

PROCESS FOR EXTRACTION OF GLUCOSINOLATES FROM BY-PRODUCTS OF WHITE CABBAGE (*Brassica oleracea* var. *capitata* f. *alba*)

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ABSTRACT

White cabbage (*Brassica oleracea* var. *capitata* f. *alba*) has high nutritional value and is considered “the magic drug for the poor.” As a member of the *Brassica* family, white cabbage contains glucosinolates that prevent the growth of some types of cancer, enhance immunity of cells, and are capable of producing antibiotics and preventing disease. The present study aimed to extract glucosinolates from by-products of the white cabbage industry to apply in the preservation of agricultural products and foodstuff, and the prevention of postharvest losses caused by microorganisms. The study focused on understanding the impact of materials, solvents, and extraction parameters to glucosinolates extraction from by-products of cabbage. Plant material particles sized 0.5 mm to 1 mm in diameter were considered the best plant material sizes to extract glucosinolates. The aqueous solution of methanol (60%), the ratio of material to solvent (g/ml) 1:10, the extraction temperature of 50°C, and the extraction time of 1 hour were the most efficient for extractions of glucosinolates from the by-products of cabbage.

Keywords: By-product of white cabbage, extraction, glucosinolates.

Quy trình tách chiết glucosinolates từ phụ phẩm bắp cải trắng (*Brassica oleracea* var. *capitata* f. *alba*)

TÓM TẮT

Bắp cải, một loại rau có giá trị dinh dưỡng cao và được xem như “thuốc chữa bách bệnh của người nghèo”. Cũng như tất cả các loại rau thuộc họ Cải, bắp cải chứa glucosinolates là hoạt chất có thể ngăn chặn sự phát triển của một số loại ung thư, tăng cường khả năng miễn dịch của tế bào và có khả năng kháng sinh, phòng chống sâu bệnh. Mục đích của nghiên cứu này nhằm chiết xuất hoạt chất glucosinolates từ phụ phẩm của bắp cải để ứng dụng bảo quản, hạn chế sự hư hỏng do vi sinh vật gây ra cho nông sản, thực phẩm. Nghiên cứu tập trung vào tìm hiểu ảnh hưởng của nguyên liệu, dung môi cũng như thông số quá trình đến khả năng trích ly glucosinolates từ phụ phẩm bắp cải. Kết quả cho thấy phụ phẩm bắp cải có kích thước 0,5mm < d ≤ 1mm là thích hợp nhất cho quá trình trích ly; dung môi methanol 60%, tỷ lệ nguyên liệu/ dung môi 1/10, nhiệt độ trích ly 50°C, thời gian trích ly 1h cho hiệu quả cao nhất trong chiết xuất glucosinolates từ phụ phẩm bắp cải.

Từ khóa: Glucosinolates, phụ phẩm của bắp cải trắng, tách chiết.

1. INTRODUCTION

Glucosinolates (GSLs) are sulfur containing secondary plant metabolites that are responsible for the pungent aromas and spicy tastes of *Brassica* vegetables. They are not only important to plants, as they act as part of their major defense system, but also to humans in many ways. GSLs-containing *Brassica*

vegetables have anticarcinogenic effects (Mithen *et al.*, 2000). Epidemiological studies suggest that the consumption of *Brassica* vegetables can reduce the risk of cancers of the stomach (Hansson *et al.*, 1993), colon and rectum (Kohlmeier *et al.*, 1997), bladder (Michaud *et al.*, 1999), lung (London *et al.*, 2000), breast (Terry *et al.*, 2001) and prostate (Giovannucci *et al.*, 2003). Another important

application that GSLs may have is their beneficial effect on controlling pests and diseases in some crops (Brown and Morra, 1995; Manici *et al.*, 1997; Tierens *et al.*, 2001; Makkar *et al.*, 2007; Góralaska *et al.*, 2009). However, studies related to the exploitation and application of GSLs in agricultural product preservation in Vietnam is still very limited.

