Evaluation of different processing methods on the nutritional composition and certain anti-nutritional factors in pea *Pisum sativum*

Erlinda S. Ganzon-Naret

University of the Philippines Visayas, College of Fisheries & Ocean Sciences, Institute of Aquaculture, Iloilo, Philippines. Corresponding author: E. S. Ganzon-Naret, erlindanaret@yahoo.com

Abstract. Green pea *(Pisum sativum*) is an annual leguminous crop which prefers a well drained sandy soil and temperate climate. It is a nutritious legume which contains 25% protein, amino acids, carbohydrate, Vitamins A and C, and minerals such as calcium and phosphorus. Apart from being an important food source, pea seeds play an important role as an alternative feed ingredient which can replace soybeans as protein source in the diet formulation for marine, brackishwater fish and crustaceans. Therefore, this study was conducted in order to evaluate the different processing methods on the nutritional and anti-nutritional factors of *P. sativum*. The highest crude protein content was observed in dehulled *P. sativum* (DGP) as 22.31%, followed by autoclaved *P. sativum* (AGP) as 21.56%, soaked green pea (SGP) 20.66% and the lowest value was reported in unprocessed raw *P. sativum* (RGP) at 19.39%. The mean values showed that the maximum trypsin inhibitor activity (TIA) was observed in the unprocessed RGP (7.39 TIU/mg) while the lowest was recorded in AGP (6.62 TIU/mg). Moreover, highest phytic acid content was observed in RGP (421.10 mg phytic acid/100 g), followed by SGP (396.73 mg phytic acid/100 g), AGP (368.30 mg phytic acid/100 g) and lowest value was in DGP (310.07 mg phytic acid/100g). The mean values for total tannin content (%) were 3.81, 4.42, 4.42 and 4.06 for RGP, DGP, AGP and SGP respectively. Likewise, it was observed that the average saponin content for processed and unprocessed pea meals ranged from 0.407 to 0.623%.
Key Words: green pea, crude protein, dehulling, autoclaving, soaking, anti-nutrients.

Introduction. Legumes belong to family "Leguminosae", also known as "Fabacea", which bear fruit in the form of pods with the seeds known as beans. They are considered as an important source of macro and micronutrients (Tharanathan & Mahadevamma 2003; Olivia & Ardythe 2013). In the Philippines, peas and other legumes including lentils, chickpeas and beans are most often sold as fresh vegetables and in dried form. Peas are often referred to as "pulses," with sweet taste and starchy texture. They are good sources of amino acids, vitamins, minerals, phenolic compound, with high energetic value (Costa et al 2006; Muzquiz et al 2012). Legumes are rich in fibre content especially in their husk fractions which contributes to the human therapeutic effects by lowering plasma cholesterol (Vasishtha & Srivastava (2013). Peas are of great nutritional value due to its high protein content which range from 21 to 32% (Nikolopoulou et al 2007; Kotlarz et al 2012). According to Valencia et al (2008) peas and their protein concentrates may be used as substitute of soy and their derivatives for growing pigs and poultry. They are found to contain anti-nutrients namely Trypsin inhibitor activity (TIA), tannins, phytic acid and saponins which reduce protein quality, amino acid availability and adversely affect the nutritive value of the seeds (Wang & Duan 2006; Anton et al 2008; Hefnawy 2011). TIA is present in many legumes in varying amount which decrease the digestibility of protein and may even cause pancreatic enlargement. The mean TIA in field pea ranged from 2.22 trypsin inhibitor unit (TIU) mg^{-1} of dry matter (Wang et al 1998) to 7.66 TIU mg^{-1} of DM. Phytic acid (phytate) is usually stored in the form of phosphorus in the seeds which impairs the absorption of iron and zinc and to a lesser extent, calcium (Morris & Ellis 1980). The antinutritional effects of phytic acid are primarily attributed to their strong chelating property to complex with protein associated

