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Investigation of the effects of extraction temperature and time on bioactive compounds content from garlic (*Allium sativum* L.) husk

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Garlic (*Allium sativum* L.) has been used as a functional food and medicine in traditional prescriptions for centuries. The extract of garlic husks contains phytonutrients and antioxidant capacity, which can be applied in the food, nutraceutical, cosmetics, and pharmaceutical industries. However, garlic husks, a by-product of the food industry, are considered agricultural wastes. Hence, this research aims at evaluating the content of several compounds in the extract of garlic husks and determining the appropriate temperature and time for the extraction processing of bioactive compounds from garlic husks. In this research, garlic husk powder was extracted at different temperatures from 40 to 80°C during time durations of 30–120 min. This study found that the optimum temperature was from 60 to 70°C and the time duration was from 60 to 90 min for the extraction process. The optimal content of total polyphenols content of 8.93 ± 0.252 mg gallic acid equivalent/g, total flavonoids content of 0.028 ± 0.002 (mg quercetin equivalent/g), total thiosulfinates content of 9.73 ± 0.071 (µmol/g), and total anthocyanins content of 0.0047 ± 0.0001 (mg/g) of dried garlic husk. Based on the finding, the study suggests that garlic husk should be utilized as a potential source of natural antioxidants in garlic extract, a food supplement, that contains antioxidants to support the cardiovascular and immune systems+ and odorless garlic products.

KEYWORDS

bioactive compound, extraction, garlic husk, temperature, time

Introduction

Garlic (*Allium sativum* L.) has been used for disease treatments due to the existence of multiple natural antioxidant compounds in the bulb, cloves, leaves, and flowers. The garlic plant's bulb is the most widely used part of the plant. Apart from the single clove type, garlic bulbs are divided into numerous fleshy sections called cloves. Along with leaves and flowers, garlic cloves are used for food consumption or medicinal purposes. Garlic is grown in various regions, including Asia (i.e. Vietnam, Thai, Myanmar, Lao, Cambodia, Singapore, China, Japan, and Korea), the Middle East, northern Africa, southern Europe, and parts of Latin America. Garlic is easy to grow and can be grown year-round in mild climates and the price is normally affordable for most people (Sikandar et al., 2022).

Garlic contains more sulfur (at least four times) than other high sulfur vegetables: onions, broccoli, and cauliflower. Garlic is also rich in carbohydrates containing fructose, protein, fiber, saponins, phosphorus, potassium, zinc, moderate amounts of selenium and vitamin C, steroid glycosides, lectins, prostaglandins, essential oils, adenosine, vitamins B_1, B_2, B_6, and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenols, and essential amino acids (Borek, 2006).

There are many studies showing the health benefits and anti-aging effects of garlic. Due to being rich in antioxidant compounds and organic sulfur, garlic has been shown to help prevent many chronic diseases and conditions related to aging, especially cardiovascular and cerebrovascular disease, cancer, and Alzheimer's disease. Garlic reduces risk factors for disease and aging, including reducing oxidative stress and inflammation, lowering cholesterol, triglycerides, and homocysteine, inhibiting coronary plaque formation, and preventing platelet aggregation. Garlic treatment increases vasodilation and blood circulation, lowers blood pressure, prevents nerve cell death and increases memory and cognitive ability, boosts immunity, boosts glutathione, promotes internal antioxidants, increases physical endurance, and improves fatigue (Watson and Preedy, 2010).

For thousands of years, garlic has been used for the treatment of many diseases thanks to the existence of several bioactive compounds with proven therapeutic effects, such as antimicrobial, antioxidant, hypolipidaemic, anticoagulant, anti-hypertensive, antitherosclerotic. and hypoglycaemic (Bautista et al., 2005). Allicin (diallylthiosulfinate) is the main thiosulfinate with two allyl groups as carbon chains in fresh garlic. Diallylthiosulfinate is responsible for the characteristic flavor of garlic (Salazar et al., 2008). Besides, anthocyanins are compounds belonging to the flavonoid, common water-soluble pigments, often exhibiting purple, burgundy, or green colors in vegetables, fruits, flowers, and roots of plants.

These compounds have antioxidant attributes, cancer prevention, and anti-aging properties for consumers. However, recent studies have found that garlic husk extraction contains phenolic compounds with high antioxidant and antibacterial activity (Kallel et al., 2014; Kimthet et al., 2017; Mohamed et al., 2022; Paula et al., 2022). Considering the huge benefits of garlic for human wellbeing, all the components of garlic should be utilized to enhance people's livelihoods and reduce the waste of agricultural products.

