Heliyon 10 (2024) e37746

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Quantitative analysis of β-ODAP neurotoxin among different varieties of grass pea (*Lathyrus sativus* L.) flour: A comparative study

Md Kawsar Miah, Md Abdul Alim [*], Md Azizul Haque, Rokeya Begum [**]

Department of Food Technology and Nutritional Science, Faculty of Life Science, Mawlana Bhashani Science and Technology University, Santosh, Tangail, 1902, Bangladesh

ARTICLE INFO

Keywords:
β-ODAP neurotoxin
Protein content
Emulsion
Grass pea flour
Antioxidant

ABSTRACT

Grass pea (*Lathyrus sativus* L.), a protein-rich pulse crop, is often overlooked due to its association with neurolathyrism and its neurotoxin, β-ODAP. The study aims to compare the β-ODAP content, chemical, and functional properties of four BARI varieties and two local varieties of grass pea seed flour. The findings presented that the β-ODAP content of BARI varieties grass pea flour was significantly ($p < 0.05$) lower than local varieties, and the least amount of β-ODAP was found in BARI-3 varieties (0.086 %), which is below the safe level (0.15 %) for consumption. The safe level of neurotoxin was also found in the BARI-1 variety (0.13 %), but local varieties grass pea flour of Pabna and Tangail showed a significantly higher ($p < 0.05$) value of 0.39 and 0.49 % β-ODAP content, respectively. There were no significant differences in protein content among BARI and local varieties, with the highest value of 26.58 % protein content found in the BARI-2 variety. In terms of functional properties, the BARI-5 variety had the highest water absorption capacity (2.92 ml/g) and oil absorption capacity (1.48 ml/g). The grass pea BARI variety, with its high oil absorption capacity and low β-ODAP content, can be utilized in food formulations for bakery products, sausages, and functional ingredients.

1. Introduction

A prospective crop, grass peas (*Lathyrus sativus* L.), is now farmed on an estimated 1.50 million hectares globally, yielding 1.20 million tons of product annually [1,2]. It's a healthy way to get protein, which may compensate for the increasing demand for protein among populations worldwide. Additionally, it is a great crop that requires little input, doesn't need fertilizer or irrigation, and is less vulnerable to insect pests than the majority of other legumes [3]. Grass pea, an ancient cultivated crop, was once a special food for kings and is now a survival food for the poor. Its tasty foods are popular in Europe, Africa, and South Asia. Local farmers sell their products outside commercial systems, and grass pea is used for animal feed, cereal supplementation, and human consumption [1,2]. In

* Corresponding author. Department of Food Technology and Nutritional Science, Faculty of Life Science, Mawlana Bhashani Science and Technology University, Santosh, Tangail, 1902, Bangladesh.
** Corresponding author. Department of Food Technology and Nutritional Science, Faculty of Life Science, Mawlana Bhashani Science and Technology University, Santosh, Tangail, 1902, Bangladesh.
E-mail addresses: kawsarftns@gmail.com (M.K. Miah), alim.food@mbstu.ac.bd (M.A. Alim), mdazizul.haque@mbstu.ac.bd (M.A. Haque), rokeya.ftns@mbstu.ac.bd (R. Begum).

https://doi.org/10.1016/j.heliyon.2024.e37746
Received 10 February 2024; Received in revised form 6 September 2024; Accepted 9 September 2024
Available online 10 September 2024
2405-8440/

the food industry, it has great value for adding protein to human food as well as in the animal food chain.

Grass peas are a popular legume in arid and semiarid regions due to their adaptability to low water conditions and poor-quality soils. They have narrow leaves, winged stems, and a deep root system, which help them thrive in various soil types [1,2,4]. Grass peas have a global impact in terms of good nitrogen fixers, high yield potential at low fertilization levels, and hardy root systems [5,6]. They are commonly used as an intercrop with wheat and rice, and their high yield potential at low fertilization levels makes them a cost-effective crop [2,6]. Grass peas are popular across Europe, Africa, and South Asia for various dishes, including cooking, boiling, roasting, making into drinks or sauces, and eating uncooked seeds [2,6,7]. In addition to being fed by humans and animals, legumes are used in medications, resins, coatings, soap, paints, and cosmetics, making them an important economic component of world trade [8,9]. Grass peas are high in lysine and have a protein level of up to 29.9 % w/w [10].

Despite being considered a promising crop with a high protein content, its uses have been limited due to the presence of β-N-oxalyl-L-α, also known as β-oxalyl amino alanine (BOAA), β-diaminopropionic acid (ODAP), or β-oxalyl amino acid. β-ODAP, a neurotoxin found in Lathyrus sativus seeds, is linked to neurolathyrism, an upper motor neuron degenerative disease characterized by spastic paraplegia of the lower limbs [11,12]. Excessive consumption of these seeds in Asia and Africa has been linked to neurolathyrism, a non-protein neurotoxic amino acid that can cross the blood-brain barrier, accumulate in the central nervous system, and produce severe convulsions. Lathyrism, characterized by spastic paraparesis, can lead to permanent disability. Historically, it has affected populations relying on Lathyrus sativus, a primary food source. Economically, communities may face decreased productivity and social stigma [13,14]. According to reports, consuming too many grass pea seeds over an extended period of time may result in neurolathyrism [15,16]. It is unfortunate that the grass pea has been disregarded up until now despite having very strong agronomic and nutritional features because of unfavorable press regarding its harmful effects. However, scientists and researchers are working to restore this crop's global dispersal [17]. Bangladesh also has a higher prevalence of protein energy malnutrition (PEM) [18]. As grass peas contain a higher amount of protein (20–30 %), by effectively extracting this protein from grass pea seeds, we can use this as an alternative source for protein enrichment in various bakery food products [19,20]. Bangladesh Agricultural Research Institute (BARI) has released high-yielding and low-level of ODAP BARI (1, 2, 3, 4, and 5) varieties of grass pea, which is lower than the detrimental level. Until now, quantitative analysis of β-ODAP neurotoxin among different varieties of grass pea flour has received very little attention for research. Although grass pea flour has several nutritional advantages, the presence of β-ODAP in it poses serious health hazards, particularly in regions where it is a main meal. There have been reports of variations in the amount of β-ODAP in various grass pea varieties, but there aren't many thorough comparison studies. The goal of the current study was to ascertain the approximate composition of the BARI-Kheshari-1,2,3,5 grass pea varieties as well as the local Pabna and Tangail variety. It also aimed to evaluate the functional characteristics of various varieties of *Lathyrus sativus* L. flour, assess the flour's antioxidant capacity, and compute the amount of neurotoxin present. The findings of the study were expected to enable both public and private policymakers to make wise decisions regarding the high amount of protein that will benefit local communities by encouraging them to consume protein from plant sources instead of animal sources. The study will raise awareness and enhance initiatives to consume grass pea as a protein source by reducing the neurotoxin content with processing, highlighting the role of nutrients in grass pea. It was anticipated that grass pea flour would find use in the creation of food items high in protein based on its functional characteristics.

