Development of Advanced Processing Techniques for Pigeon Pea Dal to Enhance *In Vitro* Protein Digestibility

Abstract

Pigeon pea (*Cajanus cajan*) is a nutritionally significant legume, rich in protein (18–25%), carbohydrates (57.6%), and essential micronutrients such as ascorbic acid, folic acid, niacin, and pantothenic acid. However, the presence of antinutritional factors such as polyphenols, phytic acid, and enzyme inhibitors adversely impacts protein digestibility and bioavailability. This study was conducted to develop advanced, energy-efficient thermal processing methods, including hydrothermal treatment, autoclaving, and infrared heating, to enhance the *in vitro* protein digestibility (IVPD) and reduce the polyphenol content of pigeon pea dals. Each treatment was applied at varying intensities, followed by different drying durations, and evaluated for moisture content, IVPD, and polyphenol levels. The moisture content across treatments ranged from 8.45% to 16.78%, with the lowest levels achieved under extended drying durations (5 hours for hydrothermal and infrared heating, and 8 hours for autoclaving). IVPD significantly increased with thermal exposure, reaching 82.81% in hydrothermal (HT30+DT5), 85.72% in autoclaving (AC60+DT8), and 85.12% in infrared heating (IRH30+DT5). Polyphenol content exhibited a decreasing trend with increasing duration of thermal treatment. This reduction is attributed to thermal degradation of polyphenols during treatment. Thermal processing disrupted protein matrices and degraded polyphenols, contributing to improved digestibility.

Keywords: Protein digestibility, polyphenol, autoclaving, infrared heating, hydrothermal, pigeon pea

1. Introduction

Pulses are the edible seeds of leguminous plants, such as chickpeas, lentils, peas, pigeon pea and beans, which are cultivated and consumed globally. They serve as vital food sources, contributing significantly to the nutritional quality of diverse human diets. Among the legumes, pigeon pea (*Cajanus cajan*) is an important legume crop, widely grown and consumed in the tropics and the semi-arid tropics of the world (Singh and Eggum, 1984). Pigeon pea contains a

significant amount of protein (18-25%), carbohydrates (57.6%) as well as other important nutritional compounds like ascorbic acid, folic acid, niacin and pantothenic acid (Pranati *et al*., 2024). In addition to its nutritional components, pigeon pea contains bioactive compounds such as polyphenols (0.3–1.83%), phytic acid (0.2–0.9%), and enzyme inhibitors, which can affect the digestibility and bioavailability of proteins (Gomezulu&Mongi, 2022; Kachare *et al*., 2018). Various processing techniques, such as heat treatments, soaking, dehulling, enzymatic hydrolysis, fermentation, and germination, have been shown to effectively reduce antinutritional factors in legumes and plant-based proteins that can otherwise hinder digestibility (Kalpanadevi & Mohan, 2013; Samtiya*et al*., 2020). Optimizing these methods and gaining insight into the biochemical interactions influencing pigeon pea proteins are essential for improving their nutritional and functional value for human consumption.

This study aims to develop advanced, energy-efficient processing methods to enhance the protein digestibility of pigeon pea dals. Three distinct thermal treatments—hydrothermal, autoclaving, and infrared heating—were applied at varying levels, followed by drying for different time durations. Post-treatment, moisture content, *in vitro* protein digestibility (IVPD), and total polyphenol content were analysed to assess the effectiveness of each processing method.

2. Materials and methods

2.1. Materials

Pigeon pea dal was procured from the supermarket in New Delhi. The chemicals utilized for the estimation of protein quality parameters such as Pepsin from Porcine gastric mucosa, pancreatin were obtained from Sigma-Aldrich (St. Louis, MO, USA), Gallic acid pure (98%) was purchased from G-Biosciences (USA).

