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Effect of cooking methods on protein content and neurotoxin (β-ODAP) concentration in grass pea (*Lathyrus sativus* L.) grains

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ABSTRACT

Grass pea (*Lathyrus sativus* L.) is an important food legume crop but notorious for plant neurotoxin β-N-Oxalyl-L-α, β-diaminopropionic acid (β-ODAP), causing neurolathyrism to human and animals. The study aimed to compare changes in physical and chemical parameters of 13 genotypes of different geographic origins in pre & post-boiling, microwaving and autoclaving. The results showed significantly different effects of pre and post-cooking treatments on genotypes and cooking methods independent of one another. The genotypes from South Asia exhibited higher β-ODAP content compared to the genotypes from other region. Significantly negative correlation was noted between protein content and β-ODAP concentration across the treatments under boiling ($r = -0.555**$) and microwave ($r = -0.342*$) treatments. Boiling was the best treatment and significantly reduced β-ODAP concentration by 70%. Its reduction percentage remained 30%, and 14% under microwaving and autoclaving, in the same order. Therefore, boiling was recommended over other treatments for safe human consumption of grass pea seeds.

Efecto de los métodos de cocción sobre el contenido de proteínas y la concentración de neurotoxinas (β-ODAP) en los granos de guisante [chícharo, arveja] (*Lathyrus sativus* L.)

RESUMEN

Aunque el guisante (*Lathyrus sativus* L.) es un importante cultivo de leguminosas alimenticias, es conocido por contener la neurotoxina vegetal β-N-Oxalil-L-α, β-ácido diaminopropiónico (β-ODAP), que causa neurolatirismo en los seres humanos y los animales. El presente estudio se propuso comparar los cambios en los parámetros físicos y químicos de 13 genotipos provenientes de diferentes orígenes al ser sometidos a hervido, previo y posterior, en el microondas y el autoclave. Los resultados permitieron constatar efectos significativamente diferentes en los genotipos ocasionados por los tratamientos previos y posteriores a la cocción y los métodos de cocción independientes entre sí. En comparación con los genotipos provenientes de otras regiones, aquellos procedentes del sur de Asia mostraron un mayor contenido de β-ODAP. Asimismo, se observó una correlación significativamente negativa entre el contenido de proteínas y la concentración de β-ODAP en todos los tratamientos de hervido (r=−0.555**) y microondas (r=−0.342*). El hervido resultó ser el mejor tratamiento; tras su aplicación se redujo significativamente la concentración de β-ODAP en 70%. El porcentaje de reducción se mantuvo en 30% y 14% bajo microondas y autoclave, en el mismo orden. Por lo tanto, para favorecer el consumo humano seguro de semillas de guisantes se recomienda el hervido sobre otros tratamientos.

1. Introduction

Grass pea (*Lathyrus sativus* L.) is an important food legume crop used as human food and animal feed in rainfed dry areas. In the recent past, the crop has received considerable attention from scientific and farming community alike due to its climate smart attributes such as tolerance to drought, waterlogging, heat, cold, and salinity (Lambein et al., 2019). Grass pea is recognized as a source of affordable protein, micronutrients, and fiber of seeds (Sen Gupta et al., 2021; Tamburino et al., 2012; Tarade et al., 2007). Although grass pea is rich in protein, its utilization is limited by the presence of a free non-protein amino acid, called β-N-Oxalyl-L-α, β-diaminopropionic acid (β-ODAP). Its presence has been associated with a disease, neurolathyrism, which is characterized by the paralysis in lower limbs of humans as a consequence of the overconsumption of grass pea in an unbalanced diet for a longer period (Rao et al., 1964). This has brought a bad reputation to grass pea despite many physiological functions with therapeutic potential (Lambein et al., 2019). One of the physiological functions with possible therapeutic potential of β-ODAP is the activation of protein kinase C, which adds a new dimension to explore grass pea potential in the treatment of Alzheimer's disease, hypoxia, and long-term potentiation of neurons essential for memory (Singh & Rao, 2013). In addition to β-ODAP, grass pea grains also contain L-homoarginine (Rao, 2011), which is a modulator of the biosynthesis of nitric oxide which, in turn, reduces the excitation of neuronal receptors (Bell, 2003).

However, β-ODAP with low dosage is also reported as neuro-protective. It has been reported as a neuro-excitatory amino acid also known as dencichine found in *L. sativus* and occur in several Panax species known for its antihemorrhage property. So, this "neuro-excitatory amino acid" may be acceptable as described in Vaz Patto and Rubiales (2014), Lambein et al. (2019), and several others. Thus, a dietary intake of grass pea grains could be valuable for human health and deserves to be studied further. Grass pea grains are consumed as dhal/curry/soup or as thickening agents in food paste and sauces, and for incorporation into foods designed for improved satiety and delayed glycemic response (Akalu et al., 1998). It is also evident that consumption of low β-ODAP grass pea does not lead to neurolathyrism (Khandare et al., 2014). Therefore, research on how to reduce β-ODAP concentration has taken the central stage in grass pea.

β-ODAP content <1.5 mg g-1 (0.15%) in *L. sativus* seeds is proposed safe for consumption of human beings (Abd El Moneim et al., 2001). However, difference in the duration and the amount *L. sativus* seeds intake matter. In pursuit to reduce β-ODAP concentration to a safe level for human consumption, efforts have been made to develop grass pea varieties with low β-ODAP concentration (Kumar et al., 2020, 2013), in addition to many low-cost agronomic practices (Sarker et al., 2018) and food processing methods (Geda et al., 1995; Getahun et al., 2005). Among major food processing methods, soaking of grass pea grains in water and boiling in hot water have been commonly been used. Geda et al. (1995) recorded 28% reduction in β-ODAP concentration in whole grains with cold-water treatment for 12 h and 37% reduction with hot water (50°C) treatment for 3 h. Supplementing with grains rich in sulfur amino acids and antioxidant containing herbs such as onion, garlic, and ginger has been proposed to prevent possible lathyrism from over-consumption (Getahun et al., 2005). Cooking process not only improves flavor and palatability but also enhances the bioavailability of nutrients and inactivates antinutritional factors (Xu & Chang, 2008). It is generally understood that the availability of quality protein with better digestibility is also enhanced by cooking besides destroying heat-labile antinutritional factors in legume grains (Wang et al., 1997).

Earlier reports show that cooking is effective in improving nutritional quality of grass pea by improving protein availability and reducing β-ODAP concentration (Abd El Moneim et al., 2001; Wang et al., 1997). However, there is no information if there is heritable variation among grass pea genotypes in this regard. Therefore, sufficient knowledge about cooking methods, its effect on physical and chemical properties, and genetic variation in grass pea germplasm would be useful for its safe consumption after processing or cooking. Past studies on food processing effect on β-ODAP concentration were based on a single or a few genotypes. Therefore, the present study aimed at exploring the genetic variation for physicochemical properties among 13 grass pea genotypes in response to cooking treatments and to explore if cooking method could help reduce the β-ODAP concentration in grass pea genotypes to a safe limit.