2. Materials and methods

The research was carried out at the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, and the General and Research Laboratory of the Food Technology and Nutritional Science Department, Mawlana Bhashani Science and Technology University (MBSTU), Tangail.

2.1. Collection of samples

A grass pea variety (BARI 1, 2, 3, and 5) sample was collected from the Bangladesh Pulse Research Institute, Ishwardi, Pabna, Bangladesh. Due to its unavailability, the BARI-4 variety of grass pea could not be collected. Local varieties were collected from the local markets in Pabna and Tangail, Bangladesh. In Tangail, Bangladesh, the local market provided the rest of the components for assessment. Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) provided all analytical reagents and standards.

2.2. Sample preparation

The collected grass pea samples were pulverized into fine flour using a blender (UD-013, India). In the flour, other materials such as husk, clay, and wood could be found. As a result, the flour was sieved through a mesh screen with a 100 μm sieve size, eliminating sawdust, husk, clay, and wood. This method produced samples that were pure. Afterwards, the grass pea flour samples were stored in a regular temperature storage unit for future research.

2.3. Chemical analysis of grass pea flour

The [21] technique was used to assess the moisture, ash, and crude fiber content of the researched kinds of grass pea flour. The Kjeldahl technique outlined in the [21] method was used to evaluate the crude protein concentration. The crude carbohydrate (CHO) content was determined, according to Ref. [22] description.

2.4. Determination of antioxidant activity

Antioxidant activity is determined by the methods of [23]. At first, a 3-g flour sample was positioned in a 50-ml beaker, and 30 ml of methanol was added to the sample and mixed well. Extract the solution in a beaker using cotton in the funnel. Then re-filter it until a clear supernatant is obtained. After that, the prepared DPPH solution (1 g of DPPH) and 50 ml of methanol were mixed well in a beaker. A micropipette was washed correctly, and 1 ml of sample extract and 3 ml of DPPH solution were taken with the micropipette to measure the absorbance in the spectrometer. Finally, the absorbance was assessed at 515 nm against the corresponding blank solution, which is ready by taking 1 ml of methanol and 3 ml of DPPH solution. The assay was performed in triplicates. The following Eq. (1) was used to determine the antioxidant activity of grass pea flour.

$$DPPH\ scavenged\ (\%) = (A - B)/Ax100 \tag{1}$$

Where, A = the absorbance of the DPPH solution.

B = absorbance of the extract of sample taken after 15 min of reaction with DPPH.

2.5. Functional properties analysis of grass pea flour

2.5.1. Water absorption capacity (WAC) and oil absorption capacity (OAC)

The technique outlined by Ref. [24] was used to calculate the WAC and OAC. A 1 g of the sample was combined with 10 ml of distilled water, allowed to sit at room temperature for 30 min, and then centrifuged at 2000 rpm for 10 min. After centrifugation, the supernatant was separated and measured. Except for substituting 10 mL of commercially refined soybean oil for the distilled water in the OAC procedure, the methodology used was identical to that of WHC. Per gram of the sample, WAC and OAC were represented as percentages of water-bound content. The following Eqs. 2 and 3. were used to analyze the WHC and OAC.

$$\text{Water absorption capacity (ml / g)} = (W_1 - W_2) / S \times 100 \tag{2}$$

Where, W_1 = ml of water added to the sample, W_2 = ml of supernatant water, and S = weight of the sample

$$\text{Oil absorption capacity (ml / g)} = (W_1 - W_2) / S \times 100 \tag{3}$$

Where, W_1 = ml of oil added to the sample, W_2 = ml of supernatant oil, and S = weight of the sample.

2.5.2. Bulk density

The bulk density was determined utilizing the technique described by Ref. [25]. Initially, a 100-ml graduated cylinder containing 50 g of the material was tapped 20–30 times. Subsequently, the sample's volume was assessed. Using Eq. (4), the bulk density was computed as the weight of the sample per unit volume.

$$Bulk\ density\ (g / ml) = weight\ of\ sample/volume\ \text{occupied}\ by\ the\ sample \tag{4}$$

2.5.3. Swelling capacity

The swelling capacity was assessed using the technique described by Ref. [25], with slight modifications. First, water was poured to a 100 ml graduated cylinder filled with the sample up to the 10 ml mark, bringing the total amount down to 50 ml. By inverting the cylinder, the graded cylinder's top was snugly covered and blended. After 2 min, the suspension was reversed once more, and it was left to stand for an additional 30 min. After 30 min, the sample's volume was measured and reported as the final volume. Swelling capacity was calculated as a percentage of the volume increase due to swelling using the following Eq. (5).

$$Swelling\ capacity = (M_2 - M_1) / M_1 \times 100 \tag{5}$$

Where, M_1 = the initial volume of the sample and M_2 = the final volume of the sample.

2.5.4. Emulsion capacity (EC)

Following the dispersion of the flour/blend (2-g) in 10 mL of distilled water, the height of the solution in the cylinder was measured. After homogenizing the solution with 5 mL of refined canola oil, the resultant emulsion was centrifuged for 5 min at 1100×*g*. The emulsifying activity was determined by measuring the height of the emulsified layer and using Eq. (6) to compute the percent increase in the height of the solution, following [26].

$$EC\ (\%) = H_2/H_1 \times 100 \tag{6}$$

Where, H_1 = initial height of the solution before emulsification and H_2 = height of the emulsified layer.

2.5.5. Emulsion stability

The approach of [27] was utilized to ascertain the emulsion stability. The centrifuged sample was heated to 80 °C for 30 min, and then it was cooled to room temperature to determine the emulsion stability (ES). The material was then centrifuged for 10 min at 4000 rpm. The emulsification stability was determined by applying Eq. (7) to the height of the emulsion layer, which was recorded.

$$Emulsion\ stability = V_2/V_1 \times 100 \tag{7}$$

Where, V_2 = volume of the emulsion layer after heating (ml), and V_1 = volume of the whole layer (ml).

2.5.6. Foaming capacity

The foaming capacity is determined using the method published by Ref. [28] (Eq. (8)). A 2 g sample was blended for 1 min in 100 mL of distilled water. The liquid was then swiftly poured into a 250 mL measuring cylinder, and the volume of foam was recorded.

$$Foaming\ capacity = (V_2 - V_1)_/ V_1 \times 100 \tag{8}$$

Where, V_1 = initial volume of foam and V_2 = final volume of foam.

2.5.7. Foaming stability

Foaming stability was measured by investigation the fall in volume of foam after every 10 min for 1 h by means of the subsequent Eq. (9).

$$Foaming\ stability = volume\ of\ foam\ after\ a\ set\ period\ of\ time / initial\ foam\ volume \times 100 \tag{9}$$

2.6. Determination of amino acids of grass pea flour

A method for evaluation of free amino acids in the flour of *Lathyrus sativus* using RP-HPLC with UV detection at 280 nm was carried out.