The wastes incurred from crop cultivation and processing are generally referred to as agricultural crop residues and food industry, respectively. The quintessentially lignocellulosic biomass is considered an important sustainable raw material for bio-refineries, the production of fuels, fertilizer, and chemicals (Balogun et al., 2018; Vivek et al., 2019; Singh et al., 2021, 2022). Out of this, garlic husks from the food processing industry made up ∼25% garlic, thereby it was considered agricultural waste (Kallel et al., 2016; Kimthet et al., 2017; Paula et al., 2022). On the other hand, currently, there are many techniques to obtain phytochemicals from plants, such as soxhlet extraction, immersion, supercritical liquid extraction, supercritical water extraction, and ultrasonic extraction (Kallel et al., 2014). However, the extraction efficiency and antioxidant activity depended not only on the extraction method but also on the extraction temperature and time. According to Spigno et al. (2007), temperature and time extraction affect the solubility, mass-to-transfer rate (diffusion coefficient), and stability of phenolic compounds. It is highly unstable at a near-neutral pH level, at elevated temperatures, or in the presence of lipids. It also rapidly degrades during processing or storage which limits its bioavailability (Kim et al., 2012). Hence, it is essential to investigate the appropriate temperature and time for the extraction processing of bioactive compounds from garlic husks by using a solvent extraction method.

To achieve sustainable development goals, the utilization of the by-products from garlic is the focus of many stakeholders in the food and medical industries. However, there are few studies focusing on the potential safe sources of natural additives with antibacterial and/or antioxidant properties for the food industry. In addition, different compounds have been extracted and isolated, some of them are known as polyphenols. There is limited reported data about the extraction of bioactive compounds from garlic husk (Kimthet et al., 2017). Therefore, this study is expected to fill the research gap.

Therefore, the objective of this research was twofold. Firstly, this study aims to determine the appropriate temperature and time for the extraction processing of bioactive compounds from garlic husks by using a solvent extraction method. Secondly, this research aims at evaluating the content of several compounds in the extract of garlic husks.

Materials and methods

Materials

Fresh garlic bulbs were harvested and selected at the maturity of 130–135 days (after planting) at Van Hai ward, Phan Rang—Thap Cham city, Ninh Thuan province, Vietnam. The dried garlic husks were separated from the garlic bulbs. Dried garlic husks were collected and dried to obtain a moisture content of 3%−5%. Next, the dried garlic husk samples were finely ground and passed through a 1 mm sieve to obtain homogeneous powder particles. The garlic husk powder was packed in aluminum-coated plastic bags and stored at 4°C before extracting.

Methods

Garlic husk powder (5 g) was mixed with 250 ml of distilled water and extracted at 40–80°C for 30–120 min. The mixture extracts were filtered through a Whatman® 41 filter paper. All extracts were kept at 4°C prior to the analysis.

Total polyphenols content [mg gallic acid equivalent (GAE)/g dry matter]

Total polyphenols content (TPC) was determined by the Folin-Ciocalteu method (Wolfe et al., 2003). Phenol reacts with phosphomolybdic acid in the Folin-Ciocalteau reagent. A blue complex is formed in an alkaline medium. The absorbance of the sample was measured at wavelength 765 nm by using a UV spectrophotometer. To determine TPC in the samples, the color intensity was measured on the spectrophotometer and the gallic acid standard curve.

Total flavonoid content

The total flavonoid content (TFC) was determined through the coloration method with $AlCl_3$ (Zhu et al., 2010). The absorbance of the reaction solution was measured at a wavelength of 415 nm. To determine TFC in the samples, the quercetin standard curve was employed. The results were expressed by using mg of quercetin equivalent (QE) per gram of dry weight (mg QE/g).

Total thiosulfinate content (μmol/g; TTC)

The measurement of absorbance was conducted at a wavelength of 412 nm of 2-nitro-5-thiobenzoate by adopting the method of Kinalski and Norena (2014).

Total anthocyanin content (mg/g)

Total anthocyanin content (TAC) was analyzed by using differential pH methods based on the color change due to a change in the structure of anthocyanins between pH = 1.0 and pH = 4.5 (The Association of Analytical Communities (AOAC) International., 2003). At pH = 1, anthocyanins exist in the form of oxonium or flavium with maximum absorption, and they are in the form of colorless carbinol at pH = 4.5. The differential pH method allows for the fast and exact determination of the TAC, even in the presence of other interfering compounds.

Statistical analysis

The data was recorded in triplicates. The data were analyzed by two-way ANOVA followed by using Fisher's Least Significant Difference (LSD) test. The reference equation was chosen based on the R^2 obtained from the multiple regression analysis, the equation obtains higher R^2 as follows:

$$TAC/TFC/TPC/TTC = \beta_0 + \beta_1 X + \beta_2 Y + \beta_3 X^2 + \beta_4 Y^2 + \beta_5 XY.$$

The significance or non-significance of results was considered statistically significant at $p < 0.05$. Moreover, the Statgraphics Centurion XVI software (version 16.0; Statpoint Technologies, Inc., USA) was used for response surface plots in this study.