2.2 Processing treatment

a. Hydrothermal treatment

Coarsely ground to particle size of 2.5 µm and steamed for 10, 15 and 30 min at 100°C. The treated dal was then dried using tray dryer (Fathom® Home Electric Dehydrator) for 3, 4 and 5 hours.Treatments are namely, HT10+DT3, HT10+DT4, HT10+DT5, HT15+DT3, HT15+DT4, HT15+DT5, HT30+DT3, HT30+DT4 and HT30+DT5.

b. Autoclaving treatment

Previously soaked (12h) dal were autoclaved for 15, 30 and 60 min 15 psi and 121°C. After the treatment the dal was dried using tray dryer (Fathom® Home Electric Dehydrator)for 5, 6 and 8 hours. Treatments are namely AC15+DT5, AC15+DT6, AC15+DT8, AC30+DT5, AC30+DT6, AC30+DT8, ACT60+DT5, AC60+DT6 and AC15+DT8.

c. Infrared heating

Previously soaked (12 h) dals spread in a uniform 0.5 cm thick layer and placed 11 cm below an NIR lamp with a power of 150 watts having a wavelength (0.7–1.0 µm); density of 480 watts m^2 and temperature 40°C was maintained in the chamber for 10, 15 and 30 mins. Following the treatment, the dal was dried using tray dryer (Fathom® Home Electric Dehydrator) for 5, 6 and 8 hoursfor 3, 4 and 5 hours. Treatments are namely, IRH10+DT3, HT10+DT4, IRH10+DT5, IRH15+DT3, IRH15+DT4, IRH15+DT5, IRH30+DT3, IRH30+DT4 and IRH30+DT5.

2.3. Determination of moisture content

Moisture content of the sample was determined using Kern MLS 50-3D Electronic Moisture Analyser (*Kern & Sohn GmbH.*)

Percentage moisture = (Loss in weight due to drying/Weight of original sample) x 100

2.4. *In vitro* protein digestibility (IVPD) determination

In vitro protein digestibility (IVPD) was assessed using the method described by Vitali et al. (2009), with slight modifications. In summary, 0.5 g of the flour sample was mixed with 12.5 mL of a trypsin solution (0.5 mg/mL pepsin dissolved in distilled water; pH 2) and incubated at 37 °C in an incubator shaker (Orbitek, Scigenics Biotech) for 2 hours. The solution was then neutralized to pH 7 using 6 M NaOH, followed by the addition of 2 mL of pancreatin solution (5 mg/mL pancreatin prepared in phosphate buffer, pH 8.2). The mixture was further incubated at 37 °C with continuous shaking for 24 hours. To terminate the enzymatic reaction, 7 mL of 10% TCA was added, and the sample was centrifuged at 4100 × g for 20 minutes. The residue was collected, washed, and analyzed for protein content using the Kjeldahl method (AOAC, 2006). The protein content in the supernatant was determined by subtracting the protein content in the pellet from the total protein content of the sample. The IVPD was determined using the formula:

IVPD = (Protein content in the supernatant / Protein content in the sample) ×100

2.5. Polyphenol extraction:

Polyphenol was extracted using method given by Fratianni *et al.* (2014). Briefly, polyphenols were extracted using Sample were homogenized with water in a 1:1 ratio (w/v). After 2 hours of incubation, four volumes of acetone were added. The mixture was incubated at 4 °C for 24 hours and centrifuged at 11,600 × g. The resulting supernatants were collected and stored at 4 °C. The pellets were further treated with one volume of acetone, followed by a 1-hour incubation at 4 °C. The supernatants from both extractions were combined, filtered, and completely evaporated before being stored at −20 °C in the dark until further analysis.

2.6. Total polyphenol content estimation

The total phenolic content was determined using the Folin–Ciocalteu reagent, following the method of Singleton and Rossi (1965). Absorbance was measured at 760 nm at room temperature using UV/Vis spectrophotometer. Quantification was based on a standard curve prepared with gallic acid, and the results were expressed as micrograms of gallic acid equivalents (GAE).

2.7. Statistical analysis

All assays and experiments were conducted in triplicate, and the results were reported as the mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA (Analysis of Variance) in IBM SPSS Statistics 19 (SPSS Inc., USA). Significant differences were evaluated using a t-test and considered significant at a 5% level ($p < 0.05$).