2.7. Quantitative estimation of β-ODAP (β-N-oxalyl-L-α, β-diaminopropionic acid) by OPT method

The process of figuring out a solution's precise concentration is called standardization. Among the analytical techniques frequently employed in standardization is titration. A titration involves reacting a precise volume of one chemical with a known volume of another. The process of standardizing 2,3-diaminopropionicacid (DAP) standard solution is carried out in compliance with Table S1.

The standard graph was constructed using a range of concentrations of the standard, ranging from 10 μl to 100 μl of standard. Fig. S1 shows the optical density vs. concentration curve. The β-ODAP content was estimated by the OPT (ophthaldehyde) method. This explains the normal ODAP concentration range of 0.176–1.760. Two portions of the seed sample, 20 and 40 μl, were collected for estimation. 200 μl of 3N KOH was added to each test tube, and they were all incubated for 20 min in a boiling water bath. After the incubation and chilling phases, distilled water was added to each test tube until the final volume reached 1 mL. The addition of 2 ml of OPT reagent came next. For the purpose of developing color, the components were incubated for 20 min at room temperature. The T60 UV–VIS spectrophotometer was used to measure the color generated at 420 nm.

2.8. Statistical analysis

SPSS (version 20) was used to conduct statistical analysis. One-way analysis of variance (ANOVA) was used to identify differences in proximate composition and functional qualities. The study employed a significance threshold of $p < 0.05$. Using Microsoft Excel 2016, all of the tests were replicated and their means and standard deviations were calculated.

3. Results and discussion

3.1. Proximate composition and energy value of grass pea flour

Table 1 summarizes the proximate composition of BARI-1, BARI-2, BARI-3, BARI-5, the Local Pabna, and the Local Tangail variety of grass pea flour (*Lathyrus sativus* L.). Among the varieties, the highest amount of moisture percent was found in the BARI-2 variety, which is 13.07 percent and significantly ($p < 0.05$) different from other varieties. The lowest amount of moisture is 10.36 percent

Table 1
Proximate composition and energy value of different varieties of grass pea flour.

Parameters	BARI-1	BARI-2	BARI-3	BARI-5	Local Pabna (LP)	Local Tangail (LT)
Moisture (%)	11.98 ± 1.04^{abc}	13.07 ± 1.72^{c}	11.50 ± 0.61^{abc}	10.91 ± 0.34^{ab}	10.36 ± 0.66^{a}	12.80 ± 1.44^{bc}
Ash (%)	2.72 ± 0.72^{a}	3.24 ± 0.74^{a}	3.09 ± 0.16^{a}	2.58 ± 0.50^{a}	2.98 ± 0.06^{a}	2.79 ± 1.19^{a}
Crude fat (%)	1.59 ± 0.52^{a}	1.91 ± 0.33^{ab}	1.60 ± 0.10^{a}	1.53 ± 0.39^{a}	2.82 ± 0.56^{c}	1.36 ± 0.12^{a}
Crude protein (%)	26.09 ± 2.71^{a}	26.58 ± 1.24^{a}	25.40 ± 0.52^{a}	26.05 ± 0.19^{a}	26.09 ± 0.80^{a}	26.06 ± 1.26^{a}
Crude fiber (%)	5.37 ± 0.58^{ab}	4.49 ± 0.88^{a}	4.56 ± 0.41^{ab}	4.99 ± 0.23^{ab}	6.6 ± 0.45^{c}	5.62 ± 0.61^{bc}
Carbohydrate (%)	57.62 ± 1.89^{bc}	55.18 ± 0.50^{a}	58.39 ± 0.70^{c}	58.91 ± 0.44^{c}	57.75 ± 0.96^{bc}	55.98 ± 1.78^{ab}
Nitrogen free extract (%)	52.26 ± 1.77^{ab}	50.7 ± 1.38^{a}	53.83 ± 0.93^{b}	53.92 ± 0.56^{b}	51.15 ± 1.27^{ab}	50.35 ± 2.16^{a}
Energy value (Kcal/100g)	349.1 ± 6.7^{ab}	344.28 ± 6.9^{a}	351.49 ± 4.5^{ab}	353.64 ± 3.3^{ab}	360.77 ± 2.53^{b}	349.43 ± 9.5^{ab}

Values are Mean ± SD of three replicates. Different superscript in the same row indicates significant differences at $p < 0.05$.

found in the local Pabna variety, but 10.91 percent in the BARI-5 variety, which is the lowest among BARI varieties. About 13 percent moisture content was reported by Ref. [29]. The flour's relatively low moisture level improves storage stability by inhibiting mold formation and minimizing biochemical processes [9]. The ash content of the local Pabna and Tangail varieties was found to be 2.98 and 2.79 percent, respectively, which is comparable to BARI-1 (2.72).

The highest ash content in the studied grass pea variety flour was 3.24 percent, which was found in BARI-2. The previous study [30] reported about 3.30 percent ash in grass pea flour. Ash content helps determine the amount and type of minerals in grass pea flour. The protein content of grass pea flour was not significantly different. Among them, only the BARI-3 variety possessed a slightly lower protein content, which was 25.4 percent. It is [30] reported 25.60 percent protein in grass peas. Recently, grass pea flour used as rich source of protein in bakery formulation and also making infant food preparation for its high emulsifying ability [31]. The highest amount of crude fat (2.82 percent) and crude fiber (6.6 percent) content is found in the local Pabna variety, which is significantly ($p < 0.05$) greater than other varieties. It is [32] reported 2.08 percent fat content in grass pea flour. The previous study [33] displayed the fiber content of grass pea flour was 5.31 percent, which is similar to the BARI-1 variety. Fiber can serve to improve the overall nutritional profile of grass peas as well as add a number of functional advantages. BARI-5 showed significantly ($p < 0.05$) greater amounts of carbohydrate (58.91 percent) and nitrogen-free extract (53.92 percent) than local varieties. It is [34] reported an amount of 55.8 percent carbohydrate in grass pea flour, which was similar to the BARI-2 variety. The energy value of the local Pabna variety was 360.77 Kcal/100 g of flour, which was higher than other studied varieties and considerably different ($p < 0.05$) from all other varieties. It is due to the highest fat value and lowest water content (10.36 %) of the local Pabna variety. The previous study [35] reported an energy value of 361 Kcal/100g in grass pea flour.

3.2. Antioxidant capacity of grass pea flour

The antioxidant capacity of grass pea flour is presented in Fig. 1. Among the BARI varieties, the maximum value for antioxidant activity was found in the BARI-2 variety, which is 11.05 percent. It is [36] reported an antioxidant activity of 7.98 percent in grass pea, which was close to the local Pabna variety but significantly different from the local Tangail variety of grass pea.

