**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**

**Faculty of Tropical AgriSciences**

# Effect of Degradation of Antinutrient Factors in Runner Bean (*Phaseolus coccineus*) Seed by Thermal and Nonthermal Treatment

MASTER'S THESIS

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## Declaration

I hereby declare that I have done this thesis entitled Effect of Degradation of Antinutrient Factors in Runner Bean (*Phaseolus coccineus*) Seed by Thermal and Nonthermal Treatment independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to the Citation rules of the FTA.

In Prague  2024

Ibitayo Esther Adeniran

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**Abstract**

Runner bean (*Phaseolus coccineus*) is a rich source of nutrition but are often limited by antinutrient factors like trypsin inhibitors, phytic acid, saponins, and α-galactosides, which reduce their nutritional value and consumer appeal. This research aims to address this by studying the effects of thermal and non-thermal treatments on antinutrient reduction in runner bean seeds. Specifically, it examines the impact of treatments on antinutrient levels, liquid-solid ratios in boiling, and functional properties of white and black runner bean seeds. Various parameters, including tannin content, phytic acid concentration, and saponin analysis, were assessed using established methodologies, and the data were subjected to analysis of variance using GENSTAT, 8edition 2016. Least significance LSD was also used to compare treatment means ($P < 0.05$).

Initial tannin concentrations decreased post-soaking, from 1.45 mg/ml to 0 mg/ml for white seeds and from 39.41 mg/ml to 23.93 mg/ml for black seeds. Saponin levels also decreased significantly during soaking, with concentrations dropping from 0.44 mg/ml to 0.04 mg/ml for white seeds and from 0.07 mg/ml to 0.02 mg/ml for black seeds.

Additionally, there was a notable decrease in phytic acid concentration after soaking, particularly at higher soaking ratios. For unsoaked seeds, the phytic acid concentration was 3.84 mg/ml for white seeds and 3.76 mg/ml for black seeds, while after soaking at a ratio of 5:1, these concentrations decreased to 2.47 mg/ml for white seeds and 2.68 mg/ml for black seeds. This study underscores the effectiveness of soaking in reducing antinutrient content, thereby enhancing the nutritional quality of runner beans.

The functional properties of runner bean seeds that indicate their water absorption, foaming, and emulsification capacities influenced by processing techniques were also explored. These findings are valuable for the food industry, offering insights into the development of bean-based products with enhanced nutritional profiles and functional attributes.

**Keywords:** Runner bean, Antinutrients, thermal and non-thermal treatment, mean ± standard deviation, food industry, tannins, phytate, saponin, trypsin inhibitors, analysis of variance.

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**List of abbreviations**

ABS: Absorbance

CIAT: International Center for Tropical Agriculture

CONC:Concentration

DW Distilled water

EC: Emulsifying Capacity

FC: Foaming Capacity

ha: Hectare

HCl Hydrochloric acid

IPK: Leibniz institute of plant genetics and crop plant research

LSD: Least significant difference

OAC:Oil absorption capacity

ONU: United Nations Organization

UN: United Nations

USDA:United States department of agriculture

WAC: Water Absorption Capacity

WHC: Water Holding Capacity

WSI: Water Solubility Index

# 1 Introduction

The United Nations (UN) predicts that more than a billion people will be added to the world's population during the next 15 years, bringing it to 8.5 billion in 2030 and 9.7 billion in 2050 (ONU 2015). Numerous factors such as socioeconomic changes, expansion of cities, and awareness of the need for protein in a balanced diet, are responsible for the expanding global demand for protein. Conversely, certain factors lower demand, such as an increased understanding of how food production and consumption affect the environment and public health (Goldstein & Reifen 2022).

Since the dawn of agriculture, cereals, starchy roots, and fruit which are poor sources of protein have made up most of the human diet. These categories of food crops have mostly comprised the human diet throughout recorded history, and they continue to do so today (Lam & de Lumen 2003). Legumes are the second-largest family in terms of human significance, behind Gramineae. 27 percent of the world's primary crop production is made up of grain and forage legumes, and just grain legumes contribute 33 percent of the human body's requirement for dietary protein nitrogen. Because of the 20–50% protein content of legume seeds, there is growing interest in using proteins from various legumes. Protein from legume plants like soybeans, and Glycine max, are almost exclusively employed in the food industry, whereas other legume species are used less frequently. Pea (*Pisum sativum*), broad bean (*Vicia faba*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), *Lupinus* spp., and chickpea (*Cicer arietinum*) are the other major grain legumes. They are typically intercropped with cereals to increase crop yield, increase nitrogen use efficiency, and decrease weed infestation (Mondal et al. 2015).

Grains and forage legumes are farmed on 12 to 15 percent of the Earth's arable land which is over 180 million ha. They produce 27% of the world's principal crops, with grain legumes alone meeting 33% of the world's population's requirements for dietary protein and nitrogen (Van Kessel & Hartley 2000). More than 35% of the processed vegetable oil consumed worldwide comes from legumes, mostly soybean (*Glycine ma*x) and peanut (*Arachis hypogeae*), which are also significant sources of dietary protein for the chicken and pork industries. The massive growth in soybean production in Brazil, with national mean soybean prices, demonstrates the promise of legume crops (Lam & de Lumen 2003).

Many nations and cultures cultivate and use legumes, which raises the possibility that they may have developed to supplement grains by providing the proteins and other nutrients that humans need. Legumes are often characterized by their distinctive floral structure, podded fruit, and the ability of 88 percent of the species investigated to create rhizobial symbioses, allowing nitrogen fixation and improving soil fertility and crop productivity (Graham & Vance 2003).

Legumes are the main source of nutrition for a sizable portion of the human population. A type of nutrient-dense food that is low in fat, high in protein, and rich in minerals and complex vitamins, common beans are one of the most significant crops in the world. However, a significant number of antinutrients present in legumes compromise their nutritional value (Sharma 2021).

Consuming legumes that have not been properly processed may alter normal metabolism and have detrimental consequences on human health. Before consumption, effective processing is required to remove these antinutritional components. Reducing antinutrients during the production of traditional dishes based on legumes may be accomplished by employing response surface methods to optimize the processing factors. The toxins contained in beans may result in neurological symptoms such as limb numbness, headache, pressure in the chest, and even severe coma, among others (Shang et al. 2016).

According to Lopez-Martinez (2020) phytic acid, lectins, trypsin inhibitors, polyphenolic compounds, and common bean fiber have been shown to have significant biological effects, including promoting the development of bifidobacteria in a product or within people's or animals' intestines.

Common beans are a type of nutritious meal that is low in fat, high in protein, and rich in minerals and complex vitamins. They are one of the most significant crops in the world. However, even though the endogenous poisons are rendered inactive by the high temperatures used in cooking, intoxication incidents still occur because of this substance.

Runner bean seed contains nutritional and antinutritional factors. These antinutritional factors like tannin, phytate, oxalate, and trypsin inhibitors reduce food intake and nutrient utilization. Oxalate, phytate, tannin, and trypsin inhibitors are major antinutritional factors in legumes (Ojiako & Igwe 2008). Tannin has a cross-link with proteins and causes a reduction in protein digestion of beans.

Oxalate is known to reduce calcium absorption, and its salts are poorly soluble at gut pH, complexes are created when oxalate binds to calcium (calcium oxalate crystals). These oxalate crystals impede the body from absorbing and using calcium (Mohan et al. 2015). This study sets out to assess the Degradation of Antinutrient Factors in runner bean *(Phaseolus coccineus)* Seed by thermal and non-thermal treatment.

# 2 Literature Review

Legumes are the seeds of dicotyledonous plants in the Leguminosae family. These have been successfully used and are widely dispersed over the world. They are categorized as grain legumes and oily yielding legumes according to consumption patterns. Pulses and dry grains of peas, chickpeas, lentils, peas, beans, and lupins are used as grain legumes, whereas soybean, peanut, groundnut, and alfalfa are used for oil extraction. (Sharma 2021) Since ancient times, legumes have been significant crop plants, used to both provide food and improve the soil.

The bean is the most effective at reviving the soil, according to Theophrastus (370–285 B.C.). Because the plant grows loosely and rots rapidly, beans are not a burden to the soil; in fact, they seem to manure it (Van Kessel & Hartley 2000). The starch-rich, tubberized taproot of the runner bean has nodules and vegetative buds in the zone of the neck. The 2-7 m stem is strong, robust, and somewhat twisted, and it fits well with pole guidance (Schwember et al. 2017).

After the common bean *(Phaseolus vulgaris*) and the lunatus (*Phaseolus lunatus*), the (scarlet) runner bean (*P. coccineus* L.) is most likely the third-most significant *Phaseolus* specie (Schwember et al. 2017) this climbing perennial is cultivated as an annual vegetable for its immature green pods. Plants are summer-appropriate long-day twiney vines. They originate from South or Central America, the regions where runner beans first appeared (Kalloo, 1993).

The function of beans in the human diet has recently drawn attention for their protein content as well as their functional qualities, and some writers have claimed that their consumption may help lower the risk of obesity, diabetes, cardiovascular disease, colon, prostate, and breast cancer (De Ron et al. 2016).

*Phaseolus coccineus*, also known as runner bean in English, ayocote bean in Spanish, and benibana-ingen in Japanese, it is a legume whose origin and domestication are best determined by archeological, historical, and botanical data In the Puebla, Oaxaca, and Chiapas states in Mexico's highlands. The economic significance of runner bean is only in some parts of the world like Central America, South America, Africa, and Europe, it is of less economic significance in the United States (Sinkovič et al. 2019). According to the large number of registered cultivars used as either food sources or ornamentals, the United Kingdom looks to be the primary grower when compared with other countries (Rodiño et al. 2007). Scarlet runner beans are often produced in the UK because they are more suited to the cool climate than ordinary beans and they consistently yield a harvest of green beans suitable for commercialization (Schwember et al. 2017).