2. Materials and methods

2.1. Grain material

The present study was carried out on 13 advanced grass pea breeding lines from the ICARDA genebank originating from diverse agroecology representing Bangladesh (IG116826, and IG116888), Nepal (B222, IG115031, and IG115429), Ethiopia (IG65107, IG65108, IG65109, and IG65171), Greece (IG64906), Cyprus (IG65245), and Turkey (IG65926, and Gurbuz) [based on their protein percentage (≥21%) and β ODAP (<0.35%) contents] (Table 1). These genotypes were grown at Tel Hadya (360 56'E, 360 01'N, 284 m AMSL), Syria as part of the field evaluation of the ICARDA grass pea improvement program. The grains for the study were harvested for individual genotypes in three replicates.

2.2. Grain sample preparation

Unhealthy, wrinkled, and hard grains were removed manually and healthy grains of similar size were separately washed in slow-running tap water in a sieve. The grain lots were dried at 25 ± 1°C temperature and stored in 2-kg craft paper bags with 3-g pack of silica gel desiccant to keep them dry during storage. Physical and biochemical properties including 100-grain weight, hydration capacity, swelling capacity, total protein content, and β-ODAP concentration of

Table 1. Physical parameters of grass pea germplasm of different origins after soaking in water overnight for 12 hour.

Tabla 1. Parámetros físicos del germoplasma de guisantes de diferentes orígenes tras ser remojados en agua durante 12 horas.

Genotype	Origin	Dry 100-seed weight (g)[$]	Soaked 100-seed weight (g)[$]	Hydration capacity (g/ seed)[$]	Swelling capacity (ml)[$]	Hydration index	Swelling index
IG116888	Bangladesh	7.59 ± 1.11[de]	16.23 ± 0.44[d]	0.08 ± 0,009 [cd]	0.06 ± 0.04[bc]	1.14 ± 0.01	1.05 ± 0.003
IG116826	Bangladesh	7.83 ± 1.13[d]	15.60 ± 0.29[de]	0.07 ± 0.005[cdef]	0.08 ± 0.001[b]	0.99 ± 0.02	1.24 ± 0.001
B222	Nepal	6.18 ± 0.1[f]	12.64 ± 1.23[g]	0.06 ± 0.006 [f]	0.05 ± 0.001c	1.05 ± 0.03	1.03 ± 0.001
IG115031	Nepal	6.75 ± 1.01[def]	13.48 ± 0.89[fg]	0.06 ± 0.005[ef]	0.05 ± 0.001 [c]	1.00 ± 0.01	0.94 ± 0.001
IG115429	Nepal	7.31 ± 0.02[def]	15.46 ± 0.91[de]	0.08 ± 0.01[cdef]	0.06 ± 0.001[bc]	1.12 ± 0.01	1.18 ± 0.002
IG65107	Ethiopia	6.44 ± 1.10[ef]	13.58 ± 1.09[fg]	0.07 ± 0.001[def]	0.06 ± 0.001[bc]	1.11 ± 0.03	1.23 ± 0.003
IG65108	Ethiopia	6.49 ± 2.22[def]	14.11 ± 0.97[def]	0.07 ± 0.005[cdef]	0.06 ± 0.001[bc]	1.17 ± 0.03	1.33 ± 0.003
IG65109	Ethiopia	7.76 ± 0.38[de]	16.15 ± 1.45[d]	0.08 ± 0.005[cde]	0.07 ± 0.005[bc]	1.08 ± 0.02	1.11 ± 0.002
IG65171	Ethiopia	9.48 ± 1.01[c]	18.71 ± 1.66[c]	0.09 ± 0.006 [c]	0.07 ± 0.004[bc]	0.97 ± 0.04	1.11 ± 0.001
IG64906	Greece	7.73 ± 0.15[de]	15.55 ± 0.50[de]	0.07 ± 0.001 [cdef]	0.06 ± 0.001[bc]	1.01 ± 0.02	1.01 ± 0.002
IG65245	Cyprus	15.20 ± 1.64[a]	30.62 ± 1.84[a]	0.15 ± 0.037[a]	0.12 ± 0.02[a]	1.01 ± 0,02	1.08 ± 0.002
IG65926	Turkey	6.50 ± 1.74[def]	13.38 ± 1.36[fg]	0.06 ± 0.03[ef]	0.06 ± 0.005[bc]	1.06 ± 0.03	1.30 ± 0.002
Gurbuz	Turkey	12.02 ± 0.11[b]	23.80 ± 0.64[b]	0.11 ± 0,056[b]	0.11 ± 0.008[a]	0.97 ± 0.02	1.35 ± 0.004
Mean		8.25	16.87	0.08	0.07	1.05	1.15
Maximum		15.20	30.62	0.15	0.12	1.17	1.35
Minimum		6.18	12.64	0.06	0.05	0.97	0.94

[$]All means with different small letter in a single column are statistically different (*p* < 0.05) as separated by Duncan's multiple range test.

[$]Todas las medias con distinta letra minúscula en una misma columna son estadísticamente diferentes (*p* < 0.05), separadas por la prueba de rangos múltiples de Duncan.

each grain sample were estimated before and after each cooking treatment.

2.3. Physical properties: dry 100-grain weight (g)

After the harvesting and threshing of seeds from each accession, they were cleaned, and air dried at room temperature (25 ± 1°C) in shade to homogenize their moisture content to ~ 10% (Ulloa & Mera, 2010). Three samples of 100 grains of equal size per genotype were weighed separately and average weight was recorded in grams.

2.4. Soaked 100-grain weight (g)

Three samples of 100 grains of equal size each genotype were weighed and transferred to a measuring cylinder of 500 ml capacity separately by adding 100 ml distilled water and retained overnight (12 h) at room temperature (25°C). Next day, the excessive water was drained out using Whatman filter paper Grade-1 and weighed for the average soaked 100-grain weight (Ulloa & Mera, 2010).

2.5. Hydration capacity and index

Soaked grains were used to measure hydration capacity per grain. The hydration index was determined using the following equations (Sood et al., 2002).

$$Hydration\ capacity = \frac{Weight\ of\ soaked\ seeds - Weight\ of\ seeds\ before\ soaking}{Number\ of\ seeds}$$

$$Hydration\ index = Hydration\ capacity\ perseed / Weight\ of\ one\ seed$$

2.6. Swelling capacity and index

Hundred grains of equal size were counted and their initial volume was noted in Thermo Scientific™ Nalgene™ 500 ml cylinder. These grains were soaked in 250 ml distilled water for 12 hours. The volume of the grains before and after soaking was measured using a graduated cylinder. Swelling capacity and index was determined using the following formula (Williams et al., 1983).

$$Swelling\ capacity = \frac{Volume\ of\ seeds\ after\ soaking - Volume\ of\ seeds\ before\ soaking}{Number\ of\ swellen\ seeds}$$

$$Swelling\ index = Swelling\ capacity\ perseed / Volume\ of\ one\ seed$$

2.7. Cooking treatments

2.7.1. Control

Triplicate samples of 100 healthy grains of 13 grass pea genotypes were carefully selected through visual observations. The samples of each genotype were soaked and stirred in distilled water at pH 7 (1:10 w/v) for 15 min at room temperature to note their pH values.