3. Results and discussion

3.1. Moisture content

The moisture content of treated and dried dal was determined and the values are represented in Figure 1. The moisture content was found in the range of 8.48±0.21-16.78±0.18 % in hydrothermal treatment, 9.43±0.17-15.12±0.03 % in autoclaving treatment and 8.45±0.05-13.87±0.19% in infrared heating. The results revealed that in case of different level of hydrothermal treatments and infrared heating treatment, drying condition of 5 hours achieved significantly ($p< 0.05$) the lowest values of moisture content. Similarly, in case of autoclaving, drying condition of 8 hours achieved the lowest moisture content at different level of autoclaving condition.

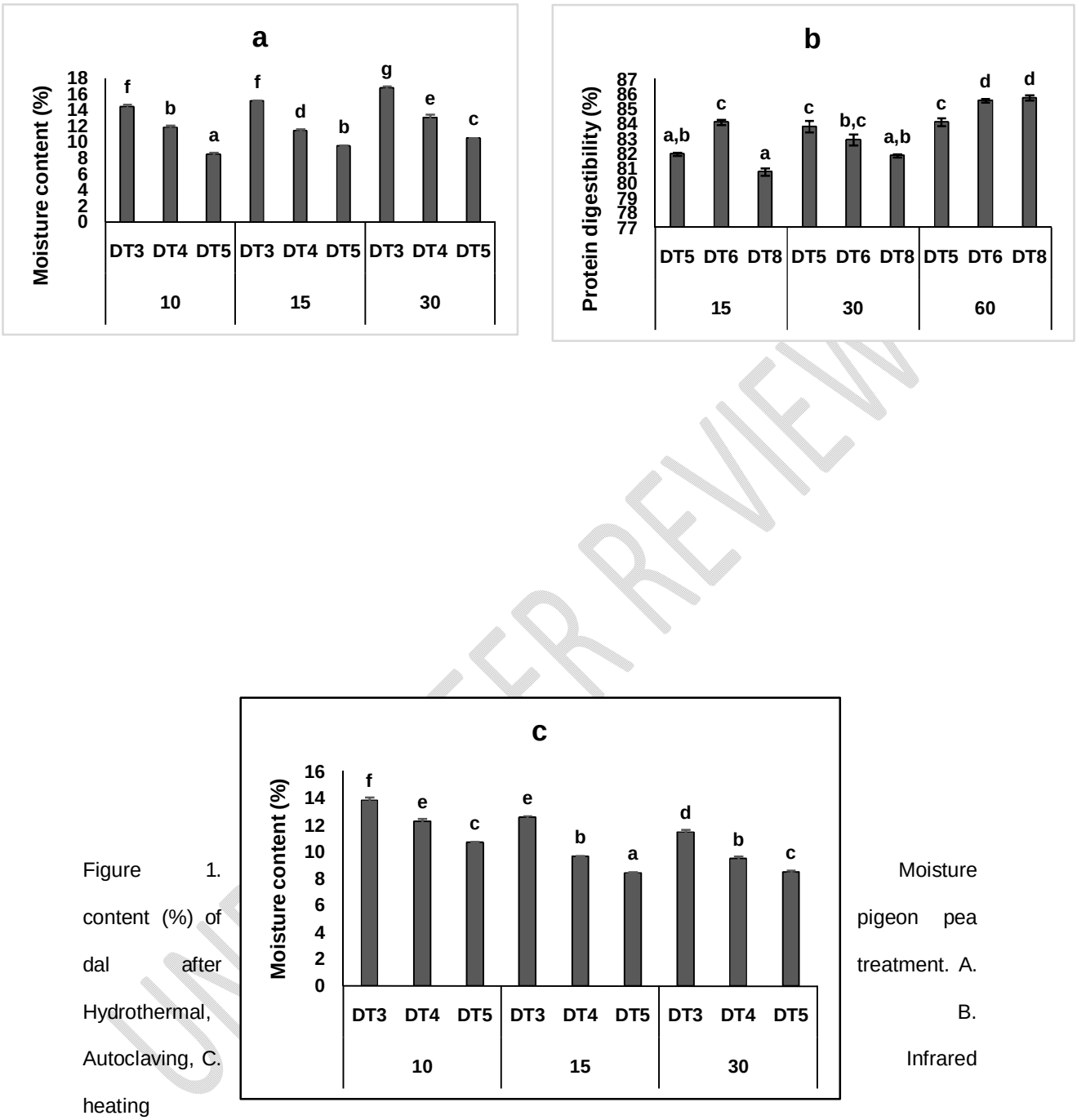
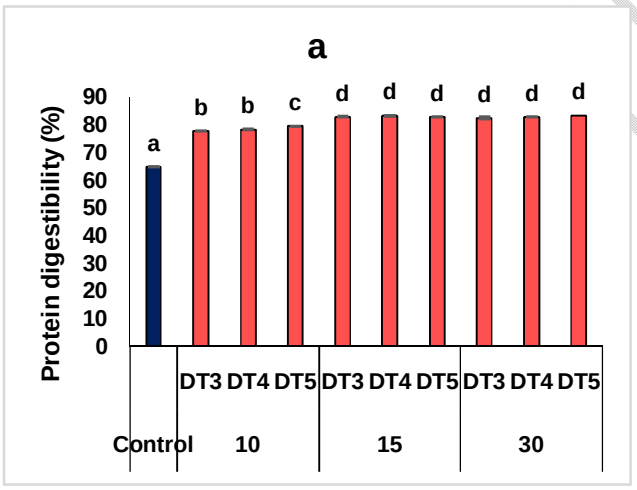


