Chemical composition and antioxidant properties of Philippine oyster (*Crassostrea iredalei*) residue

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ABSTRACT

This study evaluated the oyster residue, a by-product of oyster extract processing in terms of chemical components (proximate, total amino acid, chemical score), physical properties and its antioxidant activities (free radical scavenging activity, reducing power, and Gallic acid equivalents).The extraction process involves homogenization with water, mild heat treatment and filtration steps. The resulting residue was dried and pulverized. Analyses revealed that the product contain 73.89 + 0.40% protein, 8.92 + 0.12 % lipid, 8.65 + 0.02% ash, and 3.98 + 0.05 % moisture with an Aw of 0.24. Amino acid profile (g/100g crude protein) shows that the product contains the nine essential amino acids, having lysine (6.26g) and leucine (6.15g) as the major components. Evaluation of the chemical score (CS) of the residue showed that tryptophan (59.6) was the limiting amino acid. Essential amino acid index (EAAI) of 94.9 indicates that the EAA balance of the residue is well balanced despite being deficient in tryptophan (Trp). Antioxidant activities significantly increased with increasing sample concentration, suggesting the potency of bioactive compounds responsible for its antioxidant capabilities. Results of the study shows that oyster residue can be a good source of dietary protein and bioactive compounds.

KEYWORDS

oyster residue, essential amino acids, antioxidant activity

INTRODUCTION

Oysters are the most common bivalves farmed in the Philippines where they are cultured traditionally in natural beds in rivers and estuarine ecosystems. It is a viable economic activity in coastal communities due to low input and capitalization compared to culture of milkfish, shrimp and other species. Oyster production in the Philippines was at 179,512 MT in 2014 with Western Visayas, Ilocos, and Central Luzon as the highest producing regions (PSA 2015). Different species of oysters are cultivated namely *Crassostrea iredalei* and moderately sized *C. malabonensis*. *Crassostrea iredalei*, marketed to a length of 6-9cm, is the most commercially desirable species due to its fast growth, large size, and straight shell margins that make them easier to open (*Samsin*, 1988).

As one of the known exotic and gastronomic delicacy consumed in the Philippines, consumers eat oysters due to their perception of being tasty (e.g. meaty, flavourful, juicier) and high nutritional value(Andalecio et al 2014).

Males were believed to be fond of eating oyster as a sexual stimulant (*Laloo et al,* 2000; *Kotta et al,* 2013) since it contains elevated zinc, which increases testosterone level. Moreover, health benefits from seafood have been well documented (*McManus* et al, 2011; *Lund*, 2013) and therefore make it a good substitute for meat products. Oysters and mussels contain high quality proteins that are rich in tyrosine (an amino acid); are natural sources of zinc, high levels of calcium, iron and vitamins and are low in fat, cholesterol and calories (*Harnedy and FitzGerald,* 2012). They also have bioactive components such as potential antioxidants, which are beneficial for the development of functional foods (*Grienke et al*, 2014; *Kim and Palella*, 2012).

The food industry is now driven by factors such as nutrition, health, safety, and convenience (*Zhang et al*, 2015). Rather than producing traditional products that are bottled/canned or

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dried-smoked, dried oyster extract concentrate can serve as an alternative since it can be a good source of dietary protein and other nutritional components. Protein concentrates are low-cost and are stable products of high dietary quality that can be used in the production of new food products (*Stillings*, 1971).

Due to an increase in bacterial infections, there is now a greater demand for effective and nontoxic antibacterial therapeutics (*Ramasamy and Balasubramanian*, 2014). Studies have reported that oyster extracts of several species contain many bioactive peptides and many of these bioactive compounds show anti-tumor, antibacterial, and antiviral activities led to the greater demand. Several bioactive substances are being isolated and characterized from food derived from the marine environment with a great potential for treatment of human and fish diseases as well as through their incorporation into functional foods and nutraceuticals (*Karthikeyan et al*, 2014; *Ryan et al*, 2011)).