2.7.2. Boiling

Hundred grains of each genotype were cooked in Thermo Scientific™ Nalgene™ 1 L beaker in distilled water in 1:10 ratio (w/v) on a hot plate at 100°C for 90 min.

2.7.3. Autoclaving

The rinsed soaked 100 grains of each genotype were autoclaved using vertical autoclave [Hi clave HVA 110 – Hirayama Japan] at 1.45 kPa pressure, temperature of 121°C for 20 min in 1 L beaker containing distilled water (1:10, w/v) and appropriately covered with an aluminum foil.

2.7.4. Microwaving

The rinsed soaked 100 grains of each genotype placed in a 1 L glass beaker with distilled water (1:10, w/v) and covered with aluminum foil for 5 min. These were cooked in a microwave oven (Bosch HMV8052U) on 1450 W, 15 A current, 60 Hz frequency, at maximum number of 10 levels. It had maximum extraction rate of 385 CFM high for 5 min.

The time of cooking grains of each genotype was optimized and checked for their softness after every 5 min interval by taking them out with a metal spoon after squeezing them in between thumb and forefinger in boiling, autoclaving and microwaving to determine their cooking time. The grains were taken as cooked if the cotyledons of the respective genotype disintegrated during pressing.

2.8. Chemical analysis

The grains of each genotype from three cooking treatments (boiling, autoclaving, microwaving), along with uncooked samples were fine ground to powder separately and subjected to the following chemical analyses.

2.8.1. Total protein content

Near infrared reflectance spectroscopy (Model-5000) scanning monochromator [NIR Systems, Silver Spring, MD, USA (wavelength range of 1100–2500 nm)] offers a rapid and inexpensive method for protein analysis (Williams et al., 1978). Therefore, NIR technique was used for total protein analysis.

2.8.2. ODAP analysis (Rao, 1978)

The grass pea grains were powdered, diluted in 1:20 ratio using 60% ethanol and mixed in a horizontal shaker for 45 minutes at 27 x g. These mixtures were centrifuged at 4536 x g for 15 minutes followed by the collection of 2 ml supernatant in blank tubes. Each of the sample tubes was vortexed after adding 4 ml of 3 N potassium hydroxide (KOH) followed by heating them in water bath for 30 minutes at 100°C. Thereafter, the tubes were again centrifuged at 4536 x g for 15 minutes and pipetted to 250 µl hydrolyzed samples. These samples were mixed with 2000 µl of o-phthalaldehyde (OPT) solution and 750 µl of distilled water. Subsequently, the tubes were vortexed and incubated for 2 h at 40°C. Spectrophotometer (Cadex Canada, Model: SB038) readings were taken by setting absorbance at OD425 nm as described by the manufacturer.

2.9. Statistical analysis

The data related to physical parameters, total protein, and β-ODAP concentration were subjected to the analysis of variance using SPSS-24 statistical software. Mean values were separated by using Duncan's multiple range test for significant differences among the genotypes and treatments. Pearson's 1-tailed correlation analysis was used to establish a relationship between total protein and β-ODAP concentration under each cooking treatment.

3. Results

3.1. Physical parameters

Statistical analysis showed that grass pea genotypes differed significantly ($p < 0.05$) for dry and soaked 100-grain weight, hydration and swelling capacities, and their indices.

3.1.1. Dry and soaked 100-grain weight

Based on the mean and range, it is evident that significant variability existed among grass pea genotypes for dry and soaked 100-grain weight (Table 1). Based on the dry and soaked 100-grain weight, these genotypes formed six and seven distinct groups statistically. Dry 100-grain weight among grass pea genotypes varied from 6.18 to 15.20 g with a mean of 8.25 g. Similarly, soaked 100-grain weight ranged from 12.64 to 30.62 g with a mean of 16.87 g. A genotype originating from Nepal, B222, recorded minimum dry and soaked grain weight, and a genotype from Cyprus, IG65245, the maximum. Soaking of grains into water led to an increase in grain weight by 97 to 117%, depending on the genotype, and its origin and seed size. On an average, grass pea grains imbibed water equal to their weight. The mean dry and soaked grain weight of genotypes originating from Nepal was lowest (6.75 and 13.86 g) followed by genotypes from Ethiopia (7.54 and 15.64 g), Bangladesh (7.71 and 15.92 g), Greece (7.73 and 15.55 g), Turkey (9.26 and 18.59 g), and Cyprus (15.20 and 30.62 g). The maximum

range in 100-grain weight was observed among the Ethiopian germplasm, ranging from 6.44 to 9.48 g for dry grains and from 13.58 to 18.71 g for soaked grains. A significant and positive perfect relationship was noticed between dry and soaked 100-grain weight in grass pea.

3.1.2. Hydration and swelling capacities and their indices

Hydration and swelling capacities that reflect the capacity of each genotype to imbibe water in a reasonable length of soaking time was substantially different among the genotypes. Hydration capacity of grass pea genotypes varied between 0.06 and 0.15 g with a mean of 0.08 g per grain compared to the swelling capacity that ranged from 0.05 to 0.12 ml with a mean value of 0.07 ml per grain (Table 1). Minimum hydration and swelling capacity was observed in the germplasm from Nepal (B222 and IG115031) and maximum in the germplasm from Cyprus (IG65245). Hydration index in 13 grass pea genotypes varied from 0.97 to 1.17 with a mean of 1.05. Ethiopian germplasm displayed maximum range for hydration index with lowest hydration index in IG65171 and the highest in IG65108. Swelling index also showed a wider range from 0.94 to 1.35 ml with a mean of 1.15 ml per grain in grass pea germplasm. IG115031 of Nepal origin recorded the lowest and Gurbuz of Turkey origin, the highest.

3.2. Nutritional parameters

3.2.1. Total protein content

Protein content in 13 grass pea genotypes varied from 21.87 to 24.96% with a mean of 23.50% in uncooked samples (Table 2). A genotype from Nepal, IG115031, displayed the lowest protein content whereas Ethiopian genotype IG65108 showed the highest protein content among the tested germplasm. On an average, germplasm emanating from Ethiopia recorded the highest protein content (24.08%) followed by the germplasm from Turkey (24.02%), Greece (23.50%), and Cyprus (23.33%) whereas the germplasm

Table 2. Effects of different cooking methods on total protein content in 13 grass pea genotypes.