Cabbages are cultivated worldwide and widely consumed in the human diet. They are popular mainly due to their affordable price, availability in local markets, and consumer preference. The GSLs profile of cabbage differs depending on type. Among cabbages, the white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) appears to contain the highest level of GSLs, with a mean total value of 148 mg per 100 g fresh weight. This value is almost double the levels observed in red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) and savoy cabbage (*Brassica oleracea* var. *capitata* f. *sabauda*) (Possenti *et al.*, 2016). White cabbage (*Brassica oleracea* convar. *capitata* var. *alba*) is also a main vegetable in Vietnam. It has been reported that up to 40% of white cabbage leaves, after processing, are lost as waste, which is generally used as fertilizer or animal feed. However, the waste has been reported to contain high amounts of dietary fiber and GSLs (Nilnakara *et al.*, 2009). The idea of using the cabbage outer leaves, which are usually discarded, to produce value added products was thus proposed. Extracting glucosinolates from the by-products of white cabbage to apply in the preservation of agricultural products and foodstuff, and prevention of postharvest losses caused by microorganisms will be of great value for farmers and consumers.

Extraction of bioactive compounds from plant materials is the first important step in the utilization of phytochemicals in the preparation of dietary supplements or functional foods, food ingredients, pharmaceuticals, and cosmetic products. Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability. It is generally known that the yield of chemical extractions depends on the chemical composition and

physical characteristics of the material, the type of solvent used with varying polarities, the material to solvent ratio, as well as the extraction temperature, and extraction time. In order to obtain high yields of GSLs from the vegetal materials, it is important to determine the correlation between the extract conditions and the yield of the obtained bioactive ingredient. In this paper, we report an easy and repeatable process for extracting GSLs that is suitable for the production of food-grade GSLs.

2. MATERIALS AND METHODS

2.1. Plant material preparation

The outer leaves of *Brassica oleracea* var. *capitata* f. *alba* not used to make food were obtained from a local grocer, washed, and air dried. Plant materials (healthy, fresh outer leaves without physical damage) were cut into the constant size of 0.5 × 2.0 cm and oven-dried for 24 hours at 65°C. After drying, samples were mechanically crushed into different particle sizes. The dried ground samples were subsequently held in PE bags with desiccant inside and stored in a sealed container (dark, dry, and room temperature environment) for extractions.

2.2. Plant material particle size separation and extraction process

The dry samples were separated into 3 types of raw particle sizes: (a) powder (below 0.5 mm in diameter), (b) fine (from 0.5 to 1 mm in diameter), and (c) medium (from 1 to 2 mm in diameter). The ground material was used to perform dynamic extractions with different solvents (water, methanol, and ethanol), at different concentrations (40, 50, 60, 70 and 80%), in different volumes of extraction (in accordance to 5 different sets of material to solvent ratios from 1:6 to 1:14), at 5 different extraction temperatures (from 40 to 80°C), and for different extraction times (from 0.5 to 2 hours) in an incubator shaker with a shaking speed of 150 rounds/min. All the solutions were transferred to 50 ml falcon tubes and then centrifuged for 15 min at 6000 rounds/min. The collected supernatant was evaporated using

vacuum rotary equipment at 60°C, 330 mbar to obtain the liquid crude extract that was approximately 20% of the original volume. In this study, the impact of raw materials, solvents, and extraction parameters were set by choosing the best parameters for the extraction of GSLs from by-products of cabbage (Tables 1, 2, and 3). All the extraction processes were carried out in 3 replicates and all the analyses on each sample were done in triplicate.

2.3. Analysis the liquid crude extract

The liquid crude extract was subjected to a quantitative analysis of total GSLs using the

alkaline degradation and reaction with ferricyanide method described by Jan *et al.* (1999) with minor modifications.

The 2 mL liquid crude extract was mixed with 2 mL NaOH 1M. After 30 min, 0.15 mL HCl (37%, w/v) was added to neutralize the solution. The resulting mixture was centrifuged (13,500 rpm, 3 min) and 2 mL of the supernatant was mixed with 2 mL of ferricyanide (1 mM) prepared in phosphate buffer (pH 7, 0.2 M). The absorbance of the solution was measured within 15 s against phosphate buffer (pH 7, 0.2 M) at 420 nm.