Since the nutritional benefits of oyster extract for human health have been well documented, the utilization of by- products can help improve the economic aspect of processing by discovering their potential. At present, by- products are viewed as potential resources instead of waste (*Rustad*, 2003). The conversion of these discards into high value functional ingredients can offer a solution in the disposal of such waste (*Harnedy and FitzGerald*, 2012). Thus, the study aims to characterize the dried oyster residue, as a by-product from extract processing and to assess its possible use as food ingredient.

MATERIALS AND METHODS

Sample collection and preparation of oyster residue

Fresh oysters (Crassostrea iredalei) in shell were purchased from a local market in Iloilo City, Philippines and transported to the Institute of Fish Processing Technology laboratory of the College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Iloilo. The oysters were thoroughly washed to remove dirt and debris adhering to the shell and hot-water dipped to facilitate shell opening and meat extraction. The meat was boiled for 5 min in water with a ratio of 1: 0.5 (oyster meat: water) and homogenized. The homogenate was filtered pressing to squeeze out the liquid extract through a fine mesh net. The resulting solid residue was chopped into small parts and dried in a cabinet dryer for 8h at 60-80^{o}C, with constant stirring to attain even drying. After cooling, the dried residue was pulverized into powder for using a grinder, packed in laminated PET/Foil/PE bags, and refrigerated at 4°C prior to analyses.

Preparation of ethanol oyster residue extract

The dried oyster residue (5 g) was homogenized with 5 ml distilled water and 20 ml 95% ethanol. After centrifugation for 10 min at 1500 rpm, the upper layer was recovered. The precipitate was treated again with 20 ml 95% ethanol and centrifuged as above. The recovered upper layers were combined and adjusted to 50 ml by adding 95% ethanol (*Peralta et al*, 2005). The resulting solution, which contains approximately 85% ethanol was designated as 85% ethanol sample extract.

Determination of pH value, water activity and proximate composition (protein, moisture, crude fat and crude ash

Water activity (Aw) of the sample (10 g) was measured using a water activity meter (Novasina ms1, Axiar Ltd Pfaffikon, Switzerland) at 30^{o}C. The oyster residue (5 g) was homogenized with 50-ml triple distilled water and its pH was measured using calibrated pH meter (Hm Digital, Thermo Scientific, Waltham, MA USA) (*DFO*, 1986). The proximate composition of dried oyster residue was analyzed using standard methods (*AOAC*, 2000), while the moisture content of a 2-g sample was determined by drying in an oven at 105^{o}C until constant weight. The loss was considered as the moisture content of the sample. Cruse ash was analyzed by weighing 2 g of sample in a porcelain crucible and placed in a temperature-controlled furnace at 600^{o}C and weighed. Crude protein was determined using Tecator™ Digestor and Kjeltec™ 2100 (Foss Analytical Höganäs, Sweden). One-gram sample was digested in the presence of sulfuric acid, potassium sulfate, and copper sulfate. Sodium hydroxide was added into the digested solution and distilled. The resulting distillate was titrated with standard acid solution. The crude protein was calculated as nitrogen multiplied with the factor 6.25, and crude fat was extracted with anhydrous ether using the Soxhlet method. The resulting extract was dried at 100^{o}C to constant weight. All analyses were done in three replicates.

Total amino acids and its chemical score

The oyster residue was analyzed for its total

amino acid profile following the standard protocols for amino acid analysis (*AOAC*, 1997). Results were expressed as g 100 g^{-1} protein. The essential amino acid (A/E) ratio of each essential amino acid (EAA) was calculated as the percentage of the total EAA. The chemical score was determined using the formula (Eq 1):

$$\text{Chemical score} = \frac{\%\ \text{limiting EAA in oyster residue}}{\%\ \text{corresponding required EAA levels for human diet}} \quad /1/$$

The geometric mean of the essential amino acids (i.e. EAAI) is the ratio of all essential amino acids of the oyster residue to the EAA requirements of the human adult (Oser, 1959). It was estimated using the formula (Eq 2):

$$EAAI = EAAI = 10\log^{EAA} \text{ where: } \log EAA = 0.1[\log(a_1/a_{1s} \times 100) + \log(a_2/a_{2s} \times 100) + \log(a_n/a_{ns} \times 100)] \quad /2/$$

a_1.., an are the amino acid contents of the oyster residue while a_{1s}…a_{ns} are the required levels of these amino acids in the human adult.