Results and discussion

The experimental research results show that regression equations were constructed, using the influence of temperature (X: 40–80°C) and time (Y: 30–120 min) extraction on the TAC, TFC, TPC, and TTC (Equations 1, 2, 3, and 4, respectively) with a rather high coefficient of determination ($R^2 > 0.7$).

$$TAC = -0.0151 + 0.0005\,X + 0.00013\,Y - 0.3\,10^{-5}X^2 - 5.6667\,10^{-7}\,Y^2 - 0.1\,10^{-5}\,XY \quad (1)$$

$R^2 = 0.91$

$$TFC = -0.05128 + 0.00159\,X + 0.00075\,Y - 0.9\,10^{-4}\,X^2 - 0.3\,10^{-5}\,Y^2 - 0.5\,10^{-5}\,XY \quad (2)$$

$R^2 = 0.72$

$$TPC = -25.9 + 0.6888\,X + 0.3353\,Y - 0.0049\,X^2 - 0.0017\,Y^2 - 0.00113\,XY \quad (3)$$

$R^2 = 0.86$

$$TTC = -13.8 + 0.386\,X + 0.16\,Y - 0.00234\,X^2 - 0.0007\,Y^2 - 0.0002\,XY \quad (4)$$

$R^2 = 0.92$.

The results of the experiments were shown in Figures 1–4. The response surface models indicated that the effects of temperature and time on TAC, TFC, TPC, and TTC could help to predict extraction results, respectively. In the different garlic husk extracts, the content of total flavonoid was notably higher than it was in mature garlic bulbs (5.78 and 4.16 μg QE/g, respectively) (Bozin et al., 2008). That difference between the total polyphenol and flavonoid in the bulb and the husk could be explained by the accumulation of polyphenol compounds in the outer parts of plants such as husks, skins, and shells (Lecumberri et al., 2007). Generally, in immersion extraction, the extraction processes consist of desorption from the solid surface and diffusion of the solid matrix to the extraction phase. Therefore, the extraction rate is slow. Anthocyanins are a well-water-soluble compound. The results in Figure 1 recommended

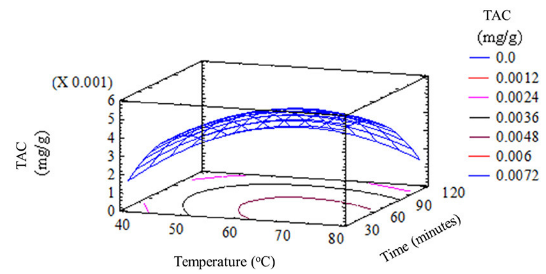

FIGURE 1
Effect of extraction temperature and time on the TAC in garlic husk.

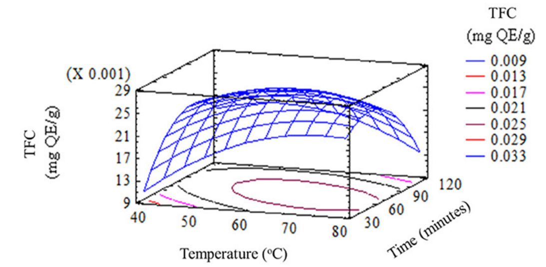

FIGURE 2
Effect of extraction temperature and time on the TFC in garlic husk.

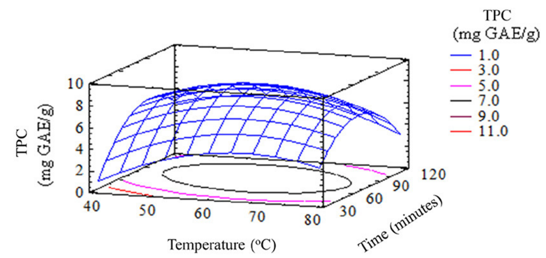

FIGURE 3
Effect of extraction temperature and time on the TPC in garlic husk.

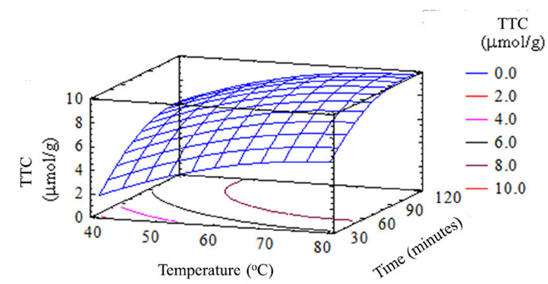

FIGURE 4
Effect of extraction temperature and time on the TTC in garlic husk.

that temperature and time of extraction had significant effects ($p < 0.05$) on TAC in garlic husk extract. Garlic husk was extracted at 80°C for 120 min, indicating the lowest TAC (0.0010 mg/g). As the extraction temperature increased, TAC also increased. However, anthocyanins would be degraded if extraction time was prolonged. TAC was 0.0055 ± 0.0003 mg/g, with the suitable temperature and time for extracting anthocyanins from garlic husks being 70°C and 60 min, respectively.