Table 1. Independent parameters involved

Factor names	Factor levels
Plant material particle size	Powder, fine, and medium particle size (mm in diameter)
Type of solvent	Water, methanol 70%, and ethanol 70%
Solvent concentration	40, 50, 60, 70, and 80 (%)
Material to solvent ratio	1:6, 1:8, 1:10, 1:12, and 1:14 (g/ml)
Extraction temperature	40, 50, 60, 70, and 80 (°C)
Extraction time	0.5, 1.0, 1.5, and 2.0 (hours)

Table 2. Controlled independent parameters

Factor names	Factor levels
Weight of plant material	2 g of dried leaves
Shaking speed	150 rounds/min
Centrifugation condition	15 min at 6000 rounds/min
Evaporation condition	60°C, 330 mbar

Table 3. Experimental design for studying the effects of different extraction parameters on the glucosinolates content of the extractions

Experiment	Extraction parameters	Fixed parameters
Plant material particle size	Powder, fine, and medium particle size (mm in diameter)	Ethanol 70%, 1:10 g/ml, 60°C, 2 hours
Type of solvent	Water, ethanol 70%, and methanol 70%	Selected plant material particle size, 1:10 g/ml, 60°C, 2 hours
Solvent concentration	40, 50, 60, 70, and 80 (%)	Selected plant material particle size and type of solvent, 1:10 g/ml, 60°C, 2 hours
Material to solvent ratio	1:6, 1:8, 1:10, 1:12, and 1:14 (g/ml)	Selected plant material particle size and solvent, 60°C, 2 hours
Extraction temperature	40, 50, 60, 70, and 80 (°C)	Selected plant material particle size, solvent, material to solvent ratio, 2 hours
Extraction time	0.5, 1.0, 1.5, and 2.0 (hours)	Selected particle size, solvent, material to solvent ratio, extraction temperature

The content of total GSLs in the by-products of cabbage was calculated from the absorbance reading using the formula:

$$c = \frac{A \cdot V \cdot K}{\epsilon \cdot l \cdot m}$$

Where:

c: glucosinolates content (mol/gam dry weight)

A: optical density (420 nm)

V: the volume of the GSLs crude extract (L)

K: the dilution factor of the extract during the alkaline treatment and reaction with ferricyanide

ϵ : the molecular absorption coefficient (23,000 M.cm⁻¹)

l: the thickness of the cuvet (1 cm)

m: dry weight of leaves used in the sample (2 g)

2.4. Statistical analysis

All experimental results in this study were expressed as mean values $\pm$ standard errors (SE) of nine measurements (n = 9). In these single factor experiments, the significant differences ($p < 0.05$) among means were

subjected to one-way analysis of variance (ANOVA) with Tukey's test using the statistical software JMP 7.0.

3. RESULTS AND DISCUSSIONS

3.1. Effect of plant material particle size

Plant material particle size (mm in diameter) affects the extraction rate by increasing the total mass transfer area when the particle size is reduced (Schwartzberg and Chao, 1982). Results, shown in Figure 1, indicate that material particle size significantly affected the rate of the extraction of GSLs compounds from samples ($p < 0.0001$).

Theoretically, it was expected that the powder particle size of plant materials would produce the highest yield of GSLs. However, the highest amount of GSLs was obtained from the fine particles with sizes of 0.5 to 1 mm in diameter. This particle size could be the most suitable for solvent movement into the gaps of the capillary system so GSLs content of the obtained extracts were the highest. This particle size was used for subsequent experiments.