3.3. Functional properties analysis of grass pea flour

The various kinds of grass pea flour's water and oil absorption capacities are shown in Fig. 2(a). The quantity of water that a food product retains following filtration and the application of a light centrifugal pressure is known as its "water absorption capacity" [34]. The findings showed that the BARI-5 variety's oil and water absorption capacities were 1.48 and 2.92 ml/g, respectively, and that these values differed considerably ($p < 0.05$) from those of the native Pabna and Tangail kinds. The ability to absorb water is impacted by the high protein content. The lowest value for water absorption capacity was observed in the local Pabna variety, which is 1.07 ml/g, and the lowest value for oil absorption capacity was 0.78 ml/g in the BARI-2 variety. The another study [37] reported a water absorption capacity of 2.70 ml/g in grass pea flour, which is near the BARI-5 variety. BARI-2 and the local Tangail variety possessed a near-similar value of oil absorption capacity, and their amounts were 0.78 and 0.89 ml/g, respectively.

According to Ref. [38], flour's relatively high oil absorption capacity suggests that it may be helpful in food compositions that require oil retention capacity, including sausage and bread goods. These qualities suggest that the protein may also find application as a functional element in dishes like angel and sponge cakes, whipped toppings, and chiffon desserts [39]. Fig. 2(b) shows the bulk densities of many types of grass pea flour. The food business uses bulk density, a measurement of flour's weight, to determine how to handle materials, package products, and apply them [34]. Bulk density did not differ significantly between BARI and local grass pea flour varieties. The previous study [40] reported 0.48 g/mL bulk density in grass pea flour, which is similar to the observed values of BARI-1 and BARI-3 varieties.

Fig. 2(c) displays the swelling and foaming capacities of many types of grass pea flour. According to Ref. [35], the swelling capacity provides an indication of the sample's starch's ability to absorb water under particular circumstances, such as temperature and water availability. A value of 120.6 percent was observed in the BARI-2 variety, which had the highest swelling capacity among BARI as well as the total studied varieties. It is the high moisture content of the BARI-2 variety that causes high swelling capacity. Relatively high

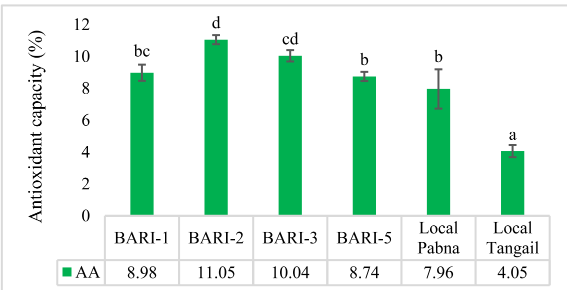

Fig. 1. Antioxidant capacity of Grass pea flour (*Lathyrus sativus* L.). Different superscript in the different bar indicates significant differences at $p < 0.05$.

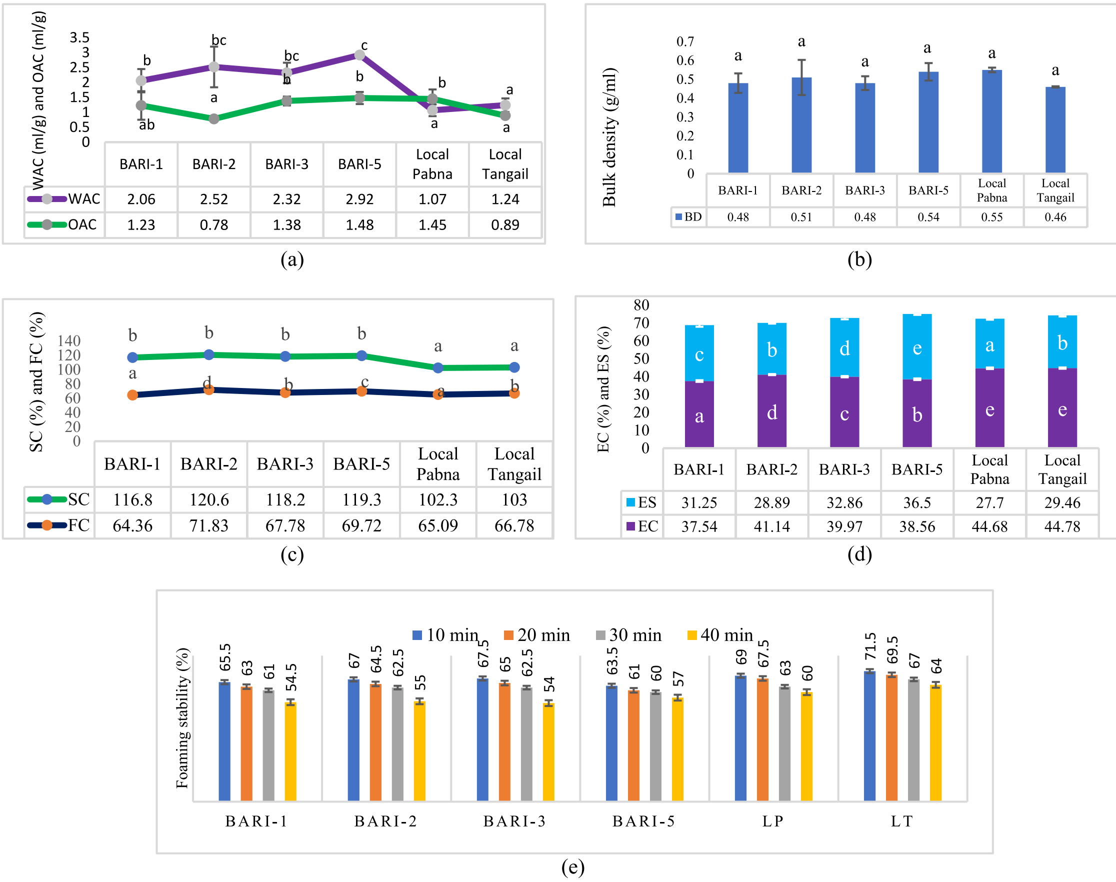

Fig. 2. Functional properties of different varieties Grass pea flour (*Lathyrus sativus* L.). (a) Water absorption capacity and oil absorption capacity, (b) Bulk density, (c) Swelling capacity and Foaming capacity, (d) Emulsion capacity and emulsion stability, (e) Foaming stability. Different superscript in the different bar and line indicates significant differences at $p < 0.05$.

swelling capacity considered as more appropriate for bakery product formulation for shaping good texture and structure [41]. In BARI-1 and the local Pabna variety, foaming capacity was observed at a near-similar value of 64.36 and 65.09 percent, respectively. The highest amount of 71.83 percent foaming capacity was observed in BARI-2, which was significantly ($p < 0.05$) different.

