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Antioxidative and Functional Properties of *Rastrineobola argentea* (*Dagaa)* Fish Protein Hydrolysate

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Abstract

Protein hydrolysates are good nutritional supplements as their bioactive ingredients can be easily absorbed and utilized for various metabolic activities. Antioxidative activity and functional properties of fish protein hydrolysate prepared from *Dagaa* (*Rastrineobola argentea)* using exogenous commercial enzyme (Alcalase) and endogenous enzymes were investigated. The degree of hydrolysis (DH) of Alcalase hydrolysate (AH) was 83 % while that of *Dagaa* endogenous enzymes hydrolysate (EH) was 45%. The hydrolysates contained 13.3 % and 12.6 % protein on a wet weight basis respectively and no detectable lipid content. The antioxidant potential was established as Oleic acid (lipid) peroxidation inhibition at 31.5 %, 49.5 % and 29% for AH, EH and the commercial antioxidant Butylhydroxytoluene (BHT) respectively. Similarly, 2, 2, diphenyl-1-picrylhydrazyl (DPPH) percentage free radical-scavenging activity was established at 90 %, 71.8 % and 89 % for AH, EH and BHT. Percentage DPPH radical scavenging ability using IC_{50} was 2.84 mg/ml, 8.00 mg/ml and 2.4 mg/ml for AH, EH and BHT, respectively. Ferric ion reducing inhibition test also showed a similar trend with AH showing significantly higher Ferric ion reducing inhibition power in comparison to EH. The functional properties tested were solubility, emulsifying capacity, fat absorption and foam stability of the hydrolysates. The relative solubility increased with increase in percentage DH. At pH 7, AH showed solubility of 43 % and EH 29 %. Consequently, it can be concluded that AH is a better antioxidant in polar systems (higher DPPH % radical scavenging ability and Ferric ion reduction inhibition) whereas the EH is a better antioxidant in non-polar systems (higher Lipid peroxidation inhibition). The promising functional and antioxidative properties of AH and EH suggests that *Dagaa* protein hydrolysate presents a promising candidate for inclusion in food systems.

Keywords: Dagaa, Antioxidant, Protein hydrolysate, DPPH, Alcalase

INTRODUCTION

Use of enzymes to recover fish protein hydrolysates (FPH) is a technology that is gaining popularity due to the bioactive properties associated with these compounds. These include immune modulatory, antioxidant, antihypertensive, neuroactive, antithrombotic, antimicrobial, and mineral or hormone regulating properties (Fujita and Yoshikawa 1999; Alasalvar *et al.,* 2002 and Samaranayaka 2010). The functional properties of the hydrolysates are also important as they influence their behaviour and utilization in food systems. The properties are related to the quantum of reacting amino acid residues, molecular size, distribution of electrostatic charges, hydrophilicity and hydrophobicity as well as carbohydrate and mineral interaction (Calvez and Berge 2013). Moreover, they are also affected by the physicochemical properties of the parent substrate protein, specificity of proteolytic enzyme used and hydrolysis conditions. Solubility is one of the most important properties of protein and protein hydrolysates as it affects other functional properties such as emulsification and whipppability of the proteins (Souissi *et al*., 2007). It also influences water holding capacity and oil holding capacity because high solubility means smaller molecular size, which is prejudicial to holding capacity. According to Kristinsson and Rasco (2000), the reduction in peptide size is what provides a high correlation rate between solubility and the DH (Santos *et al*., 2011). Enzymatic hydrolysis of native fish proteins has

been shown to improve their functional properties, including solubility, emulsifying capacity and foaming characteristics hence offering interesting opportunities for food applications (Shahidi, 1994). *Catla catla, Umbrina canosai, Sardinella aurita* among other fish species have been utilized for production of fish protein hydrolysate with antioxidant activities (Elsavaran et *al*., 2012, Centenaro et *al*., 2011 and Souissi *et al*., 2007).

