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Original Article

Optimization of Microwave Assisted Extraction of Total Phenolic Contents from *Triticum aestivum* L. Whole-grain Cereal

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ABSTRACT

Whole-grain cereals have unique blend of bioactive constituents and antioxidants which are proposed to be responsible for the health profits of whole-grain consumption. Nevertheless, phytochemicals and antioxidants in whole-grains have not got as much attention as the phytochemicals in fruits and vegetables as many of these compounds are bound to the matrix of the grain, making their extraction difficult. Since the investigation of different constituents in the plant is limited by the extraction step; this paper essentially manages the design and use of microwave assisted extraction (MAE) apparatus for the extraction of *Triticum aestivum* L. (Wheat) grains as an example of whole-grain cereals. The performance of the method (MAE) is compared to that of other established techniques like soxhlet, stirring extraction and maceration for their extracts total phenolic content (TPC) and statistically by Student's t-test. The ideal extraction states were established according to several experimental conditions to enhance the extraction productivity as 1000 W microwave power, 95% v/v ethanol as the extraction solvent, 10 minutes extraction time, 10:1 ml/gm as the solvent to material loading ratio and 20 minutes preleaching time. It can be concluded that MAE provides significant advantages in terms of extraction efficacy and time savings.

Key words: microwave assisted extraction (MAE), cereals, *Triticum aestivum* L. (Wheat), total phenolic content (TPC) and gallic acid equivalent (GAE).

INTRODUCTION

Whole-grains are a good resource of dietary fiber, vitamins, minerals and bioactive compounds. Wheat (*Triticum aestivum*) is an imperative agricultural item and essential nourishment worldwide and contains important profitable healthful ingredients. Wheat and wheat-based nourishment abundant in natural antioxidant agents can ideally act as the basis for advancement of functional foods expected to enhance the wellbeing of a huge number of clients [1]. Growing evidence point to that intake of whole wheat foods may be associated with potential health benefits as well as the reduced risk of coronary heart diseases and certain sorts of cancer [2, 3]. These significant impacts are contributed to the bioactive elements in wheat grain, for example, phytochemicals and non-digestible carbohydrates [2, 4].

Extraction is the first fundamental step in plant research as plant's crude extracts preparation is the essential point for the isolation and refinement of chemical substances existing in plants [5]. Phytochemicals and antioxidants in whole-grains are bound to the matrix of the grain, making their extraction difficult so many of these compounds have not received as much attention as the phytochemicals in fruits and vegetables [6].

Various investigations on the profitable effects of MAE for therapeutic plants have been issued, with broad updates over conventional extraction methods offering entirely enhanced suitability and brought down extraction time [7, 8]. Contrasted with the conventional techniques, MAE has numerous favorable advantages, for instance higher extraction rate, shorter extraction time, better products with lower expense and lessened solvent utilization.

In the vascular frameworks and glands in the plant cell, the free water atoms with the immediate interaction of microwaves causes an amazing increment in inward pressure as a result of the inner moisture content evaporation which bring about the accompanying split of the plant tissue and the active constituents discharging to the organic solvent [8]. Hence, in case of botanical extractions,

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MAE is an attractive substitute to routine extraction strategies.

In this study, a capable efficient extraction apparatus is designed to utilize the microwave energy for the enhanced yield of total phenolic content (TPC) from *Triticum aestivum* L. grains. The offered extraction model has been appeared differently in relation to several routine extraction methods and the impacts on the extraction yield of several experimental conditions are considered (extraction time, microwave power, solvent concentration and volume, preleaching time and extraction cycle) systematically. To date, the MAE technique of total phenolic content from *Triticum aestivum* L. grains has not been reported. This represented study intends to build up a cheap, green, quick MAE strategy for the productive huge scale extraction of bioactive compounds, and to assess the viability of the proposed extraction system in contrast to routine extraction methods.

MATERIALS AND METHODS

PLANT MATERIAL: Certified samples of species of wheat *Triticum aestivum* L., was acquired from National Research Center (NRC), Agriculture and Biology Research Division, Dokki, Egypt. The authenticity of the certified samples of wheat was additionally confirmed by contrasting their morphological characters with description specified in various standard floras and texts [9].

CHEMICALS: Folin ciocalteu regent and gallic acid were supplied from Sigma-Aldrich, Egypt. Sodium carbonate, methanol, ethanol were purchased from Al-Gomhuria company for Chemicals, Egypt.