Evaluation of Antioxidant activities

Free radical scavenging activity was determined by using various concentrations (mg d.w.) of the ethanol oyster extract, diluted with ethanol and incubated with 0.25 ml of 0.5 mM DPPH (1,1-diphenyl- 2- picrylhydrazyl) for 20 min at room temperature. Absorbance was read at 517 nm using a UV- Vis spectrophotometer (Sunny UV-7802 Sunny Optical Technology Com Ltd, China) (*Peralta et al*, 2008). The blank was prepared as above without the sample. The DPPH radical scavenging activity was calculated using (Eq. 3):

$$\text{Radical scavenging activity (\%)} = \frac{Abs_{blank} - Abs_{sample} \times 100}{Abs_{blank}} \quad /3/$$

The reducing power was measured based on the method of Oyaizu (*1986*). Different concentrations of 85% ethanol sample extract (mg, dw) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferric cyanide (K3F(CN)6). The mixture was incubated in a water bath at 50°C for 20 min. After cooling, 2.5 ml 10% trichloroacetic acid (TCA) was added and centrifuged for 10 min at 3000 rpm (Hettich Rotofix 32 A, Hettich Lab Technology, Tuttlingen, Germany). The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml freshly prepared 0.1% (w/v) ferric chloride ($FeCl_3$). The reducing power was determined by measuring the formation of Perl's Prussian blue at 700 nm using a UV- Vis spectrophotometer (Sunny UV-7802 PC, Sunny Optical Technology Com Ltd, China). The blank was prepared by adding the solvent without extract.

The phenolic content of the ethanol sample extract was determined by the Folin- Ciocalteu method (*Slinkard and Singleton*, 1977). Varying concentrations (mg,dw) of the 85% ethanol extract solution was used and added to 1.58 ml of distilled water and 100 µl of the 2N Folin-Ciocalteu reagent (Sigma Chemical Co, St Loius MO USA) in a test tube. The contents were mixed well by vigorous shaking and allowed to react for 8 min before adding 300 µl of 20% (w/v) Na_2CO_3. The resulting solution was stirred well and incubated in the dark for 2 h at 23°C. The absorbance was read at 765 nm using a spectrophotometer (Sunny UV-7802 PC Sunny Optical Technology Com Ltd, China). A blank solution was prepared as above without the sample extract. Gallic acid, a known phenolic compound, was used as standard. The stock solution was prepared by dissolving 0.500 g of dry gallic acid in 10 ml ethanol in a 100-ml volumetric flask and marked to volume with distilled water. Increasing concentrations of gallic acid were used and analyzed as above. The standard curve was used to extrapolate the phenolic content of the sample extract and expressed as gallic acid equivalents (GAEs, mg/L).

Statistical Analysis

The results on proximate composition, pH and antioxidant properties (DPPH scavenging , reducing power, total phenol content) were expressed as mean values ± standard deviation (S.D.). Significant differences ($p<0.05$) between the mean values of the various concentrations for antioxidant activities were calculated through one- way analysis of variance (ANOVA) and Post hoc analysis using Duncan's multiple range tests (DMRT) and SPSS 16.0 for Windows. Pearson coefficient test (r) was used to determine the relationship between reducing power and total phenol content.

