DOI: 10.31276/VJSTE.65(1).32-37

Effect of thermal treatments (roasting, microwaving, steaming) on anti-nutritional factors and physical and antioxidant properties of black gram (*Vigna cylindrica* L. Skeels)

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Received 17 June 2021; accepted 14 July 2021

Abstract:

The aim of this research was to investigate the effect of thermal treatments, including roasting, microwaving, and steaming, on anti-nutritional factors and physical and antioxidant properties of black gram (*Vigna cylindrica* L. Skeels). Regarding its physical properties, thermal treatment increased the water-holding capacity (WHC) and decreased the oil-holding capacity (OHC) of the black gram samples. No significant difference was observed between the roasted sample and the control. Regarding the chemical properties, anthocyanins accounted for the vast majority of total phenolic content (TPC). The steamed sample had the lowest TPC and anthocyanin values, which were 272.46 mg/100 g dry basis and 121.20 mg/100 g dry basis, respectively. Among the three types of thermal treatments, roasting resulted in the highest TPC content, which was 424.35 mg/100 g dry basis. Particularly, the steaming treatment led to the lowest anthocyanin content and antioxidant capacity (AC) in the flour sample; furthermore, there were no significant differences in the TPC and AC values between the microwaved and roasted samples. The phytate content was significantly reduced in all heat-treated samples and there were no significant differences among these cooked samples. The tannin content of the steamed sample was recorded to be the lowest, which was 164.19 mg/100 g dry basis. In summary, roasting, microwaving, and steaming significantly influenced the nutritive values of black gram (*Vigna cylindrica* L. Skeels).

Keywords: **anthocyanin, black gram, heat treatments, physical properties, phytate, tannin.**

Classification numbers: **2.2, 3.1**

1. Introduction

Black gram is widely cultivated in many areas with tropical climates like India, Asia, and Africa. Specifically, *Vigna cylindrica* is a breed of black gram that is broadly cultivated in Vietnam. Black gram is rich in protein and contains several components with desirable proportions including a low sodium content, high potassium content, complex carbohydrates, high fibre content, and a high content of essential, polyunsaturated fatty acids such as linoleic and linolenic acids with the ability to lower serum cholesterol in humans [1].

Despite having high nutritional values, the in-use proportion of nutrients in black gram is restrained by the presence of several anti-nutritional factors. According to H. Umaru, et al. (2007) [2], anti-nutritional factors (ANFs) can impair the function of the gastrointestinal tract, thereby interfering with the metabolic process. There are two types of ANFs that are the focus of this research: tannins and phytates. Tannins have been reported to block digestive enzymes and hence decrease the digestibility of most nutrients, particularly carbohydrates and proteins [3]. Meanwhile, phytates have been reported to bind with proteins and starch as well as crucial dietary minerals, thus lowering the bioavailability of these nutritional components in humans [4].

It is widely accepted that significant AC of food is related to high TPC [5]. Black gram has a rich content of antioxidants in its seed coat and contains high amounts of phenolic compounds that are believed to play an important role in promoting a healthy human diet [6]. This is especially true for anthocyanins, which are one

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subclass of phenolic compounds. These water-soluble pigments are also present in seed coat of black gram. The health-related functions of antioxidants are diverse. T.M. Djordjevic, et al. (2011) [7] demonstrated that antioxidants have the ability to reduce the occurrence of aging-related chronic diseases including heart disease and some types of cancer.

Black gram must experience an appropriate heat treatment before being consumed. Thus, this processing step results in changes to black gram's nutritional attributes, particularly, anti-nutritional factors and antioxidant properties. Therefore, this study was designed to compare the influences of different heat treatments on anti-nutritional factors like tannin and phytate contents, physical properties like oil and water holding capacities, and antioxidant properties of black gram. Comparisons among the three heat treatments could indicate which heat-applied treatment provides the most desirable results. Thus, this work can be used for further industrial scale and for potential health-concerned products on the Vietnam market.