Tabla 2. Efectos de diferentes métodos de cocción en el contenido de proteína total de 13 genotipos de guisantes.

| Genotype | Total protein content (g per 100 g grains) | | | |
	Uncooked grains	Boiled grains	Microwaved grains	Autoclaved grains
IG116888	23.43 ± 0.65[b*A**]	22.96[b]±0.97[cB]	23.20 ± 0.88[b B]	23.96 ± 1.81[abcA]
IG116826	22.30 ± 2.00[cB]	23.36 ± 3.03[abA]	23.16 ± 1.33[bB]	24.00 ± 2.08[abcA]
B222	23.33 ± 1.00[bB]	25.40 ± 2.25[abA]	25.23 ± 3.12[abB]	25.63 ± 1.52[abA]
IG115031	21.87 ± 1.52[dC]	23.23 ± 1.52[aA]	23.23 ± 3.42[bA]	22.80 ± 1.04[bcB]
IG115429	23.37 ± 2.00[bB]	23.63 ± 1.47[bB]	23.26 ± 1.73[bB]	25.96 ± 1.15[abA]
IG65107	24.27 ± 3.00[aB]	26.53 ± 2.39[aA]	22.86 ± 1.33[bC]	20.23 ± 2.51[cD]
IG65108	24.96 ± 1.52[aB]	28.23 ± 1.33[aA]	27.06 ± 2.28[aB]	27.80 ± 1.28[aB]
IG65109	23.67 ± 1.00[bC]	25.10 ± 2.62[bcB]	25.86 ± 3.28[aaB]	27.63 ± 2.25[aA]
IG65171	23.40 ± 2.00[bB]	23.10 ± 2.40[bB]	23.13 ± 2.71[bB]	23.66 ± 3.37[abcA]
IG64906	23.50 ± 4.24[bB]	24.03 ± 0.72[aB]	23.90 ± 0.72[aA]	23.53 ± 1.41[bB]
IG65245	23.33 ± 2.01[bB]	25.36 ± 2.51[aA]	24.96 ± 1.11[abB]	25.26 ± 4.56[abA]
IG65926	23.60 ± 1.10[bB]	23.63 ± 3.37[bB]	24.50 ± 2.91[bA]	24.36 ± 2.02[abcA]
Gurbuz	24.43 ± 4.16[aB]	26.16 ± 1.28[aA]	22.73 ± 1.46[bB]	21.36 ± 1.33[bcB]
Mean	23.50	24.67	24.08	24.32
Maximum	24.96	28.23	27.06	27.80
Minimum	21.87	22.96	22.73	20.23
%*** increase in total protein content after cooking		4.99[a]	2.49[c]	3.50[b]

*All values with small letters in a column are significantly different ($p < 0.05$) using Duncan's multiple range test.

**All values with capital letters in a row are significantly different ($p < 0.05$) using Duncan's multiple range test.

***All values with capital letters in a row are significantly different ($p < 0.05$) using LSD test for **increase in total protein content after cooking.**

*Todos los valores con letras minúsculas en una columna son significativamente diferentes ($p < 0.05$) mediante la prueba de rango múltiple de Duncan.

**Todos los valores con letras mayúsculas en una fila son significativamente diferentes ($p < 0.05$) mediante la prueba de rango múltiple de Duncan.

***Todos los valores con letras mayúsculas en una fila son significativamente diferentes ($p < 0.05$) utilizando la prueba LSD para el **aumento del contenido de proteína total después de la cocción**.

Table 3. Effects of different cooking methods on β-ODAP content in 13 grass pea genotypes.

Tabla 3. Efectos de diferentes métodos de cocción en el contenido de β-ODAP de 13 genotipos de guisantes.

Genotypes	Uncooked grains	Retention time (min)	Boiled grains	Retention time (min)	Microwaved grains	Retention time (min)	Autoclaved grains	Retention time (min)
IG116888	0.26[bcd]* [A]**	15 min	0.08[abcC]	90 min	0.21[bcB]	5 min.	0.23[bcAB]	20 min
IG116826	0.35[aA]		0.08[abcD]		0.21[bcC]		0.24[bB]	
B222	0.24[bcdeA]		0.08[bcC]		0.18[cdB]		0.19[bcdB]	
IG115031	0.33[aA]		0.12[abC]		0.24[abB]		0.31[aA]	
IG115429	0.35[aA]		0.12[aC]		0.25[aB]		0.34[aA]	
IG65107	0.26[bcdA]		0.08[abcD]		0.18[cdC]		0.23[bcB]	
IG65108	0.22[deA]		0. 07[cC]		0.13[eB]		0.19[bcdA]	
IG65109	0.22[cdeA]		0.06 [cD]		0.14[eC]		0.18[cdB]	
IG65171	0.27 [bA]		0.08[abcD]		0.21[bcC]		0.23[bcB]	
IG64906	0.21[eA]		0.05[cC]		0.14[eB]		0.16 [dB]	
IG65245	0.27 [bA]		0.08[abcC]		0.19[cdB]		0.21[bcdB]	
IG65926	0.22[deA]		0.07[cC]		0.16[deB]		0.21[bcA]	
Gurbuz	0.27[bcA]		0.08[abcD]		0.18[cdC]		0.23[bcB]	
Mean	0.27		0.08		0.19		0.23	
Maximum	0.35		0.12		0.25		0.34	
Minimum	0.21		0.05		0.13		0.16	
% reduction in ODAP content after cooking			70.04		30.30		14.43	

*All values with small letters in a column are significantly different ($p < 0.05$) using Duncan's multiple range test.

**All values with capital letters in a row are significantly different ($p < 0.05$) using Duncan's multiple range test.

*Todos los valores con letras minúsculas en una columna son significativamente diferentes ($p < 0.05$) utilizando la prueba de rango múltiple de Duncan.

**Todos los valores con letras mayúsculas en una fila son significativamente diferentes ($p < 0.05$) utilizando la prueba de rango múltiple de Duncan.

from Bangladesh (22.87%) and Nepal (22.86%) recorded the lowest protein content.

3.2.2. β-ODAP concentration

The results showed significant variation for β-ODAP concentration in uncooked grains of the genotypes tested (Table 3). Based on the mean (0.27%) and range (0.21 to 0.35%), it is evident that in spite of sufficient genotypic variation, β-ODAP concentration in all 13 genotypes was higher than the safe limit postulated for its consumption. On an average, germplasm emanating from Greece (0.21%) recorded the lowest β-ODAP concentration in its uncooked grains followed by the germplasm from Ethiopia (0.24%), Turkey (0.25%), and Cyprus (0.27%). Germplasm from Bangladesh and Nepal recorded more than 0.30% β-ODAP concentration in its uncooked grains. These results followed the well-established trend that the grass pea germplasm from drier regions of South Asia displayed higher β-ODAP concentration in their grains compared to the germplasm collected from the Mediterranean region of West Asia and Southern Europe.

3.3. Effect of cooking methods on protein content and β-ODAP concentration

Three methods of cooking were tested to observe if a cooking method could be deployed as an effective means to reduce β-ODAP concentration in grass pea to a safe limit. The results on the effect of cooking methods are presented for protein content in Table 2 and β-ODAP concentration in Table 3. The results indicated significant differences among cooking treatments with regard to their effect on protein content and β-ODAP concentration in grass pea genotypes. In general, the magnitude of cooking effect was genotype-specific. The direction of cooking effect was positive on protein content and negative on β-ODAP concentration. Among cooking treatments, boiling grains was superior with 4.99% protein advantage over uncooked grains, followed by autoclaving (3.50%) and microwaving (2.49%) (Table 2). Similarly, β-ODAP concentration was reduced by 70%, 30%, and 14% under boiling, microwaving, and autoclaving, respectively.