RESULTS AND DISCUSSION

Proximate composition, water activity (Aw) and pH of the oyster residue

The composition using proximate analyses can support the potential of the powdered oyster residue by providing an estimate of its quality and gross composition. Results reveals the following chemical and physical composition of oyster residue; protein (73.89± 0.40 %), lipid (8.92±

0.12 %), ash content (8.65± 0.02 %), and moisture (3.98± 0.05 %) having a pH value of 6.54 ± 0.02 and water activity (Aw) of 0.24. The low moisture content and water activity of the product can inhibit growth of most bacteria, molds, or yeast, thus suggesting its shelf life stability. The relatively high ash content indicate its presence of rich macro elements and trace minerals. Oysters are known to contain significantly high proportions of trace metals such as zinc (Zn) and iron (Fe) which are one of the important components in human diet (*Asha et al*, 2014). The lipid content (8.92%) of oyster residue is comparable with the reported value (7.9 g/100g edible portion) of dried oyster product (*FNRI,* 2002). Oyster is said to be poor source of fat but it is a good dietary sources of essential polyunsaturated fatty acids (PUFA) (*Ahsa et al*, 2014; *Ackman*, 1995). Toda *et al* (2015) reported that oyster-extract by-product (OEBP) contained 9.6g /100g crude lipid, which is composed of main fatty acids (% w/w) DHA (3.3%), EPA (3.7%), palmitic acid (26.8%) and oleic acid (1.8%).

Oyster residue's main component was protein at 73.89%, brought about by the extraction and drying process. The process developed was a simple water extraction without the use of enzyme or chemical intervention. With the application of mild heat during the process, some protein coagulates could form as a result of the reduction of protein solubility (*Sun-Waterhouse et al*, 2014) thus, becoming part of the water insoluble component. Similar results was obtained by Toda *et al* (*2015*) where they found high protein remaining in the oyster extract by product (OEBP) in the processing of oyster (*Crassostrea gigas*) extract powder.

Fish protein concentrate is defined as any stable fish preparation in which the protein is more concentrated than that in the original fish (*Windsor,* 2001). The addition of oyster protein concentrate in foods can be beneficial for consumers' nutrition especially children and young adults. There is a large potential in utilizing the product as ingredient for value added products, but this would still warrant further research.

Total amino acids and its chemical score

Protein quality can be evaluated by the ratio of essential amino acids (EAAs) content to human amino acid needs (FAO, 2001). Table 1 shows a total of 18 amino acids composed of essential and non-essential amino acids, and glutamic acid (11.89 g /100g) and aspartic acid (8.04 g/100 g) as the predominant components of oyster residue (Table 1). It contains all the nine EAAs required by human for growth, however these cannot be synthesized in human cells and therefore must be provided in the diet (*Wu*, 2010). Lysine (6.26 g/100g) and leucine (6.15 g/100g) were the most abundant among the EAAs in the residue, while other EAAs content (histidine, methionine, isoleucine, valine, threonine, phenyalanine, and tryphophan) ranged from 0.92-4.66g/100g. Such results are similar to that found by Asha et al (2014) in oyster (*Crassostrea madrasensis*) where essential amino acid lysine was most abundant (14.3g/100 g).

Protein quality can be assessed by comparing the EEAs content of oyster residue with reference to amino acid needs of human adults set by the FAO/ WHO/UNU (*30*) (Table 2). The determination of the biological value of proteins for human is a long and tedious process, which may require animal models in the experimentation. Thus, quotients from amino acid analysis of the protein in question have been used to predict its biological value.

One of these quotients is the "chemical score (CS)" which is based on the assumption that growth will be limited by the EAA in the diet since the ratio to its content in the human adult requirement level is the lowest. The CS of the oyster residue for human adult was estimated to be 59.6 (Table 2) corresponding to that of tryptophan, which was considered as the most limiting EAA. This score indicates that if the oyster residue was used as the sole source of protein in the nutrition of the human adult, protein synthesis would proceed only up to 59.6% of the ideal 100% maximum synthesis rate. A supplementation of 41.4% tryptophan to the diet would make up for this deficiency. However, CS is not considered as the definitive predictor of protein nutritive value since the limiting EAA plus other EAAs may also have some effect on it. The essential amino acid index (EAAI) gives additional information on a protein's nutritive value. The CS is the geometrical mean of the ratio of all EAAs in the evaluated protein relative to their content in a highly nutritive reference protein, viz., whole egg (*Oser*, 1959) and better still, human adult EAA requirement. The EAAI of the oyster residue for human consumption was estimated to be 94.9; a perfect index is 100. This indicates that despite the low CS, the balance of EAA was very good. These predictors, however, are useful only under the assumption that the oyster residue is used as a sole source of protein in the human diet.

