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Optimization Studies on Ohmic-Assisted Extraction of Bioactive Compounds from Garlic

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Ohmic heating technique, a high-temperature short-time process (HTST), where heat is generated by controlling the electrical conductivity of the food components. In this study, garlic was extracted by ohmic heating technique at different voltage gradients (13.33 to 26.66 V/cm) for different extraction times (5, 7.5 and 10 min). Similarly, garlic was conventionally extracted in different heating rate (low, medium, high) for different extraction times (5, 7.5 and 10 min). The process parameters were optimized based on the physico-chemical analyses such as, total phenol content, DPPH radical scavenging activity and diallyl disulphide content by high performance liquid chromatography. Resulted data were compared with the data obtained from conventional extraction done at (35-82 ºC). Though there were no significant differences ($p < 0.05$) found in physico-chemical properties of garlic extract, while the samples treated with ohmic heating has significant levels of diallyl disulphide content. Diallyl disulphide content of about 67.1% was obtained in ohmic extract whereas only 33.8% was resulted by conventional extract.

Keywords: Garlic, Ohmic heating, Antioxidant activity, Diallyl disulphide, Extraction, Total phenol content.

INTRODUCTION

A bulbous crop garlic is one of the popular spices used as a spice throughout the world. For centuries, garlic has been cultivated for its medicinal and culinary properties. It is primarily used as a seasoning mix in various food formulations such as mayonnaise, flavoring agents, ketchup, meat sausages, chutney, pickles and salad dressings, *etc*. India is the second largest producer of garlic, after China. However, about 50-60% of the total production is wasted due to improper post-harvest practices and supply chain management. This can be controlled by making various processed garlic products such as garlic flakes, oil, paste, dehydrated garlic powder, *etc*. The processing techniques paved the way for developing new products and improvising the product providing more employment and income [1].

Garlic is a source of various biologically active phytomolecules, including organosulfur compounds, phenolic acids, allylthiosulfinates, flavonoids and vitamins. The health properties of garlic depend on its bioactive compounds. Biological effects of garlic are attributed to its organosulphur compounds. Some of the organosulphur compounds are diallylsulphide, diallyldisulphide, diallyltrisulphide, allyl methyl trisulphide, dithiins and ajoene are some of the organosulphur compound. The activity of these compounds occur when the garlic bulb is crushed thereby causing the release of allinase enzyme which play major role in enzymatic reaction required for the production of thiosulfinates [2].

Garlic is well known for its odour, which emanates from alliin and other sulphur compounds. Diallyl disulphide is one of the distinctive volatile compounds which are present in garlic essential oil and crushed garlic. It is responsible for the odour and flavour. When a cell is broken, allinase triggers the breakdown of alliin to give allicin, which has a strong aroma and is converted to diallyl disulfide by reduction [3]. Many scientific evidences recommend the usage of garlic in controlling blood pressure, atherosclerosis, serum cholesterol and platelet aggregation [4]. In recent times, coronavirus (Covid-19) has infected and killed millions of people. Though there are many drugs developed to control the disease, the need to lookout for safe

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and natural medicine is a major concern for scientists all over the world. Coronavirus comes from a rotavirus family of viruses that often affects respiratory system in humans.

In general, extraction is done to enhance potentiality and wipe out displeasing features of food components such as acidic, irritating and oxidizing compounds. Most of the extraction techniques use organic solvents which are not only causes environmental pollution but also get extracted into the raffinate and affecting the product purity [5]. Traditional methods of extraction have many disadvantages including long extraction time, degradation of thermally stable constituents, *etc*. To overcome the ever increasing demand for a pure product from an efficient process, ohmic heating technique was adopted in this study for extraction of garlic. This method enhances the extraction process by increasing the transfer between the solvent and plant material, shorten the extraction time, improve the extraction yield and reduce the operational costs.