OPTIMIZATION OF EXTRACTION PARAMETERS: In this study, the impacts of numerous critical extraction parameters (irradiation time, microwave power, solvent type, solvent composition, loading ratio, preleaching time and extraction cycle) were efficiently examined to establish the ideal extraction states for the most extreme yield of total phenolic content (TPC).

CONVENTIONAL EXTRACTION TECHNIQUES: For contrast with MAE system, three routine extraction techniques specifically, maceration, Soxhlet and stirring extraction were used. TPC extraction yield was expressed as mg/g of gallic acid equivalent (GAE) utilizing calibration curve of gallic acid (Figure 1).

Soxhlet Extraction: Comprehensive Soxhlet extraction was done utilizing a conventional Soxhlet device with precisely measured 100 g of the powdered grains for 24 h. The extracting solvent was 95% v/v ethanol. The solvent was evaporated under reduced pressure after extraction.

Stirring extraction: Stirring extraction was done in a closed conical flask by persistent stirring with the assistance of a magnetic stirrer for 48 h. The extracting solvent was 1 L 95% v/v ethanol and 100 g powdered grains was utilized. After stirring, the solvent was evaporated under reduced pressure.

Maceration: For 48 h, maceration was carried out in a closed conical flask. 1 L 95% v/v ethanol was utilized as the extracting solvent and using 100 g powdered grains. The solvent was evaporated under reduced pressure after maceration.

MICROWAVE ASSISTED EXTRACTION (MAE): A household microwave oven was modified in our laboratory with the addition of a magnetic stirrer, water condenser and a condensate pump as shown in Figure 2 & 3 with water running from the condensate pump through the water condenser of the microwave assisted extraction system, 100 g *Triticum aestivum* L. grains were mixed with a suitable solvent (1L). To get the desired temperature of around 35°C, the suspensions were irradiated with 1 min of power on and after that heating for 1 min of power on and cooling for 10 s of power off and so on, to the pre-setting extraction time, without permitting super-boiling of the suspensions. After extraction, the samples were evaporated under reduced pressure.

RESULTS AND DISCUSSION

OPTIMIZATION OF EXTRACTION PARAMETERS

Impact of microwave power: Figure 4 highlighted the common yield power plots for the extraction yield of TPC expressed as mg/g of gallic acid equivalent (GAE). Generally, the extraction productivity was enhanced by rising the microwave power from 600 to 1100 W. Within short irradiation time, (6 and 8 min) yield of TPC was upgraded with microwave power expanding. Exactly when heating the extraction solutions adequately long (12 min), under various powers the yields were comparative. The distinction of the TPC yield between 600 to 1000 W seems more critical with short irradiation times differentiated to extended irradiation times. Since critical increment in extraction yield was seen for all extraction time at 1000 W microwave power, so it was viewed as ideal. The revived extraction of TPC by expanding microwave force can be credited to the quick impacts of microwave energy on phytomolecules by ionic conduction and dipole rotation which offer power dissipation inside the solvent and plant material and create molecular movement and after that heating [10]. Extra electromagnetic power was transmitted rapidly to the extraction framework and enhanced the extraction adequacy when the microwave power expanded from 600 to 1100 W. Comparable clarifications were also represented to support the impact of microwave power, for example, the MAE of flavonoids from *Saussurea medusa maxim* cultured cells [11], MAE of notoginseng saponins from cultured cells of *Panax notoginseng* [12] and MAE of flavonoids from *Radix astragali* [13].

Impact of extraction time: Figure 5 shows the impact of the duration of microwave radiation of 6, 8, 10, 12, and 14 min at 1000 W microwave power on the extraction yield of TPC. Three stages were remarked during the microwave extraction process. The principal stage (1) between 6 min and 8 min is described by the ascent in extraction that addresses to the first amounts extracted, situated at the surface of grains. This is trailed by the second stage (2) between 8 min to 10 min described by the expansion in extraction yield speaking to the understudy dispersion of the objective analyte from the focal point of the grains towards the outside medium occupied by the natural moisture warming situated in the plant cells. Finally, the third stage (3) that begin after 10 min denote the termination of the extraction procedure. MAE achieved 283.95 mg/g of gallic acid equivalent (GAE), the most elevated extraction yield, when irradiation time was 10 min. Besides, more irradiation time increment brought about no advancement in the extraction performance. Comparable perceptions were also informed for MAE of artemisinin from *Artemisia annua* L. [14], silybinin from *Silybum marianum* [15] and tanshinones from *Salvia miltiorrhiza* [16]. In view of the fact between 10 min and 12 min of the extraction time, there was no critical contrast in extraction yield gotten; the previous was viewed as ideal for greatest extraction.