Antioxidant Activities

Results of the assay on antioxidant activities show that DPPH radical scavenging activity, reducing power, and total phenol content significantly increased ($p<0.05$) as a function of concentration (Tables 3 and 4).

The free radical scavenging activity of the ethanol

Table 1. Amino acid content of oyster (*Crassostrea irreddalei*) residue powder

Analyte	Amount (g/100 g protein)
Essential Amino Acids	
Histidine	4.66
Methionine	3.26
Lysine	6.26
Isoleucine	3.77
Valine	4.07
Leucine	6.15
Threonine	3.76
Phenylalanine	3.26
Tryptophan	0.92
Non-essential amino acids	
Arginine	5.99
Cysteine	1.03
Tyrosine	3.05
Aspartic Acid	8.04
Serine	3.56
Glutamic Acid	11.89
Glycine	3.35
Alanine	4.04
Proline	3.49

Table 2. Comparison of EAAs of oyster (*Crassostrea irreddalei*) residue to EAAs human adult requirements (% protein)

EEAs	Oyster residue		Human adult requirement		(A/E oyster)/(A/E human req)x100
	% protein	A/E*1	% protein	A/E*1	
Histidine	4.66	11.88	1.6	12.60	94.33
Isoleucine	3.77	9.61	1.3	10.2	93.93
Leucine	6.15	15.68	1.9	15.0	104.84
Lysine	6.26	15.97	1.6	12.6	126.72
Methionine +	4.29	10.94	1.7	13.4	81.74
Phenylalanine+	6.31	16.09	1.9	15.0	107.57
Threonine	2.78	7.09	0.9	7.1	100.05
Tryptophan	0.92	2.35	0.5	3.9	59.60
Valine	4.07	10.38	1.3	10.2	101.40
Σ EAA	39.21		12.7		
CS*2	59.60				
EAAI*3	94.9				

*[1]A/E = ratio of each % EAA divided by the total % EAA multiplied by 100.

*[2]Chemical score (CS) = lowest (A/E oyster)/(A/E human adult requirement) = 59.60. This value corresponded to that of Trp which is considered as the most limiting EAA in the oyster residue

*[3]EAAI = EAAI=$10^{\log EAA}$ where: log EAA=0.1[log(a_1/a_{1s} X 100) + log(a_2/a_{2s} X100) + log(a_n/a_{ns} X 100)];
a_1.., a_n are the amino acid contents of the oyster residue while a_{1s}…a_{ns} are the required levels of these amino acids in the human adult.

Table 3. DPPH radical scavenging activity (%) and reducing power (Abs) of 85% ethanol extract from oyster (Crassostrea iredalei) residue as a function of concentration

Concentration (mg, dw)	% DPPH radical scavenging activity	Reducing Power (Abs)
10	25.5 ± 0.02[a]	0.091 ± 0.017[a]
20	32.2 ± 0.01[b]	0.257 ± 0.008[b]
30	35.9 ± 0.01[c]	0.375 ± 0.018[c]
40	40.4 ± 0.00[d]	0.533 ± 0.086[d]
50	46.4 ± 0.01[e]	0.604 ± 0.041[d]

Results are expressed as mean ± S.D (n=3). Values in the same column with different letters are significantly different (p<0.05)

Table 4. Total phenol content, expressed as GAEs, of 85%ethanol oyster extract of varying concentration

Concentration (mg, dry wt.)	GAEs (mg/l)
15	5.78 ± 0.78 a
20	10.23 ± 0.78 b
30	26.33 ± 1.57 c
40	28.60 ± 0.71 c
50	38.57 ± 1.59 d