with its six reactive phosphate groups that prevents them to become available for monogastric animals (Carnovale et al 1987; Urbano et al 2000; Gupta et al 2015). Tannins are polyphenolic compounds that bind enzymes and other proteins to form insoluble complexes under specific environmental condition. Previous studies (El-Shemy et al 2000) showed that tannins were almost completely eliminated without changes in the protein composition and amino acids in soybean and fababean seeds due to the removal of the cortex. Omnes et al (2017) reported that tannin ingestion resulted in significantly decreased cumulative feed intake, growth, feed and protein efficiencies, apparent digestibility coefficients, hepatosomatic index, and carcass lipids in juvenile European seabass (*Dicentrarchus labrax*).

Saponins are glycosides that are widely distributed in all cells of legume plants with foaming properties, bitter taste and reduce palatability of livestock feeds (Shi et al 2004). They are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. Clinical studies have shown that saponins have health promoting components that help protect against cancer and also lower cholesterol (Shutler et al 1989; Duane 1997; Guclu-Ustundag & Mazza 2007). Heng et al (2006) investigated that the different varieties of peas differed significantly in their saponin content from 0.0 to 1.5 g kg^{-1} (dry matter). Today, the latest trend is to focus on the utilization of different processing and feed preparation techniques for the effective utilization of legumes in order to maximize their nutritional value, improved palatability and digestibility (Akande & Fabiyi 2010).

So far, little research has been done on the various processing methods for the improvement of the nutrient composition and reducing the anti-nutritional factors present in *Pisum sativum*. The objectives of this study were to analyze the proximate chemical composition of different processed and unprocessed *P. sativum* as source of plant protein for sea bass; to determine which processing method is the most effective in reducing the anti-nutrients (ANFs) in mature *P. sativum* such as tannins, phytic acid, TIA and saponins either by dehulling, soaking or autoclaving in order to improve the nutritional value, digestibility and palatability of *P. sativum* as an alternative feed ingredient for *Lates calcarifer*.

Material and Method

Materials. One batch consisting of 25 kg of RGP was procured from the local market in Iloilo Ciiy, Philippines. The seeds were cleaned thoroughly to be free from dust, dirt and molds. The samples were kept in refrigerator at 4°C until further analyses.

Sample preparation: physical and heat –treatment methods. This study was conducted from February to May 2017 at the University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Miag-ao, Iloilo, Philippines. Samples of RGP were subjected at different processing methods at the Wet Laboratory of the Institute of Aquaculture.

Soaking. RGP seeds about 5 kg were soaked in distilled water for 12 h at room temperature (24°C) at a ratio of 1:10 (W/V). The soaked seeds were then rinsed five times with 500 mL distilled water and dried in air convection oven at 60°C to about 10% moisture content. Seeds were milled to pass through # 60 mesh sieve.

Dehulling. Another portion of the whole dry seeds consisting of 5 kg were dehullled using traditionally mortar and pestle. The seed coats/testa was removed from the kernels of *P. sativum* after processing and dehulled peas were then ground to pass through a 60 mesh nylon sieve.

Autoclaving. Five batches of green peas were autoclaved. Each batch containing 1 kg was spread thinly at a depth of 1-2 cm in a stainless aluminum tray and heat-treated at 15 psi for 60 min. at 121°C. The seeds were oven dried, kept cooled and stored at 4°C.

Unprocessed pea. Samples of unprocessed raw kernels of *P. sativum,* about 5 kg, were ground and stored in polyethylene bags at 4°C until use.