This species has several advantageous agronomic characteristics, including disease resistance, lodging resistance (due to strong stem bases), cold tolerance, lengthy epicotyls and racemes, and the existence of a tuberous root system that allows a perpetual (Santalla et al. 2004)

2.1 Uses, botanical and anatomical characteristics of Runner Bean (*Phaseolus coccineus)*

The Phaseolus genus includes more than 70 species, all of which are native to the Americas, as well as five domesticated taxa, *P. vulgaris L.*, *P. lunatus L.*, *P. acutifolius A. Gray, P. coccineus L., and P. dumosus,* each of which has a unique geographic distribution, life history, and reproductive system (Schwember et al. 2017). Gregor Mendel was the first researcher to conduct crosses with runner bean, which was known as *Phaseolus multiflorus* at the time. Mendel also conducted crossings with runner bean and *Phaseolus vulgari*s in his pea cross-breeding experiments (Mendel, 1866).

*Phaseolus coccineus* and *Phaseolus costaricensis* are closely related to each other as well as to *Phaseolus dumosus Macfad* commonly known as runner bean (Schwember et al. 2017). These two species can hybridize naturally, and *P. coccineus* has produced hybrids with both of them. Common bean can be crossed with *P. coccineus*, *P. dumosus*, and *P. costaricensis* as the female parent, albeit the progeny is only partially viable and fertile depending on the parental combinations (Singh & Schwartz, 2011).

2.2 Collections of Genetic Material of Runner Bean (*Phaseolus coccineus)*

About 4685 *P. coccineus* accessions have been documented worldwide (Schwember et al. 2017).With around 36,000 *Phaselous spp*. accessions to date, representing 44 taxa from 110 countries, the International Center for Tropical Agriculture's (CIAT) Germplasm Bank and Genetic Resources Program in Cali, Colombia, maintains the largest and most diverse collection of beans in the world. It also contains about 958 accessions of *Phaseolus coccineus* (CIAT 2015). The Agricultural Institute of Slovenia (AISLJ) preserves 995 runner bean accessions (Mkwaila et al. 2011). In addition, the National Germplasm Bank of INIFAP (Instituto Nacional de Investigaciones Forestales, Agrcolas y Pecuarias) in Chapingo, Mexico, holds 798 accessions of the Mexican *P. coccineus* collection. (Rodiño et al. 2007). Additional organizations that conserve P. coccineus include the United States Department of Agriculture (USDA) with 374 accessions, the Leibniz Institute of Plant Genetics and Crop Plant Research

(IPK) in Germany with 439 accessions, and other germplasm repositories in Europe and South America (Santalla et al. 2004).

## 2.3 Agronomic characteristics of Runner Bean *(Phaseolus coccineus)*

### 2.3.1 Production techniques of Runner Bean

According to Graham and Vance (2003) there are five general categories of cropping systems for *Phaseolus coccineus.*

- Bush or (rarely) climbing bean crops as the only crops.
- Relay the intercropping of maize with bush, indeterminate, or climbing beans. In such setups, the maize primarily serves as an inanimate support and the beans are typically sown after the maize is physiologically ripe.
- Bush or indeterminate beans are inter-planted in rows with maize. Although planting is frequently done simultaneously, certain regions have maize and bean planting dates that range by up to 30 days.
- Maize and beans with growth habits III or IV intercropped together. In these systems, maize and beans are frequently planted simultaneously into the same hill.
- Beans can be inter-planted with other grains, as well as with bananas, cassava, coffee, or sugarcane. Such intercropping frequently serves as a means of generating income while waiting for the main crop to mature.

### 2.3.2 Management techniques of Runner Bean

The runner bean is grown on trellises, poles, fence lines, plants (such as maize), or other support structures to produce high-quality pods (Damme et al. 2007). If the leading branches of climbing (indeterminate) varieties of runner beans are nipped out to encourage bushy growth, they can yield without assistance (Schwember et al. 2017). Three runner bean ecotypes were tested in Chile with and without trellis support; without support, seed output reached 3.4 t/ha, yielding just a little less than common bean and runner bean with trellis support. (Tay et al. 2000). These scientists concluded that 153 kg/ha was the most cost-effective seeding rate and suggested sowing seeds with a 20 cm intra-row and 60 cm inter-row spacing (corresponding to 83,300 plants/ha).

### 2.3.3 Intercropping of runner bean seed

In order to maintain sustainability in agricultural ecosystems, it is crucial that such farming systems remain profitable and environmentally friendly with higher producing nutritious foods and higher economical value (Javanmard et al. 2020). Intercropping is the practice of growing many crops concurrently in the same field at the same time. Small-scale farmers demonstrated that adding three different climbing beans, including *Phaseolus coccineus* to maize boosted crude protein while also affecting the fermentation profile and fiber concentration. However, maize-bean silage's nutritional value was comparable to that of maize silage, showing that maize-bean silage mixtures might be employed in dairy cow feeds.

## 2.4 Nutritional Profile of runner bean seed (*Phaseolus coccineus)*

Proximate Composition**:** This is known as the expected structure; it is used to describe the main components that make up *Phaseolus coccineus* a food mass. This also includes the content of ash and moisture in the crop that accounts for different sets of the variable from reported macronutrient quantities, they are designated as proteins, carbohydrates, fat, and fiber (Lopez-Martinez 2020).

Carbohydrates**:** *Phaseolus coccineus* is an excellent source of carbohydrate 53–68%). This legume makes up the majority of grains and its major constituent is dietary fiber constituting of starch and non-starch polysaccharides, with lower but still important amounts of oligosaccharides. The starch in this legume is acceptable for ingestion by diabetic patients and those at a high risk of developing diabetes since it digests more slowly than starch from cereals and tubers and has a low glycemic index (GI) rating for blood glucose management (Lopez-Martinez, 2020).

Oligosaccharides: It is well-recognized that pulses contain important components of oligosaccharides. Important oligosaccharides are -galactosides such as verbascose, raffinose, and stachyose (tetrasaccharides) (pentasaccharide). According to Kosson (2014), it was detailed that stachyose was the main galactooligosaccharide of runner bean from 2.29 to 2.50 g 100 g1 followed by raffinose (from 0.29 to 0.51 g 100 g1) and low amounts of verbascose (0.04 g 100 g1).

Proteins: According to Lopez-Martinez (2020), legumes in general are known to be excellent sources of quality protein (17 to 30%)., this quality is linked to the ability of legumes to fix

Nitrogen into the soil through nitrogen-fixing bacteria in their roots that converts excess Nitrogen to ammonium.

Minerals: Minerals are known to maintain the body's fluid osmotic equilibrium, lower blood pressure, control excitability in muscles and nerves, and increase the normal retention of protein throughout growth (Mondal et al. 2015). According to Lopez-Martinez (2020), potassium is the most prevalent mineral in *Phaseolus coccineus* seed. It was followed by magnesium and calcium, while copper was the least abundant mineral.

### 2.4.1 Bioactive Components and Antinutrients

Polyphenols: The presence of polyphenolic chemicals affects the color of bean seeds (Beninger & Hosfield, 2003). The primary polyphenolic substances are flavonoids, which comprise anthocyanins, flavanol glycosides, and condensed tannins (proanthocyanidins). However, proanthocyanidins are the flavonoid class that is most prevalent in beans (Lopez-Martinez 2020).

Trypsin Inhibitors: Trypsin inhibitors as the name implies can reduce the stomach's trypsin activity, they serve as a barrier between the trypsin-containing bodies and the cytoplasm. To protect themselves from invasive germs and insects. However, their presence decreases the ability of legumes to digest (Kosson 2014). Trypsin inhibitor levels were assessed in two varieties of Mexican runner beans by Corzo-Ros *et al,* who reported values of 6.90 and 6.73 TIUmg1 for purple and brown, respective. The amount of trypsin inhibitors in cultivars varies and is dependent on factors like genotype, growing and environmental conditions.

Phytic Acid: Myo-inositol hexaphosphate, also known as phytic acid, is a naturally occurring molecule found in legumes and the seeds of most plant species. Because its salts can reduce the bioavailability of minerals, they are frequently referred to as anti-nutrients. Phytic acid acts as the principal source of phosphorus in runner beans, the cotyledons contain the acid in common beans. Gonzalez and Paredes-Lapez (1993) noted that phytic acid was present in both water-soluble and insoluble forms (80% and 20%, respectively).

Oxalate: According to Libert and Franceschi (1987) Runner beans are typically classified as food high in oxalate (half of the varieties of runner beans have 23.8 mg of oxalate per half cup) This is with the exception of one bush variety of runner beans called the Roma beans, they are medium oxalate food with (8.4 mg. per half cup when boiled). Chipps et al. (2005) researched on runner beans and the results showed different *P. coccineus* lines have varying degrees of *S. sclerotiorum* and susceptibility to mold resistance, which is influenced by oxalic acid. The partially resistant lines' infected stem tissues had oxalate concentrations that were lower compared to those of the wolven

Tannins: According to Gonzalez and Paredes-Lapez (1993) tannins are located mainly in the seed coats of runner beans, they are water-soluble phenolic compounds. According to Ojiako and Igwe (2008) the most prevalent form of tannin present in runner bean is proanthocyanidins. Based on their structural characteristics, tannins can be divided into two groups: hydrolyzable and condensed tannins (Hatew et al. 2016).

2.5 Effects of soaking on compositions of common beans (*Phaseolus vulgaris* L.)

Dry matter: dry matter content exhibited significant decreases under various soaking conditions. Specifically, for CAL 98, a substantial reduction was observed after 10 hours of soaking across all seed-to-water ratios. In contrast, RI 52 experienced significant reductions after only 2 hours of soaking at a 1/10 ratio. Similarly, DRKF showed notable losses following 2 hours of soaking across all ratios. This decline in dry matter content can be attributed to seed rehydration and the diffusion of water-soluble compounds from the seed into the soaking water, as elucidated by Ravoninjatovo et al. (2022). During soaking, water penetration into the seed occurs through the micropyle, facilitating the exchange of soluble compounds between the seed and the soaking water. The extent of compound loss is influenced by the volume of soaking water, with lower quantities limiting compound loss due to complete seed absorption, leading to equilibrium, and minimal compound diffusion. Conversely, higher water volumes promote greater compound diffusion into the soaking water, potentially due to alterations in the seed coat structure facilitating the diffusion of water-soluble compounds. This leaching process entails the loss of both antinutrients and beneficial soluble nutrients such as carbohydrates, minerals, and vitamins, ultimately impacting the nutritional composition and density of the beans, as documented in studies by (Blöchl et al. 2008; Carmona-García et al. 2007: Nakitto et al. 2015).