Dagaa (*Rastrineobola argentea*) presents the second highest (63 %) fish landings in Lake Victoria (Witter and winter 1995; Wanink 1999 and Nyeko 2008). Postharvest losses are usually between 20-30 % and during rainy season they can rise to 50 % due to ineffective drying (Ofulla *et al*., 2007, Dampha, 1992). The value of the catch is usually very low due to physical losses, color change, bacterial and mould spoilage despite the high *Dagaa* landings (Mndeme *et al.,* 1998). Consequently, *Dagaa* is mainly used for production of fishmeal in the animal feed industry, rather than for human consumption (Bille and Shemkai, 2006). Alternative methods of using this under-utilized *Dagaa* fish species are therefore needed to increase its potential utilization and market value. For example production of fish protein hydrolysates with antioxidative properties which have recently found use in pharmaceutical, nutraceutical and food processing/preservation industries (Alasalvar *et al.,* 2002; Hagen and Sandnes, 2004). Production of fish protein hydrolysate from *Dagaa* has not been reported. This study was therefore done to investigate the antioxidative capacity and functional properties of Alcalase *Dagaa* protein hydrolysate.

MATERIALS AND METHODS

Sampling

Fresh *Dagaa* (1 kg each) was sampled in labeled polythene bags from various landing sites: Dunga, Nduru, Paga, Rota and Usari from four fishermen around the shores of Lake Victoria. *Dagaa* representative samples were obtained by mixing 200g of mince from each individual site to constitute a 1kg representative mince. This was well mixed and stored frozen at (-20 °C) until use.

Preparation of Dagaa protein hydrolysate

A 1 Kg portion of *Dagaa* mix was thawed at 4 °C overnight and homogenized in a blender for about 2 min. The homogenate was put in a reactor and mixed with a buffer (pH 7) at a ratio of 2: 1 (w/v). The contents were allowed to attain a temperature of 56°C, with stirring at minimum rpm (Stuart stirrer, UK). A predetermined optimized ES ratio of 2% (v/w) of Alcalase was added and hydrolysis was allowed to proceed for 6 hours. The enzyme activity was then terminated by placing the hydrolysate in a water bath at 100 °C for 15 min. The hydrolyzed *Dagaa* was centrifuged at 10000 ×*g* for 20 min. After decantation and removal of sludge, the soluble fraction was stored in airtight plastic container at -20 °C for further use. This fraction was labeled Alcalase *Dagaa* protein hydrolysate (AH). For hydrolysis with endogenous enzymes, 1 Kg of *Dagaa* mix underwent the same process conditions as described above except that temperature was set at 52°C and pH 7 as hydrolysate produced using these conditions was observed to have high antioxidative potential after a one- hour autolysis for cathepsin L-like endogenous proteases in Pacific Hake muscle (Samaranayaka *et al*., 2006, 2008 and An *et al*., 1994). This fraction was labeled Endogenous *Dagaa* protein hydrolysate (EH).

Determination of Degree of Hydrolysis (DH)

Degree of hydrolysis (% DH) was calculated according to percent of trichloroacetic acid (TCA) ratio method as described by Hoyle and Merritt (1994). After hydrolysis, 20 ml of protein hydrolysate was added to 20 ml of 20 % (w/v) TCA to produce 10 % TCA soluble material. The mixtures were then left to stand for 30 min to allow precipitation, followed by centrifugation (7800 xg, 15 min). The supernatant was analyzed for protein content using Biuret method. DH was then computed as shown in the formula below:

$$\text{DH (\%)} = \frac{\text{10\% TCA soluble nitrogen in the sample}}{\text{Total nitrogen in the sample}} \times 100\%$$

Proximate Composition

Dagaa hydrolysates (AH and EH) were analyzed for moisture and ash, using standard methods AOAC 930.15 and 942.05, respectively (AOAC, 1995). Lipid was determined by the method of Bligh and Dyer (1959) and soxhlet extraction

method according to AOAC method 991.36 (AOAC, 1995). Crude protein content was determined from nitrogen content by Kjedahl method (AOAC, 1995). The proximate content was expressed on a wet weight basis.