The higher the protein level of the flour, the higher the foaming capability, which is why BARI-2 has a larger protein content. It is [35] reported a foaming capacity of 56.32 percent in grass pea flour. The higher foaming capacity positively impacts the appearance, consistency and volume of the baked goods (sponge cake, cookies etc.) by causing the flour protein to cohesively surround the air bubble [42]. The emulsion capacity and emulsion stability of different varieties of grass pea seed powder are presented in Fig. 2(d). The emulsion capacity of local varieties was significantly higher than that of BARI varieties. The local Pabna and local Tangail varieties showed 44.68 and 44.78 percent emulsion capacity, respectively, whereas BARI-2 showed 41.14 percent, which was the highest among BARI varieties. A larger protein content might be the cause of a better emulsion capacity [43]. Grass pea flour has an emulsion capability of 41.04 percent, according to Ref. [40]. Grass pea flour's increased emulsion capacity indicated that it was more digestible, making it a potential addition for baby food recipes. In terms of the stability of the emulsion, BARI-5 variety grass pea flour showed the highest value, which was 36.50 percent, and BARI-3 showed 32.86 percent stability. The lowest emulsion stability was observed in the local Pabna variety, which was 27.70 percent, and this value is considerably different ($p < 0.05$) from the BARI-5 variety. The reason for the maximum emulsion stability is the examined grass pea flour's high protein and low-fat content. It is [37] stated the lowest value of 29.75 percent emulsion stability in grass pea flour and the highest value of 81.74 percent reported by Ref. [27].

The foaming stability of different varieties of grass pea flour is presented in Fig. 2(e). Foaming stability of the local Tangail variety was always found to be high compared to other studied local and BARI varieties.

High foaming stability is due to the high protein content. The values for foaming stability of the local Tangail variety were 71.5 percent for 10 min, 69.5 percent for 20 min, 67 percent for 30 min, and finally 64 percent for 40 min. It is [27] reported a foaming stability of 90 percent at 10 min, 89 percent at 20 min, 84.5 percent at 30 min, and 82 percent at 40 min.

3.4. Amino acid of grass pea flour

The amino acid content of different varieties of grass pea flour is presented in Table 2 and chromatogram of amino acid of different varieties is presented in Fig. 3. The amino acid composition in food denotes the nutritional quality of food proteins [44]. Glutamic acid is found to be the most abundant in the local Tangail variety, followed by aspartic acid, arginine, leucine, lysine, and serine. It is [45] reported 4 percent glutamic acid in grass pea flour. No cysteine was found in the BARI variety, whereas 0.02 and 0.03 percent were noted in the local Pabna and local Tangail varieties. It is [7] reported a significant level of cysteine content, which was 1.79 percent.

3.5. Determination of β-ODAP content of grass pea flour

The β-ODAP content of different varieties of grass pea flour is presented in Fig. 4 β-ODAP content of local varieties was significantly ($p < 0.05$) higher than that of BARI varieties. It is due to the breeding and research strategy for the high-yielding and low β-ODAP-content variety developed by BARI. When the samples from the Rajshahi Division and the coastal districts in Bangladesh were assessed at BARI in 1993–1994, the β-ODAP content of the seed varied from 0.04 to 0.75 % with a mean of 0.32 % [46]. Locality, growth circumstances, and environment all affect β-ODAP levels [16]. For local Pabna and local Tangail varieties, the observed values for neurotoxin β-ODAP were 0.39 and 0.49 percent, respectively. It was 0.086 percent for BARI-3, which was similar to the value of 0.08 percent described by Ref. [47]. Among BARI varieties, the highest β-ODAP content of 0.22 percent was observed in the BARI-5 variety, which was similar to Ref. [48]. In the cases of BARI-1 and BARI-2, the observed values were 0.139 and 0.195 percent, respectively. It is [46] reported β-ODAP content of 0.06 percent in the BARI-1 variety and 0.0137 percent in the BARI-2 variety. β-ODAP content in *Lathyrus sativus* L. *is* 0.02–2.59 percent, as reported by Refs. [45,49]. It is [50] reported a β-ODAP content of 0.16–0.34 percent in grass pea flour. It is [51] reported that β-ODAP content of <1.5 mg/g (0.15 %) in *L. sativus* seeds is safe for human consumption. So, the result revealed that the amount of β-ODAP content in BARI-1 and BARI-3 varieties is below the safe level of consumption. High β-ODAP content responsible for lathyrism, a degenerative motor neuron syndrome [50]. People should avoid high β-ODAP contained varities.

To reduce β-ODAP concentration to a safe level for human consumption, many efforts have been made including effective agronomic practices (breeding Low-ODAP genotype), and food processing techniques such as soaking in water, cold water treatment, and hot water treatment [52]. Low β-ODAP varieties from BARI will have a positive impact on farmers, local communities, the food industry and all group of people in the world by providing safe and nutritious food.

In Bangladesh, people have a strong reliance on BARI (Bangladesh Agricultural Research Institute) and their new varieties for cultivation. Therefore, it is essential to conduct comprehensive tests and comparisons among all BARI varieties. The exclusion of the BARI-4 variety of grass pea from the current study was a notable limitation.

4. Conclusion

This study analyzed the neurotoxin content of nutrient-rich grass pea (*Lathyrus sativus* L.) and compared it to BARI and two local varieties. The results showed that all BARI and local varieties were high in protein, but two local varieties had high crude fiber content. The functional properties of local varieties were significantly different than BARI varieties, suggesting they could be used in food like whipped toppings and sponge cakes. The emulsion capacity of local varieties was significantly higher than BARI varieties, making them suitable for infant food formulations. The low neurotoxin content of BARI varieties makes them more suitable for consumption and prepared for producing various food formulations and making the food nutrient dense which can be techno-economically feasible and can be exploited commercially. Thus, it is expected in the current study that farmers and local people will have to more attention in low neurotoxin content of BARI varieties (BARI-1 and BARI-3) for safe agricultural practices. For future research direction, genetic

Table 2
Amino acid content of different varieties of grass pea flour.

Amino acid	BARI-1(%)	Local Pabna (LP) (%)	Local Tangail (LT) (%)
Asp	2.88	2.64	2.93
Thr	0.89	0.85	0.94
Ser	1.49	1.26	1.43
Glu	5.18	4.78	5.27
Pro	1.10	0.95	1.10
Gly	1.16	1.02	1.14
Ala	1.23	1.04	1.18
Cys	0.00	0.02	0.03
Val	1.21	1.13	1.24
Met	0.00	0.00	0.00
Ile	1.08	1.03	1.10
Leu	1.91	1.77	1.93
Tyr	0.92	0.83	0.88
Phe	1.24	1.13	1.25
His	0.70	0.55	0.61
Lys	1.81	1.67	1.84
Arg	2.48	2.24	2.42