Boiling method of cooking showed significant ($p < 0.05$) positive effect on protein content (Table 2). Protein content in grass pea genotypes ranged from 22.96 to 28.23% with an overall mean of 25% under boiling method of cooking. Boiling showed an overall 4.99% higher protein content compared to uncooked grains. All genotypes except two, namely, IG116888 and IG65171 showed 1 to 13% protein gain/advantage over the uncooked grains. Genotype IG65108 from Ethiopia was the biggest gainer of protein content (>13%) in response to boiling method. Autoclaving of grass pea grains indicated an overall increase of 3.5% in protein content. The total protein content of two genotypes IG65107 (16.65%) and Gurbuz (12.57%) was significantly decreased under autoclaving while remaining genotypes showed up to 17% increase in protein content over their uncooked grains. Three genotypes, namely IG115429, IG65108, and IG65109 showed more than 10% increase in protein content over their uncooked grains. Microwave treatment also showed negative effect (0.5 to 7% decrease) on protein content in five genotypes, namely, IG116888, IG115429, IG65107, IG65109, and Gurbuz while the remaining eight genotypes displayed 1.7 to 9.2% increase in protein content over their uncooked grains.

Cooking treatments showed significant effect ($p < 0.05$) on β-ODAP concentration in grass pea genotypes, with highest reduction by boiling (70%) followed by microwaving (30%) and autoclaving (14%). The β-ODAP concentration among genotypes ranged from 0.05 to 0.12% with overall mean of 0.08% under boiling treatment. All genotypes except two from Nepal (IG115031 and IG115429) recorded less than 0.10% β-ODAP concentration after boiling, bringing its concentration within the safe limit of consumption. As compared to uncooked grains, all genotypes recorded 64 to 77% reduction in β-ODAP concentration in boiled grains. The second-best method of cooking was microwaving, under which β-ODAP concentration in grass pea genotypes reduced by 18 to 41%, with a mean of 0.19% and a range of 0.13 to 0.25%. Similarly, significant ($p < .05$) reduction in

Table 4. Correlations analysis among different physical and chemical properties of 13 genotypes of grass pea.

Tabla 4. Análisis de correlación entre diferentes propiedades físicas y químicas de 13 genotipos de guisantes.

Correlation Parameters	Total protein	Total ODAP	Dry 100-Seed weight	Soaked 100-seed weight	Hydration Capacity	Hydration index	Swelling capacity	Swelling index
Total protein	1							
Total ODAP	−0.180	1						
Dry 100-seed weight	0.030	0.059	1					
Soaked 100-seed weight	0.046	0.042	0.989**	1				
Hydration capacity	0.061	0.024	0.954**	0.988**	1			
Hydration index	0.145	−0.124	−0.368*	−0.228	−0.076	1		
Swelling capacity	0.084	0.031	0.914**	0.902**	0.869**	−0.339*	1	
Swelling index	0.077	−0.067	0.064	0.059	0.052	−0.037	0.404*	1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

**. La correlación es significativa al nivel de 0.01 (2 colas); *. La correlación es significativa al nivel de 0.05 (2 colas).

Table 5. Correlation between total protein content and β-ODAP concentration in grass pea under different cooking methods.

Tabla 5. Correlación entre el contenido de proteína total y la concentración de β-ODAP en el guisante bajo diferentes métodos de cocción.

	Total protein content			
Parameter	Uncooked	Boiled	Microwaved	Autoclaved
β-ODAP	− 0.217	− 0.555*	− 0.342*	− 0.115

*Values are significantly different ($p < 0.01$) using LSD test.

*Los valores son significativamente diferentes ($p < 0.01$) mediante la prueba LSD.

β-ODAP concentration in all genotypes was observed under autoclave treatment compared to uncooked grains. The β-ODAP concentration ranged from 0.16 to 0.34% with an overall mean of 0.23% under autoclaving. Five genotypes, namely, IG65108, IG115031, IG115429, IG65926, and IG116888 did not show significant reduction under autoclaving when compared to uncooked grains ($p < .05$). The remaining genotypes displayed reduction of 3 to 31% under autoclaving compared to their uncooked grains.

In the present study, water pH after boiling, microwaving and autoclaving treatment ranged from 4.19 to 9.27, 4.81 to 9.37, and 4.54 to 9.65, respectively, depending on the genotype and its grain constituents (Data not shown).

3.4. Correlation among the traits

Pearson's correlation coefficient results (Table 4) showed a significant positive correlation of soaked 100 seed weight and dry 100 seed weight (0.989**); hydration capacity and dry hundred seed weight (0.954**), hydration capacity and soaked 100-seed weight (0.988**), swelling capacity and dry 100-seed weight (0.914**), swelling capacity and soaked 100-seed weight (0.902**), swelling capacity and hydration capacity (0.869**). Whereas, hydration index and dry 100-seed weight (−0.368*) and swelling capacity and hydration index (−0.399*) showed a significant negative correlation. However, all other traits showed non-significant positive or negative correlations.

3.5. Correlation between protein and β-ODAP before and after cooking treatments

In the present study, negative relationship was observed between protein content and β-ODAP concentration across the treatments (Table 5). However, correlation coefficient was significantly negative between protein content and β-ODAP concentration only under boiling (r = −0.555**) and microwaving (r = −0.342*) treatments whereas it was

negative but non-significant among the uncooked grains (r = −0.217) and autoclaving (r = −0.115).

4. Discussion

Generally, grass pea grains are cooked whole or split as dhal based on the convenience and taste without considering the effect of cooking on antinutrient compounds like β-ODAP and nutritive value (Tarade et al., 2007). Soaking and cooking induce several changes in nutritional value of food legumes (Urga et al., 2006; Xu & Chang, 2008). Knowing these changes helps the consumer to prepare and cook food to maximize its nutritional value. Soaking grass pea grains in water before cooking is a common practice to soften texture and hasten the cooking process. In the present study, we assessed hydration and swelling capacity of grass pea grains soaked in water overnight, and the changes in protein content and β-ODAP concentration of 13 genotypes under three different cooking methods, namely, boiling, microwaving, and autoclaving. The study showed significant effects of genotypes and cooking methods on protein content and β-ODAP concentration in grass pea grains, and significant variation in their hydration and swelling capacities in response to overnight soaking in water.