ANTIOXIDTIVE PROPERTIES OF DAGAA PROTEIN HYDROLYSATE

2, 2, Diphenyl picryl hydrazyl (DPPH) radical scavenging capacity assay

The method described by Kitts and others (2000) was used to determine the DPPH % (radical scavenging activity) RSA of the *Dagaa* hydrolysates (AH and EH) and commercial antioxidant BHT. DPPH solution (1.8 mL, 0.1 mM in 80 % ethanol) was mixed with AH or EH solution (0.20 mL, at 3 mg/mL final assay concentration in 50 % ethanol). Absorbance (Abs.) of the solution was then read at 517 nm after 30 minutes of incubation at room temperature. For the control assay, 0.20 ml of 50 % ethanol was used in the assay instead of the *Dagaa* hydrolysate solution. Sample controls were also made for AH and EH by mixing 0.20 ml either hydrolysate solution with 1.8 mL of 80 % ethanol. RSA of *Dagaa* protein hydrolysates (AH and EH) was calculated as follows:

$$\text{DPPH radical scavenging capacity (\%)} = \left\{ \frac{\text{Abs. of sample - Abs. of sample control}}{\text{Abs. of assay control}} \right\}$$

Ferric ion reducing antioxidant capacity

The method described by Oyaizu (1988) was used to measure the ferric ion reducing capacity of AH and EH. A 2.0 mL aliquot of AH/ EH stock solution in phosphate buffer {PB (0.2M, pH 6.6)} was mixed with 2.0 mL of the same buffer and 2.0 mL of 1 % potassium ferricyanide to yield final *Dagaa* protein hydrolysate concentration of 3 mg/mL. After incubation at 50 °C for 20 min, 2.0 mL of 10 % Trichloroacetic acid was added. A 2.0 mL aliquot was then mixed with 2.0 mL of distilled water and 0.4 mL of 0.1 % ferric chloride. Absorbance at 700 nm after 10 minutes was measured as an indication of reducing power.

Lipid peroxidation in a oleic acid model system

This was done according to the method described by Osawa and Namiki (1985) with modification. Oleic acid was used to measure the inhibition of lipid peroxidation in acid/ethanol/water emulsion system using AH and EH samples (5mg) and BHT at final assay concentration of 0.2 mg/ml.

FUNCTIONAL PROPERTIES OF DAGAA HYDROLYSATES

Solubility

The solubility of AH and EH was tested according to the method described by Tsumura *et al.*, (2005). A gram of sample was dissolved in 100 ml dH_2O. The pH was adjusted in the range of 3 - 10 using either 2 M HCL or 2M NaOH. The solution was then stirred for 10 minutes at room temperature before centrifugation at 8000 xg for 10 minutes. The supernatant was then diluted and protein content determined by biuret reagent. Solubility of the hydrolysates was calculated as follows:

$$N_2 \text{ solubility} = \frac{\text{supernatant } N_2 \text{conc.} * 100}{\text{Sample } N_2 \text{ conc.}}$$

Emulsification

The emulsifying property of hydrolysates (AH and EH) was investigated according to the method described by Kinsella *et al.*, (1976). 5 ml of 5 % hydrolysate in dH_2O was homogenized at 20,000 rpm for 90s at room temperature. This was then centrifuged at 2400 Xg for 3 minutes. The volume of each fraction (oil, emulsion, and water) was then determined.
The emulsifying capacity = ml of emulsified oil/g FPH

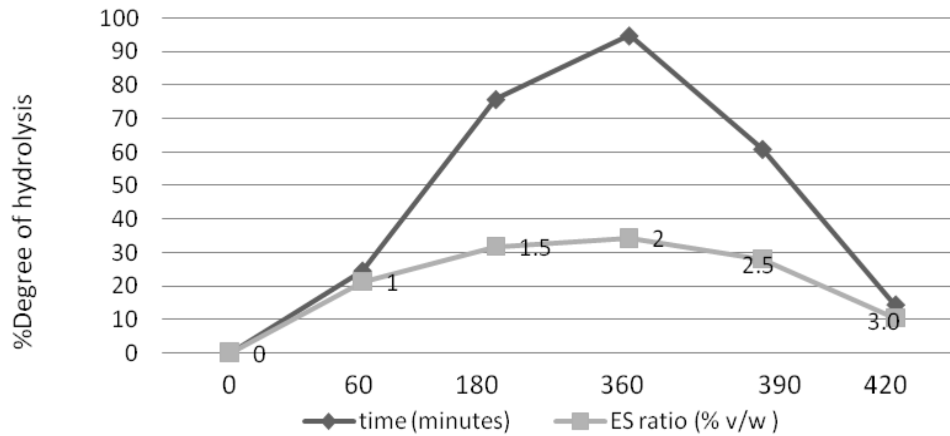

Figure1. Effect of time and enzyme substrate (%) ratio on % degree of hydrolysis (%DH)