Results are expressed as mean ± S.D (n=3). Values in the same column with different letters are significantly different (p<0.05); nd= not detected

oyster extract was measured using a stable free radical, DPPH. The ethanol solution of DPPH exhibits a deep purple color that diminishes in intensity in the presence of an antioxidant. The reduction of DPPH is then monitored by the decrease in absorbance at 517 nm, reflecting an increasing scavenging activity (*Sivakumar*, 2012; *Brand-Williams et al*, 1995). Free radicals are generally reactive and attack molecules such as lipids, proteins and sugar to induce oxidative damage. In the presence of antioxidant molecules, it can quench free radicals by providing hydrogen atoms or by electron donation, which inhibits the production of catalyst of lipid peroxidation (*Biswas et al*, 2010; *Noguchi and Niki*, 1999). Reducing power can serve as a significant indicator of the potential antioxidant activities of the 85% ethanol oyster extract. The assay resulted in the reduction of Fe^{3+} (ferricyanide complex) to Fe^{2+} in the presence of antioxidants in the sample (*Behera et al*, 2006). The test solution changed color from yellow to various shades of blue or green, or termed as Perl's Prussian blue, depending on the reductive capabilities. Increasing absorbance of the test reaction mixture at 700 nm (Table 3) indicates an increase in reducing power.

Studies have showed high radical scavenging activity (85.7%) and significant reductive capabilities in *Saccostrea. cucullata hydrolysate* (*Umayaparvathi et al*. 2014) and peptide fractions in *Crassostrea. talienwhanensis meat hydrolysate* (*Wang et al*, 2014; *Dong et al*, 2010). The result of the assays imply that oyster residue can possibly contain substances, which can act as electron donor and react with free radicals to convert them into stable products to terminate radical chain reaction (*Yen and Chen*, 1995; *Chanda and Dave*, 2009). Amino acids such as tyrosine, methionine, histidine, lysine and tryptophan are generally known to exhibit antioxidant capabilities (Kitts and Weiler, 2003) and its presence in oyster residue (Table 1) can be responsible for the observed activity. Marine- derived protein hydrolysates and peptides arising from processing by- products, mollusks, and crustaceans have been shown to exhibit bioactivities in vitro (*Lee et al*, 2014; *Zhao et al*, 2011).

Total phenol content, expressed as gallic acid equialents (GAEs, mg/L), increased with oyster extract concentration, as measured by the Folin-Ciocalteu method (Table 4). The manifestation

of phenolic compounds in oyster can be related to its utilization of plant-derived materials, such as microalgae and microalgae, as food (*Kidd*, 2011; *Fuda et al*,2015) since they are filter-feeders and strain phytoplankton (microalgae), bacteria and organic matter as it passes through its gills. Phenolic compounds are a group of low and medium molecular weight secondary metabolites which are divided into flavonoids, phenolic acids, gallic acids and tannins and are known to be effective antioxidants that can act as scavenger of singlet oxygen and free radicals (*Aligiannis et al*, 2003; *Jorgensen et al*, 1999). The total phenol content in the ethanol oyster extract can be responsible for the observed antioxidant activities in addition to other compounds exhibiting antioxidant capabilities such as peptides and amino acids. It has been reported that antioxidant activity is well correlated with the content of phenolic compounds (*Balakrishnan et al*, 2014; *Aligiannis et al*, 2003). Correlation tests showed a positive relationship between total phenol content and DPPH radical scavenging activity (r=0.943) and reducing power (r=0.939).

The study revealed that the oyster residue can be a potential source of natural antioxidant that could serve as a replacement for synthetic antioxidants therefore preventing free-radical-induced lipid peroxidation, which is accountable for the development of off- flavors and the undesirable chemical compounds in food.

Conclusion

Oyster residue, as a by-product of oyster extract processing, was found to contain high protein content, essential amino acids needed by the human body and bioactive compounds exhibiting antioxidant activities. This product can be considered as a natural cheap source protein concentrate of high dietary quality and a potential functional ingredient that can be used in a variety of food products. This does not only makes the nutrients from oyster available to a wide variety of consumers, but it also maximizes the utilization of the resource and increases its market value.

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