Hydration capacity is usually considered necessary to decrease cooking time and increase drained weight in legumes (Rehman et al., 2001; Taiwo et al., 1997). In the present study, hydration and swelling capacity that reflect the capacity of a genotype to imbibe water was significantly different among grass pea genotypes. Soaking grass pea grains in water overnight for 12 hours showed 97 to 117% increase in seed weight, depending on the genotype, its origin, and seed size. On an average, soaked grains of grass pea almost doubled in their weight and size after imbibing water overnight. Ethiopian germplasm displayed the maximum variation for hydration and swelling capacity. These results agree with earlier findings of increase in grain weight of Ethiopian grass pea when soaked in water for 12 hours (Urga et al., 2006). Small-seeded grass pea germplasm of South Asia showed lower hydration and swelling capacity than the large seeded germplasm of Mediterranean countries. Similar results were reported in chickpea in which desi genotypes with lower hydration index required more time for cooking compared to Kabuli genotypes with high hydration index (Malunga et al., 2012). Hydration and swelling indices depend on the fiber content in grains (Wang et al., 1997). During soaking, the water dispersed into the starch granules and protein fractions, which facilitate gelatinization and protein denaturation leading to softening of the texture (Siddiq &

Uebersax, 2013). Soaking in water allows the seeds to absorb water, to decrease and eliminate anti-nutritional factors in legumes. However, soaking for long periods has been found to reduce nutritional quality of legumes through leaching of nutrients into the soaked water (Taiwo et al., 1997).

Protein content of cooked grains was compared with that of uncooked grains of 13 grass pea genotypes. It is evident from the narrow range (21.87–24.96%) that the grass pea genotypes included in the present study did not differ significantly for protein content. This range in protein content in grass pea germplasm agree with earlier report on germplasm of Turkey origin (Basaran et al., 2012). Among cooking treatments, boiling was superior with 4.99% increase in protein content over uncooked seeds, followed by autoclaving (3.5%) and microwaving (2.49%). One genotype from Ethiopia, IG65108 with highest protein content was also the biggest gainer for protein availability (8 to 13%) in response to cooking methods. The results suggested that microwaving, autoclaving, and boiling treatments increased protein content that depended on the kinetic energies provided by the respective heating treatments (Tarade et al., 2007). The results confirm the findings of Urga et al. (2006) and Hailu et al. (2015) that blanching and soaking affected total protein and physical characteristics of grass pea grains in a genotype-dependent manner. Past studies on soaking and thermal processing showed improvement in protein digestibility of kidney beans (Abd El-Hady & Habiba, 2003; Rehman et al., 2001). Protein content improved by 3–5% under different cooking methods, suggesting that appropriate cooking method may improve the bioavailability of grass pea protein. There are also reports of significant loss in protein content of different legume crops. In the present study, two genotypes each under boiling (IG116888 and IG65171) and autoclaving (IG65107 and Gurbuz), and five genotypes under microwaving (IG116888, IG115429, IG65107, IG65109, and Gurbuz) displayed 0.5 to 16.7% reduction in protein content over their uncooked grains. The amino acids are building blocks of proteins, which are made up of organic molecules consisting of alpha carbon atoms linked to a hydrogen atom, an amino and, a carboxyl group, along with variable components or a side chain. Multiple amino acids link within a protein, using peptide bonds, form a long protein chain (Wilson, 2003). Amino acid composition generally indicates the nutritive value of the protein source (Bodwell et al., 1980). Observed protein nutritive value, in raw samples could be lower compared to the actual value due to unavailability of some amino acids due to no digestion. Furthermore, number of antinutritional factors in grass pea including trypsin inhibitors oppose the protein digestibility (Monsoor & Yusuf, 2002; Wang et al., 1998). Therefore, total protein contents of grass pea seeds depends on complete digestion of the seeds (Monsoor & Yusuf, 2002). Therefore processing or boiling treatments of the grass pea seeds carry special value. Previous studies showed that the type of heat during cooking dominantly influences the destruction of protein content by rupturing and breakage of cell walls in seed coats and cotyledons that govern tenderness quality of the grains (Chandrasekaran et al., 2013; Sahaf et al., 2018). The level of tenderness due to boiling variably affected protein content of some genotypes used in the study. Therefore, gain or loss of protein content in grass pea germplasm during cooking is valuable information for future breeding program.

In the present study, grass pea germplasm showed a wide range of 0.21–0.35% for β-ODAP concentration that was higher than the safe limit postulated for its consumption. On an average, germplasm emanating from Greece recorded the lowest β-ODAP concentration in its uncooked grains followed by germplasm from Ethiopia, Turkey, and Cyprus. Germplasm from Bangladesh and Nepal recorded more than 0.30% β-ODAP concentration in its uncooked grains. These results followed the well-established trend that the grass pea germplasm from drier regions of South Asia displayed higher β-ODAP concentration in their grains compared to the germplasm collected from the Mediterranean region of West Asia and Southern Europe (Kumar et al., 2011).

In order to bring β-ODAP concentration below its safe limit that is 0.15%, three methods of cooking were tested if any of the cooking method could be deployed as an effective means to reduce β-ODAP concentration in grass pea genotypes. β-ODAP is known to be relatively heat stable and water-soluble compound. We observed marked reduction in β-ODAP concentration when grass pea grains were cooked. The magnitude of reduction depended on the genotype, its origin and the method of cooking. β-ODAP concentration in grass pea reduced significantly under different cooking treatments with highest reduction under boiling (70%) followed by microwaving (30%) and autoclaving (14%). Boiling treatment was effective in bringing down β-ODAP concentration within the safe limit of consumption in all grass pea genotypes except two from Nepal (IG115031 and IG115429). As compared to uncooked grains, all genotypes recorded 64 to 77% reduction in β-ODAP concentration in boiled grains. Reduction of this magnitude in β-ODAP concentration could be due to subsequent isomerization of β-ODAP during heating. Microwaving and autoclaving methods of cooking did not reduce β-ODAP concentration to the safe limit of consumption. Autoclaving grass pea grains was not effective in reducing β-ODAP in the previous study as well (Ramachandran & Ray, 2008). In alkaline medium, loss of β-ODAP was more than in acidic medium (Akalu et al., 1998; Tarade et al., 2007). The water pH after cooking treatment changed variably in different genotypes in the present study. An increase or decrease in pH of water depends on the genetic background-based cell texture of the grain material subject to variable wear and tear with heated water based on the cooking method (Tarade et al., 2007). As the temperature increased during boiling, water molecules from three methods of cooking reacted with grain tissue cells variably with water influx through imbibition, adsorption, and differential osmotic movements (Tarade et al., 2007).

The results suggest that selection of plants based on soaked 100-seed weight and dry 100-seed weight; hydration capacity and dry 100-seed weight, hydration capacity and soaked 100-seed weight, swelling capacity and dry 100-seed weight character would be beneficial. The results of study confirm that all positive Pearson's correlation coefficient value between the parameters will improve cooking quality of seeds in agreement with Urga et al. (2006); who confirmed that hydration of grass pea seeds reduced cooking time and increased the weight and texture of the cooked seeds. The results further suggest that selection of plants based on these parameters will also have positive impact on the reduction of β-ODAP in indirect manner.