Proximate composition. Proximate analysis of unprocessed and processed samples of RGPs were determined at the Laboratory Facilities for Advanced Aquaculture Technologies (LFAAT) of SEAFDEC, AQD in Tigbauan, Iloilo. The moisture content of unprocessed and processed GPs were determined using the Infra Red Moisture Analyzer. Total nitrogen was analyzed using Kjeltec 2300 and the nitrogen-protein conversion factor 6.25 is used for the computation of crude protein (CP). The percentage protein content was calculated as follows:

$$\text{Protein \%} = \text{Nitrogen \%} \times 6.25$$

Crude fat and crude fiber were determined using Soxtec 2055 and Fibertec respectively. Samples for ash analyses were placed in the crucibles into the muffle furnace at a temperature of 600^{o}C following the procedure as described by the Association of Official Analytical Chemists (AOAC 2000). Nitrogen Free Extract (NFE) is calculated as:

$$\text{NFE} = 100\% - (\%\text{CP} + \%\text{Crude Fat} + \%\text{Crude Fiber} + \text{Ash})$$

The samples for proximate analyses were expressed as g/100 g dry matter (DM).

Analysis of anti-nutrients (ANFs)

TIA. TIA was determined according to the modified standard analytical procedure of Hamerstrand et al (1981). About 0.5 g of prepared sample in powder form was extracted using 20 mL of 0.01N NaOH. The mixture was stirred homogeneously for 1 h and centrifuged at 3000 rpm for 10 min. A 0.2, 0.4, 0.6, 0.8 and 1 mL aliquot of the supernatant was collected in separate test tubes and the volume was adjusted to 1 mL using distilled water. One mL of trypsin solution was added to the mixture and incubated at 37^{o}C water bath for 10 min. Benzoyl-alpha argenine-p nitroanilide (BAPNA) solution and 0.5 mL acetic acid were added after 10 min. The absorbance was read at 410 nm.

Trypsin inhibitor unit (TIU) was calculated using the equation below:

$$TIU/mg = (Y intercept\ of\ Sc)\ x\ DF\ x\ Vf)\ /mass\ of\ sample$$
$$DF = Dillusion\ factor$$
$$VF = final\ volume$$

Phytic acid. Phytic acid was determined using the modified method of Wheeler & Ferrel (1971). Twenty (20) mL of 3% trichloroacetic acid was used to extract 1.0 g of dried sample with constant agitation. The mixture was centrifuged at 3000 rpm for 10 min and 10 mL of the supernatant aliquot was transferred to a centrifuge tube. A 2.0 mL of saturated $FeCl_3$ was rapidly added to the aliquot using pipet. The mixture was heated for 45 min in water and then centrifuged at 3000 rpm for 10-15 min. The supernatant was discarded and the precipitate was washed twice with 10 mL of 3% trichloroacetic acid and finally with 10 mL hot distilled water. The precipitate was then dispersed with a little amount of water and 2 mL of 1.5 N NaOH was added and diluted to 15 mL with distilled water. The diluted solution was heated to boiling for 30 min and quantitatively filtered while was still hot. Again, the supernatant was discarded and the precipitate was washed with 30 mL of hot distilled water. The precipitate was dissolved in 10 mL of 3.2 N phosphoric acid and diluted to 20 mL with distilled water. The sample was allowed to cool at room temperature and about 4.0 mL aliquot was transferred into the test tube and added with 1.0 mL of 1.5 M KSCN (potassium thiocyanate) within a minute and diluted to 10 mL with distilled water. The absorbance of the sample was read at 480 nm within 1 min. A standard curve was prepared using $FeCl_3$ as standard and the phosphorus phytate was calculated using the phosphorus:iron ratio (4:6).

Tannin. Total tannin content was analyzed using the modified standard procedure of Price et al (1978). Twenty (20) mL of 50% ethanol was used to extract 2.0 g of powder sample. The mixture was vortexed for 10 min and centrifuged at 3000 rpm for 10 min.

About 0.5 mL aliquot of the supernatant was added with 0.5 mL of 8% HCl in methanol and 2.0 mL of 1% vanillin solution. The absorbance was read at 500 nm. A standard curve was prepared using catechin as standard. Total tannin content was calculated from the standard curve using interpolation method.