Ohmic extraction works on the principle of generating heat within the fluid due to the application of alternating electric current. The applied voltage gradient causes electroporation and enhancing mass transfer of components into the solvent. In ohmic extraction, when high voltage gradient is applied, the electrons are stimulated and hence attain more energy to excite water molecules. An avalanche of electrons, which known as streamer is formed. The streamer disseminate from the positive to negative electrode. When the streamer reaches negative electrode, electrical breakdown occurs which causes bubble cavitation, pressure shock waves and liquid turbulence. This process leads to fragmentation of particles and damage of cell structure that stimulate the extraction of intracellular compounds [6]. Ohmic extraction technique has advantages of rapid uniform heating in short time and enhancing the quality attributes of the product. There are only few research studies are available on the extraction of bioactive compounds by ohmic assisted technique [7,8]. Hence in this study, it was attempted to extract garlic bioactive principles by ohmic heating and also to compare its efficiency with conventional heating techniques. The process parameters such as voltage gradients, heating rate and extraction time are optimized based on physico-chemical analyses. A comparative analysis on the quantification of diallyl disulphide content was done for the extract obtained from optimized process.

EXPERIMENTAL

Differential extraction of garlic: Extraction of garlic pulp was done using ohmic and conventional techniques. The flowchart depicting the process of extraction is illustrated in Fig. 1. All trials were performed in triplicates.