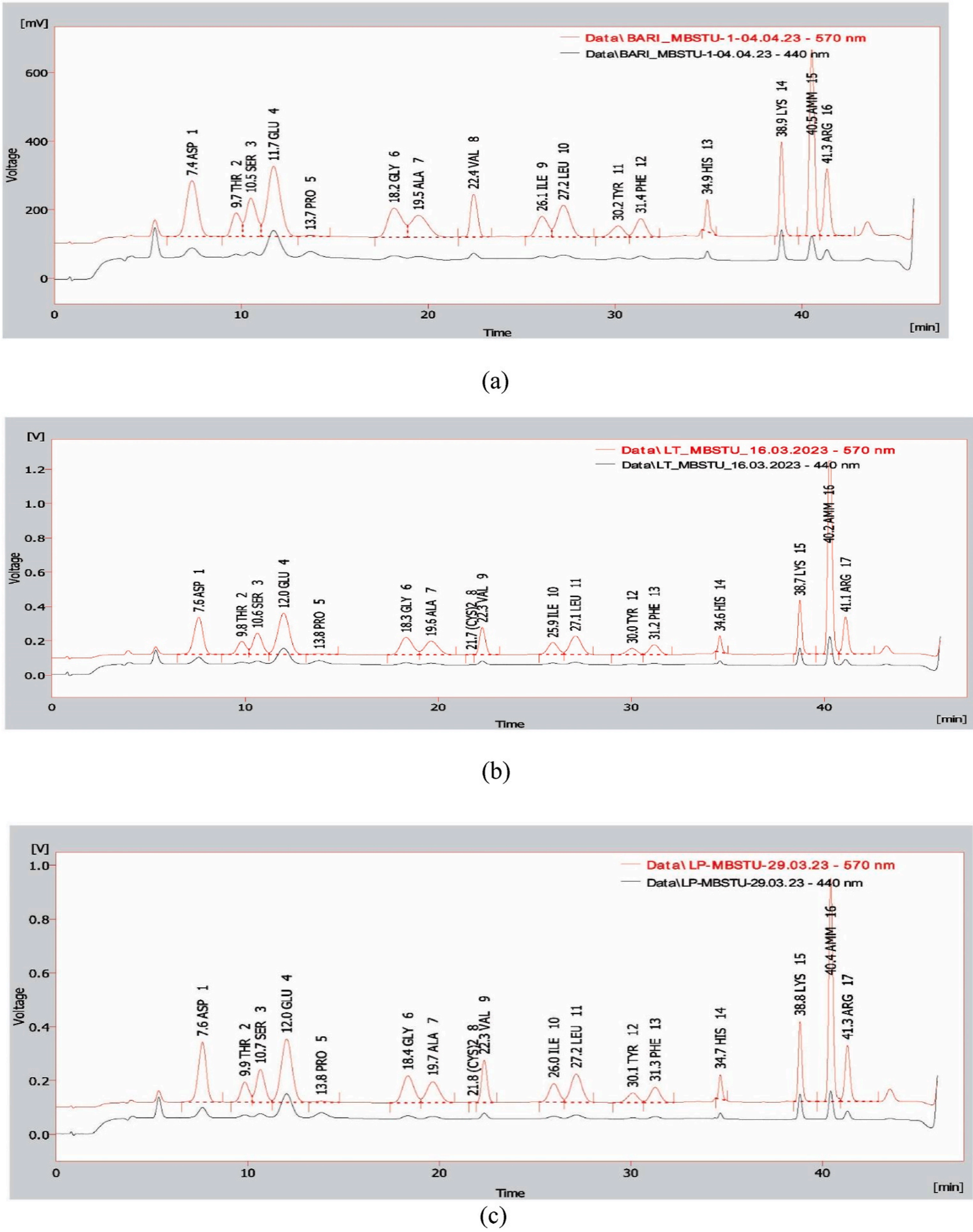

Fig. 3. Chromatogram of amino acid of different varieties of grass pea flour. (a) BARI variety, (b) Local Tangail variety, (c) Local Pabna variety.

diversity, molecular genetics, gene editing, identify low-ODAP genotypes, breeding Low-ODAP genotype and also precision agriculture technique that could be helpful for improving cultivation practices of grass pea. Explore other anti-nutritional and toxic substances such as phytates, tannins. Explore the potential incorporation of grass pea flour as protein ingredient in bakery formulation in broad range such as bread, tortilla, and cookies etc. Bio-fortification and biotechnological approach might be increased nutritional content of grass peas.

Ethical statement

This manuscript does not contain any studies with human participants or animals performed by any of the authors. Ethics approval or patient consent was not required for this study.

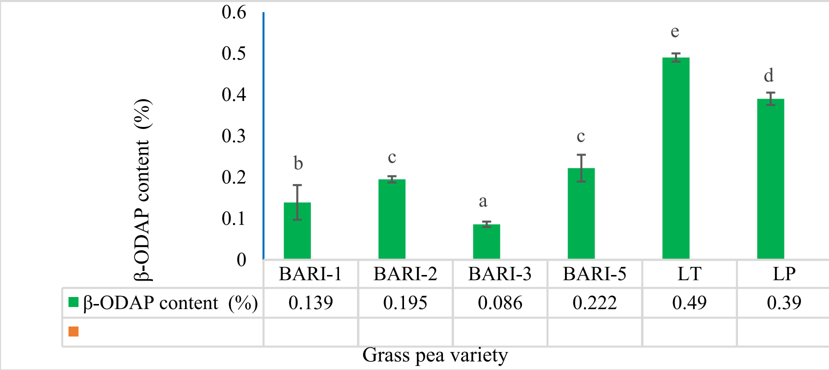

Fig. 4. β-ODAP content (%) of different varieties of grass pea flour.

Data availability statement

Data will be made available upon request.

CRediT authorship contribution statement

Md Kawsar Miah: Writing – original draft, Methodology, Formal analysis, Data curation. **Md Abdul Alim:** Writing – review & editing, Formal analysis, Data curation. **Md Azizul Haque:** Writing – review & editing, Methodology. **Rokeya Begum:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The Ministry of Science and Technology of the People's Republic of Bangladesh provided financial support for the research project titled "Nutrient composition and functionality of grass pea seeds available in Bangladesh (Gr. No.BS-289)" which was the basis for this study. The authors would like to thank them for their generosity and also thank the Department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University in Bangladesh, for providing laboratory facilities for this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37746.