Temperature is one of the important parameters which significantly influence the total polyphenol compound content of extraction. At high temperatures, the solubility of compounds could be increased because of decreasing the polarity of the solvent, which facilitated the solvent penetration into the material (Williams et al., 2009). In addition, the cell tissues of the material may be disrupted during extraction processing which makes the mass transfer rate increase (Maran et al., 2013; Mokrani and Madani, 2016). Besides, the lignin (this is the component of the plant cell wall) might be decomposed into polyphenol compounds at high temperatures (Wahyu et al., 2008; Brebu and Vasile, 2010). Brebu and Vasile (2010) trusted that the functional group of lignin was cleaved at high temperatures. The results expressed the low molecular weight product. Rearrangement of the backbone is complete when it leads to the release of the volatile product. Besides, the cleavage of the aryl-ether linkages results in the formation of highly reactive and unstable free radicals. Then these products have further reactions through rearrangement, electron abstraction, or radical–radical interaction to form stable products. So, the decomposition of the polymer structures in lignin results in the formation of aromatic hydrocarbons, polyphenol, hydroxyphenolics, and guaiacyl-syringyl type compounds (Brebu and Vasile, 2010).

Similarly, the temperature and time of extraction also had a significant effect on the remaining three compounds (polyphenols, flavonoids, and thiosulfinates) in garlic husks by ($p < 0.05$). The TPC in garlic husks extracted at 60°C for 90 min was 8.93 ± 0.25 (mg GAE/g), which was higher than other extraction conditions. It was lower than the content present in the dry skin of red onion (80 mg GAE/g) (Nuutila et al., 2003), but was similar to or higher than the contents in several vegetables and plants, such as garlic bulb extracts (0.05–0.98 mg GAE/g) (Bozin et al., 2008), onion (2.5 ± 0.1 mg GAE/g), carrot leaf and peel (7.4 ± 0.1 and 6.6 ± 0.1 mg GAE/g, respectively) and potato peel (4.3±0.2 mg GAE/g) (Kähkönen et al., 1999; Meneses et al., 2013). Therefore, the results implied that garlic husk is a raw material with a significant content of polyphenol compounds. In parallel, water dissolves a wide range of compounds and is inexpensive, nontoxic, and nonflammable which is commonly used in food processing.

On the other hand, thanks to the polarity and good solubility of polyphenol components from various plant materials, methanol was a better solvent than others in extracting phenolic compounds (Siddhuraju and Becker, 2003). For example, the extraction of phenolic compounds from walnut green husk (Fernández-Agulló et al., 2013), cacao bean husk (Kim et al., 2004), and wild rice hulls (Asamarai et al., 1996).

To a limited extent, high temperatures increased the efficiency of garlic husk extraction by enhancing the diffusivity and solubility of the substance. Beyond this limit, high extraction temperature would reduce the content of polyphenols, flavonoids, and thiosulfinates in extracts. The time of extraction affected the extraction of active ingredients. When the extraction time was short, the bioactive compounds were not completely extracted. On the contrary, when the time was too long, some bioactive compounds would be oxidized and decomposed. The quality and quantity of the phytonutrients would also decrease because most of these compounds were sensitive to high temperatures. Besides that, total polyphenol content (14.47 mg GAE/g of dried weight garlic husk) and antioxidant activity (1.13 mg/ml) were attained by using methanol rather than using ethanol soxhlet as the solvent in soxhlet extraction. This result can be explained by the fact that methanol could extract more polar compounds as well as polyphenol compounds than ethanol (Lapornik et al., 2005).

Conclusions

The results obtained in this work showed that garlic husks can be used as a source of phytonutrients. The extraction of polyphenol compounds and various bioactive compounds could be considered as an alternative of great interest for the change in the value of this food processing by-product. Research results expressed that both factors, namely extraction temperature and time affected the extraction of healthful compounds from garlic husks. The high amount of TPC of 8.93 ± 0.252 mg GAE/g, TFC of 0.028 ± 0.002 (mg QE/g), TTC of 9.73 ± 0.071 (μmol/g), and TAC of 0.0047 ± 0.0001 (mg/g) of dried garlic husk which was achieved at a temperature of 60–70^{o}C for a period of 60–90 min. This suggested that garlic husks could be a potential source of antioxidants to be used for different biological applications, health promotion, and disease prevention.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

NT contributed to conception and design of the study who organized the database, performed the statistical analysis, wrote the manuscript, contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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