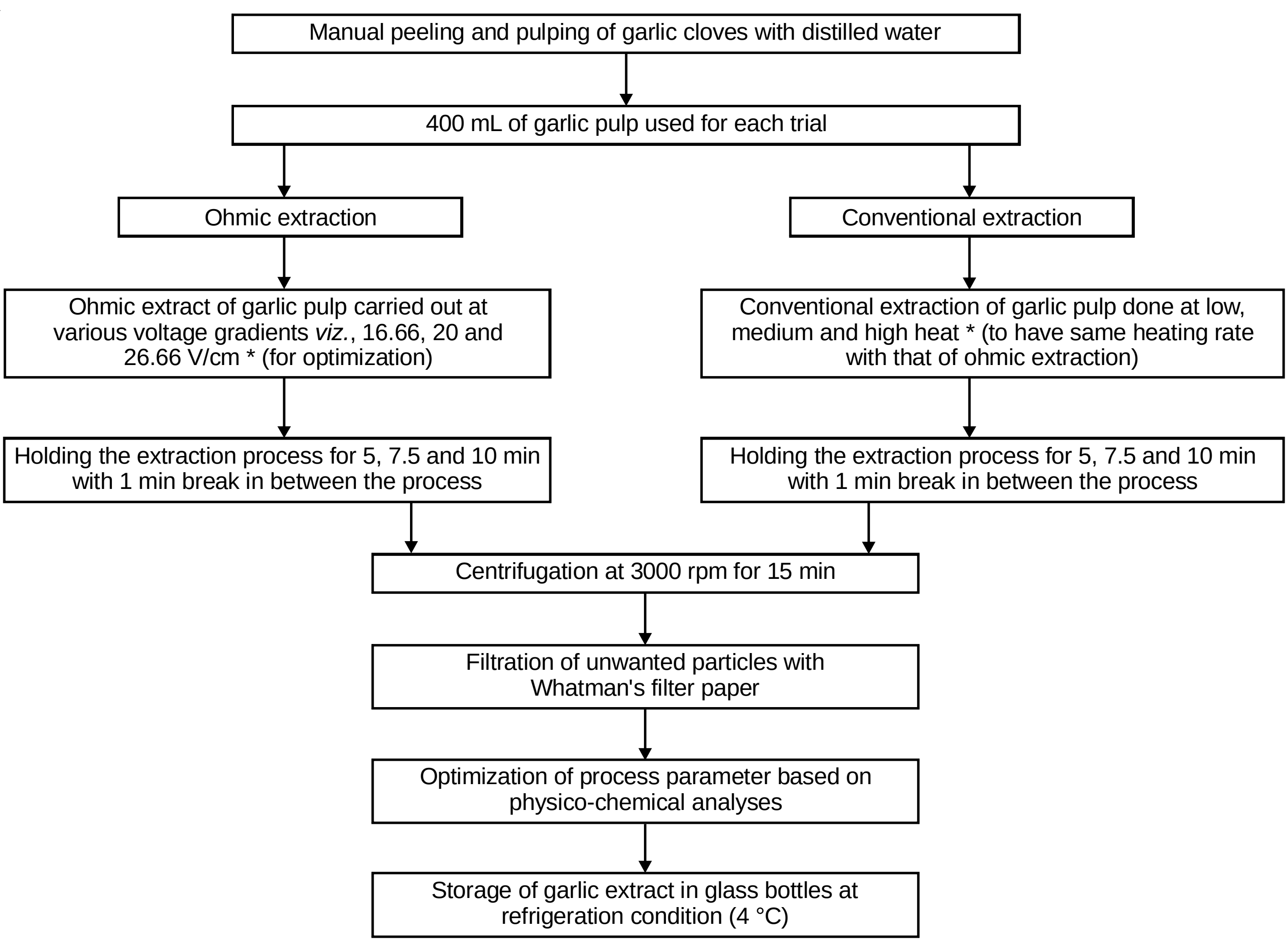

Fig. 1. Flow diagram or diffferential extraction of bioactive compounds in garlic [Conditions: 13.33 V/cm and low heat (35-82°C), 20 V/cm and medium heat (38-82 °C), 26.66 V/cm and high heat (47-82°C)]

Total phenol content: Differentially processed garlic extract was analyzed for total phenol content [9]. The garlic sample (0.125 mL) was added into a vial containing 0.5 mL of distilled water and 0.125 mL of Folin-Ciocalteu′s reagent was mixed to the solution and kept stationary for 3 min. Addition of 1.25 mL of 7% Na_2CO_3 was done and the total volume was made up to 3 mL using distilled water. The reaction mixture was incubated for 60 min and the absorbance was noted at 760 nm using UV spectrophotometer. The linear regression equation was obtained from the calibration of standard gallic acid and used to evaluate the total phenol content in garlic extract obtained by differential techniques. The final concentration of total phenol content was expressed in terms of mg Gallic acid equiv./g dry weight.

DPPH radical scavenging activity: The radical scavenging activity of garlic was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [10]. In a test tube containing 3 mL of DPPH (0.1 mM), 1 mL of garlic extract was added and incubated in a dark place for 30 min and absorbance was noted at 517 nm using UV-spectrophotometer. The radical scavenging activity was computed with the formula mentioned below and expressed in terms of percent inhibition. All tests were performed in triplicates.

$$\text{Radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{Blank}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Blank}}} \times 100$$

HPLC analysis of diallyl disulphide content: Garlic extracts obtained by the differential processing were analyzed for a bioactive compound, diallyl disulphide. Quantification of diallyl disulphide content was done by HPLC [11]. In brief, 200 mL of garlic extract was poured in a plate and pretreated in a deep freezer for 48 h. Freeze drying was done using Lyodel benchtop lyophilizer. The drying occurred with a vacuum pressure of 0.012 mbar at -60 ºC for 48 h. Dried garlic extract (1 g) was added into 30 mL of 90% methanol containing 0.01N HCl. The mixture was then agitated for 30 min and made up to 50 mL with the solvent mixture. It was centrifuged for 15 min at 105 m/s using bench-top micro-centrifuge. The supernatant (1 mL) was pipetted out into vials for HPLC analysis.

A Shimadzu UFLC (LC-20AD, Japan) system with a C18 column was used. HPLC conditions are as follows (i) column temperature: 25 ºC; (ii) mobile phase: methanol/water (86:14 v/v); (iii) injection volume: 20 µL; (iv) flow rate: 1 mL/min; and (v) running time: 14 min. The analyte was detected at 210 nm.

Statistical analysis: All the data under the measurements to study the impact of various processing techniques on the physico-chemical attributes and functional properties were computed for statistical evaluation by ANOVA along with post-hoc multiple pairwise Tukey test. One-way ANOVA was done in MINITAB version 17.0 statistical software. Non-linear and linear regressions were done by employing GRAPH PAD PRISM statistical software.

RESULTS AND DISCUSSION

In this study, garlic was ohmically extracted at various voltage gradients (16.66, 20 and 26.66 V/cm) and different processing time (5, 7.5 and 10 min). The efficiency of ohmic extraction was compared with conventional extraction (low, medium and high heat). Optimization of process parameters was done based on physico-chemical analyses such as colour, total solids, antioxidant activity, total phenol content, *etc*. The extract obtained from optimum parameters were freeze-dried to analyze the retention of diallyl disulphide by HPLC technique.

