Mini Review: Extraction of Allicin from *Allium sativum* using Subcritical Water Extraction

A S Zaini\(^1\), N R Putra\(^1,2\), Z Idham\(^1,2\), N S Md Norodin\(^1,2\), N A Mohd Rasidek\(^2\), M A Che Yunus\(^2\)

\(^1\) Centre of Lipids Engineering and Applied Research (CLEAR), Ibnu Sina Institute for Scientific & Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia
\(^2\) School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia

Email: azizi@cheme.utm.my, asyahmi22@graduate.utm.my

Abstract. Garlic or *Allium sativum* have a lot of health benefits, especially on reducing blood pressure, fighting against the common cold and improve cholesterol in which lower the risk of heart attack problem. It is considered as one of the best disease preventive foods as it exhibits high biological activity when the fresh garlic is cut or crushed that attributed to sulfur compounds and thiosulphates. One of the active compounds in thiosulphates group namely allicin. Generally, allicin is not presence in garlic and to produce the allicin, enzyme alliinase is need to activate with the presence of water. The common technique to extract allicin is using solvent extraction, UAE, PLE and SCCO\(_2\). The drawbacks of using these technique includes the use of organic solvent, long extraction time, and required two type of processes which is enzymatic process and extraction process. SWE is introduced to enhance the conventional process with high purity of product, water poses a mimic the properties of organic solvent and the enzymatic and extraction process undergo in one system. Therefore, this mini review aims to discuss the allicin in garlic, the literature on allicin extraction, the principle of SWE, and the application of SWE on allicin extraction.

1. Introduction

Garlic (*Allium sativum L.* ) belongs to the *Alliaceae* family has been cultivated around the world for centuries and used for both culinary and therapeutic properties [1, 2]. Garlic exhibits high biological activity when the fresh garlic is cut or crushed that attributed to sulfur compounds and particularly to thiosulphates and it is considered to be one of the best disease-preventive foods [3-5]. These compounds are responsible for garlic’s therapeutic properties, such as cardiovascular disease, hypertension, cholesterol, and diabetic [6-8]. Organosulfur compounds like alliin, allicin, diallyl disulfide, and S-allylcysteine are found in garlic [9] while allicin is the most dominant among others active compound [10]. Allicin is produced from alliin when the enzyme alliinase is released by chopping and crushing the garlic bulb [11] or homogenized the dried flakes with distilled water [12].
The enzymatic process of allicin as provide by Ilić et al., [4], the enzyme alliinase is formed in the presence of water to form allyl sulfenic acid and elimination of water from two molecules of allyl sulfenic acid form allicin. The weight of epoxide allicin in garlic is approximately about 60% [13]. Several studies have been conducted related on extraction of allicin from garlic includes solvent extraction [10, 14], supercritical fluid extraction (SFE) [12, 15], Ultrasonic-Assisted Extraction (UAE) [11] and pressurized liquid extraction (PLE) [16] The drawback of using solvent extraction is the concentration of allicin contain high toxicity with low purity. The used of SCOC₂ inhibit the enzymatic process, results the extraction and enzymatic process undergo separately. With the intention to raise these difficulties, a high selectivity and safe extraction technique such as subcritical water extraction (SWE) is introduced as a single-step process involving the enzymatic and extraction process in a one close system.

SWE has the potential to extract the allicin since the formation of allicin from alliin also require water to activate the enzyme. SWE offers numerous benefits being a completely green extraction method that involves water as a solvent and also economically operating cost besides it pursue high selectivity to extract different classes of compounds [17]. The operating condition for this subcritical water extraction is at a temperature over boiling point (100 °C) and below its critical point (371 °C) by maintaining the high pressure (1 – 221) bar so that the water in liquid phase [18, 19]. Adjusting the temperature in range of subcritical phase can alter the polarity of water and manipulated the water behavior to act as an acid, base catalyst and organic solvent properties. Therefore, water at this condition can be used to extract compounds with low and medium polarity [20].

1.1. Allicin in Garlic

The primary precursor for the flavor of garlic is allicin (C₆H₁₀S₂O) with the molecular weight of 162.273 g/mol, density of 1.1 g/cm³ and boiling point of 248.6 °C at 1 atm. The appearance of allicin is colorless liquid [21]. Allicin or diallyl thiosulfinate in the scientific name is the most abundant of thiosulfinate that found in garlic bulb, typically accounting 70% w/w of total thiosulfinate [22]. Although allicin is the main bioactive compound produced by garlic, it is not physically present in a whole garlic bulb. Allicin is only formed when the cell wall membrane of garlic bulb was ruptured, or the simple word is crushed, chopped, or cut. The enzymatic reaction through alliinase produces allicin from alliin [4, 11, 23]. Figure 1 shows the enzymatic reaction of alliin and biosynthesis of allicin.