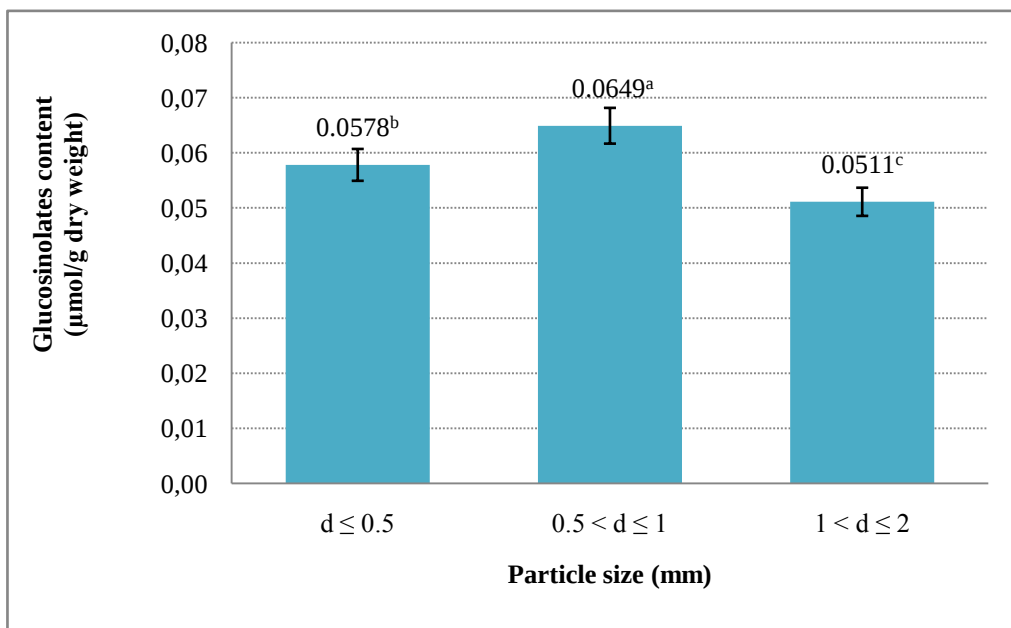


Figure 1. Effect of particle size on the glucosinolates content of the extract

Note: Values marked by different letters indicate significant difference ($p < 0.0001$)

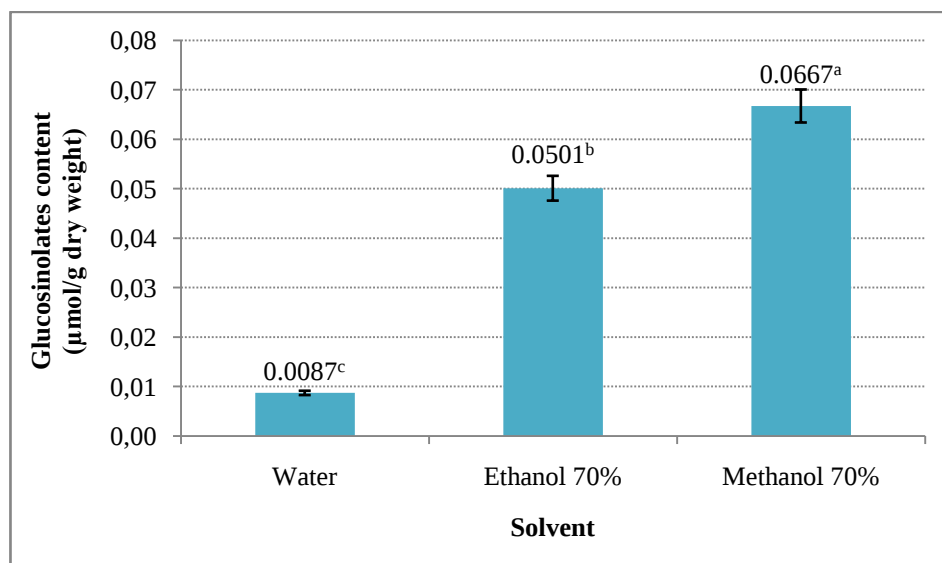


Figure 2. Effect of solvent type on the glucosinolates content of the extract

Note: Values marked by different letters indicate significant difference ($p < 0.0001$)

3.2. Effect of type of solvent

Extraction yield is strongly dependent on the solvent. Hence, the selection of extraction solvents is critical for an extraction study. An extraction solvent generally is selected according to the purpose of the extraction, polarity of the interested components, polarity of undesirable components, overall cost, safety, and environmental concerns (Wang *et al.*, 2008). Due to the strong polarity of GSLs, the solvents water, ethanol 70%, and methanol 70% were used.

Among the three types of solvents tested, aqueous methanol (70%, v/v) showed a significantly higher extraction capacity for GSLs from cabbage by-products ($p < 0.0001$) (Figure 2). These results are in accordance with the polarity of the solvent used for the extractions and the solubility of GSLs in them. It is interesting to note that the polarities of water, ethanol, and methanol are 1.000, 0.654 and 0.762, respectively (Reichardt, 2003), hence the polarity of ethanol 70% is 0.7578 and the polarity of methanol 70% is 0.8334.