Saponin content. The determination for saponin content was analyzed according to the study of Oleszek (2002). About 5.0 mL of 80% ethanol was used to extract 500 mg of ground sample. The sample was then refluxed at 50°C on the Evapomix Evaporator for 15 min. The mixture was then centrifuged at 3000 rpm for 10 min. The extraction was repeated more than twice and the pooled extract was adjusted to 15 mL with 80% ethanol. The supernatant was made to pass through a pre-hydrated PVPP (polyvinylpolpyrollidone) mini columns. A 2 mL eluate was collected and 10 mL of the aliquot was placed in the test tube. The sample was pre-treated with 0.5 mL glacial acetic acid and mixed using the vortex mixer. Three (3) mL of freshly prepared Liebermann-Buchard reagent was added to the sample and shaked. The resulting mixture was read at 450 nm and a standard curve was prepared using condensed saponin as standard. The saponin content was calculated from the standard curve using interpolation method. Due to the inavailability of highly sophisticated equipment at UPVisayas, samples of processed and unprocessed green peas were sent to the Analytical Service Laboratory of the Institute of Plant Breeding, College of Agriculture and Food Science at the University of the Philippines Los Banos, College, Laguna, Philippines for more advanced procedures and accuracy of results.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) using completely randomized design (CRD). Means were compared by Duncan's Multiple Range Test ($p<0.05$) using SPSS (Statistical Package for the Social Science) version 16 (SPSS Inc., Illinois, USA) released in 2008.

Results and Discussion. The proximate composition of unprocessed (raw) and processed green pea seeds are presented in Table 1. There was a significant ($P<0.05$) differences in moisture contents between the unprocessed and processed treatments of green pea seeds. The unprocessed RGP had a moisture content of 10.21% which is similar to the result obtained by Offia-Olua & Madubuike Agugo (2015) on raw mungbean flour at 10.14%. However, autoclaving had the lowest moisture content as compared with other samples, this could be attributed to the high temperature during the heat treatment processing at 121°C for 60 min. Autoclaving and soaking significantly ($P<0.05$) decreased the moisture content at 4.74 and 5.67% respectively. The same trend was also observed in the ash content where AGP (2.79) and SGP (3.11%) were considerably low as compared with DGP and RGP which ranged from 3.16 to 3.17%, however no significant ($P>0.05$) differences in the ash content were observed among the treatments. Similar findings were also reported by Ataga & Ota-Ibe (2006), Udensi et al (2010) and Ramadan (2012) that ash content of wild mango, *Mucuna flagellipes* ("ukpo") and soybeans respectively decreased due to the leaching of minerals in the water. The highest crude protein level was observed after dehulling (22.31%) which resulted in a significant ($P<0.05$) increase as compared with other treatments such as autoclaving (19.39%) and soaking (20.66%) respectively.

Table 1

Effect of processing on the proximate analysis of *Pisum sativum* seeds (g/100 g DM)*

Treatment	*Crude protein*	*Crude fat*	*Crude fiber*	*Ash*	*Moisture*	*NFE***
RGP	21.56±0.13^{c}	1.06±0.01^{a}	5.92±0.17^{c}	3.17±.002^{a}	10.21±0.08^{c}	70.46±0.35^{b}
DGP	22.31±0.35^{c}	1.08±0.12^{a}	1.23±0.06^{a}	3.16±0.07^{a}	10.69±0.06^{d}	72.22±0.34^{c}
AGP	19.39±0.17^{a}	1.31±0.13^{a}	6.77±0.06^{d}	2.79±0.49^{a}	4.74±0.12^{a}	67.58±0.18^{a}
SGP	20.66±0.11^{b}	1.18±0.09^{a}	5.17±0.04^{b}	3.11±0.01^{a}	5.67±0.04^{b}	69.88±0.05^{b}

* Mean of two replicate samples±standard error of the mean (SEM). Treatment means within a column followed by different superscripts are significantly different ($P<0.05$). ** NFE – Nitrogen Free Extract.