![Figure 1. Biosynthesis of Allicin from Alliin](image)

Upon the cell wall broken, alliin hydrolyzed by enzyme alliinase, and in this case, alliin reaction leads to the production of allyl sulfenic acid, pyruvic acid, and ammonia. Two molecules of allyl sulfenic acid condense spontaneously, by removing water become one molecule of allicin. A study by Touloupakis and Ghanotakis [24] shows the allicin yielded approximately 2.5mg/g of fresh garlic or about 5-20 mg per clove. Aside of enzymatically process [25], allicin can be prepared chemically [4, 26], and biologically [26]. Nonetheless, allicin at the molecular level is challenging to carry out due to its chemical instability [21] and decomposes to others organosulfur compound such a diallyl sulfide, diallyl disulfide, and ajones [26]. Therefore, the review on the chemical properties as well as the
breakthroughs on the overall process, including sample preparation, extraction, analysis, and preservation of allicin is needed.
1.2. Methods of Garlic Extraction

The review on the extraction of allicin from garlic is necessary for better understanding of the plant materials itself. Table 1 shows the summarize of the extraction allicin from garlic using different methods.

**Table 1. Summarize on allicin extraction using different method of extraction**

| Extraction Process                  | Solvent                     | Conditions                          | Results                                      | Author(s)                                      |
|-------------------------------------|-----------------------------|-------------------------------------|----------------------------------------------|------------------------------------------------|
| Ultrasound-assisted extraction (UAE) | Deionised water             | t = 30, 60, 90, 120, 150 mins       | 112 µg/ml concentration allicin at optimum conditions (sliced garlic, 25 °C, 90 min) | Mathialagan et al., [11]                        |
|                                     |                             | T = 25, 30, 34 °C                  |                                              |                                                 |
|                                     |                             | Size = grinded and sliced          |                                              |                                                 |
| Pressurized-liquid extraction (PLE)  | Ethanol                     | T = 40 °C                          | 332 µg/ml concentration of allicin           | Farías-Camomanes et al., [16]                   |
|                                     |                             | P = 6 MPa                          |                                              |                                                 |
|                                     |                             | F= 2 x 10^{-3} ml/min              |                                              |                                                 |
|                                     |                             | t = 5 min                          |                                              |                                                 |
| Supercritical CO₂ Extraction (SCCO₂)| Carbon Dioxide              | T = 35, 45, 55, 65 °C              | 0.31 – 1.76 µg/g concentration of allicin    | del Valle et al., [12]                          |
|                                     |                             | P = 15, 30, 45 MPa                 |                                              |                                                 |
|                                     |                             | F = 12 – 14 ml/min                 |                                              |                                                 |
| Ultrasonic assisted extraction (UAE), Salting-out extraction (SOE) | UAE = Ethanol, SOE = Ethanol, ammonium sulfate | t = 20 min Optimum SOE: 22.57% (w/w) ethanol concentration 22.11% (w/w) ammonium sulfate concentration | 94.17% allicin obtained in alcohol-rich phase under this condition. | Li et al., [10]                                |
| Homogenizer and sonicator           | Tap water                   | V = 60ml                           | 0.48 mg/ml of allicin in whole green garlic plant | Arzanlou and Bohlooli [27]                      |
|                                     |                             | t = 5 min                          |                                              |                                                 |
|                                     |                             | 100% amplitude                     |                                              |                                                 |

UAE is the one of modern extraction technique commonly use in laboratories and industries due to its fast and efficient extraction method, need no heating and most important is environmentally technique. This technique was interpret by Mathialagan et al., [11] where the used of UAE using water as solvent gives the optimum yield of allicin at 25 °C, 90 minute of extraction and using slice garlic with 112 µg/mL concentration of allicin. Meanwhile, Arzanlou and Bohlooli [27] on the introducing the new source of allicin from green garlic plant show this whole garlic plant contain 0.48 mg/ml of allicin by using homogenizer and sonicate with tap water as solvent as the medium of extraction. However, the extraction efficiency increase when pressure is applied since the solvent is force to carry out the allicin by using PLE technique and this approved by Farias-Camomanes et al., [16] where the extraction occurred at 6 MPa results the concentration of allicin in garlic extract was found to be 322 µg/g sample higher than allicin content in fresh garlic with 189 µg/g sample. On the other hand, Supercritical carbon dioxide extraction has a potential on the extraction of allicin from garlic flakes where the allicin yield from 1.03 mg/kg to 1.76 mg/kg when increasing temperature from 35 °C to 55 °C at constant pressure of 30 MPa [12]. However, the garlic flakes do not contain allicin and undergo synthesis process enzymatically first before extraction occurs. In overall, the extraction of allicin from garlic bulb were dominated by PLE followed by UAE, and SCCO₂.
2. Fundamentals on Subcritical Water Extraction

SWE technique is widely used for the extraction of plants and herbs [28, 29]; waste products such as onion skin [30], cocoa shell [31]; microalgae [32]; and environmental [33]. The criteria to choose the extraction technique was green in which the extract yield produced high purity of bioactive compound from solute content, free from organic solvent and shorter extraction time [34, 35]. The extracted yield must be free from the toxic solvent in order to make sure the purity of the bioactive compound is not interposed with the toxic solvent. Thus, the chosen extraction technique in this study is essential in order to preserve the high bioactive compound extracted.

