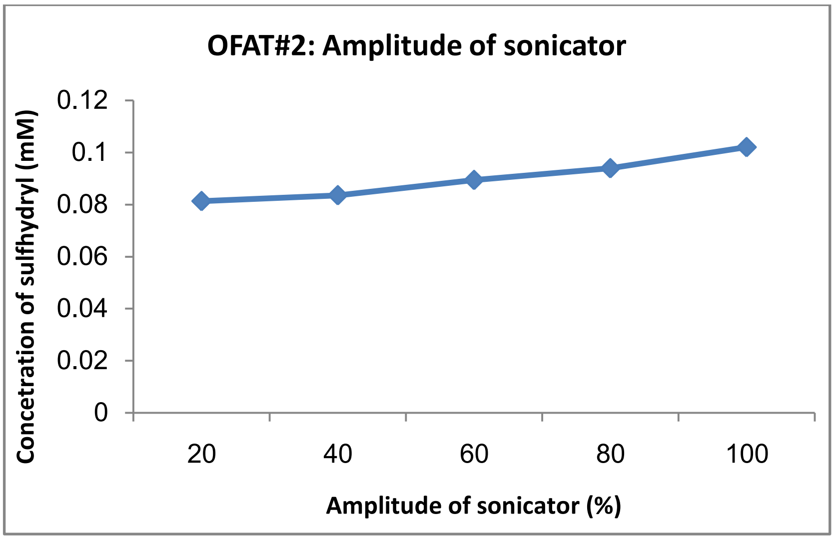

Fig. 2: Effect of sonicator's amplitude. Extraction process was performed at 20, 40, 60, 80 and 100% amplitude of sonicator. The 10% (w/v) garlic concentration was used, temperature was at room temperature with 15 seconds extraction time.

Conclusions:

In conclusion, simple, safe and cost effective water-based ultrasonic-assisted method of extractingthiols from garlic bulbs was successfully performed. Quantification of thiols was done by using Ellman's reagent method. The optimal garlic concentration of 10 % (w/v) was able to extract 0.170 mMthiols and 100 % amplitude of sonicator corresponds to the highest value of thiol compounds obtained.

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