Impact of solvent composition: Figure 6 demonstrates that the aqueous ethanol concentration was extraordinarily impact the yield of TPC. Most elevated yield was acquired with 95% v/v ethanol concentration. Fall in extraction yield is brought about by further water content rising. The mass exchange process can be expanded by the presence of some measure of water through expanding the relative polarity of the solvent. In this manner, enhancing its solubilizing limit and through successful swelling of the plant material, subsequently expanding the surface area for interaction between the solute and the solvent. Excess thermal stress can be brought about by the presence of abundance measure of water, because of the solution was quickly heated as a consequence of powerful water absorption of microwaves.

Impact of preleaching time: The contact time between the extracting solvent and the sample matrix before microwave irradiation is defined as preleaching time. Fig. 7 uncovers that extraction process continued enhancing till preleaching time achieved 20 min that was viewed as best for upgrading the yield of extraction. Further preleaching time expansion did not demonstrate any encouraging impact on the progress of extraction. When the preleaching time reached 20 min, thus licensed adequate swelling of the plant matrix. The cell wall was blasted by the aids of this expanded hydrated status, because of internal thermal stress and growth of the cell pores, in this way helping draining of the objective analyte. Parallel perceptions were likewise made in the MAE of tanshinones; from *Salvia miltiorrhiza* [16] and silybinin; from *Silybum marianum* [15].

Impact of solvent to material ratio: To certify that the whole sample is soaked, particularly while having a matrix that will swell through the extraction procedure, the solvent volume ought to be adequate. Normally, a higher volume of solvent will expand the extraction performance in traditional extraction strategies; however in MAE lower yield may be given by an extra solvent volume [14, 17]. Numerous loading ratios (5:1, 10:1, 15:1 and 20:1 ml/g) were inspected to research the impact of solvent to material ratio on the yield of TPC. Fig. 8 demonstrates that with the expanding solvent volume between 5:1 ml/g and 10:1 ml/g, the yield of TPC expanded and with the expanding solvent volume past 15:1 ml/g, the TPC yield diminished. As between 10:1 ml/g and 15:1 ml/g loading ratio, no noteworthy contrast in the yield of TPC was commented, so the previous was chosen as the ideal. This was most likely as a consequence of a lacking blending of the solvent once the microwaves are connected at bigger solvent volumes. Besides, more absorption of microwave energy will be brought about by bigger volume of solvent (95% v/v ethanol). Henceforth, adequate microwave power may not be accessible for encouraging the cell breakage for viable filtering out of the objective analyte [18]. Undifferentiated from impacts were additionally recorded within the MAE of tea polyphenols and tea caffeine from green tea leaves [19] and the MAE of artemisinin from *Artemisia annua* L. [14].

Impact of extraction cycle: In this analysis, the impact of recurrent and progressive extractions of the residue (extraction cycle) was examined. The ideal parameters accomplished in the examination study were set for the extraction conditions. Further 5.5 mg/g of gallic acid equivalent was delivered in a second progressive extraction of the residue, taking the last extraction respect 283.95 mg/g of gallic acid equivalent. From the above information, in the first extraction cycle 97% of the extraction was over. So the progressive second extraction did not demonstrate any significant extraction.

Thus, the last ideal extraction conditions as got from the study are summarized in table (1).

Proposed extraction mechanism: The structure of the cell is influenced by the microwave treatment that leads to raising the internal pressure and the sudden increment of temperature. Among MAE, cellular wall cellulose lacks its hydration and decreases its mechanical quality due to the higher temperature carried out by the cell wall, which permits the solvent to achieve a simple passage inside the cell channels [8, 20]. Amongst the cell wall rupture handle, the chemical substance quickly exudates inside the cell into the adjacent solvents. Unlike in relation to that of heat reflux extraction which relies on a progression of penetration and solubilization procedures to bypass the analytes outside the matrix, MAE mechanism built on subjecting the analytes to the solvent throughout cell rupture.