Moreover, there is a growing interest in the use of the sustainable and environmentally friendly technique as the water used is one of the alternatives to organic solvent where it reduced the organic solvents in favor of clean solvent [36, 37]. Besides, SWE has a series of advantages including clean, shorter extraction time, efficient, and excellent selectivity due to modifying polarity of solvent by altering the temperature [38]. However, the extraction process uses water in conditions of pressure and temperature so-called as subcritical phase conditions, and it is required specific equipment to withstand the subcritical phase conditions.

This study has the potential to develop one-step process of allicin and the concept of this study as compare to SCCO$_2$ as shown in Figure 2. Even though, the extraction of allicin using UAE and PLE are clarified as one-step process since the extraction did not require the sample preparation, but the use of ethanol in PLE technique is one of the weaknesses in the study compared to UAE which use deionised water as solvent. However, the weakness of UAE is the technique unable to use pressure to enhance the extraction process. Thus, the use of SWE fulfil the disadvantageous of previous study (PLE and UAE) where the solvent use can be altering the polarity as well as control pressure.

![Figure 2](image_url)

**Figure 2.** A schematic representation processes of allicin from garlic

SWE is specific for high reactivity in which can be exploited for recovery valuable molecules or compounds, including peptides and polysaccharides [39]. Furthermore, the advantage of using subcritical water extraction evade from multiple steps of the process to one-step process in order to acquire selected bioactive compound from the plant samples. For example, a study by Maksimenko et al., [40] reveals the potential of subcritical water extraction where the development of one-step process for the production of the antioxidant quercetin from the flower buds of the *Sophora japonica* was succeed. In order to produce quercetin, the traditional method requires the extraction of rutin from flower buds using methanol and perform a hydrolysis process to produce the target compound, quercetin. This weakness has been solved by using SWE where the extraction and hydrolysis undergo in one closed system.
2.1. Properties of Water in Subcritical Phase

Water is the greenest of all others solvent in the context of the extraction field. Water is the most abundant existing liquid molecule on the earth, covering approximately 70% of its surface. It has tremendous benefits not only inexpensive, and environmentally benign but it is non-toxic and non-flammable solvent and also often recognized as a green extraction solvent because of providing clean extraction processing, pollution prevention and hazards free [41]. On the other hand, changing the temperature and pressure conditions for water may reduce the polarity of water. Various studies have shown that increasing the temperature and applied pressure; the water polarity can be varied to those of organic solvents [42].

Figure 3 shows a concept of SWE phase region parameters of pressure and temperature. The region is below the critical point; temperature in the range of 100 to 374 °C and pressure from 1 up to 221 bar. Appropriate pressure is needed to maintain the liquid state of water at high temperature, thus avoiding the superheated steam [43]. In addition, the chemical and physical properties of water are different in every desired temperature. For example, water viscosity and surface tension declined dramatically due to increasing temperature, and this phenomenon led to the increase in mass transfer and suitable for extraction efficiency [44] The influenced of physical parameters, including temperature and pressure in water may possess many thermodynamic properties especially dielectric constant. According to García-Marino et al., [38], water has capabilities on different extraction classes of compound depending on the temperature used due to the facility of modifying the polarity of extraction medium. Figure 4 shows the polarity of water above the boiling point and poses equivalent to the organic solvent dielectric properties.

![Figure 3. Subcritical phase region in the water phase diagram](image1)

![Figure 4. Dielectric constant of water at 20 MPa that poses equivalent to organic solvent](image2)

The dielectric constant for water at room temperature, 25 °C is approximately 80, making it an extremely polar solvent. However, the polarity of water can be altered and mimic the organic solvent polarity by increasing the temperature due to the breaking of hydrogen bonding [44, 45]. For example, the dielectric constant of water at temperature 250 °C and 50 bar is $\epsilon = 27$ which falls between methanol and ethanol as tabulated in the table above [46]. The dielectric constant of water not only effect at high temperature, but also viscosity and surface tension properties differ from water at atmospheric pressure conditions. In general, increasing the temperature leads to the decreasing in surface tension and viscosity results in better mass transfer and penetration to a solid matrix with less resistant of solvent properties [47]. In addition to this, the capacity of solvents to solubilize solutes increases and faster diffusion rates occur when using a higher temperature. Thus, enhance the extraction process with faster and more complete of extraction [48]. Without a doubt, water is a powerful solvent that can pose a polar and non-polar behavior based on the temperature and pressure controlled. The most important things are water is environmentally friendly, inexpensive, non-toxic, non-flammable and pollution prevention. Water behavior at high temperature enhances the extraction
process and increase the compound recovery because of the decreasing in surface tension and viscosity that build up the solvent penetration to the solid matrix.

3. Conclusion

In this mini review, the interest in the application of subcritical water extraction for garlic has been discussed. Apparently, research on SWE of garlic especially on extraction of allicin is lack of study. The aim of this review is to summarize all available literature on the application of this green, promising and advanced technique to extract from garlic sample which is known to contain various beneficial health compounds as well as to discuss related matters including principle and advantages of subcritical water extraction. Information presented in this short review may provide insightful details and current information regarding subcritical water extraction on garlic for future development and improvement.

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