Hydration index and dry 100-seed weight (−0.368*) along with hydration index and swelling capacity (−0.399*) showed a significant negative correlation. This argument suggests that selection of accessions for low hydration index will improve 100-seed weight and swelling capacity values. Grass pea as indicated in this study has low hydration capacity in all accessions that is why they need long time in cooking to get desirable texture for eating. The results are in agreement with Malunga et al. (2012), who point out that desi chickpea accessions had lower hydration capacity compared to Kabuli chickpea accessions and need more time to cooking comparatively.

All other traits showed non-significant positive or negative correlations, suggesting that selection of accessions based on these characters is not desirable. As these parameters are possibly controlled by more than one gene; therefore; these should be studied more carefully before making any decision. This implies that protein and β-ODAP may be under the control of different genes or group of linked genes. These genes are needed to be identified and could be silenced or deleted using biotechnological techniques effectively that is not possible using conventional breeding techniques.

The present study recorded significantly negative correlation between protein content and β-ODAP concentration under boiling and microwave methods of cooking as also reported in earlier studies for grass pea germplasm (Basaran et al., 2012). This augers well for improving protein content and reducing β-ODAP concentration concurrently through breeding. The present study indicates that method of preparation and cooking can improve the nutritive value of grass pea.

5. Conclusion

The present study showed significant genetic variability for hydration and swelling capacity, and β-ODAP concentration in grass pea germplasm. In general, small-seeded germplasm from South Asia showed less hydration and swelling capacity with higher β-ODAP concentration as compared to the large-seeded germplasm from Mediterranean countries. Germplasm from Ethiopia with large variability for protein content holds promise in improving protein content of South Asia germplasm. The present study indicates that method of preparation and cooking can improve protein availability and make grass pea food safe to consume. In general, the magnitude of cooking effect was genotype-specific. Among cooking treatments, boiling grains was superior followed by autoclaving and microwaving. To minimize nutrient loss during processing of grass pea, optimization of processing conditions are recommended for investigation.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Abd El Moneim, A. M., van Dorrestein, B., Baum, M., Ryan, J., & Bejiga, G. (2001). Role of ICARDA in improving the nutritional quality and yield potential of grass pea (*Lathyrus sativus* L.) for subsistence farmers in dry areas. *Lathyrus Lathyrism Newsletter*, *l2*, 55–58. https://www.business.uwa.edu.au/__data/assets/pdf_file/0007/919726/Abd_El_Moneim.pdf

Abd El-Hady, E. A., & Habiba, R. A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. *LWT-Food Science & Technology*, *36*(3), 285–293. https://doi.org/10.1016/S0023-6438(02)00217-7

Akalu, G., Johansson, G., & Nair, B. M. (1998). Effect of processing on the content of beta ODAP in grasspea, seeds and flour as determined by flow injection analysis. *Food Chemistry*, *62*(2), 233–237. https://doi.org/10.1016/S0308-8146(97)00137-4

Basaran, U., Zeki, A. C. A. R., Karacan, M., & Onar, A. N. (2012). Variation and correlation of morpho-agronomic traits and biochemical contents (Protein and ODAP) in Turkish Grasspea (*Lathyrus sativus* L.) landraces. *Turkish Journal of Field Crops*, *18*(2), 166–173. http://www.field-crops.org/assets/pdf/product52bd8d94c5515.pdf

Bell, E. A. (2003). Nonprotein amino acids of plants: significance in medicine, nutrition, and agriculture. *Journal of Agricultural and Food Chemistry*, *51*(10), 2854–2865. https://doi.org/10.1021/jf020880w

Bodwell, C. E., Satterlee, L. D., & Hackler, L. R. (1980). Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymic digestion methods. *The American Journal of Clinical Nutrition*, *33*(3), 677–686. https://doi.org/10.1093/ajcn/33.3.677

Chandrasekaran, S., Ramanathan, S., & Basak, T. (2013). Microwave food processing-a review. *Food Research International*, *52*(1), 243–261. https://doi.org/10.1016/j.foodres.2013.02.033

Geda, A. K., Rastogi, N., & Pandey, R. L. (1995). New processing approaches of detoxification for low toxin Lathyrus. In R. Tekle-Haimanot & F. Lambein (Eds.), *Lathyrus sativus and human lathyrism: A decade of progress* (pp. 117–120). University of Ghent.

Getahun, H., Lambein, F., Vanhoorne, M., & Van-der Stuyft, P. (2005). Neurolathyrism risk depends on type of grass pea preparation and on mixing with cereals and antioxidants. *Tropical Medicine and International Health*, *10*(2), 169–178. https://doi.org/10.1111/j.1365-3156.2004.01370.x

Hailu, D., Abera, S., & Teka, T. A. (2015). Effects of processing on nutritional composition and anti-nutritional factors of Grasspea (*Lathyrus sativus* L): A review. *Food Science and Quality Management*, *36*, 61–70. https://www.iiste.org/Journals/index.php/FSQM/article/view/19781/20143

Khandare, A. L., Babu, J. J., Ankulu, M., Aparna, N., Shirfule, A., & Rao, G. S. (2014). Grasspea consumption & present scenario of neurolathyrism in Maharashtra State of India. *Indian Journal of Medicial Research*, *140*(1), 96–101. https://www.ijmr.org.in/text.asp?2014/140/1/96/139999

Kumar, S., Gupta, P., Barpete, S., Sarker, A., Amri, A., Mathur, P. N., & Baum, M. (2013). Grass pea. In M. Singh, H. D. Upadhyaya, & I. S. Bisht, (Eds.), *Genetic and genomic resources for grain legume improvement* (pp. 269–293). Elsevier. http://dx.doi.org/10.1016/B978-0-12-397935-3.00011-6

Kumar, S., Gupta, P., Barpete, S., Choukri, H., Maalouf, F., & Sarkar, A. (2020). Grass pea. In A. Pratap & S. Gupta (Eds..), *The beans and the peas, from orphan to mainstream crops* (pp. 273–287). Elsevier. https://www.elsevier.com/books/the-beans-and-the-peas/pratap/978-0-12-821450-3

Kumar, S., Bejiga, G., Ahmed, S., Nakkoul, H., & Sarker, A. (2011). Genetic improvement of grass pea for low neurotoxin (ODAP) content. *Food Chemical and Toxicology*, *49*(3), 589–600. https://doi.org/10.1016/j.fct.2010.06.051

Lambein, F., Travella, S., Kuo, Y. H., Montagu, M. V., & Heijde, M. (2019). Grass pea (*Lathyrus sativus* L.): Orphan crop, nutraceutical or just plain food? *Planta*, *250*(3), 821–838. https://doi.org/10.1007/s00425-018-03084-0

Malunga, L. N., Zinal, E., Shoubi, I., Barel-Dadon, S., Berkovich, Z., Abbo, S., & Reifen, R. (2012). Effect of combined germination, dehulling and boiling on ineral, sucrose, stachyose, fibrulose, and phytic acid content of different chickpea cultivars. *African Journal of Food, Agriculture, Nutrition and Development*, *12*(7), 6853–6867. https://www.ajol.info/index.php/ajfand/article/view/84199