Fat absorption

The ability of the hydrolysates (AH and EH) to absorb fat was investigated by the methods described by Shahidi *et al.,* (1995) and Slizyte *et al.,* (1995). A sample (0.5 g) of each hydrolysate (AH and EH) was mixed with 10 ml of corn oil in 50 ml centrifuge tube. This was left to stand for 30 minutes at room temperature while mixing for 30s after every 10 minutes. This was then centrifuged for 25 minutes at 2000 xg. The volume of supernatants was then recorded.

$$\text{Fat absorption} = \frac{\text{volume of fat absorbed}}{\text{Gram of } \textit{Dagaa} \text{ protein hydrolysate}}$$

Foaming/Whippability properties

Determination of foaming capability of hydrolysates was done according to Watanabe *et al.*, (1981). A sample (0.25 g) of *Dagaa* protein hydrolysates (AH or EH) was dispersed in 25 ml dH_2O. The pH was then adjusted to 4, 6 and 7 with 2M HCl. This was then homogenized for 1minute at room temperature. The sample was then poured into a graduated cylinder and let to drain water from the foam phase for 30s. The drained water volume was determined.

$$\text{Whippability} = \frac{\text{Total volume} - \text{Drainage volume}}{\text{Initial volume}}$$

Statistical analysis

All analyses were performed in triplicate. Statistical analyses were performed using the statistical program (SPSS v.16.0). Post hoc test (Tukey HSD test) was used to evaluate differences among mean values for treatments at $p< 0.05$. Data was presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Alcalase Hydrolysis of *Dagaa*

During Alcalase hydrolysis, the *Dagaa* fish tissues were rapidly converted from a viscous mince into a free flowing liquid. Figure 1 shows that maximum % DH was achieved at 360 minutes of incubation with Alcalase at pH 7 and 56 °C at minimum stirring speed (Stuart overhead stirrer, UK).

Table 1. Proximate composition of *Dagaa* hydrolysates and fresh unhydrolysed *Dagaa*

Sample	Dry weight %	Ash content %(wwb)	Lipid content % (wwb)	Protein content % (wwb)
Fresh unhydrolysed *Dagaa* (FD)	21.6±0.89	4.3±0.79	3.1±0.45	18.8±0.53
EH	89.4±0.15	5.0±0.00	0.0000	12.6±0.12
AH	94.5±0.006	4.4±0.41	0.0000	13.3±0.85

Table 2. combined DPPH% radical scavenging ability of hydrolysates(AH and EH) and BHT.

Concentration (g/ml)	BHT	AH	EH
0.002	61.7	31.8	10.5
0.004	71.3	72.1	14.8
0.006	72.3	87.7	19.0
0.008	77.3	88.0	50.1
0.01	89.1	90.0	71.8

According to Mackie (1982), the enzyme was absorbed into the suspended particles, where the hydrolysis of the enzyme-susceptible peptide linkages took place simultaneously. After an initial rapid phase of hydrolysis, the rate of hydrolysis tended to decrease, entering a stationary phase. Hoyle and Merritt (1994), using Alcalase, reported a 44.7 % hydrolysis for raw herring after 60 min. The higher percent of hydrolysis (83 %) may be attributed to the difference in primary protein sequence of *Dagaa* from other fish substrates previously investigated, differences in the methods used to measure and express the % DH and extended hydrolysis time of 6 hours in comparison to the 60 minutes hydrolysis time by Hoyle and Merritt (1994).