Ghavidel & Prakash (2007) also reported that both protein and its digestibility increased significantly following dehulling of cowpea, lentil and chickpea in range of 2.2-5.1 and 13.2-16.7% respectively. This may be due to the fact that the removal of the hull portion (testa) of the seed with dehulling can increase the protein content. However, the crude protein content of RGP, 21.56% is not significantly different from DGP ($P>0.05$). According to Ukachukwu & Obioha (2000) decrease in the protein content of the legume seeds during soaking are presumably due to the progressive solubilisation and leaching out of the nitrogen into the water. The crude fat content of unprocessed GP did not differ significantly ($P>0.05$) from all the processed samples, however the autoclaved sample had the highest crude fat content (1.31%). The result indicates that the different processing methods did not affect the fat content of *P. sativum* which is due to the insoluble nature of fat. These observations are in agreement with those reported by Hefnawy (2009) in lentils (*Lens culinaris*) and Oke et al (2013) for lima beans (*Phaseolus lunatus*).

The crude fiber content of all the samples ranged from 1.23 to 6.77%. The result shows that DGP (1.23%) had significantly ($P<0.05$) lower crude fiber content than AGP, SGP and RGP. This is because, the soluble and insoluble fiber present in the seed coats of the seeds were significantly removed during dehulling. The present findings are also comparable with the work of Ramadan (2012) that the crude fiber content of soybeans was improved through soaking and autoclaving. Carbohydrates (NFE) are main source of energy in human body and they are considered major parts of cereals and pulses. The effect of dehulling significantly ($P<0.05$) improved the NFE content of GP in the present study and this is attributed to the reduction of the fiber contents in dehulled seeds. These observations are in agreement with that reported by Kerr et al (2000) and Raghuvanshi et al (2011) on cowpea and mungbeans respectively. The apparent increase in NFE could be due to the reduction of the fiber contents in dehulled seeds. It was also observed that samples of both autoclaved (67.58%) and soaked peas (69.88%) have the least values for NFE, although SGP is not significantly ($P>0.05$) different from the unprocessed RGP at 70.46%. The antinutritional factors of raw and processed green pea seeds are shown in Table 2.

Table 2

Effect of different processing methods on the ANFs of *Pisum sativum* (dry weight)*

Treatments	*Trypsin inhibitor (TIU/mg)*	*Phytic acid (mg/100 g)*	*Tannins (%)*	*Saponins (%)*
RGP	7.40 ± 0.11^{c}	421.10 ± 0.007^{b}	3.81 ± 0.53^{a}	0.571 ± 0.01^{c}
DGP	6.99 ± 0.11^{b}	310.07 ± 0.003^{a}	4.42 ± 0.39^{a}	0.623 ± 0.007^{d}
AGP	6.62 ± 0.06^{a}	368.29 ± 0.001^{b}	4.42 ± 0.58^{a}	0.407 ± 0.008^{a}
SGP	7.05 ± 0.15^{bc}	396.73 ± 0.008^{b}	4.07 ± 0.40^{a}	0.525 ± 0.001^{b}

ANFs - antinutritional factors. * Mean of three replicate samples±standard error of the mean (SEM). Treatment means within a column followed by different superscripts are significantly different ($P<0.05$).

TIA was significantly ($P<0.05$) decreased by heat-treatment method after autoclaving. It was observed that AGP have the least protease inhibitor of 6.62 TIU/mg followed by DGP (6.99 TIU/mg)) and these values were significantly ($P<0.05$) lower than the unprocessed RGP (7.40 TIU/mg). SGP (7.05 TIU/mg) was not significantly ($P>0.05$) different from RGP. Wang et al (1997) reported a higher reduction in trypsin inhibitor activity in cowpea using steam blanching rather than water blanching. Similarly, a maximum level of reducing TIA under autoclaving was also reported in some previous studies on kidney beans (*Phaseolus vulgaris*) and in white faba beans (*Vicia faba*) by Shimelis & Rakshit (2007) and Luo & Xie (2012) respectively.