Monsoor, M. A., & Yusuf, H. K. M. (2002). In vitro protein digestibility of lathyrus pea (*Lathyrus sativus*), lentil (*Lens culinaris*), and chickpea (*Cicer arietinum*). *International Journal of Food Science and Technology*, *37*(1), 97–99. https://doi.org/10.1046/j.1365-2621.2002.00539.x

Ramachandran, S., & Ray, A. K. (2008). Effect of different processing techniques on the nutritive value of grass pea, *Lathyrus sativus* L., seed meal in compound diets for Indian major carp rohu, *Labeo rohita* (Hamilton), fingerlings. *Fisheries and Aquatic Life*, *16*(2), 189–202. https://doi.org/10.2478/s10086-008-0016-2

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

Rao, S. L. N. (2011). A look at the brighter facets of β-N-oxalyl-L-α, β-diaminopropionic acid, homoarginine and the grass pea. *Food Chemical and Toxicology*, *49*(3), 620–622. https://doi.org/10.1016/j.fct.2010.06.054

Rao, S. L. N., Adiga, P. R., & Sarma, P. S. (1964). The isolation and characterization of β-N-Oxalyl-L-α,β-Diaminopropionic acid: A neurotoxin from the seeds of lathyrus sativus. *Biochemistry*, *3*(3), 432–436. https://doi.org/10.1021/bi00891a022

Rehman, Z., Salariya, A. M., & Zafar, S. I. (2001). Effect of processing on available carbohydrate content and starch digestibility of kidney beans (*Phaseolus vulgaris* L.). *Food Chemistry*, *73*(3), 351–355. https://doi.org/10.1016/S0308-8146(00)00311-3

Sahaf, S. A., Zarghi, H., & Golian, A. (2018). Effect of raw or autoclaved grass pea seeds in diet on performance and egg quality of laying hens. *Journal of Animal Production*, *20*(1), 131–142. https://doi.org/10.22059/jap.2018.245080.623237

Sarker, A., Fikre, A., El-Moneim, A. M. A., Nakkoul, H., & Singh, M. (2018). Reducing anti-nutritional factor and enhancing yield with advancing time of planting and zinc application in grasspea in Ethiopia. *Journal of the Science of Food and Agriculture*, *98*(1), 27–32. https://doi.org/10.1002/jsfa.8433

Sen Gupta, D., Barpete, S., Kumar, J., & Kumar, S. (2021). Breeding for better grain quality in *Lathyrus*. In D. S. Gupta, S. Gupta, & J. Kumar (Eds.), *Breeding for enhanced nutrition and bio-active compounds in food legumes* (pp. 131–156). Springer. https://doi.org/10.1007/978-3-030-59215-8_6

Siddiq, M., & Uebersax, M. A. (2013). *Dry beans and pulses production, processing, and nutrition*. Wiley Online Library. https://doi.org/10.1002/9781118448298.ch1

Singh, S. S., & Rao, S. L. N. (2013). Lessons from neurolathyrism: A disease of the past & the future of *Lathyrus sativus* (Khesari dal). *Indian Journal of Medical Research*, *138*(1), 32–37. https://www.ijmr.org.in/text.asp?2013/138/1/32/116175

Sood, M., Malhotra, S. R., & Sood, B. C. (2002). Effect of processing and cooking on proximate composition of chickpea varieties. *Journal of the Science of Food and Agriculture*, *39*(1), 69–71. https://doi.org/10.1002/jsfa.1001

Taiwo, K. A., Akanbi, C., & Ajibola, O. O. (1997). The effects of soaking and cooking time on the cooking properties of two cowpea varieties. *Journal of Food Engineering*, *33*(3–4), 337–346. https://doi.org/10.1016/S0260-8774(97)87278-0

Tamburino, R., Guida, V., Pacifico, S., Rocco, M., Zarelli A., Parente, A., & Maro A. D. (2012). Nutritional values and radical scavenging capacities of grass pea (*Lathyrus sativus* L.) seeds in valle agricola district, Italy. *Australian Journal of Crop Science*, *6*(1), 149–156. http://www.cropj.com/di_6_1_2012_149_156.pdf

Tarade, K. M., Singhal, R. S., Jayram, R. V., & Pandit, A. B. (2007). Kinetics of degradation of ODAP in *Lathyrus sativus* L. flour during food processing. *Food Chemistry*, *104*(2), 643–649. https://doi.org/10.1016/j.foodchem.2006.12.018

Ulloa, P., & Mera, M. (2010). Inheritance of seed weight in large-seed grass Pea *Lathyrus sativus* L. *Chilean Journal of Agricultural Research*, *70*(3), 357–364. https://doi.org/10.4067/S0718-58392010000300002

Urga, K., Fufa, H., Biratu, E., & Gebretsadik, M. (2006). Effects of blanching and soaking on some physical characteristics of grass pea (*Lathyrus sativus*). *African Journal of Food Agriculture Nutrition and Development*, *6*(1), 1–17. https://doi.org/10.4314/ajfand.v6i1.19174

Vaz Patto, M. C., & Rubiales, D. (2014). Lathyrus diversity: available resources with relevance to crop improvement L. sativus and L. cicera as case studies. *Annals of Botany*, *113*(6), 895–908. https://doi.org/10.1093/aob/mcu024

Wang, N., Lewis, M. J., Brennan, J. G., & Westby, A. (1997). Effect of processing methods on nutrients and anti-nutritional factors in cowpea. *Food Chemistry*, *58*(1–2), 59–68. https://doi.org/10.1016/S0308-8146(96)00212-9

Wang, X., Warkentin, T. D., Briggs, C. J., Oomah, B. D., Campbell, C. G., & Wood, S. (1998). Trypsin inhibitor activity in Field pea (Pisum sativum L.) and grass pea (Lathyrus sativus L.). *Journal of Agricultural and Food Chemistry*, *46*(7), 2620–2623. https://doi.org/10.1021/jf971007b

Williams, P. C., Nakkoul, H., & Singh, K. B. (1983). Relationship between cooking time and some physical characteristics in chickpeas (*Cicer arietinum* L.). *Journal of the Science of Food and Agriculture*, *34*(5), 492–496. https://doi.org/10.1002/jsfa.2740340510

Williams, P. C., Stevenson, S. G., Starkey, P. M., & Hawtin, G. C. (1978). The application of near infrared reflectance spectroscopy to protein-testing in pulse breeding programmes. *Journal of the Science of Food and Agriculture*, *29*(3), 285–292. https://doi.org/10.1002/jsfa.2740290315

Wilson, R. P. (2003). Amino acids and proteins. In J. E. Halver & R. W. Hardy (Eds.), *Fish nutrition* (pp. 143–179). Academic Press. https://doi.org/10.1016/B978-012319652-1/50004-5

Xu, B., & Chang, S. K. C. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry*, *110*(1), 1–13. https://doi.org/10.1016/j.foodchem.2008.01.045