Aqueous methanol had highest extraction efficiency, suggesting the use of aqueous methanol as an extraction solvent for the following steps in this study. However, there

was still a need to check if using a different water percentage in methanol (% v/v) could be used to increase the extraction efficiency of GSLs from cabbage by-products in the present experiment.

3.3. Effect of methanol concentration

As can be seen from Figure 3, the GSLs content as a function of methanol concentration follows a parapol shape, and the methanol concentration had a significant effect ($p < 0.0001$) on the extraction efficiency of GSLs from by-products of cabbage.

Methanol has a lower polarity than water, hence, with the addition of water to methanol, the polarity of the complex solvent will increase continuously. So the GSLs in the cabbage by-product extracts increased with increasing water content according to the “like dissolves like” principle (Chirinos *et al.*, 2007). The GSLs content of the extracts from cabbage by-products reached a maximum at 60% methanol (v/v) followed by a significant decrease at higher concentrations of methanol in the extraction medium.

A solvent of 60% methanol (v/v) was chosen to determine the effect of the ratio of material to solvent on the extractions.

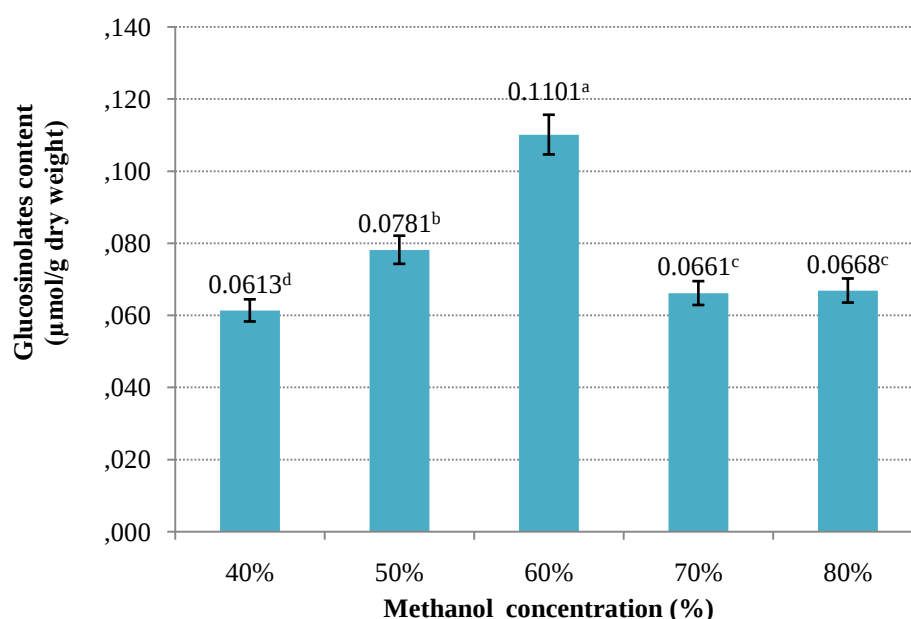


Figure 3. Effect of methanol concentration on glucosinolates contents of the extracts

Note: Values marked by different letters indicate significant differences ($p < 0.05$)