Tannins: Tannins: The study of how soaking and heat treatment affected the nutritional value of three types of Madagascar-grown common beans (Phaseolus vulgaris L.) provided important new information about the evolution of tannins. Initial observations indicated that soaking seeds for 2 hours did not yield a significant reduction in overall tannin content. However, a substantial decrease was observed after 10 hours of soaking, particularly with a high seed-to-

water ratio, except for the CAL 98 variety, where no significant decrease was observed under any soaking conditions. Notably, the most significant reduction in tannin content was observed with the RI 52 variety. Furthermore, the study emphasized the importance of soaking seeds for a duration of 10 hours with a high seed-to-water ratio to achieve the maximum reduction in tannin content across all varieties. This recommendation aligns with findings from Ravoninjatovo et al. (2022).
The research also delved into the nuanced effects of soaking on tannin levels under different conditions. While some studies such as those by Helbig et al. (2003) and Yasmin et al. (2008) reported no significant reduction in tannin levels (Ndiritu et al. 2014; Barampama & Simard 1994; Shimelis & Rakshit 2007) rather noted a decrease of 17%–30% in various common bean varieties. Furthermore, the study highlighted the complexity of tannin behavior during soaking. Condensed tannins, which comprise soluble (8%–28%) and insoluble (1%–5%) compounds in the seed coat, were found to decrease during soaking, likely due to the diffusion of soluble tannins into water. However, variability in reduction may arise because certain tannins bind with proteins in the cotyledon endosperm, limiting their solubility. Additionally, challenges in detection arise when tannins bind to proteins, rendering them insoluble in typical solvents.

The puzzling increase in tannin content observed in the RI 52 variety at a 1/1 ratio after 10 hours suggests the intricate behavior of tannins, indicating the potential for unique responses among different tannin compounds. Tannins encompass diverse compounds, each potentially exhibiting distinct behaviors during soaking. Their extraction is influenced by factors such as vacuolar location, cellular decompartmentalization level, and molar mass. Moreover, chemical lability and weak bonding to other cell constituents further complicate their extraction process. Given the complexity of tannin behaviour during processing, the study emphasizes the necessity for further research to achieve a comprehensive understanding of their evolution. Such research is vital for optimizing processing methods to effectively reduce tannin content in common beans while preserving their nutritional quality.

Saponins: investigated the saponin content and composition of chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris)* following soaking in various solutions including distilled water, citric acid, and sodium bicarbonate. Additionally, the impact of cooking durations ranging from 30 to 120 minutes after presoaking the seeds in distilled water was examined. The results revealed that soaking did not alter the saponin content or composition of chickpeas and lentils, regardless of the pH of the soaking solution. However, native saponin, soya-saponin VI, underwent partial degradation during cooking, transforming into soya-saponin I, with both saponins leaching into the cooking solution at rates of 2-5% and 6-14% for chickpea and lentil, respectively. While lentils experienced an overall loss of saponin content (15-31% loss), no such reduction was observed for chickpeas.

Further studies by Sathya and Siddhuraju (2015) investigated the effects of soaking on the nutritional and antinutritional components of the underutilized tree legume *Parkia roxburghii*. Their findings revealed that soaking led to enhancements in protein (15-36%) and lipid content (11-69%), accompanied by reductions in other proximal components. Moreover, all soaking methods significantly reduced antinutrients such as condensed tannins, phytate, saponins, trypsin inhibitors, chymotrypsin inhibitors, and lectins. Notably, an increase in total phenolics (117-207%) and tannins (171-257%) content was observed. These reductions in antinutritional components resulted in increased protein (9-20%) and starch digestibility (75-254%)

Phytate: Research has demonstrated that soaking treatments have a notable impact on the composition and characteristics of common beans, potentially influencing factors like phytase content (Fernandes et al. 2010). The effects of soaking on the composition of common beans (*Phaseolus vulgaris L.)* encompass alterations in nutritional quality, reduction of antinutrients, and modifications in specific compounds such as α-galactooligosaccharides. Similarly, observations by Escobedo et al. (2019) indicated that thermal treatments (e.g., autoclaving for 3 minutes) resulted in both market classes exhibiting reductions greater than 49% ($p < .05$) in α-GOS concentration and an 80% reduction in tannin levels, while still preserving nutritional attributes.

Protein: The protein contents of the seeds were unaffected by soaking under various conditions across all varieties. This finding is consistent with prior research by Ravoninjatovo et al. (2022) as well as studies conducted by Pujolà et al. (2007) and Shimelis & Rakshit (2007). It appears that the structure of proteins prevents them from being altered during soaking, unlike other compounds that become solubilized during this process.

Anthocyanins: The examination of anthocyanin evolution in coloured varieties (CAL 98 and DRKF) during soaking was detailed in the study, revealing notable findings. The investigation uncovered the leaching of anthocyanins into the soaking water across all varieties, irrespective of the seed-to-water ratio (Ravoninjatovo et al. 2022). The most significant decrease was observed after a soaking duration of 10 hours. It was found that short-duration soaking with a high seed-to-water ratio was imperative to prevent a reduction in anthocyanins. This reduction in anthocyanin levels during soaking can be attributed to their high solubility in water, with the most substantial reduction typically occurring after 10 hours (Rodriguez-Amaya, 2018). Notably, for the CAL 98 variety, anthocyanin levels were higher when soaked with a 1/10 seeds-to-water ratio compared to other ratios, hinting at the possibility of additional reactions beyond simple diffusion (Dangles & Fenger 2018). Furthermore, the phenomenon of co-pigmentation, influenced by concentration and seed-to-water ratio, may also play a role in anthocyanin reactivity (Dangles & Deluzarche 1994).

α-Galactosides: The investigation demonstrated that soaking had a discernible impact on the concentrations of α-galactosides in the samples. Specifically, when subjected to soaking at 30°C, a decrease in α-galactosides was observed, which was attributed to a combination of diffusion in water and enzymatic hydrolysis processes. The typical structure of α-galactosides in common beans, such as stachyose and raffinose, suggests that endogenous α-galactosidases may catalyze the conversion of stachyose into raffinose. Consequently, an increase in raffinose levels might occur if its production surpasses diffusion losses during soaking.

This observation supports the findings of Siddiq et al. (2006), who reported analogous results in red kidney beans following a 12-hour soaking period. Despite variations in bean varieties, stachyose consistently exhibited the greatest reduction during soaking. Additionally, Shimelis & Rakshit (2007) documented a significant decrease in raffinose and stachyose levels after soaking red kidney beans for 12 hours. It is noteworthy that the duration of soaking profoundly influences the efficiency of α-galactosides extraction; longer soaking durations facilitate increased diffusion and α-galactosides metabolism.

Further corroborating evidence is provided by Coffigniez et al. (2018), who observed that enzymatic degradation contributed to the reduction in α-galactosides content in cowpea seeds subjected to a 12-hour soaking period at 35°C. These findings collectively highlight the intricate dynamics involved in α-galactosides alterations during soaking, emphasizing the significance of soaking conditions and duration in influencing α-galactosides content in leguminous seeds.

Phytic acid: The alterations observed in phytic acid content indicate a significant reduction in all varieties following soaking, though no definitive trend was discerned. Interestingly, longer soaking durations did not consistently lead to the highest reductions. Notably, CAL 98 exhibited an increase in phytic acid across all seed-to-water ratios after 2 hours of soaking, albeit to a lesser extent compared to other varieties. Notably, phytic acid levels in white and red varieties were nearly eliminated. To achieve maximum reduction, soaking for 2 hours with a high seed-to-water ratio was optimal for RI 52 (99%) and DRKF (99%), whereas, for CAL 98, a 1-hour soak with a limited seed-to-water ratio resulted in an 87.13% reduction.

Furthermore, the discussion delves into conflicting findings regarding phytic acid reduction during whole-seed processing, as noted by . Previous studies such as Deshpande et al. (1982) reported a 31% increase in phytic acid content post-processing. While soaking is generally believed to decrease phytic acid levels due to water solubility and leaching, instances of post-soaking phytic acid increase have been observed. This discrepancy may stem from altered bean components, particularly proteins, facilitating enhanced extractability (Greiner & Konietzny 1998). Additionally, inconsistencies in trends could result from selective phytic acid measurement without considering all phytates.

2.6 Effect of Thermal Treatment on Compositions of common beans (*Phaseolus vulgaris*.)

Dry matter: Studies conducted by Ravoninjatovo et al. (2022) explored the impact of thermal treatment on the composition of beans, revealing notable alterations in dry matter content—thermal treatment led to the removal of approximately 50% of the dry matter across all varieties studied. Interestingly, employing a high seed-to-water ratio resulted in a significant reduction in dry matter. Notably, short-time thermal treatment with a low seed-to-water ratio demonstrated greater effectiveness in preserving maximum dry matter content at specific temperatures for different bean varieties. The reduction in dry matter during thermal treatment was attributed to the thermal degradation of seed compounds and their subsequent diffusion into the heating water. Key components such as proteins, starch, and pectin underwent denaturation, gelatinization, and solubilization during thermal treatment, leading to losses in nutritional quality. Furthermore, thermal treatment was observed to increase seed coat pore diameter and water permeability, consequently enhancing diffusivity (Chigwedere et al. 2019).

Proteins: Thermal treatments applied under various conditions did not exhibit any significant impact on the protein contents across all varieties studied. This finding contrasts with the observations made by Rehman and Salariya (2005) who reported a reduction in protein contents following thermal treatment. The behaviour of proteins in the present study can be elucidated by the understanding that heat processing has the potential to enhance protein digestibility by inducing structural modifications in globulins, inactivating protease inhibitors, and altering protein conformation through denaturation (Clemente et al. 1998). Additionally, factors such as protein insolubility, which prevents diffusion, or the leaching of soluble nutrients and certain antinutrients, could contribute to the observed increase in protein concentrations (Obong &

Obizoba 1996). Moreover, Khattab et al. (2009) noted that processing treatments such as soaking and cooking led to an augmentation in the total essential amino acids content, further emphasizing the dynamic nature of protein behaviour under different processing conditions.