Proximate composition

The proximate content on wet weight basis (wwb) of AH, EH and unhydrolysed *Dagaa* (FD) are shown in Table 1. The relative soluble protein content increased with increase in % DH. The lipid content decreased in the hydrolysate (AH and EH) compared to unhydrolysed sample (FD). This may be due to the fact that, as the hydrolysis proceeded, the membranes tended to round up and form insoluble vesicles, which could have allowed the removal of membrane structural lipids (Shahidi *et al*., 1995). The insoluble fraction (vesicles) was separated from the soluble hydrolysate in the form of a pellet by centrifugation.

Antioxidative properties

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging Ability

The free RSA of the hydrolysate and synthetic antioxidants was assessed using the scavenging effect on DPPH free radical. Although there are numerous ways to study antioxidative activity, the use of DPPH is common due to its simplicity and the minimal time required compared to other methods (Wang *et al.,* 2004). Concentration dependent % RSA followed the order; AH > BHT> EH (Table 2). AH showed a concentration dependent antioxidant activity of between 31.8-90.0 %. This was higher than those reported for EH (10.5-71.8%). AH showed a significant ($p<0.05$) increase in % RSA with increase in concentration. The scavenging ability obtained in the current study was better than that reported using protein hydrolystae of mackerel (*Scomber austriasicus*) and from Sardinella (*Sardinella aurita*) which had scavenging activity of 15.4 and 41 %, respectively (Wu *et al.,* 2003 and Souissi *et al.,* 2007). The increased % RSA is possibly due to increased solubility within the aqueous medium due to increased % DH. A possible mechanism for DPPH scavenging is the protonation of DPPH to its more stable DPPHH form. Because of its unpaired electrons DPPH has its maximum absorbance at 520nm. As it gets reduced (electrons get paired off, in the presence of the radical scavenger) the absorbance stoichiometrically decreases with respect to the number of electrons taken up (Geckil *et al.,* 2005). Reports by Calvez and Berge (2013) showed that protein hydrolysates with good % RSA are rich in Histidine, Leucine, Tyrosine, Methionine and cysteine. It is therefore likely that AH is rich in these amino acids.

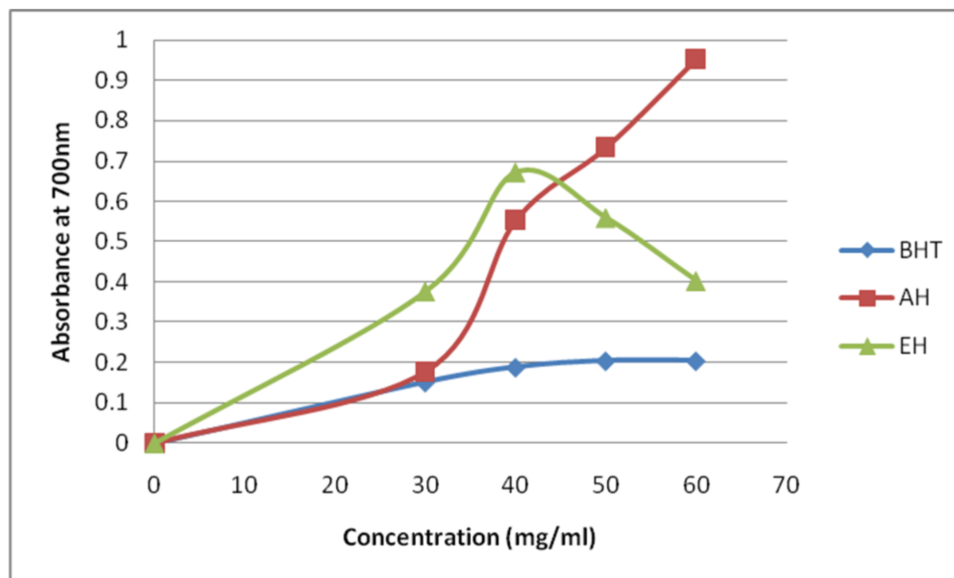

Figure 2. Ferric ion reducing antioxidant capacity of protein hydrolysates (AH andEH) and BHT.

Ferric iron Reduction Inhibition.