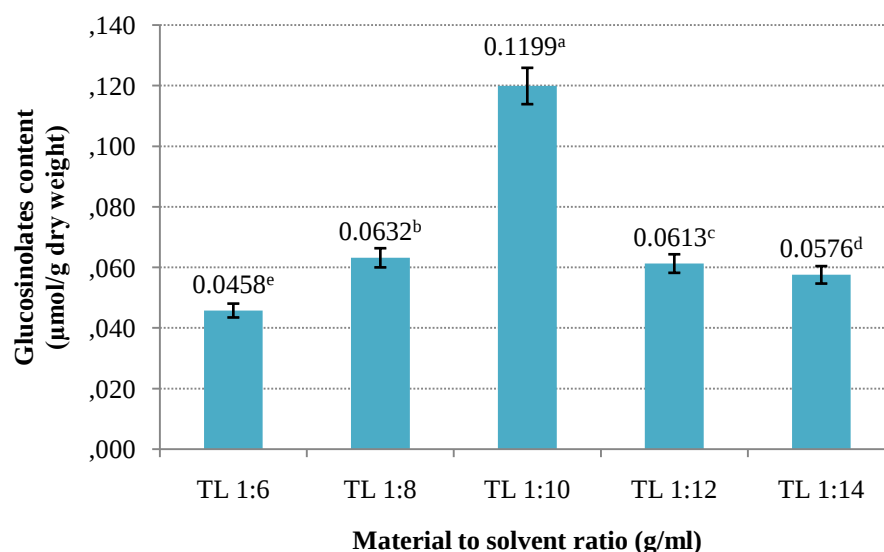


Figure 4. Effect of material to solvent ratio on glucosinolates content of the extracts

Note: Values marked by different letters indicate significant differences ($p < 0.0001$)

3.4. Effect of the ratio of material to solvent

The material to solvent ratio showed a significant effect ($p < 0.0001$) on the GSLs content in the extracts as shown in Figure 4. There was an increase of the GSLs yield from the cabbage by-product extracts when the material to solvent ratio (g/ml) increased. The material to

solvent ratio of 1:10 (w/v) showed the highest amount of GSLs and a further increase in material to solvent ratio 1:12 significantly decreased the level of GSLs in the extracts.

A high material to solvent ratio could promote an increased concentration gradient, producing a higher chance of bio-active

components coming into contact with the extraction solvent, resulting in an increase of the diffusion rate that allows for greater extraction of materials by solvent (Cacace and Mazza, 2003). These results were consistent with the mass transfer principle. However, active component yields will not continue to increase once equilibrium is reached (Herodež *et al.*, 2003).

Overall, the main effect of the material to solvent ratio was to modify the solubility and equilibrium constant, and thus, increase the total GSLs yields to the maximum at the most suitable material to solvent ratio. An equilibrium constant trend was observed at the material to solvent ratio of 1:10 (w/v) indicating a sufficient amount of extracting solvent was used in the extraction GSLs from the by-products of cabbage, and this ratio was chosen for the determination of extraction temperature and extraction time.

3.5. Effect of extraction temperature

The GSLs extraction yields as a function of the extraction temperature are shown in Figure 5. Results indicated that there was a significant

increase in the extraction of total GSLs when the temperature increased from 40 to 50°C, but further increases in temperature significantly decreased the level of GSLs in the extracts ($p < 0.0001$). This was due to the increased solubility and diffusion coefficient of the solutes, as well as enhanced mass transfer and penetration of the solvent into the plant matrix (Al-Farsi and Chang, 2007), thus, accelerating the whole extraction.

However, since 60% methanol (v/v) was used for the extractions in this study, the temperature should not exceed 65°C, the boiling point of methanol, since the evaporation of methanol from the aqueous methanol solution would decrease the concentration of aqueous methanol and that would lead to lower levels of extracted GSLs. Moreover, increasing of the extraction temperature would increase the extraction costs.

Considering the above facts, a moderate extraction temperature of 50°C was selected as the optimal extraction temperature for the subsequent steps due to practical and economical considerations.