COMPARISON OF MAE WITH OTHER TRADITIONAL EXTRACTION STRATEGIES ON TPC YIELD: The points of interest and difficulties of the procedures principally impact the decision of an extraction strategy; for example, extraction yield, complication, generation cost, time and the ecological impact. In general, the most usually utilized extraction systems are heat reflux extraction and maceration. The expansive measures of solvent and prolonged extraction time involved are the disadvantages of maceration and heat reflux extraction. These extraction techniques are not good from a business perspective considering the costly solvent utilization and the long extraction time. Interestingly, MAE strategy demonstrated recognizable penetration power, less exposure to organic solvents, high extraction productivity, decreased extraction time that can prompt better items with lower cost. MAE was contrasted with the other routine extraction systems to get quantitative data regarding the extraction of TPC from *Triticum aestivum* grains in the present study. The states of various methods and their outcomes are compressed in Table 2. All the extraction strategies were utilized under their upgraded conditions. Table 2 showed that as far as yield of TPC, the best results were gotten by MAE, which gave essentially higher qualities. On extraction time, MAE was additionally the speediest extraction technique with just 10 min of extraction time with preleaching time of 20 min mixing while heat reflux extractions are tedious procedures depend on heat or blending to enhance the mass transfer rate. The statistical difference between the MAE and each routine extractive technique was determined by Student's t-test. The results revealed that MAE had a significant effect on the amount of TPC ($P< 0.01$). These components would point MAE as a practical and important innovation fitting for today's exceedingly aggressive commercial projects with developing interest for expanded profitability, enhanced productivity and lessened process duration.

CONCLUSION

The viability and productive genuine nature of MAE system for the extraction of bioactive compounds has been communicated. The strategy can likewise be helpful for the extraction of bioactive constituents which are frequently get harmed when extraction is undertaken through ordinary systems and available in trace quantity. Appear differently in relation to traditional extraction strategies uncovered that MAE could spare a considerable measure of electrical vitality and time. In addition, the amount of solvent expended in MAE was the slightest which demonstrates its ecofriendly highlight or environment friendly. This connotes, it would spare the creation cost extraordinarily. From this point forward the proposed extraction strategy could be called as green extraction method with an ecological point. A productive MAE process has been created for quick extraction of TPC from *Triticum aestivum* L. grains. MAE method can be utilized on extensive scale to separate all the bioactive constituents present in these grains as a further research.

Table 1. Ideal conditions for MAE extraction of *Triticum aestivum* grains

Microwave Power	Extraction time	Extraction solvent	Preleaching time	The solvent to material loading ratio
1000 W	10 min	95 % v/v	20 min	10:1 (ml/g)

Table 2. Parallel sketch of MAE with conventional extraction methods

Extraction technique	Extraction time	Solvent volume	TPC yield (mg/g of gallic acid equivalent)
MAE	10 min	1 L	283.95
Maceration	48 h	2 L	198.95
Soxhlet extraction	24 h	2 L	250.45
Stirring extraction	48 h	2 L	210.95

*Data presented as means ±SD (n=3); yield of TPC expressed as mg/g of gallic acid equivalent; the powdered grains sample weight= 100 g; MAE was performed at the ideal extraction conditions as got from the study.