Effect of differential extraction techniques on total phenol content (TPC) of garlic extract: The total phenol content decreased with respect to the treatment time, applied voltage gradient and heating rate. However, ohmically extracted garlic has yielded significantly higher levels of total phenol content than that of conventional extraction as shown in Fig. 2. About 28.5% reduction was observed in TPC content of garlic extracted at 16.66 V/cm (10 min) when compared to that extracted at 20 V/cm (10 min). There was no significant difference found between ohmic extraction at 20 and 26.66 V/cm. This variation in the total phenol content might be due to less treatment time. Additionally, ohmic heating permeabilizes plant cells to enhance the intracellular metabolites extraction. The TPC increased slightly but not significantly with respect to applied voltage gradients. During ohmic heating, the alternating electric current has a significant effect on the discharge of total phenol content. Even so, with increase in extraction time, the heat treatment had reduced the effect on total phenol content. On the other hand, conventionally extracted garlic also showed decrease in phenolic content with respect to extraction time. When garlic was conventionally extracted at high heat, the residence time was low, which prevented the loss of phenolic compounds. In conventional technique, garlic extracted in low heat for 10 min showed 32.8% degradation than that extracted in high heat for 10 min.

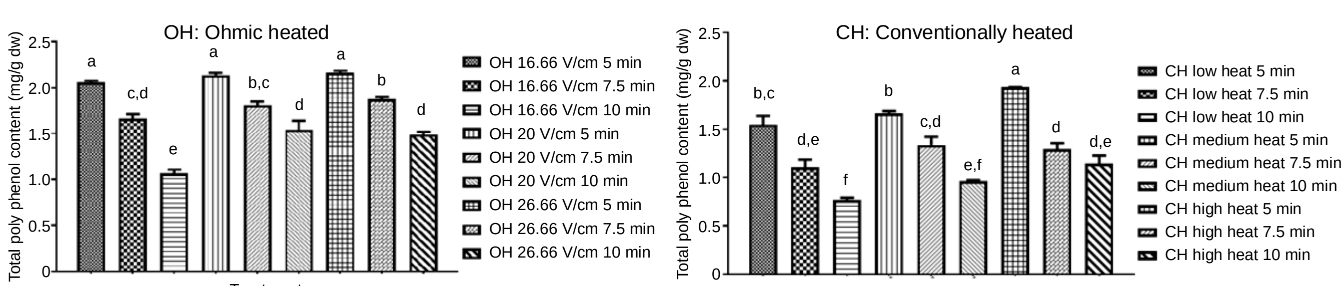

Fig. 2. Effect of differential extraction techniques on total phenol content of garlic extract[#] [[#]All the values are means of triplicate determination; [a-f]Significant differences ($p < 0.05$) by post hoc multiple pairwise Tukey test. Standard deviations are shown as error bars]

This study corroborates with the research conducted in Croatian domestic garlic by hot water extraction. The decrease in total phenols was defended that it could be attributed to phenol solubility in boiling water [12]. Applied procedures presumably formed various sorts of phenolic bonds and the decrease in TPC is most likely induced by breakdown of heat-labile phenolic compounds, chemical oxidation induced by temperature and hydrolytic catalyst release that could annihilate the antioxidant components in vegetables [12].

Effect of differential extraction techniques on DPPH radical scavenging activity of garlic extract: The garlic extract thus obtained by differential extraction techniques was studied for DPPH scavenging efficiency. The DPPH radical scavenging activity of the garlic extract obtained by ohmic and conventional method has decreased with increase in extraction time. Radical scavenging activity of garlic ohmically extracted (16.66, 20 and 26.66 V/cm) ranged from 8.83 -13.3% (Fig. 3). This decrease in antioxidant activity could be due to long residence time when garlic was ohmically extracted at 16.66 V/cm for 10 min. In ohmic extraction, no significant difference in radical scavenging activity was observed between 26.66 V/cm and 20 V/cm. Similarly radical scavenging activity of conventionally extracted garlic ranged from 4.87-9.49%, with lowest being extracted in low heat for 10 min and highest being extracted at high heat for 10 min. Loss in DPPH radical scavenging activity could be due to extensive thermal treatment (>82 ºC), which affected thermally unstable flavonoids such as ascorbic acid [13].

Effect of different extraction techniques on diallyl disulphide content of garlic: Garlic extract obtained by ohmic heating technique at 20 V/cm for 10 min and conventional heating technique in high heat for 10 min was found to be the optimized parameter with high yield of solid content, total phenol content, antioxidant property and colour. The optimized garlic extract was analyzed for diallyl disulphide content by HPLC. To quantify the bioactive compound, garlic extract was made free of moisture. The optimized garlic extract was freeze dried for 48 h to obtain garlic extract crystals. The crystals were then ground into fine powder and stored in glass bottles in refrigeration condition (4 ºC) for diallyl disulphide quantification.