2. Materials and methods

2.1. Materials and chemicals

Black gram (*Vigna cylindrica* (L.) Skeels) was purchased from the Big C Vietnam Supermarket in Ho Chi Minh city. All chemicals used for the project were supplied by several local distributors. For example, polyvinyl-polypirrolidone, sodium sulfate, hydrogen chloride, ferric chloride, sulphosalicylic acid, glycine, ethylenediamine tetraacetic acid, methanol, Folin-Ciocalteu reagent, sodium bicarbonate, gallic acid, potassium chloride, sodium acetate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Trolox were purchased from local chemical distributors.

2.2. Sample preparation

Unqualified beans were hand-removed, which included broken beans, damaged beans, and small wrinkled beans. Selected beans then were washed by tap water to completely remove foreign materials such as sand, dust, and soil.

2.3. Thermal treatment methods

Roasting: A coffee bean roaster model CBR-101 with a maximum chamber capacity of 0.6 lb/250 g was used. Selected beans were roasted for 10 min at 160°C and then were ground into flour [8]. This treatment was conducted with three replications. The final moisture content of the roasted beans was approximately 7%.

Microwave: Selected beans were soaked in tap water at room temperature for 18 h in a ratio of 1:5 (w/v) to reduce cooking time [8, 9]. They were then washed twice with tap water and left to drain in air. A microwave (Sharp R-209VN, China) was preheated to achieve standard uniform temperature by heating a porcelain bowl containing 300 ml of water for 30 s. The soaked beans were cooked at a power of 640 W for 5 min and then allowed to rest for 1 min in the microwave. The cooked gram was dried in the oven at 50°C for 24 h to reach a moisture content of approximately 9.5% before it was finally ground into flour. This treatment was also conducted with three replications.

Steaming: Black gram was soaked for 18 h and steamed at 100°C for 30 min [8]. The following steps, including draining, drying, cooling, and grinding, were carried out similarly to the microwave preparation. The moisture content of the dried samples was approximately 7-8%. The treatment was conducted with three replications.

Control sample: The washed bean was dried at 50°C for 1 h and ground into flour. The moisture content of the control sample was approximately 14%.

2.4. Analytical methods

The WHC and OHC of the black gram flour were both determined by the method of C. Chau and P. Cheung (1998) [10]. The TPC was evaluated using a Folin-Ciocalteu reagent by the method of V.L. Singleton and J.A. Rossi (1965) [11]. A spectrophotometric pH differential method was used to analyse the monomeric anthocyanin content of the black gram flour extract [12]. The AC was evaluated through DPPH radical scavenging capacity according to W. Brand-Williams, et al. (1995) [13]. Phytic acid content was determined by the colorimetric method according to R. García-Villanova, et al. (1982) [14]. Analysis of tannin content was carried out using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins [15].

2.5. Data analysis

Collected data was subjected to analysis of variation (ANOVA) using the SPSS software version 22.0. Tukey's test was used for multiple comparisons. Differences were deemed significant at the $p<0.05$ level.

3. Results and discussion

3.1. Phytate content and tannin content

At a first glance, the thermal treatments did not significantly affect the phytate content of the samples (Fig. 1). It is clearly observed that there were no significant differences in phytate content among the steaming, microwaving, and roasting treatments. Phytate content of the control sample was 15.31 mg/100 g dry solid and those of the heat-treated samples were 13.84 mg/100 g dry solid, 14.19 mg/100 g dry solid, and 13.91 mg/100 g dry solid for steaming, microwaving, and roasting, respectively. The observed reduction in phytate content of black gram during different heat treatments might be partly due to the heat-labile nature of phytate as well as the formation of insoluble complexes between phytate and other components [16]. For boiling and microwave, the loss of phytate content was also affected by the soaking period as phytate ions leach into the soaking water as a result of the concentration gradient.