Anthocyanins: The concentration of anthocyanins in both varieties significantly decreased over time, especially with higher seed-to-water ratios. For CAL 98, anthocyanins were eliminated after 1 hour of thermal treatment at 65°C with seed-to-water ratios of 1/5 and 1/10, and after 2 hours at 100°C, particularly with a high seed-to-water ratio. Similarly, DRKF anthocyanins vanished after 1 hour at 65°C with 1/5 and 1/10 ratios, and after 2 hours with a 1/1 ratio, while at 100°C, complete removal occurred after 2 hours with 1/5 and 1/10 ratios.

The findings indicate a notable decrease and eventual removal of anthocyanins. This aligns with a prior study demonstrating that boiling black beans resulted in a reduction of anthocyanins by 78.2% to 96.5% (Xu & Chang 2009). Increased temperature accelerates the degradation of anthocyanins through hydrolysis of the glycosidic bond upon contact with water, leading to the leaching and loss of both anthocyanins and colour.

Tannins: Thermal treatment significantly reduced tannin levels in all bean varieties except for RI 52 when subjected to limited seed-to-water ratio conditions, where no reduction was observed. The maximum reduction in tannins for RI 52 and DRKF varieties was achieved with a high seed-to-water ratio over 2 hours at 100°C. CAL 98 exhibited the most significant reduction after 2 hours of thermal treatment at a 1:5 seed-to-water ratio and 65°C. This reduction in tannin content over time can be attributed to the impact of thermal treatment on the permeability of cell membranes, facilitating the diffusion of tannins out of the cells (Schwimmer 1972). Additionally, the high processing temperatures may cause chemical alterations in polyphenols, leading to the decomposition of aromatic tannins and the formation of insoluble associations with seed proteins, which may not be detectable through conventional chemical methods such as soaking (Sharma & Sehgal 1992).

Saponins: The study by Hazzam et al. (2020) sheds light on the impact of heating temperature on saponins, suggesting that heat treatment can lead to their degradation, affecting sensory attributes and pharmacological properties. Strategies such as extrusion and roasting are proposed as effective means to mitigate the bitter taste associated with saponins. Similarly, findings from Liu et al. (2020) highlights how thermal processing influences the saponin profiles of *Momordica charantia L*, potentially altering bioactive components or bitter flavours. Overall, these studies collectively indicate that thermal processing can modulate the composition and characteristics of saponins across various plant sources.

Additionally, our research investigated the effects of different processing methods on antinutrients and in vitro protein and starch digestibility of *Phaseolus angularis and Phaseolus calcaratus* seeds. Techniques such as soaking (12 and 18 hours), cooking, autoclaving, and germination (24 and 48 hours) were employed. Results demonstrated a significant reduction ($p < 0.05$) in antinutrient levels, including phytates, tannins, trypsin inhibitors, and amylase inhibitors, alongside a notable improvement ($p < 0.05$) in protein and starch digestibility.

Furthermore, a significant negative correlation (ranging from $-0.80$ to $-1.00$) was observed between antinutrient levels and the digestibility of both protein and starch, suggesting that the reduction in antinutrients may contribute to enhanced digestibility. Cooking and autoclaving were found to elicit more substantial ($p < 0.05$) improvements in protein and starch digestibility compared to soaking and germination. Notably, all processing methods were more effective in enhancing starch digestibility than protein digestibility.

Phytates: Various processing techniques, including soaking, cooking, autoclaving, and germination, were evaluated to understand their effects on antinutrients and the digestibility of protein and starch in *Phaseolus angularis and Phaseolus calcaratus* seeds. These methods notably decreased the presence of antinutrients like phytates, tannins, trypsin inhibitors, and amylase inhibitors, while also significantly enhancing the digestibility of protein and starch (Chau & Cheung 1997). A clear inverse relationship was observed between the levels of antinutrients and the digestibility of protein and starch, indicating that lower antinutrient levels contribute to improved digestibility. Cooking and autoclaving emerged as particularly effective methods for enhancing both protein and starch digestibility compared to soaking and germination. Additionally, all processing methods demonstrated greater efficacy in improving starch digestibility compared to protein digestibility (Chau & Cheung 1997).

Phytic acid: Thermal treatment led to a significant decrease in phytic acid levels across all bean varieties studied, except for CAL 98 in some instances where increases were observed, notably at 100°C (1/10 ratio after 1 hour). Variability in phytic acid levels among different legume varieties was noted. Previous studies by Shimelis and Rakshit (2007) reported a 25% reduction in kidney beans' phytic acid following 35 minutes of thermal treatment, while observed a 29% reduction in chickpeas after 90 minutes of thermal treatment. Conversely Lestienne et al. (2005) observed an increase in soybean Phytic acid during thermal treatment.

The increase or decrease in phytic acid levels may be influenced by various factors such as temperature elevation, which could enhance phytic acid reduction through heat transfer within the seed, leaching into the treatment medium, or forming insoluble complexes. According to Gamel et al. (1998), increments in phytic acid levels were attributed to enzyme activation during thermal treatment, leading to increased phytic acid formation. Further research is warranted to fully understand this phenomenon and devise strategies to mitigate it effectively.

### 2.7 Effects of Thermal and on-thermal treatment on the functional properties of runner bean (*Phaseolus coccineus*).

Rezaei et al. (2019) found that plasma-treated beans exhibited a notable increase in the rate of water absorption, suggesting that non-thermal treatments can influence the water absorption properties of beans. Similarly, Granito et al. (2007) reported an increase in the water absorption index of *Phaseolus lunatus* following processing, indicating that various treatments can affect the water absorption capacity of legumes. In our study, we observed an 85% and 161.5% increase in the water absorption index with processing, highlighting the significant impact of thermal treatment on water absorption properties.

Furthermore, Miranda et al. (2013) investigated the effects of traditional soaking and cooking, storage post-cooking and freezing (-18°C, 21 days), and autoclaving on the starch digestibility of two varieties of runner beans. The results were compared with the digestibility of isolated starch subjected to similar treatments. Following thermal treatment, there was a notably higher content of rapidly digestible starch (RDS) observed in both seeds and starch, accompanied by reduced contents of resistant starch (RS) and slowly digestible starch (SDS). Interestingly, in flours derived from cooked seeds, the RDS content was higher compared to flours from autoclaved seeds, despite similar changes in other constituents such as ash and protein. This discrepancy may be attributed to improved starch gelatinization resulting from prolonged soaking of seeds.

Examining the foaming and emulsifying capacity of runner beans lays the groundwork for exploring their functional properties under various processing conditions, including both thermal and non-thermal treatments. Marki et al. (2006) delved into this area by studying protein isolates from two Phaseolus cultivars: common bean *(Phaseolus vulgaris L.)* and scarlet runner bean *(Phaseolus coccineus L.)* Using wet extraction methods, they prepared the isolates and characterized them using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), followed by an evaluation of solubility.

Emulsions were created with the protein isolates at different concentrations and pH levels (7.0 and 5.5), and their stability was assessed based on parameters such as droplet size diameter, viscosity, and creaming. Notably, emulsions containing 1% protein content exhibited instability during storage, while those with a 3% protein isolate concentration, obtained via ultrafiltration at pH 5.5 from both cultivars, displayed flocculation, particularly evident in the coccineus isolates.

Furthermore, the foaming properties of the protein isolates were scrutinized, revealing that foams generated with a 1% protein concentration showed limited foaming ability. In contrast, ultrafiltration isolates produced more robust and stable foam, especially at pH 5.5. These findings shed light on the functional characteristics of runner beans' protein isolates, offering insights into their potential applications in various food and industrial contexts.

Gamel et al. (2006) explored the impact of various seed treatments, including cooking, popping, germination, and flour air classification, on the functional properties and antinutritional factors of *Amaranthus caudatus* and *Amaranthus cruentus* seeds. Thermal treatments notably increased water absorption, reaching maximum values in the flour of popped seeds for both species. Fat absorption generally increased post-treatment. Flour foam stability saw improvement after air classification and germination followed by low-temperature drying, while it diminished following thermal treatment and germination coupled with high-temperature drying. Emulsion stability decreased with all treatments except air classification, and flour dispersibility increased with all treatments except germination followed by drying at 300°C. Air classification raised phenolic compound and phytate contents but lowered enzyme inhibitor levels, while thermal treatments, especially cooking, markedly reduced phenolic compound, phytate, and enzyme inhibitor levels compared to popping.

Both thermal and non-thermal treatments have been found to enhance the functional properties of legumes, such as their oil-absorbing capacity, emulsifying capacity, and water solubility index. Non-thermal processes like soaking, germination, and fermentation have shown promise in reducing anti-nutritional factors and enhancing legume digestibility (Patto et al. 2014). Furthermore, thermal processing can induce ultrastructural changes in legumes, affecting their functional characteristics and physiological properties, including glycemic response and hypocholesterolemic effects (Aguilera et al. 2009). Additionally, technological processes, encompassing both non-thermal and thermal treatments, have the potential to modify legume functional properties, such as emulsifying activity, stability, foaming properties, and water-holding capacity, as well as influence the activity of bioactive compounds present in legume seeds (Amarowicz 2020).

# 3 Aims of the Thesis

Runner bean have low protein digestibility this is due to the presence of anti-nutrients, which also reduces the bioavailability of proteins and trace elements (Shimelis & Rakshit 2007).The nutritional value and organoleptic acceptability must be improved to fully realize their potential as food and animal feed. This can be done by inactivating or removing unwanted components. several kinds of processing, including boiling, hydration, and germination (Matella et al. 2005).

Antinutrients like trypsin inhibitors, phytic acid, saponins, phytohaemagglutinin, and tannins) and a-galactosides (e.g. raffinose, stachyose, and verbascose) are some of the unfavorable elements in beans that may restrict how they utilize protein and carbohydrates. Assessing the effect of degradation of antinutrient factors in runner bean seed by thermal and non-thermal treatment is needed to allow us to evaluate the degradation of tannin, phytate, oxalate, trypsin inhibitor by thermal and non-thermal treatment influence germination.

Therefore, the main aim of this study is to assess the effect of degradation of antinutrient factors in runner bean seed by thermal and non-thermal treatment. The specific objectives are to:

1. Evaluate the effect of thermal and non-thermal treatment affecting antinutrients in runner bean
2. Effect of liquid-solid ratio in boiling to degradation of antinutrient factors in runner bean seeds
3. Evaluate the effect of thermal and non-thermal on the functional properties of white and black runner bean seed

# 4 Materials and Methods

## 4.1 Experimental location

The experiment was conducted at the laboratory of the Faculty of Tropical Agrosciences at the Czech University of life sciences, Prague.