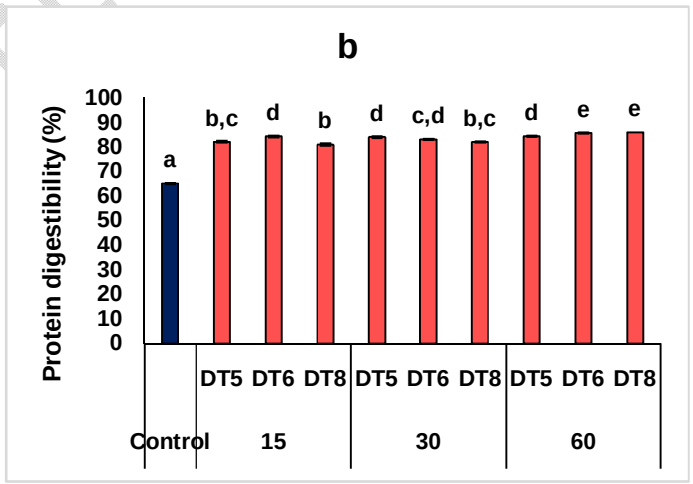
Figure 1. Moisture content (%) of pigeon pea dal after Hydrothermal, B. Infrared Autoclaving, C. heating treatment. A.

3.2. In vitro protein digestibility (IVPD)

The *in vitro* protein digestibility of the pigeon pea dal was determined after different level of hydrothermal, autoclaving, infrared heating treatment followed by different level of drying time and the results are represented in Figure 2. The results revealed the IVPD in the range of 77.72±0.15-82.81±0.11 % for different levels of hydrothermal treatment. The highest digestibility was found in

case of HT30+DT5. However, there was no significant difference was observed between HT15 and HT30 at different levels of drying time. In case of autoclaving treatment, the IVPD was found in the range of 80.75±0.25-85.72±0.12%. The significantly highest IVPD was observed in AC60+DT8. However, there was no significant difference was observed between AC60+DT6 and AC60+DT8. Whereas, in case of infrared heating the IVPD was observed in the range of 80.42±0.33-85.12±0.19%. The highest increase in IVPD was observed in IRH30+DT5. However, there was no significant difference was observed in IVPD in IRH15+DT5 and IRH30+DT5. It was also observed a increase in IVPD with increasing duration of thermal treatment. This improvement can be attributed to the disruption and loosening of protein matrices, which enhanced the accessibility of protease enzymes, leading to a significant increase in IVPD. Similar enhancements in IVPD following thermal processing have been reported by Embaby (2010), Park *et al*. (2010), and Rehman & Shah (2005) in various legumes and seeds, including peanuts, sesame seeds, peas, black grams, chickpeas, lentils, and red and white kidney beans. These studies observed improved digestibility after different thermal treatments such as boiling, autoclaving, microwave cooking, and roasting.