From the HPLC analysis (Fig. 4), it was observed that garlic extracted by ohmic heating technique at 20 V/cm was found to yield the highest concentration (0.149 g/100 g) of diallyl disulphide. While, only 0.075 g/100 g was yielded by conventional extract. Ohmic processing helps in enhancing the availability of bioactive compounds by increasing the cell membrane permeability. Research studies on efficiency of ohmic heating on the sustainability of bioactive compounds are meagerly reported. Similar studies were observed in electric field extraction of beet root bioactives [14].

Conclusion

The implication of differential extraction process on the sustainability of diallyl disulphide content was studied. Garlic pulp was ohmically extracted at various voltage gradients (16.66, 20.00 and 26.66 V/cm) and different processing time (5, 7.5 and 10 min). The efficiency of ohmic extraction was compared with conventional extraction (low, medium and high heat). The process parameters were optimized based on total phenol content and antioxidant activity. Based on the findings, it is to be concluded that the parameters *viz*., ohmic extraction at 20 V/cm (10 min) and conventional extraction in high heat (10 min) were very much optimum parameters in extraction of the compound diallyl disulphide in garlic. Furthermore, the garlic subjected to differential extraction of ohmic heating has yielded two fold increase in diallyl disulphide content (0.149 g/100 g) when compared to conventional extraction treatment (0.075 g/100 g). Thus, this ohmic heating technique can be extended for other spices for the efficient extraction bioactive principles in near future.

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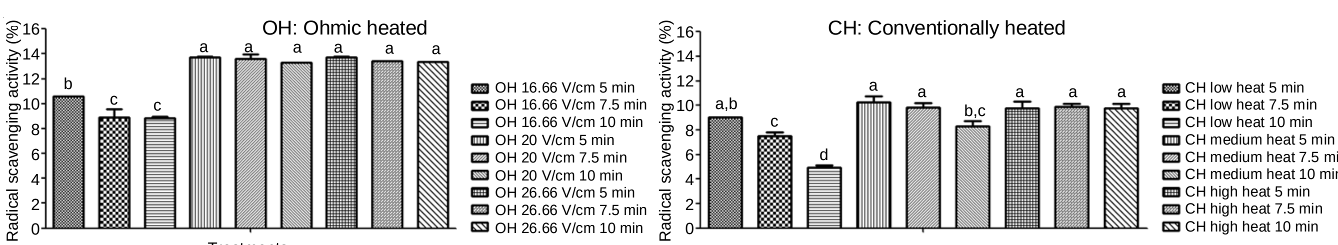

Fig. 3. Effect of differential extraction techniques on DPPH-radical scavenging activity of garlic extract# [#All the values are means of triplicate determination; a-cSignificant differences ($p < 0.05$) by post hoc multiple pairwise Tukey test. Standard deviations are shown as error bars]

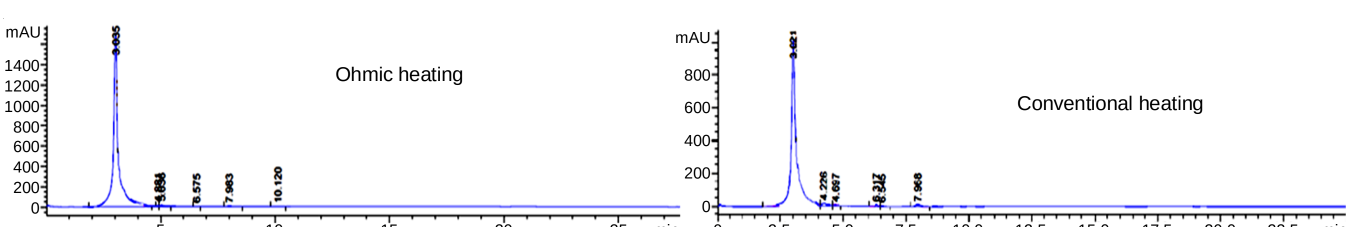

Fig. 4. Chromatogram of DADS content in garlic extract obtained by differential extraction techniques

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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