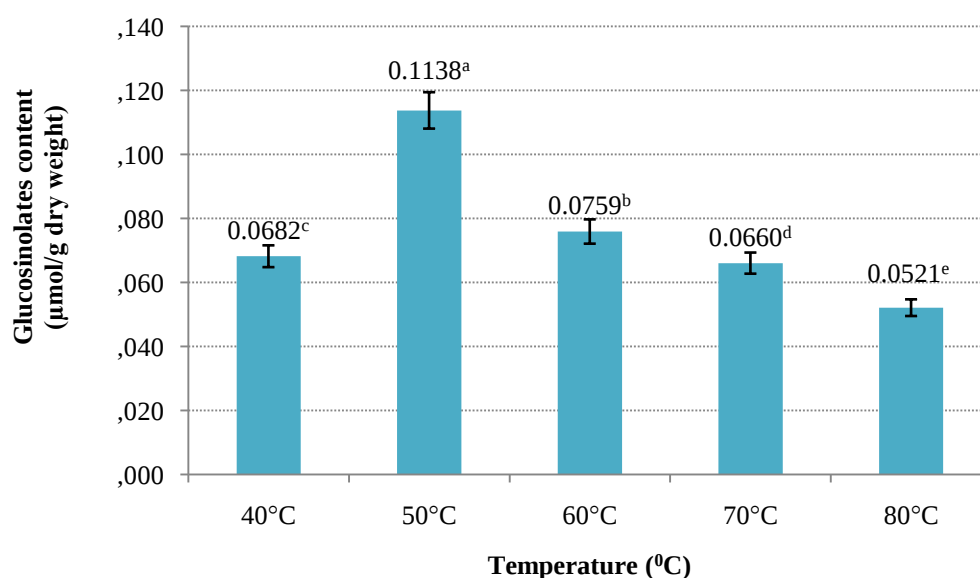


Figure 5. Effect of extraction temperature on glucosinolates contents of the extracts

Note: Values marked by different letters indicate significant differences ($p < 0.0001$)

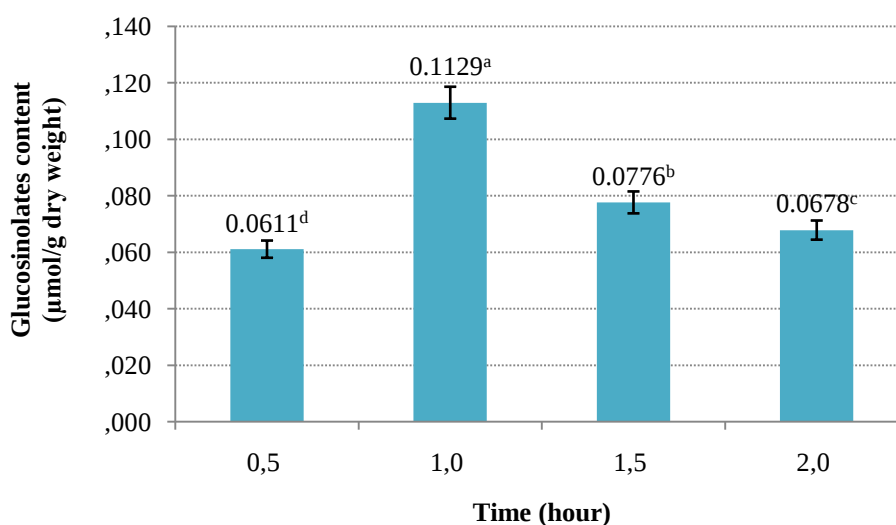


Figure 6. Effect of extraction time on glucosinolates contents of the extracts

Note: Values marked by different letters indicate significant differences ($p < 0.0001$)

3.6. Effect of extraction time

Extraction time is crucial in minimizing energy and costs of the extraction process. Figure 6 shows that the maximum concentration of GSLs was achieved with an extraction time of 1 hour. After this point, the GSLs contents decreased.

These phenomena could be well explained by Fick's second law of diffusion, which predicts that a final equilibrium between the solute concentration in the plant matrix and in the solvent might be reached after a certain time. An increase in the extraction time could increase the chance of oxidation of GSLs which would decrease the yield of GSLs in the extracts. It could also potentially increase the loss of the solvent by vaporization, which directly affects the loss of solvent to material ratio of the extractions. In addition, the increased extraction time is uneconomical and time consuming from the industrialization point of view. Thus, an extraction time of 1 hour was selected as the optimum point for extracting glucosinolates from by-products of cabbage.

4. CONCLUSIONS

This study demonstrates that is essential to systematically optimize the plant material

particle size, the extraction solvent composition, the material to solvent ratio, temperature, and time extraction for an easy and repeatable process of extracting GSLs that is suitable for production of food-grade GSLs. The GSLs yields of the extracts varied considerably as a function of plant material particle size, type of solvent (water, ethanol, or methanol), solvent composition (water/ organic solvent), extraction temperature, and extraction time. This study confirmed that using fine particle sized plant material from 0.5 to 1 mm, an aqueous solution solvent of methanol 60%, a material to solvent ratio of 0.1 g/ml, an extraction temperature of 50°C, and an extraction time of 1 hour were the most efficient for the extraction of GSLs from dry by-products of cabbage.

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