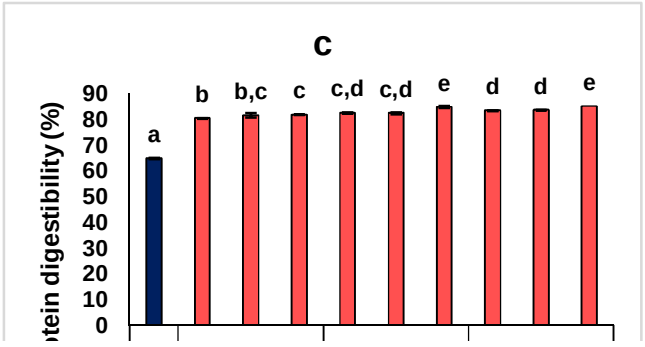


Figure 2. IVPD (%) of pigeon pea dal after thermal treatments. A. Hydrothermal treatment, B. Autoclaving, C. Infrared heating

3.3. Total polyphenol content

Polyphenols are secondary metabolite found in legumes which was reported to bind with proteins and hinder protein digestibility (Lamothe et al., 2014). The total polyphenol content in the hydrothermal, autoclaved and infrared heated samples were determined. The polyphenol content was found in the range of 60.96±0.35-73.08±0.53 mg GAE/ 100g for hydrothermal treatment, 43.34±.0.99-53.93±0.60 mg GAE/ 100g for autoclaving and 61.70 ± 0.049-70.89±0.31 mg GAE/ 100g for infrared heating. The decrease in total polyphenol content after the treatments is shown in Figure 3. In case of hydrothermal the highest decrease was observed in HT30+DT4, however, there was no significant difference was observed between HT15 and HT30 as well as different levels of drying. Whereas in autoclaving treatment AC60+DT6 showed the lowest content of polyphenol. In case of infrared heating, IRH15+DT5 showed the lowest content of polyphenol. The results revealed a decreasing trend of polyphenol content with increase in exposure of thermal treatment. This decrease in polyphenol content can be explained by thermal degradation of the polyphenol compound with exposure to high temperature for longer duration. Similarly, decrease in polyphenol content after thermal treatment were reported earlier in different studies. Djabali*et al*. (2020) reported a decrease in total polyphenol compound in lentil after heat treatment. Rocchetti*et al*. (2017) reported a decreased level of polyphenol in dry pasta after cooking.

From the findings it was also observed a negative correlation between IVPD and total polyphenol content. With increasing exposure to thermal treatment decreased polyphenol content however increasing the IVPD. This negative correlation can be explained by the negative impact of polyphenol on protein digestibility. Polyphenols can bind with proteins as well as proteases and hence can potentially reduce the protein digestibility (Velickovic and Stanic-Vucinic, 2018).

Thermal treatment possibly caused degradation of the polyphenol compound and as well as interaction of polyphenol with proteins and in turn increased the IVPD.