The level of PA in *P. sativum* was significantly ($P<0.05$) reduced by 27% (310.07 mg PA/100 g) by dehulling as compared to AGP by 12.54% (368.29 mg PA/100g) and 5.79% by SGP (396.73 mg PA/100 g). Means revealed that more phytates were present in RGP (421.10 mg PA/100g). The highest reduction in PA might be attributed to the dissociation of phytate (salts of phytic acid) complexes during dehulling thereby

minimizing the phytate content in the green peas. Moreover, dehulling produced valuable effects on the ANFs of legumes. The current findings are similar to the work conducted by Bishnoi et al (1994) on *P. sativum* and Mubarak (2005) who illustrated that the activity of phytic acid was reduced by 20.7% after dehulling of mungbean sedes.

Tannin content for both unprocessed and processed GPs ranged from 3.81 to 4.42% and these were not significantly ($P>0.05$) different among treatments. According to Sathya & Siddhuraju (2015) most of tannins are concentrated on the seed coats/testa rather than in the cotyledons and they have adverse effects on protein digestibility. However the processing methods such as dehulling, autoclaving and soaking employed in the present study were not efficient for the tannin activity. Similar result was obtained by Osman (2007) for heat treated *Dolichos* lab lab bean. Results are contrary with the work of Alonso et al (2000) and Egounlety & Aworh (2003) who reported that dehulling substantially reduced the levels of tannins in beans. Saponins are secondary compound present in pulses and oil seeds such as kidney beans, chick peas and soybeans. They have a carbohydrate moiety attached to the triterpenoid/steroid aglycon (Sathya & Siddhuraju 2015) with health promoting benefits such as anti-cancer and anti-cholesterol activities in human (Guclu-Ustundag & Mazza 2007). As a result of the various processing methods, AGP contained 0.407% which significantly reduced saponin by 28.72%, followed by SGP (0.525%) which registered a significant decrease of 8.06% ($P<0.05$) as compared to RGP (0.571%). However, the value for saponins during dehulling process is higher than RGP. Francis et al (2001) recommended moist heat treatment (autoclaving) of processing would remove most of the saponins from the feed ingredients because of the high solubility of saponins in water and aqueous extraction. These results are in accordance with the report of Alogbaoso et al (2015) who observed that all of the autoclaved samples of *Canavalia plagiosperma* piper seeds at 35 min at 121^{o}C have resulted to a significant ($P<0.05$) decrease of saponin content.

Conclusions. This study shows that dehulling improved significantly the nutritional composition of *P. sativum* in crude protein and in NFE contents and caused a significant decrease in the crude fiber due to the removal of seed coats during processing whereas autoclaving caused a significant loss in moisture content and slight loss on ash followed by soaking the seeds for 12 h. Autoclaving brought about a more significant ($P<0.05$) improvement by reducing the levels of antinutrients including trypsin inhibitors, phytic acid and saponins thus increasing the bioavailability of the nutrients in *P. sativum*. Therefore, it is recommended that combined processing of soaking, dehulling and autoclaving to be employed in order to reduce effectively tannins in *P. sativum*.

Acknowledgements. The author is very grateful to the University of the Philippines Visayas for funding this in-house research project entitled: "Evaluation of different processing methods on the nutritional composition and certain anti-nutritional factors in peas *P. sativum*: utilization of raw, dehulled, autoclaved and soaked peas in seabass diets". The author also wishes to thank the administrative and academic personnel of Institute of Aquaculture, College of Fisheries and Ocean Sciences for their encouragement and supportin implementing this study.

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*** AOAC 2000 Official Methods of Analysis of AOAC International, 17th edition, Association of Official Analytical Chemists, Washington, DC.

Received: 19 March 2018. Accepted: 06 June 2018. Published online: 13 June 2018.
Authors:
Erlinda Souribio Ganzon-Naret, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Iloilo, 5023 Miag-ao, e-mail: erlindanaret@yahoo.com
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How to cite this article:
Ganzon-Naret E. S., 2018 Evaluation of different processing methods on the nutritional composition and certain anti-nutritional factors in pea *Pisum sativum*. ABAH Bioflux 10(1):18-26.