## 4.2 Number of Replication

The treatments were replicated three times.

## 4.3 Test Crop used

The test crop used for the experiment was runner bean seed

## 4.4 Sample Preparation

After the samples were collected, they were stored in a dry place before boiling or incubation.

## 4.5 Name of Seed Variety

*Phaseolus coccineus* bean seed (black runner bean) and *Phaseolus coccineus* Albena variety, (white runner bean)

## 4.6 Source of sample used

Seeds were ordered from SEMO a.s. Smržice, Czech Republic and the black were from Senger's Genussmanufaktur, Austria.

## 4.7 Pre-treatment procedure

Seeds were kept in a dark place at room temperature.

## 4.8 Chemical Reagents

The chemical reagents that were used are Hydrochloric acid that was produced at VWR International S.A.S France, Methanol from Carl Roth GmbH CO. Kg Germany, Vanillin from Merck spol SRA Czech republic, and tannic acid from Sigma-aldrich, CO, Germany, Acidic ammonium iron (III) Sulphate deodecahydrate from Merck KGaA, Germany, 2.26ipyridine from Merck spol sro, Czech republic, Thioglycolic acid from Merck spol sro, Czech republic, Phytic acid from Sigma Aldrich, co, Switzerland, sulphuric acid from VWR International S.A.S

France. Ethanol from VWR International S.A.S France, Diosgenin from Sigma, Aldrich.co China. Oil from Albert Prague, Czech Republic, sro.

4.9 Laboratory equipment used

The equipments used in the experiments are a spectrophotometer UV- 1900i, Shimodzu corp, a centrifuge machine MPW med instrument, water bath from Memmert, vortex mixer, oven, electric cooker, and ice bath.

4.10 Experimental Procedure

4.10.1 Tannin Analysis

Tannin content was determined following the methodology described by Onyango et al.(2005) with modifications from Yadav et al. (2021) and Kheto et al. (2023).

Sample Preparation: One gram of the sample was combined with 10 mL of acidified methanol (1 mL concentrated hydrochloric acid per 100 mL methanol).

**Extraction and Centrifugation Procedure:**

The mixture was incubated at 30°C for 15 hours. After incubation, the mixture was centrifuged at 10,000 rpm for 15 minutes, and at 7000 rpm for 15 minutes. 1mL of the supernatant (extract) was transferred to a test tube.

**Vanillin-hydrochloric acid reagent**: This was made by combining equal parts acidified methanol (8 ml concentrated hydrochloric acid/100 ml methanol) and vanillin solution (4 g vanillin/100 ml methanol).

**Combined sample and Reagents**: 5 mL reagent was added to the extract (1 mL) and kept in a dark environment for 20 min. The samples were incubated and kept in a dark environment for 20 minutes.

**Measurement of absorbance:** Using a vanillin-hydrochloric acid reagent as a blank, absorbance was measured in 1-cm cuvettes after 20 minutes at 500 nm using an Ultrospec 1000 spectrophotometer. To correct for the interference of natural pigments, sample blanks were prepared by subjecting the original extract to the conditions of the reaction but without the vanillin-hydrochloric acid reagent.

The absorbance was measured using a UV–Vis Spectrophotometer (AU 2701, Systronics India Ltd.) at **500 nm** against Vanillin-HCl reagent as blank. The tannin content is expressed as mg of tannic acid equivalent per 100 g of sample.

**Standard curve:**

Prepared by adding 1 g tannic acid (Fluka Chemie AG, Buchs, Switzerland) to 100 ml acidified methanol and this stock solution was used at various dilutions from 1:10 to 1:50

(a) Tannic acid solution (1 g of tannic acid in 100 ml of acidified methanol) was used as the standard (R2 = 0.9969)

(b) the standard curve was prepared using tannic acid

Phytates - Phytic acid: This was tested using the method of (Onyango et al. 2005; Yadav et al. 2021; Kheto et al. 2023).

**Extraction and Centrifugation**: 0.1 g of sample and 10 mL of HCl (0.2 N) solution were taken into a centrifuge tube and kept in a shaker at room temperature for 1 h. After that, the suspension was centrifuged at 5000 rpm for 15 min to collect the supernatant.

**Sample processing**: The supernatant (0.5 ml) was pipetted into a test tube fitted with a ground-glass stopper before adding 1 ml ammonium iron sulphate (0.2 g of ammonium iron sulfate in 100 ml HCl and made up to 1000 ml distilled water) was added to the test tube

**Boiling and cooling**: The prepared mixture was boiled in a water bath for 30 min and rapidly cooled down to 25 °C. After that, 2 mL of Bipyridine solution (1 g Bipyridine in 1 mL of thioglycolic acid mixed with 99 mL DW) was added to the mixture and incubated for 2 minutes.

**Absorbance:** Absorbance was read after 1 min using a spectrophotometer UV- 1900i at 519nm against distilled water as blank

**Standard Curve:**

(a) 125 mg of sodium phytate (Sigma Aldrich GmbH, Steinheim, Germany) was added to 100 ml of 0.2 mol/l hydrochloric acid to create a standard curve. This stock solution was then utilized at several dilutions to provide final phytate phosphorous concentrations ranging from 3 to 30 mg/ml.

(b) As a standard, sodium salt hydrate phytic acid was used. The standard solution (R2 = 0.9925) was prepared by taking 130 mg of phytic acid sodium salt hydrate in 100 ml of

0.1 N HCl. To obtain various concentrations, this stock solution was diluted several times.

(c) The standard curve was prepared using phytic acid solution (mixture of 100 mL HCL (0.2 N) in 130 mg phytic acid sodium salt hydrate) and expressed as mg of phytic acid/100 g of d.m.

4.10.3 Saponin Analysis

Extraction and Centrifugation: The sample extract was prepared by adding 10 mL of methanol to 1 g of sample and incubated for 12 hours at ambient temperature.

Sample Preparation: Adding vanillin and sulphuric acid, incubation and rapid cooling

(a) To 250 µl of Saponin solution, an equivalent amount of Methanolic vanillin was added. Sulphuric acid was added to the resulting solution and kept at 60 °C. After 10 min of incubation, it was rapidly cooled by keeping it in an ice bath

(b) 0.5 mL sample extract was taken into a test tube, and 5 mL vanillin solution (800 mg vanillin into 10 mL of ethanol) followed by 5 mL of H2SO4 (12 mL concentrated H2SO4 in 38 mL of DW) was immediately added. After that, the mixture was incubated for 10 min at 60 °C and rapidly cooled by keeping it in an ice bath.

Absorbance: Spectrometer (AU 2701, Systronics India Ltd) was used to measure the absorbance value of the mixture at 540 nm and expressed as mg diosgenin/g of d.m.

Standard curve: Saponin was analysed using the diosgenin method as described by Kheto et al., 2023 with slight modifications. The standard curve was prepared by weighing 5mg of diosgenin and topped up with methanol to a volume of 10ml (Stock solution), this solution was kept in a water bath for few minutes as the diosgenin was difficult to dissolve. 0.8g of vanillin was also weighed and topped up to 10ml with ethanol. 72% sulphuric acid was prepared. Dilutions were prepared from the stock solution in the concentrations of 0mg/ml, 0.01mg/ml, 0.05mg/ml, 0.1mg/ml, 0.25mg/ml and 0.5mg/ml. From these dilutions, 250ul as taken and combined with 250ul vanillin solution and 2.5ml 72% sulphuric acid. These solutions were then put in a water bath for 21 minutes with shaking at regular intervals of 7 minutes. After this the absorbance of these known concentrations were taken at 448nm to plot the standard curve. sample extract was prepared by adding 10 mL of methanol to 1 g of sample (blended seeds) and incubated overnight at ambient temperature. 0.5 mL sample extract was taken into a test tube, and 5 mL vanillin solution (800 mg vanillin into 10 mL of ethanol) followed by 5 mL of H2SO4 (72%) was immediately added. After that, the mixture was kept in a water bath for 21 minutes with shaking at regular intervals of 7 minutes. Absorbance was measured at 448 nm against a reagent blank(solvent).

4.11 Functional properties

Water absorption capacity (WAC), oil absorption capacity (OAC), foaming capacity (FC), and emulsifying capacity (EC) of control and roasted samples were determined according to the method described by Sharanagat et al. (2019) and Jogihalli, Singh, and Sharanagat (2017).

4.11.1 Water absorption capacity

For water absorption capacity (WAC), 1 g of sample was mixed with 15 mL of distilled water (DW) and vortexed for 5 min. After that, the suspension was kept for 30 min, and centrifuged (5000 rpm; 30 min). Then, the supernatant was decanted, and WAC (g/g) was calculated by Eq. (4).

$$WAC\ or\ OAC\ (\mathrm{g/g}) = \frac{Sample\ weight\ after\ ecentrifugation - Initial\ sample\ weight}{Initial\ sample\ weight} I$$

4.11.2 Oil absorption capacity

To measure oil absorption capacity (OAC), 600 mg of sample was added to 10 mL of soybean oil. The suspension was vortexed for 5 min and left to stand for 30 min followed by centrifugation (7500 rpm; 30 min). Then, the supernatant was discarded, and OAC (g/g) was calculated by equation:

$$WAC\ or\ OAC\ (\mathrm{g/g}) = \frac{Sample\ weight\ after\ ecentrifugation - Initial\ sample\ weight}{Initial\ sample\ weight} I$$

4.11.3 Water solubility index

The water solubility index (WSI) of the samples was determined according to the method of Jhonsy *et al.* (2022) with slight modifications. In brief, 0.25 g of sample and 10 mL of DW were taken in a centrifuged tube. Then, the suspension was vortexed for a few minutes and kept in a water bath (85 °C) for 30 min. Subsequently, the suspension underwent rapid cooling and was centrifuged for 15 minutes at 6000 rpm in order to extract the supernatant. Finally, the supernatant was transferred in Petri-dishes and kept at 105 °C for 3 hr. The WSI of the samples was calculated as g per 100 g using the equation:

$$WSI\ \left(\frac{\mathrm{g}}{100}\mathrm{g}\right) = \frac{Final\ weight\ of\ supernatant\ after\ drying\ (Ws)}{Initial\ sample\ weight\ (Wi)} \times 100$$

4.11.4 Foaming capacity

For foaming capacity (FC), 50 mL of DW was added to 1 g of the sample and shaken for 5 min. The contents were immediately shifted in a measuring cylinder and the foam volume was noted. The FC (%) was calculated by equation:

$$FC\ (\%) = \frac{Volume\ after\ shaking - Initial\ volume\ of\ suspention}{Initial\ volume\ of\ suspention} \times 100$$

4.11.5 Emulsifying capacity

Likewise, emulsifying capacity (EC) was estimated by taking 50 mg of sample, 5 mL of DW, and 5 mL of soybean oil into a centrifuge tube. Then, the suspension was vortexed (5 min) and centrifugation was performed (1100 g; 5 min). After that, the suspension was carefully transferred into a measuring cylinder, and the emulsion layer and total volume were noted.

$$EC\ (\%) = \frac{Emulsion\ volume}{Total\ volume} \times 100$$

4.12 Data Collection:

Data collected was subjected to analysis of variance using GENSTAT, 8$^{th}$ edition 2016. Least significance LSD was also used to compare treatment means ($P < 0.05$). The data would be presented as mean ± standard deviation.