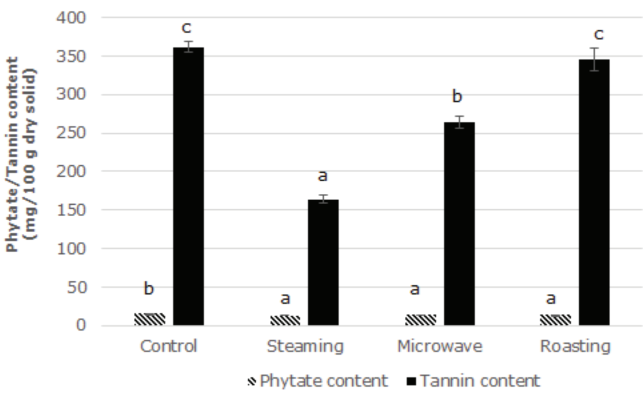

Fig. 1. Phytate content and tannin content of black gram flour after different heat treatments.

As for the values of tannin content, the steamed and microwaved sample experienced significant ($p<0.05$) reductions. However, there were no significant differences between the values of the raw and roasted ones. Particularly, the tannin contents of the control, roasted, steamed, and microwaved samples were 361.55 mg/100 g dry solid, 346.21 mg/100 g dry solid, 164.19 mg/100 g dry solid, and 264.68 mg/100 g dry solid, respectively. Thus, steaming caused the lowest value of tannin content, followed by microwave and roasting. The significant ($p<0.05$) reductions by steaming and microwaving could be explained by the nature of tannins. Tannins are mostly located at the seed coat [17] and are known to be polyphenols; moreover, all polyphenolic compounds are water-soluble [16]. Therefore, tannin losses may be caused by the leaching of tannins into soaking water under the influence of the concentration gradient [18].

The loss of tannin content was more obvious when black gram was cooked in a condition requiring a high amount of water like steaming. The explanation for this observation is clearly mentioned in a study by A.C. Laurena, et al. (1987) [19], which stated that the mechanism of polyphenol removal by steaming was similar to that by soaking in water, i.e., leaching. Furthermore, steaming provides a condition with a higher temperature, which aids the leaching process of polyphenolic compounds and causes it to occur at a faster rate. The steaming process also resulted in easier access to a protein and polyphenol interaction that promotes the inactivation of the latter due to the rapid disruption of cellular compartments. In this study, the total loss of tannin content in the steamed sample was 54.59%. Meanwhile, dry heat carried out by microwaving obtained a tannin loss of 26.79%. Roasting, however, only caused a 4.24% loss of tannin content. The reduction in tannin content of the microwaved and roasted samples agreed with earlier investigations of microwaved and roasted plant foodstuffs [20, 21]. Tannin losses during dry heat treatments might be due to the loss of compounds while treating at a high temperature. Furthermore, the loss of tannins may be due to a degradation or interaction with other components of the seeds, such as proteins, to form insoluble complexes [22].

3.2. WHC and OHC

Figure 2 indicates the effect of different heat treatments on the physical properties of black gram flour. As can be seen from the chart, the highest WHC was found for the steamed sample (2.36 g water/g flour) and the lowest WHC was found for the roasted sample (1.77 g water/g flour). There was no significant difference between the WHC values of the steamed sample and microwaved sample and these values were higher than that of the control sample. In previous studies, B.A. Acevedo, et al. (2017) [23] observed the same results on Dolichos bean and jack bean flours treated by steaming and microwaving. In general, these results are also supported by U. Singh (1988) [17], who demonstrated that during cooking and dehydration, protein denaturation and unfolding lead to the exposure of previously hidden peptide bonds and polar side chains, which can hold more water molecules. Meanwhile, the WHC of the roasted flour (1.77 g water/g flour) did not differ much from the control flour (1.61 g water/g flour). This result was explained by S. Ekanayake, et al. (2006) [24] as the degree of gelatinization (DG) of the roasted flour being lower than that of the wet-processed flours (soaked and cooked). Under low moisture content and high temperature, gelatinization of the roasted sample was considered more as a melting of crystallites. In other words, melting corresponds to the disappearance of native starch crystallinity at low hydration without a prior disappearance of granular shape.