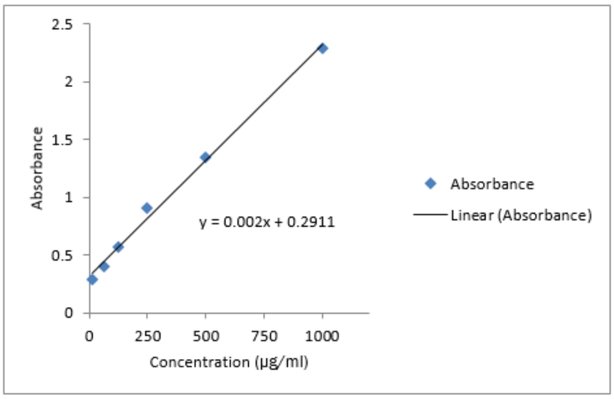

Figure 1. Calibration curve of gallic acid

Figure 2. Colored photo of MAE apparatus

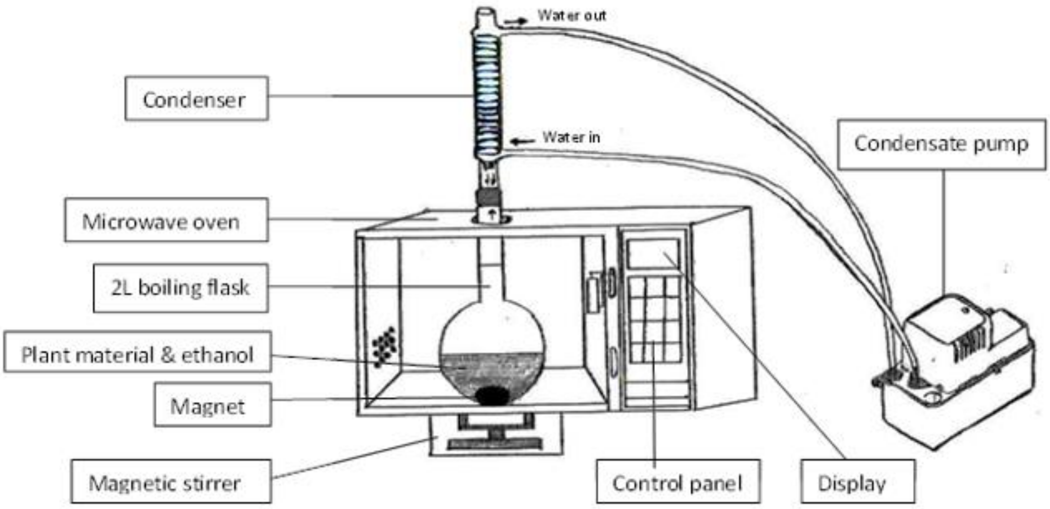

Figure 3. Diagram of MAE apparatus

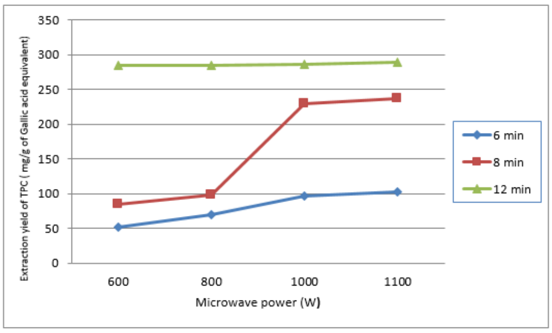

Figure 4. Impact of microwave power on the yield of TPC expressed as mg/g of gallic acid equivalent (GAE)
*Extraction states: 1 L 95% v/v ethanol as extraction solvent and 20 min of preleaching time

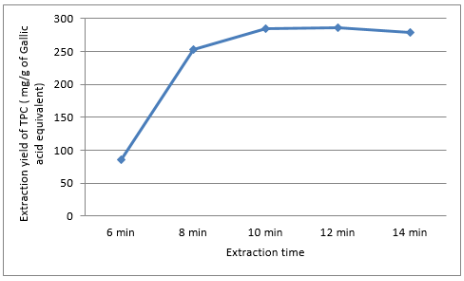

Figure 5. Impact of extraction time (irradiation time) on the yield of TPC expressed as mg/g of gallic acid equivalent (GAE)
*Extraction states: microwave power: 1000 W, extraction solvent: 1 L 95% v/v ethanol and 20 min of preleaching time

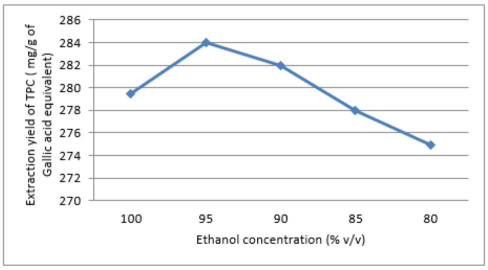

Figure 6. Impact of ethanol concentration on the yield of TPC expressed as mg/g of gallic acid equivalent (GAE)
*Extraction states: microwave power: 1000 W, 10 min extraction time, 1 L extraction solvent and 20 min of preleaching time

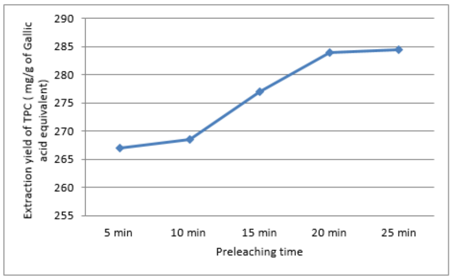

Figure 7. Impact of preleaching time on the yield of TPC expressed as mg/g of gallic acid equivalent (GAE)
*Extraction states: microwave power: 1000 W, extraction time: 10 min, and extraction solvent: 1 L 95% v/v ethanol

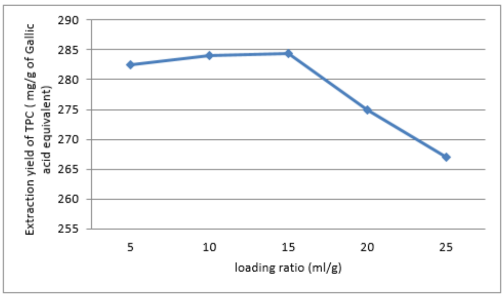

Figure 8. Impact of solvent to material ratio on the yield of TPC expressed as mg/g of gallic acid equivalent (GAE)
*Extraction states: microwave power: 1000 W, extraction solvent: 1 L 95% v/v ethanol, extraction time: 10 min and 20 min of preleaching time

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