4.13 Statistical analysis

The relationship between the varieties of seed with tannins was evaluated using analysis of variance (ANOVA) in the GENSTAT software to compare the means of different treatments in estimating the effect of thermal and non-thermal treatment affecting antinutrients in runner bean. Furthermore, using analysis of variance, the effect of thermal and non-thermal processes on the functional properties of white and black runner bean seed () was evaluated, the least significant difference (LSD) test was utilized for pairwise comparison of means, with a significance level set at $P>0.05$. The data were presented as mean ± standard deviation.

# 5 Results

## 5.1 Effect of thermal and non-thermal treatment on thermal treatment on antinutrients in runner bean

Table 1 shows the absorbance and concentration of various antinutrient factors (tannins, phytase and saponins) in white and black runner bean seeds before and after soaking. The results indicate that both white and black runner bean seeds exhibit a decrease in absorbance values after soaking compared to unsoaked conditions which also means that the absorbance values for tannins, phytase, and saponin were higher in the unsoaked seeds compared to the soaked seeds.

In the unsoaked condition for tannins, the concentration values for white and black seeds are 1.45 and 39.41, respectively. After soaking at a ratio of 5:1, the concentration values decreased to 0 and 23.93 respectively. Correspondingly, findings from the results revealed that the concentration of tannins decreases as well. In the unsoaked condition, the concentration for white and black seeds is 1.45 mg/ml and 39.41 mg/ml, respectively. After soaking at a ratio of 5:1, the concentrations decrease to 0.18 mg/ml and 23.93 mg/ml, respectively. This reduction suggests a decrease in the concentration of tannins, which are known as anti-nutrients.

Unlike tannins, the absorbance values for phytase do not exhibit significant changes between unsoaked and soaked conditions. However, there is a decrease in phytase concentration after soaking, particularly at higher soaking ratios. For instance, in the unsoaked condition, the concentration of phytase for white and black seeds is 3.84 mg/ml and 3.76 mg/ml, respectively. After soaking at a ratio of 5:1, the concentrations decrease to 2.47 mg/ml and 2.68 mg/ml, respectively.

In saponins, similar to tannins, the absorbance values for saponin decrease after soaking, indicating a reduction in saponin content. For example, in the unsoaked condition, the absorbance values for white and black seeds are 0.57 and 0.08, respectively. After soaking at a ratio of 5:1, the absorbance values decrease to 0.04 and 0.02, respectively. Correspondingly, the concentration of saponin decreases as well. In the unsoaked condition, the concentration for white and black seeds is 0.44 mg/ml and 0.07 mg/ml, respectively. After soaking at a ratio of 5:1, the concentrations decrease to 0.04 mg/ml and 0.02 mg/ml, respectively.

Table 1: **Effect of thermal and non-thermal treatment affecting antinutrients in runner bean**

| | ABS | | CONC (mg/ml) | |
|---|---|---|---|---|
| | **WHITE SEED** | **BLACK SEED** | **WHITE SEED** | **BLACK SEED** |
| UNSOAKED TANNINS | 0.01c ± 0.00 | 0.33b ± 0.02 | 1.45b ± 0.10 | 39.41a ± 2.93 |
| Soaked Tannins 3:1 | 0.01c ± 0.00 | 0.31b ± 0.00 | 1.21b ± 0.04 | 37.33a ± 0.45 |
| Soaked Tannins 4:1 | 0.01c ± 0.00 | 0.23b ± 0.01 | 0.71b ± 0.14 | 26.91a ± 0.21 |
| Soaked Tannins 5:1 | 0.00c ± 0.00 | 0.20b ± 0.01 | 0 ± 0.00 | 23.93a ± 0.72 |
| Unsoaked Phytase | 2.50a ± 0.02 | 2.51a ± 0.01 | 3.84a ± 0.03 | 3.76b ± 0.01 |
| Soaked Phytase 3:1 | 2.47a ± 0.01 | 2.44a ± 0.00 | 3.80a ± 0.01 | 3.76b ± 0.00 |
| Soaked Phytase 4:1 | 1.55b ± 0.06 | 1.96a ± 0.00 | 2.39b ± 0.09 | 3.02b ± 0.00 |
| Soaked Phytase 5:1 | 1.60b ± 0.06 | 1.74a ± 0.00 | 2.47b ± 0.09 | 2.68b± 0.00 |
| Unsoaked Saponin | 0.57b ± 0.04 | 0.08c ± 0.01 | 0.44b ± 0.03 | 0.07c ± 0.01 |
| Soaked Saponin 3:1 | 0.24b ± 0.01 | 0.04c ± 0.01 | 0.19c ± 0.01 | 0.04c ± 0.01 |
| Soaked saponin 4:1 | 0.18b ± 0.03 | 0.04c ± 0.04 | 0.14c ± 0.02 | 0.04c ± 0.06 |
| Soaked saponin 5:1 | 0.04b ± 0.01 | 0.02c ± 0.05 | 0.04c ± 0.01 | 0.02c± 0.08 |

Mean ± standard deviation of three replications, means are significantly different ($p<0.05$).

a,b,c indicates level of significance where a is highly significant, b is significant and c is the least significant (ABS. Absorbance, CONC Concentration)

Equation of the calibration curve for Tannin

Concentration = $K_1A+K_0$

$K_1$= 1.1535e+02

$K_0$=1.0975E+02

Square of Correlation coefficient $r^2$ = 0.99820

Equation of the calibration curve for Saponin

Concentration = $K_1A+K_0$

$K_1$= 7.6600e-01

$K_0$=6.0886e-03

Square of Correlation coefficient $r^2$ =0.99870

Equation of the calibration curve for Phytate

Concentration = $K_1A+K_0$

$K_1$= 1.5032e_00

$K_0$=2.3064e-02

Square of Correlation coefficient $r^2$ =0.99660

5.2 Evaluate the effect of thermal and non-thermal on the functional properties of white and black runner bean seed

Parameters in table 2 how the functional properties of white and black runner bean seeds where results revealed the ability of the two bean varieties to absorb water Unsoaked white beans have the highest water absorption index (4.15), followed by soaked beans at different ratios (3:1, 4:1, 5:1), with soaked white beans 3:1 having the lowest water absorption index (1.17)

Unsoaked black beans also show good water absorption (1.60) indicating their strong ability to absorb water, with soaked beans exhibiting slightly lower values, with soaked black beans 5:1 having the lowest (1.20). Soaking reduces the water absorption index in both white and black beans, with higher soaking ratios leading to lower indices. White beans generally exhibit higher water absorption indices compared to black beans, regardless of soaking.

Unsoaked white beans exhibit a higher foaming capacity (0.15) compared to unsoaked black beans (0.02). Soaking appears to decrease foaming capacity in both white and black beans. Soaking decreases foaming capacity in both white and black beans. However, the impact of soaking on white beans seems more pronounced.

Further results revealed that unsoaked white beans have higher oil absorbance (6.27) compared to the soaked beans at varying ratios with soaked white beans (5:1) at 1.73 having the highest thermal treatment. In contrast, soaked black beans (5:1) had the highest at 1.53 compared to unsoaked black beans (1.47), demonstrating that soaking does not significantly affect oil absorbance in either white or black beans.

Also, the experiments considered the Emulsifying capacity of the white and black runner bean seed where results revealed that white beans generally exhibit higher emulsifying capacity compared to black beans across different soaking conditions. Unsoaked white beans have the highest emulsifying capacity (49.50), indicating their strong ability to form and stabilize emulsions. Soaking at different ratios affects the emulsifying capacity differently in white beans. For instance, soaking at a ratio of 4:1 leads to a significant increase in emulsifying capacity (47.67) while soaking at a ratio of 5:1 further increases the emulsifying capacity (50.60). Furthermore, unsoaked black beans also exhibit good emulsifying capacity (50.00), comparable to unsoaked white beans (49.50). Soaking black beans does not significantly affect their emulsifying capacity, as the values remain relatively stable across different soaking ratios.

The parameter further indicates that unsoaked black beans have the highest water solubility index (40.6), followed by unsoaked white beans (24.0). Soaking generally increases the water solubility index in both white and black beans, with higher ratios leading to higher solubility as in the case of soaked white bean 5:1 having the highest value at 40.6 and soaked black bean 5:1 having the highest value at 48.9.