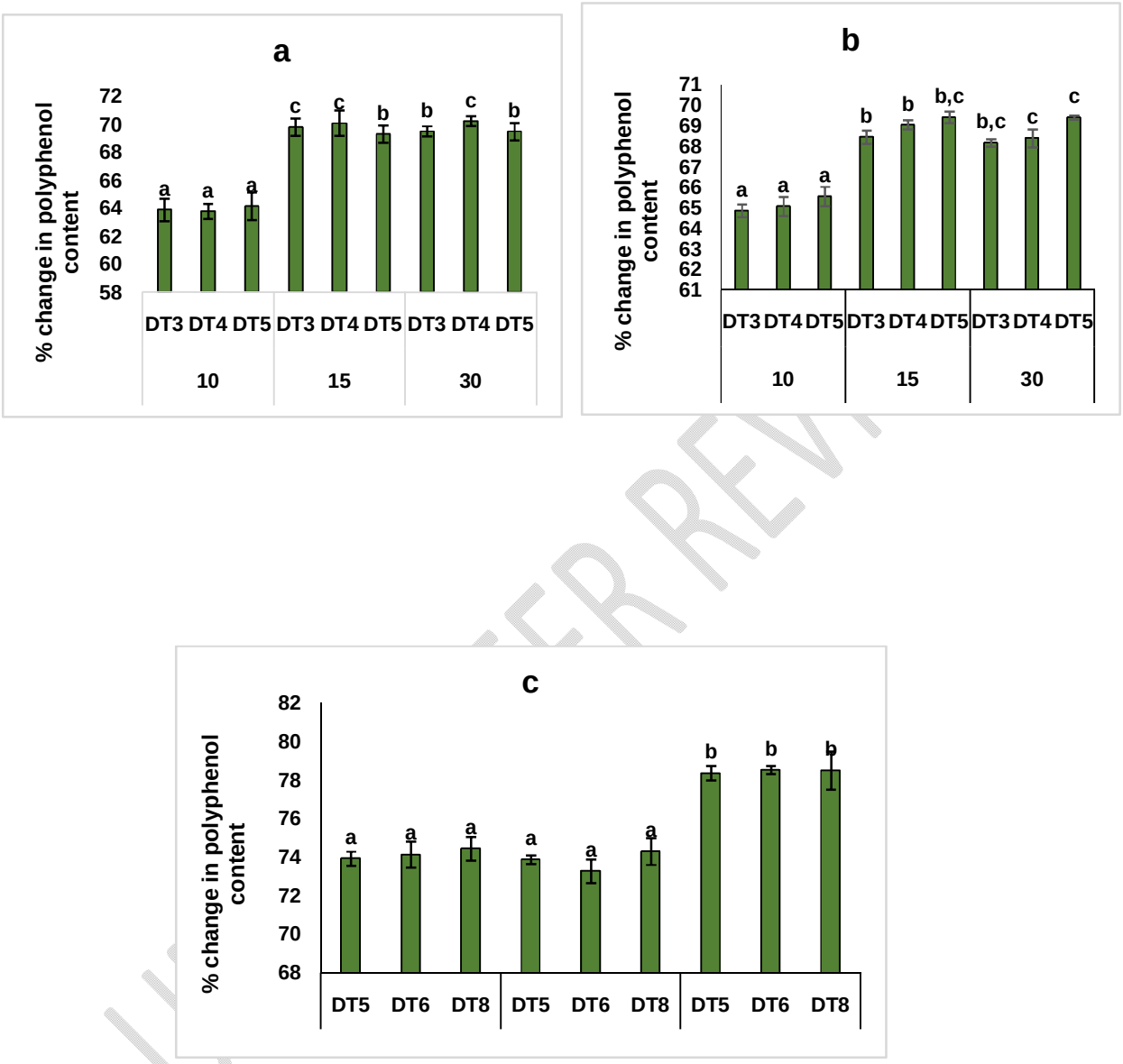


Figure 3. Percent change in total polyphenol content after thermal treatments. A. Hydrothermal B. Autoclaving, C. Infrared heating.

3.4. Conclusion

The study demonstrated that thermal processing significantly enhanced the protein digestibility of pigeon pea dal while reducing its polyphenol content. Among the hydrothermal treatments, the HT15+DT3 method was identified as the most energy-efficient, yielding the highest IVPD and the lowest polyphenol content. Similarly, for autoclaving and infrared heating treatments, the methods AC60+DT6 and IRH15+DT5, respectively, proved to be the most efficient, achieving improved IVPD and reduced polyphenol levels. Furthermore, all these treatments maintained a moisture content below 14%, making them suitable thermal processing techniques for enhancing the protein digestibility of pigeon pea dal.

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