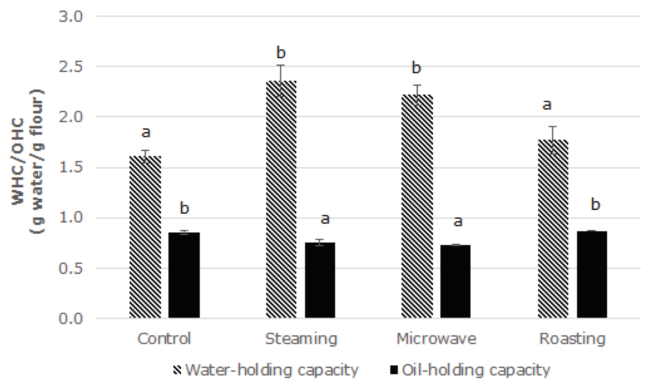

Fig. 2. WHC and OHC of black gram flour with different heat treatments.

Meanwhile, it was clear from the results that the OHCs of the steamed (0.76 g oil/g flour) and microwaved (0.73 g oil/g flour) samples were both lower than that of the control sample (0.85 g oil/g flour). Y. Aguilera, et al. (2009) [25] and K. Adebowale, et al. (2004) [26] demonstrated that the factor affecting OHC values of samples was the presence of the protein's non-polar side chains that bind with the hydrocarbon side chain of oil. When the protein was denatured by heat treatments, its secondary structure was lost, as well as the binding oil activity [27]. The results observed from this study were similar to the results of C.W. Hutton and A.M. Campbell (1981) [28] which indicated that the OHC of soya protein decreased after heat treatments were applied.

3.3. TPC and anthocyanin content

Compared to the control samples, all heat-treated samples had significant ($p<0.05$) decreases in TPC index (Fig. 3). It is explicitly observed that steaming caused the greatest TPC loss in the black gram samples. Particularly, the TPC of the control sample showed a value of 488.68 mg/100 g dry basis while 272.46 mg/100 g dry basis was obtained for the steamed one. This significant loss could be explained by B. Xu and S.K. Chang (2008) [29], who demonstrated that thermal processing caused water-soluble phenolics to leach out and some phenolic compounds to be broken down. In addition, during soaking, some polyphenols in the seed coat were hydrolysed and diffused into the soaking water, which reduced the TPC of the sample. As compared to microwave cooking and roasting, steaming required the longest time for black gram to be cooked well; moreover, a large amount of water was needed to perform the treatment successfully. The observed reduction in TPC could be mainly due to leaching of these compounds from the seed coat into the soaking or steaming water. Therefore, the boiling method was believed to be the most favourable condition for the reduction of TPC among the applied heat treatments. In the present study, it was found that about 44.25% of phenolics leached into the soaking and cooking water. Similar to the trend of steaming, microwave cooking was observed to cause a TPC reduction to 379.92 mg/100 g dry basis while roasting reduced the TPC value to 424.35 mg/100 g dry basis. Particularly, microwave cooking lost about 22.26% of TPC and roasting lost about 13.16% of TPC. The significant reduction in TPC level could be attributed to an increase in the release of phytochemicals from the cell matrix as phenolic acids. In other words, cell membranes and cell walls were disrupted during thermal processing, which caused a release of soluble phenolic components from insoluble ester bonds.

Similar to the trend of TPC shown in Fig. 3, the anthocyanin content of all the heat-treated samples were significantly reduced ($p<0.05$). The steaming treatment caused the highest total anthocyanin loss in black gram flour, followed by the two remaining thermal treatments. Particularly, the loss of anthocyanin content in the steamed sample was 64.53%, while the loss in the microwaved and roasted sample were 32.92 and 32.15%, respectively. The anthocyanin content for the steamed, microwaved, and roasted samples were 220.47 mg/100 g dry basis, 112.48 mg/100 g dry basis, and 109.85 mg/100 g dry basis, respectively. Similar results were found from yellow soybeans treated with steaming [29], roasting, and microwaving [30]. For the steamed sample, a high content of anthocyanin was lost as it diffused into the cooking water. Moreover, the treatment method required a water condition for black gram to be cooked, which made anthocyanin leach out during the process. These reasons could explain the lowest value of anthocyanin content reported in the steamed sample.