Table 2: Effect of thermal and non-thermal on the functional properties of white and black runner bean seed

| | WAC | FC | OAC | E C | W.S.I |
|---|---|---|---|---|---|
| Unsoaked White Beans | 4.15a ± 0.10 | 0.15a ± 0.05 | 6.27a ± 0.12 | 49.50b ± 3.77 | 24.00b ± 13.86 |
| Soaked White Beans 3:1 | 1.17c ± 0.06 | 0.05b ± 0.05 | 1.43b ± 0.23 | 44.17a ± 2.22 | 30.67ab ± 16.17 |
| Soaked White Beans 4:1 | 1.27bc ± 0.06 | 0.08ab ± 0.03 | 1.49b ± 0.42 | 47.67b ± 2.30 | 40.00a ± 0.00 |
| Soaked White Beans 5:1 | 1.32b ± 0.07 | 0.06b ± 0.01 | 1.73b ± 0.35 | 50.60b ± 1.04 | 40.60a ± 1.04 |
| Unsoaked Black Beans | 1.60a ± 0.10 | 0.02b ± 0.02 | 1.47b ± 0.12 | 50.00b ± 0.00 | 40.60a ± 1.04 |
| Soaked Black Seeds 3.1 | 1.27bc ± 0.12 | 0.01b ± 0.01 | 1.43b ± 0.12 | 47.90b ± 0.52 | 41.33a ± 2.31 |
| Soaked Black Seed 4:1 | 1.27bc ± 0.06 | 0.01b ±0.01 | 1.43b ± 0.12 | 48.23b ± 1.01 | 42.83a ±2.47 |
| Soaked Black Seed 5.1 | 1.20bc ± 0.00 | 0.07ab ±0.11 | 1.53b ± 0.25 | 48.90b ± 0.52 | 40.00a ± 0.00 |

Mean ± standard deviation of three replications, means are significantly different ($p<0.05$)

a,b,c indicates level of significance where a is highly significant, b is significant and c is the least significant

(WAC- Water Absorption Capacity, OAC-Oil Absorption Capacity, EC – Emulsifying Capacity, FC-Foaming Capacity, WSI-Water Solubility Index)

5.3: Relationship between Water Absorption and Runner bean seed

The result in (Figure 1) shows the relationship between water absorption and the white and black beans variety of runner bean seed in soaked and unsoaked conditions. For water absorption, unsoaked white beans rose highest and significantly reduced in the soaked white beans in varying units of water to solid ratio while unsoaked black beans rose highest in its group above other varying units of water to solid in the soaked group suggesting the ability of unsoaked white and black runner bean seed to be able to hold water more than soaked white and black runner bean seed.

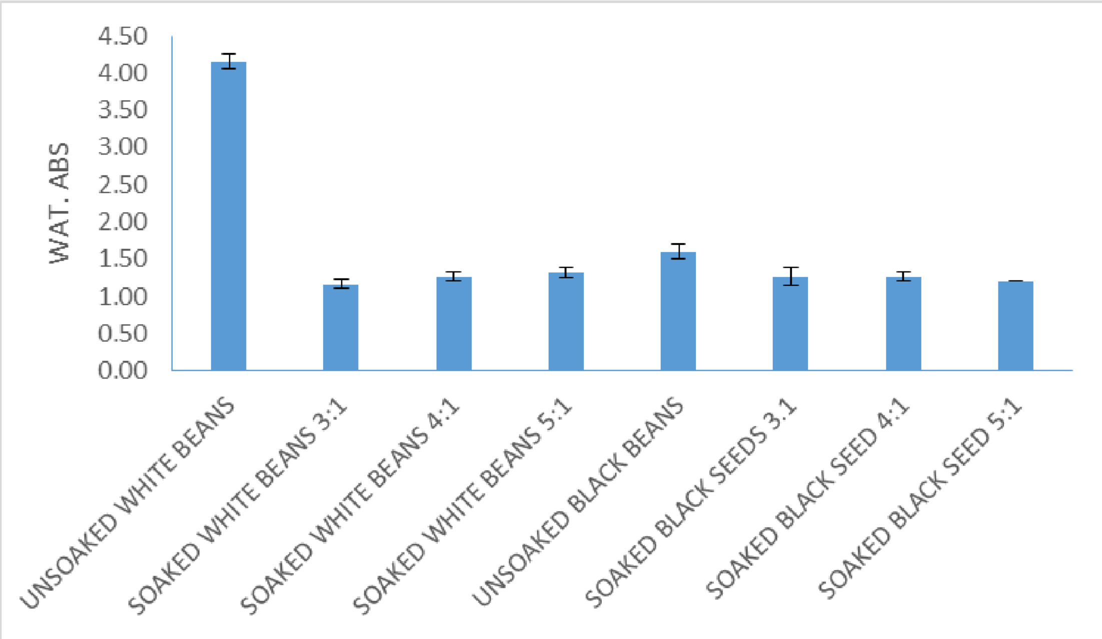


Figure 1 Relationship between water Absorption and runner bean seed
(WAT ABS- Water absorption)

I on top of each bar = error bar

5.4: Relationship between foaming capacity and Runner bean seed.

The result in (Figure 2) shows the relationship between foaming capacity and white and black beans variety of runner bean seed in soaked and unsoaked conditions. For foaming capacity, unsoaked white beans rose highest in its group significantly above other varying units of water to solid ration in soaked condition while soaked black seed 5:1 rose highest in the black runner bean seed suggesting the ability of unsoaked white beans and soaked black bean (5:1) to exhibit higher foaming capacity.

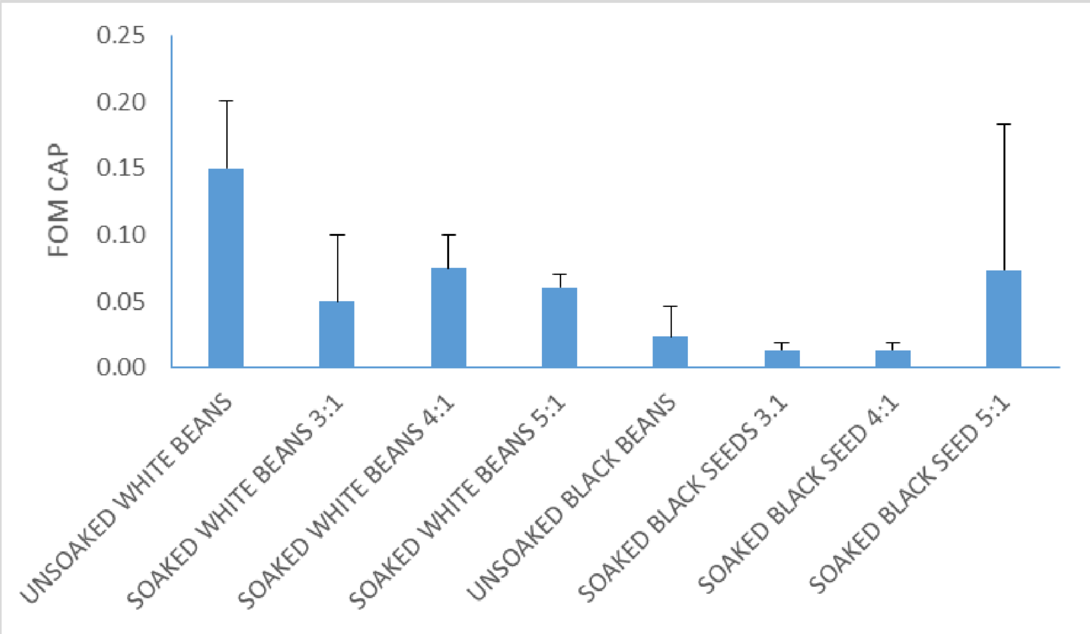


Figure 2 Relationship between foaming capacity and runner bean seed.
(FOM CAP - Foaming capacity)

I on top of each bar = error bar

5.5 Relationship between Oil Absorption and varieties of runner bean seed

Figure 3 shows the relationship between oil absorption and the white and black beans variety of runner bean seed in soaked and unsoaked conditions. For oil absorption, it rose highest in unsoaked white beans and significantly reduced in the soaked white beans in varying units of water to solid ratio while soaked black beans rose highest in its group above other varying units of water to solid in the soaked group.

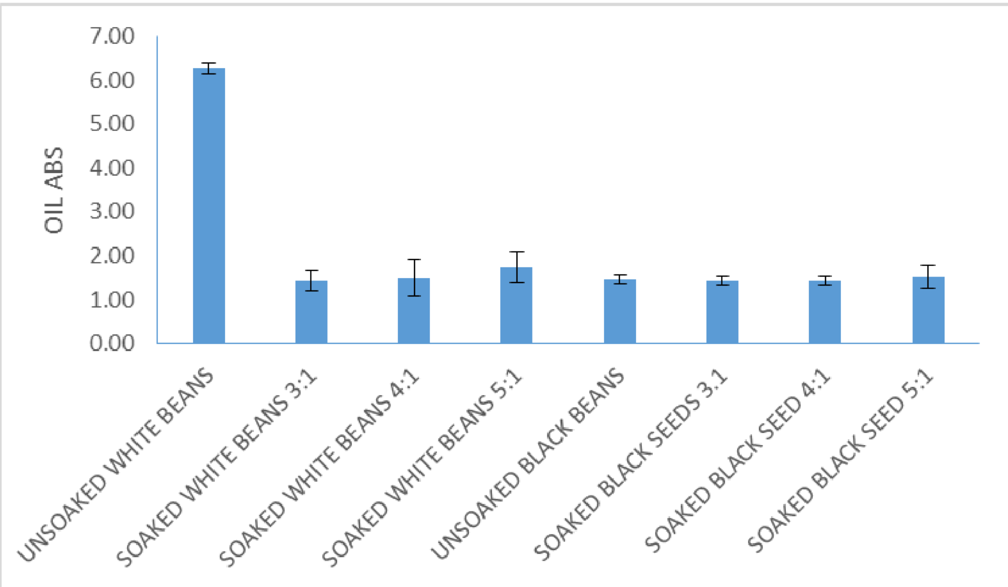

Figure 3 Relationship oil absorption and varieties of runner bean seed
(OIL ABS- Oil absorption)

I on top of each bar = error bar

5.6Relationship between water solubility index and varieties of runner bean seed

Figure 4 shows the relationship between water solubility index and white and black beans variety of runner bean seed in soaked and unsoaked conditions. For oil-water solubility index, unsoaked white beans at the water to solid ratio 4:1 and 5: 1 rose highest in its group significantly above other units while all varying units(3:1, 4:1, 5:1) of soaked black runner bean seed generally increased above the unsoaked unit.