References

[1] S. Kumar, G. Bejiga, S. Ahmed, H. Nakkoul, A. Sarker, Genetic improvement of grass pea for low neurotoxin (β-ODAP) content, Food Chem. Toxicol. 49 (3) (2011) 589–600, https://doi.org/10.1016/j.fct.2010.06.051.
[2] F. Lambein, S. Travella, Y.H. Kuo, M.V. Montagu, M. Heijde, Grass pea (Lathyrus sativus L.): orphan crop, nutraceutical or just plain food? Planta 250 (2019) 821–838, https://doi.org/10.1007/s00425-018-03084-0.
[3] L. Gonçalves, D. Rubiales, M.R. Bronze, M.C.V. Patto, Grass pea (Lathyrus sativus L.)—a sustainable and resilient answer to climate challenges, Agronomy 12 (6) (2022) 1324, https://doi.org/10.3390/agronomy12061324.
[4] G.P. Dixit, A.K. Parihar, A. Bohra, N.P. Singh, Achievements and prospects of grass pea (Lathyrus sativus L.) improvement for sustainable food production, Crop J 4 (5) (2016) 407–416, https://doi.org/10.1016/j.cj.2016.06.008.
[5] F.J. Muehlbauer, W.J. Kaiser, Expanding the production and use of cool season food legumes: a global perspective of peristent constraints and of opportunities and strategies for further increasing the productivity and use of pea, lentil, faba bean, in: Chickpea and Grasspea in Different Farming Systems, vol. 19, Springer Science & Business Media, 2012.
[6] E. Wiraguna, A.I. Malik, T.D. Colmer, W. Erskine, Waterlogging tolerance of grass pea (Lathyrus sativus L.) at germination related to country of origin, Exp. Agric. 56 (6) (2020) 837–850, https://doi.org/10.1017/S0014479720000356.
[7] E.R. Grela, W. Rybiński, R. Klebaniuk, J. Matras, Morphological characteristics of some accessions of grass pea (Lathyrus sativus L.) grown in Europe and nutritional traits of their seeds, Genet. Resour. Crop Evol. 57 (2010) 693–701, https://doi.org/10.1007/s10722-009-9505-4.
[8] P. Henderson, Grass pea (lathyrus sativus). Understudied Indig Crops, 2023.
[9] R.J. Singh, Landmark research in oilseed crops, in: R.J. Singh (Ed.), Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Oilseed Crops, vol. 4, CRC Press, Inc., Boca Raton, Fla, 2006, pp. 1–12, https://doi.org/10.1201/9781420005363.ch1.

[10] F. Stagnari, A. Maggio, A. Galieni, M. Pisante, Multiple benefits of legumes for agriculture sustainability: an overview, Chem Biol Technol Agric 4 (2017) 1–13, https://doi.org/10.1186/s40538-016-0085-1.

[11] V. Bisignano, Variation for protein content and seed weight in grass pea (Lathyrus spp.) germplasm, Bull Resso Phytogénétiques. (2002).

[12] S.L.N. Rao, P.R. Adiga, P.S. Sarma, The isolation and characterization of β-N-oxalyl-L-α, β-diaminopropionic acid: a neurotoxin from the seeds of Lathyrus sativus, Biochemistry 3 (3) (1964) 432–436, https://doi.org/10.1021/bi00891a022.

[13] P. Spencer, A. Ludolph, M.P. Dwivedi, D. Roy, J. Hugon, H.H. Schaumburg, Lathyrism: evidence for role of the neuroexcitatory aminoacid BOAA, Lancet 328 (8515) (1986) 1066–1067, https://doi.org/10.1016/S0140-6736(86)90468-X.

[14] S.L.N. Rao, A sensitive and specific colorimetric method for the determination of α, β-diaminopropionic acid and the Lathyrus sativus neurotoxin, Anal. Biochem. 86 (2) (1978) 386–395, https://doi.org/10.1016/0003-2697(78)90762-5.

[15] T. Mehta, A.J. Parker, P.K. Cusick, N.S. Zarghami, B.E. Haskell, The Lathyrus sativus neurotoxin: evidence of selective retention in monkey tissue, Toxicol. Appl. Pharmacol. 52 (1) (1980) 54–61, https://doi.org/10.1016/0041-008X(80)90247-1.

[16] C.J. Jiao, J.L. Jiang, L.M. Ke, W. Cheng, F.M. Li, Z.X. Li, Factors affecting β-ODAP content in Lathyrus sativus and their possible physiological mechanisms, Food Chem. Toxicol. 49 (3) (2011) 543–549, https://doi.org/10.1016/j.fct.2010.04.050.

[17] Q. Xu, F. Liu, P. Chen, J.M. Jez, H.B. Krishnan, β-N-Oxalyl-l-α, β-diaminopropionic acid (β-ODAP) content in lathyrus sativus: the integration of nitrogen and sulfur metabolism through β-cyanoalanine synthase, Int. J. Mol. Sci. 18 (3) (2017) 526, https://doi.org/10.3390/ijms18030526.

[18] Chandrashekharaiah, P.S., Varshney, A., Kumar, A., Kangade, S., Kushwaha, S., & Sanyal, D. Grass Pea (Lathyrus Sativus) a Crop of Future for Sustainable Agriculture in the Changing Environmental Conditions.

[19] T. Ahmed, M. Mahfuz, S. Ireen, A.S. Ahmed, S. Rahman, M.M. Islam, Nutrition of children and women in Bangladesh: trends and directions for the future, J. Health Popul. Nutr. 30 (1) (2012) 1, https://doi.org/10.3329/jhpn.v30i1.11268.

[20] P. Shanthakumar, J. Klepacka, A. Bains, P. Chawla, S.B. Dhull, A. Najda, The current situation of pea protein and its application in the food industry, Molecules 27 (16) (2022) 5354, https://doi.org/10.3390/molecules27165354.

[21] W. Horwitz, G. Latimer, AOAC-Association of official analytical chemists, Off Methods Anal AOAC Int 18th Ed Gaithersburg Md USA 45 (2005) 75–76.

[22] A.I. Ihekoronye, P.O. Ngoddy, Integrated Food Science and Technology for the Tropics, Macmillan, 1985.

[23] G. Miliauskas, T.A.V. Beek, P.R. Venskutonis, J.P. Linssen, P. de Waard, E.J. Sudhölter, Antioxidant activity of Potentilla fruticosa, J. Sci. Food Agric. 84 (15) (2004) 1997–2009, https://doi.org/10.1002/jsfa.1914.

[24] A.R. Shah, V.S. Gour, S.L. Kothari, P. Sharma, K.B. Dar, S.A. Ganie, Antioxidant, nutritional, structural, thermal and physico-chemical properties of psyllium (Plantago ovata) seeds, Curr. Res. Nutr. Food Sci. J 8 (3) (2020) 727–743, https://doi.org/10.12944/CRNFSJ.8.3.06.

[25] J.C. Okaka, N.N. Potter, Functional and storage properties of cowpea powder-wheat flour blends in breadmaking, J. Food Sci. 42 (3) (1977) 828–833, https://doi.org/10.1111/j.1365-2621.1977.tb12614.x.

[26] K. Jakobson, A. Kaleda, K. Adra, M.L. Tammik, H. Vaikma, T. Kriščiunaite, Techno-functional and sensory characterization of commercial plant protein powders, Foods 12 (14) (2023) 2805, https://doi.org/10.3390/foods12142805.

[27] S. Sethi, D.N. Yadav, S. Snigdha, A. Gupta, Optimization of process parameters for extraction of protein isolates from Khesari dhal (Lathyrus sativus L), LWT 137 (2021) 110368, https://doi.org/10.1016/j.lwt.2020.110368.

[28] M. Naczk, L.L. Diosady, L.J. Rubin, Functional properties of canola meals produced by a two-phase solvent extraction system, J. Food Sci. 50 (6) (1985) 1685–1688, https://doi.org/10.1111/j.1365-2621.1985.tb10565.x.