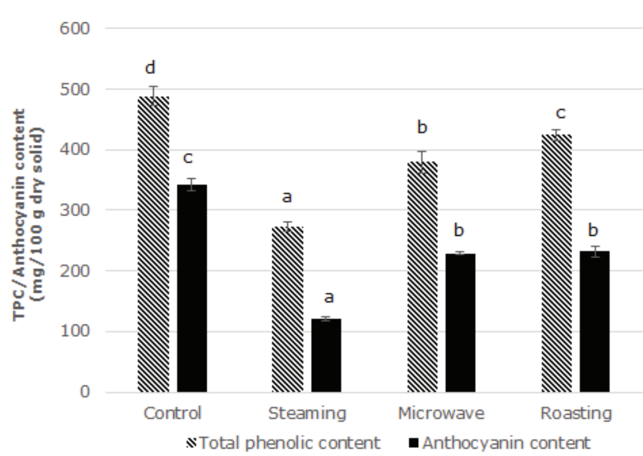

Fig. 3. TPC and anthocyanin content of black gram flour with different heat treatments.

3.4. AC

Under thermal processing conditions, the AC value of the control sample was significantly reduced ($p<0.05$) (Fig. 4). Previous studies reported similar results on roasted eclipse black beans (*Phaseolus vulgaris L.*) [29], roasted pinto and black beans (*Phaseolus vulgaris L.*) [31], and microwaved or roasted yellow soybeans (*Glycine max L.*) [30]. Particularly, steaming led to the lowest AC value in the flour sample with 157.30 mg/100 g dry solid, followed by microwaving with 281.33 mg/100 g dry solid, and roasting with 265.13 mg/100 g dry solid. Furthermore, there was no significant difference in AC between the microwaved sample and roasted sample. These results had a similar trend as the anthocyanin content shown in Fig. 3.

Moreover, as previously discussed about Fig. 3, anthocyanins accounted for the majority of the TPC in the black gram sample. Thus, the reduction in anthocyanin content after applying different heat treatments corresponded with the decrease of AC in the sample. In other words, cyaniding-3-glucoside was broken down via all three different cooking methods, which led to the reduction in the content of total phenolic acids as well as anthocyanins and, hence, the overall AC of cooked black gram samples decreased.

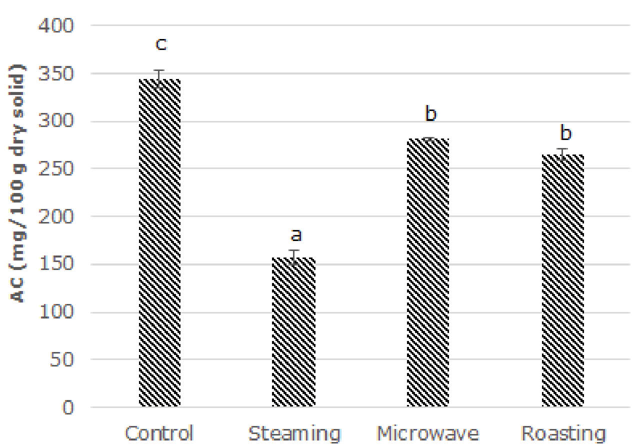

Fig. 4. AC of black gram flour with different heat treatments.

4. Conclusions

This study showed that roasting, microwaving, and steaming significantly affected anti-nutritional factors, as well as the physical and antioxidant properties, of black gram (*Vigna cylindrica* (L.) Skeels). For the most part, steaming reduced the anti-nutritional factors and antioxidant properties to the lowest levels while roasting caused the least decrease to these values. Therefore, the results from the three different heat treatments can be applied for further industrial scale and used for potential health-concerned products on the Vietnam market.

CRediT author statement

Ngoc Lieu Le: Conceptualization, Methodology, Supervision, Reviewing and Editing; My Thi Huyen Le: Experiment, Writing-Original draft preparation; Nguyen Hoang Khoa Nguyen: Data curation, Validation; Linh Tran Khanh Vu: Conceptualization, Writing- Reviewing and Editing.

ACKNOWLEDGEMENTS

This research is funded by Vietnam National University, Ho Chi Minh city under grant number C2019-28-08.

COMPETING INTERESTS

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

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