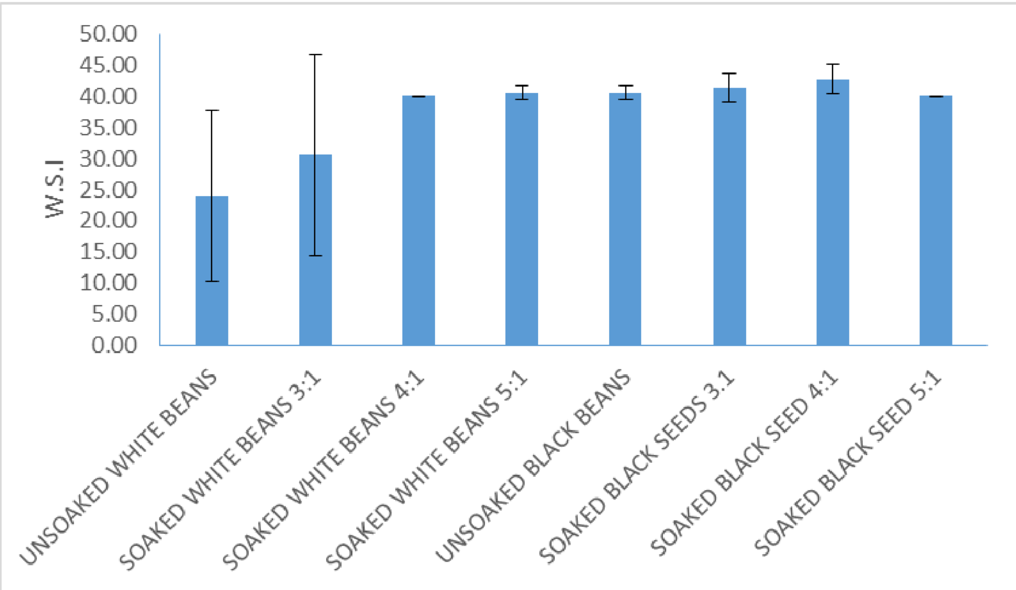


Figure 4 Relationship between water solubility index and varieties of runner bean seed
(WSI- Water solubility index)

I on top of each bar = error bar

**5.7 Relationship between Emulsifying capacity and varieties of Runner Bean Seed**

The results in figure 5 shows the relationship between emulsifying capacity and white and black beans variety of runner bean seed in soaked and unsoaked conditions. For emulsifying capacity, unsoaked black runner bean seed (50mL/g) exhibits good emulsifying capacity compared to unsoaked white runner bean seed (49.5). Soaking at a ratio of 4:1 leads to a significant increase in emulsifying capacity while soaking at a ratio of 5:1 further increases the emulsifying capacity in both varieties of runner bean seed.

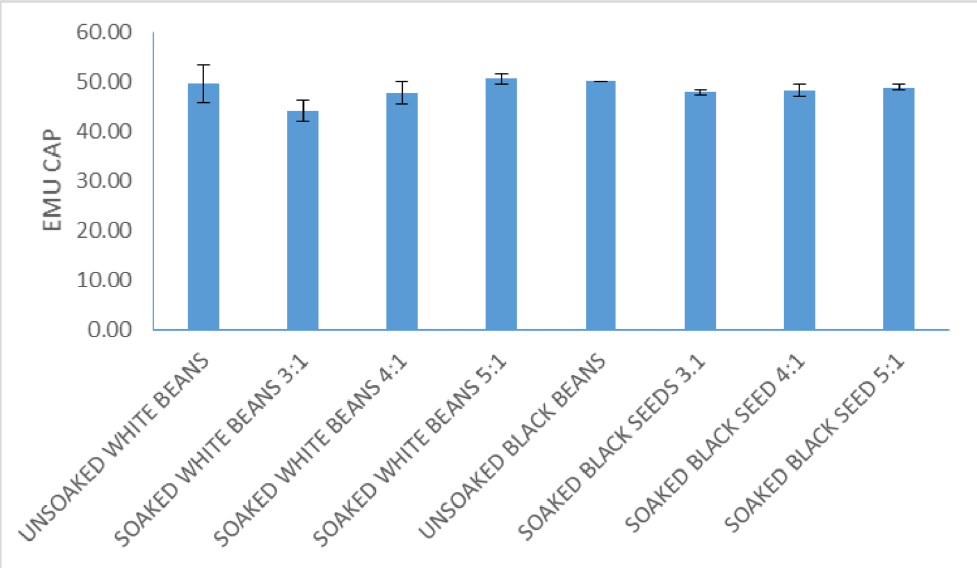


Figure 5 Relationship between Emulsifying capacity and varieties of runner bean seed (EMU CAP. Emulsifying capacity)

I on top of each bar = error bar

# 6 Discussion

This study assessed thermal and non-thermal treatments' impact on antinutrients in runner beans. Both white and black runner bean seeds showed reduced concentration of tannin after soaking. Initial tannin concentrations were 1.45 mg/ml for white and 39.41 mg/ml for black seeds, decreasing to 0 mg/ml and 23.93 mg/ml respectively after soaking. Control of these treatment processes is crucial to minimize dry matter loss while reducing antinutrients. Similarly, Granito et al. (2007) proved that the content of total polyphenols, tannins and antioxidant capacity decreased with thermal processing. The study by Ravoninjatovo et al. (2022) recommends soaking at 30°C for 1 hour followed by heat treatment at 65°C with a 5:1 water-to-seed ratio for 2 hours. This approach is also supported by Ralison (2022) which underscores the importance of soaking and thermal treatment in reducing antinutrients and preserving nutrients in common dry beans.

Saponin levels decrease during soaking. Initially, white and black seed concentrations were 0.44 mg/ml and 0.07 mg/ml. Post-soaking (5:1 water to seed ratio), levels drop to 0.04 mg/ml and 0.02 mg/ml, respectively, consistent with studies showing thermal and non-thermal treatments reduce legume antinutrients. Thermal treatments, as shown by Ranila et al. (2009), enhance legume nutritional value by reducing phytic acid and tannins, improving protein and starch digestibility. Thermal processing and high-pressure processing are effective in lowering antinutrient levels and enhancing food quality (Waseem et al. 2022; Redondo-Cuenca et al. 2022). This research aligns with previous studies emphasizing the importance of processing methods in altering legume nutrition and reducing antinutrients.

This research study also investigated the functional properties of white and black runner bean seeds,where results revealed the ability of the two bean varieties to absorb water. Unsoaked white beans have the highest water absorption index (4.15). Unsoaked black beans also show good water absorption (1.60) indicating their strong ability to absorb water which was also supported by findings by Chau & Cheung (1997) with research showing that processing techniques like soaking and thermal treatments can influence water absorption index and antinutrient levels in beans. Soaking has been found to reduce the water absorption index in both white and black beans with higher soaking ratios leading to lower indices which aligns with previous findings. Additionally, processing plays a crucial role in modifying the nutritional composition of legumes, enhancing their digestibility and overall nutritional value (Ravoninjatovo et al. 2022). The foaming capacity of white and black beans was also evaluated with results revealing that unsoaked white beans exhibit a higher foaming capacity (0.15) compared to unsoaked black beans (0.02) which supports the findings by Chau & Cheung (1997) related to the foaming capacity of white and black runner bean seeds, which explains the lower foaming capacity observed after soaking compared to before soaking. Additionally, Sahni et al. (2020) explored the effects of extrusion processing on the nutritional and functional characteristics of beans, which shed light on the alterations in foaming capacity due to soaking.

Soaked white and black beans generally increase the water solubility index in both white and black beans compared to the unsoaked beans at varying ratios contrary to claims from Batista et al. (2010) that Water solubility index, Emulsifying capacity and Oil absorption were reduced by the extrusion process. Results from this research study revealed that soaking legumes generally increases the water solubility index which is backed up by studies from Handa et al. (2017) that revealed soaking can lead to an increase in total protein content, ascorbic acid, and a decrease in anti-nutritional factors, thereby improving the nutritional value and organoleptic qualities of legumes. Additionally, soaking processes have been found to increase the hydration coefficient, total protein, ash, fat, and fibre content of legume seeds (El-Adawy et al. 2000).

# 7 Conclusion

The findings of this study demonstrate that both thermal and non-thermal treatments have a significant impact on reducing antinutrient factors in runner beans seeds. Soaking in particular led to a notable decrease in the absorbance and concentration of tannins, as well as a reduction in saponin levels. These results are consistent with previous research on different bean varieties, indicating the effectiveness of soaking in reducing antinutrients. Moreover, thermal treatments have been shown to further enhance the nutritional value of beans by reducing phytic acid, tannins, and saponins, consequently improving protein and starch digestibility. The effectiveness of these treatments underscores the importance of appropriate processing methods in preserving the nutritional quality of beans while minimizing antinutrient content.

The research conducted on the degradation of antinutrient factors in runner bean seeds through both thermal and non-thermal treatments revealed several significant findings. The study explored the functional properties of white and black runner bean seeds, focusing on water absorption, foaming capacity, and water solubility index. Results indicated that both white and black beans possess the ability to absorb water, with unsoaked white beans exhibiting the highest water absorption index, followed by unsoaked black beans. This finding underscores the importance of processing techniques such as soaking and thermal treatments in influencing water absorption indices and antinutrient levels in beans. Moreover, the foaming capacity of the beans was evaluated, showing that unsoaked white beans had a higher foaming capacity compared to unsoaked black beans. This observation aligns with previous research findings and suggests a correlation between processing methods and alterations in foaming capacity. Additionally, soaking beans generally increased the water solubility index, contradicting some previous claims regarding the effects of extrusion processing on functional properties.

Conclusively, the study emphasizes the potential of soaking processes to enhance the nutritional composition, organoleptic qualities, and functional properties of legumes, contributing to their overall nutritional value and consumer acceptability. By implementing appropriate processing methods, it is possible to effectively reduce antinutrient factors in runner beans, thereby improving their nutritional quality and overall health benefits.

## 7.1 Recommendations

It is recommended to implement a soaking protocol involving soaking at 30°C for 1 hour followed by heat treatment at 65°C with a 5:1 seed-to-water ratio for 2 hours. This protocol has shown promising results in reducing antinutrients while preserving the nutritional content of beans.

Furthermore, it is essential to optimize soaking and other processing methods to maximize the desired changes in functional properties while minimizing any potential negative effects. This could involve experimenting with different soaking ratios, durations, and combinations of thermal and non-thermal treatments. Future research should delve deeper into the mechanisms behind the observed changes in functional properties due to soaking and other processing methods to maximize the desired changes in functional properties while minimizing any potential negative effects. The findings of this research could be valuable for the food industry, particularly in the development of bean-based products with improved nutritional and functional characteristics while also educating consumers about the benefits of soaking and other processing methods in enhancing the nutritional value and functional properties of legumes could help promote their consumption.

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