[29] S. Tömösközi, R. Lásztity, R. Haraszi, O. Baticz, Isolation and study of the functional properties of pea proteins, Food Nahrung 45 (6) (2001) 399–401, https://doi.org/10.1002/1521-3803(20011001)45:6<399::AID-FOOD399>3.0.CO;2-0.

[30] R. Tamburino, A. Chambery, A. Parente, A.D. Maro, A novel polygalacturonase-inhibiting protein (PGIP) from Lathyrus sativus L. seeds, Protein Pept. Lett. 19 (8) (2012) 820–825.

[31] K. Kamaljit, S. Baljeet, K. Amarjeet, Preparation of bakery products by incorporating pea flour as a functional ingredient, Am. J. Food Technol. 5 (2) (2010) 130–135, https://doi.org/10.3923/ajft.2010.130.135.

[32] M.H. Zeidanloo, R.A. Ghavidel, M.G. Davoodi, A. Arianfar, Functional properties of Grass pea protein concentrates prepared using various precipitation methods, J. Food Sci. Technol. 56 (11) (2019) 4799–4808, https://doi.org/10.1007/s13197-019-03930-3.

[33] K. Urga, H. Fufa, E. Biratu, A. Husain, Evaluation of Lathyrus sativus cultivated in Ethiopia for proximate composition, minerals, β-ODAP and anti-nutritional components, Afr J Food Agric Nutr Dev 5 (1) (2005).

[34] K.O. Falade, C.A. Okafor, Physical, functional, and pasting properties of flours from corms of two Cocoyam (Colocasia esculenta and Xanthosoma sagittifolium) cultivars, J. Food Sci. Technol. 52 (2015) 3440–3448, https://doi.org/10.1007/s13197-014-1368-9.

[35] M. Bala, S. Handa, D. Mridula, R.K. Singh, Physicochemical, functional and rheological properties of grass pea (Lathyrus sativus L.) flour as influenced by particle size, Heliyon 6 (11) (2020) e05471, https://doi.org/10.1016/j.heliyon.2020.e05471.

[36] A. Starzyńska-Janiszewska, B. Stodolak, B. Mickowska, The effect of germination on antioxidant and nutritional parameters of protein isolates from grass pea (lathyrus sativus) seeds, Food Sci. Technol. Int. 16 (1) (2010) 73–77, https://doi.org/10.1177/1082013209353355.

[37] S. Feyzi, E. Milani, Q.A. Golimovahhed, Grass pea (Lathyrus sativus L.) protein isolate: study of extraction optimization, protein characterizations, structure and functional properties, Food Hydrocoll 74 (2018) 187–196, https://doi.org/10.1016/j.foodhyd.2017.07.031.

[38] A.M. Ahmed, J. Lydia, J.L. Campbell, Evaluation of baking properties and sensory quality of wheat-cowpea flour, World Acad Sci Eng Technol. 70 (2012) 2012.

[39] S.C. Suresh Chandra, S. Samsher, Assessment of Functional Properties of Different Flours, 2013, https://doi.org/10.5897/AJAR2013.6905.

[40] O. Aletor, C.E. Onyemem, V.A. Aletor, Nutrient constituents, functional attributes and in vitro protein digestibility of the seeds of the Lathyrus plant, WIT Trans Ecol Env. 152 (2011) 145–155, https://doi.org/10.2495/FENV11051.

[41] Z. Šmídová, J. Rysová, Gluten-free bread and bakery products technology, Foods 11 (3) (2022) 480, https://doi.org/10.3390/foods11030480.

[42] H. Nawaz, M.A. Shad, R. Mehmood, T. Rehman, H. Munir, Comparative evaluation of functional properties of some commonly used cereal and legume flours and their blends, Int J Food Allied Sci 1 (2) (2015) 67–73, https://doi.org/10.21620/IJFAAS.2015267-73.

[43] M.O. Iwe, U. Onyeukwu, A.N. Agiriga, Proximate, functional and pasting properties of FARO 44 rice, African yam bean and brown cowpea seeds composite flour, Cogent Food Agric. 2 (1) (2016) 1142409, https://doi.org/10.1080/23311932.2016.1142409.

[44] E. Pastor-Cavada, S.R. Drago, R.J. González, R. Juan, J.E. Pastor, M. Alaiz, Physical and nutritional properties of extruded products based on whole grain with the addition of wild legumes (V icia lutea subsp. lutea var. hirta and V icia sativa subsp. sativa), Int. J. Food Sci. Technol. 48 (9) (2013) 1949–1955, https://doi.org/10.1111/ijfs.12175.

[45] M. Arslan, Diversity for vitamin and amino acid content in grass pea (Lathyrus sativus L.), Legume Res- Int J. 40 (5) (2017) 803–810, https://doi.org/10.18805/LR-369.

[46] M.A. Malek, C.D.M. Sarwar, A. Sarker, M.S. Hassan, Status of grass pea research and future strategy in Bangladesh, in: *Lathyrus Genetic Resources In Asia: Proceedings Of a Regional Workshop*, 27-29 December 1995, Indira Gandhi Agricultural University, Raipur, India IPGRI Office for South Asia, New Delhi, India, 1996, p. 7.

[47] F. Wang, X. Chen, Q. Chen, X. Qin, Z. Li, Determination of neurotoxin 3-N-oxalyl-2, 3-diaminopropionic acid and non-protein amino acids in Lathyrus sativus by precolumn derivatization with 1-fluoro-2, 4-dinitrobenzene, J. Chromatogr. A 883 (1–2) (2000) 113–118, https://doi.org/10.1016/S0021-9673(00)00264-8.

[48] B.S. Dahiya, Seed morphology as an indicator of low neurotoxin in Lathyrus sativus L, Qual Plant 25 (3) (1976) 391–394, https://doi.org/10.1007/BF02590314.

[49] C.D. Hanbury, K.H.M. Siddique, Registration ofChalus' lathyrus cicera L, Crop Sci. 40 (4) (2000) 1199, 1199.

[50] S. Barpete, P. Gupta, K.M. Khawar, S. Kumar, Effect of cooking methods on protein content and neurotoxin (β-ODAP) concentration in grass pea (Lathyrus sativus L.) grains, CyTA-J Food. 19 (1) (2021) 448–456, https://doi.org/10.1080/19476337.2021.1915879.
[51] M. Sacristán, A. Varela, M.M. Pedrosa, C. Burbano, C. Cuadrado, M.E. Legaz, Determination of β-N-oxalyl-l-α, β-diaminopropionic acid and homoarginine in Lathyrus sativus and Lathyrus cicera by capillary zone electrophoresis, J. Sci. Food Agric. 95 (7) (2015) 1414–1420, https://doi.org/10.1002/jsfa.6792.
[52] A.K. Geda, N. Rastogi, R.L. Pandey, New Processing Approaches of Detoxification for Low Toxin Lathyrus, Lathyrus Sativus Hum Lathyrism Decade Prog Ghent Univ Ghent., 1995, pp. 117–120.