The reducing power of the hydrolysates (AH and EH) was compared to that of BHT *in vitro*, using the ferric ion reducing inhibition test. There was a significant ($p<0.05$), increase in Ferric ion assay absorbance with increase in concentration of both hydrolysate (AH and EH). However, there was no significant ($p>0.05$), increase in absorbance with increase in BHT concentration (Figure 2.). The concentration dependent reducing power of the samples followed the order: AH>EH>BHT. At lower concentrations, EH had higher reducing power than BHT and AH. However, with increase in concentrations above 40 mg/ml the reducing power of AH was better than that of both EH and the commercial antioxidant BHT (Fig.2.). The reducing power characteristic of a compound is a support feature for its antioxidant activity (Geckil *et al*., 2005). The reducing activity (converting Fe^{3+} to Fe^{2+}) is as a result of reductones which show antioxidative power by donating hydrogen atoms. In addition; they react with certain precursors of peroxides to inhibit peroxide formation (Geckil *et al.,* 2005). The difference in reducing power of AH and EH could be attributed to the difference in solubility between the hydrolysates. Extensively hydrolysed *Dagaa* Alcalase hydrolysate (AH) was seen to be more soluble than less extensively hydrolysed EH (Table 4). This is also true for previous studies on Sardinella by-product hydrolysates. Sardinella hydrolysate produced using Alcalase enzyme with 10.16 % DH showed highest solubility than those with 9.3 % and 6.6 % degree of hydrolysis. The hydrolysate with the highest % DH also showed highest antioxidant capacity (Souissi *et al.,* 2007).

Lipid peroxidation inhibition

Oleic acid peroxidation inhibition followed the order; EH>AH>BHT>Control. The control (without antioxidant) had the highest absorbance value, indicating the highest pro-oxidative effect. EH had the lowest absorbance. AH with the highest % DH (83 %) possessed less effective antioxidative activity (31.5 %) than EH (49.5 %). This observation was similar to previous studies by Elavarasan *et al.,* (2013).The results however differed with those obtained by Souissi *et al.,* (2007) where peptide with highest % DH was reported to be more effective in lipid peroxidation inhibition activity. Je *et al.,* (2005) isolated a peptide from Alaska Pollock (*Theragra chalcogramma*) and showed that this peptide exhibited about 85% inhibition of linoleic acid peroxidation.

The relatively low percent lipid peroxidation inhibition (29-49.5%) of hydrolysate in this current study compared to those obtained by Elsavaran *et al.,* (2013) could be explained by the relatively low concentration of hydrolysates (32 µg/ml) used for the assay. Elsavaran *et al.,* (2013) used higher hydrolysate concentrations of 40 mg/ml which recorded an inhibition of 36-54%. Moreover, the low lipid peroxidation inhibition values reported could be attributed to the substitution of single double bond oleic acid for the two double bond linoleic acid. Hydrophobic amino acids proline and hydroxyproline seem to participate in inhibition of lipid peroxidation (Mendis *et al*., 2005 and Sarmach and Ismail 2010).

Table 3. Oleic acid peroxidation inhibition by hydrolysates (AH and EH) and BHT

Set	Absorbance	% Inhibition
Control	0.184 ± 0.0005	0
BHT	0.130 ± 0.0005	29 ± 0.0005
AH	0.126 ± 0.0005	31.5 ± 0.0005
EH	0.093 ± 0.001	49.5 ± 0.001

Table 4. Solubility of protein hydrolysates

pH	EH	AH	Fresh *Dagaa* digested by NaOH
3	29.50±0.0005	39.63±0.02	74.74±0.001
4	31.87±0.0005	39.29±0.02	89.9±0.02
5	32.00±0.001	7.65±0.007	81.81±0.005
6	31.12±0.0005	24.32±0.002	77.78±0.02
7	29.87±0.0005	43.53±0.02	75.76±0.02
8	27.75±0.001	43.36±0.005	86.87±0.001
9	29.00±0.0005	41.67±0.02	79.80±0.005
10	28.50±0.0005	38.09±0.003	87.88±0.005

EH is therefore most likely to be rich in these amino acids.
As observed in this study, the highly hydrolysed AH, showed a higher % RSA than EH, which had a lower % DH and a higher lipid peroxidation. Similar observations have been reported by Mendis *et al*., 2005; Sarmach and Ismail 2010 and Calvez and Berge (2013) and was attributed to conversion of hydrophobic groups to hydrophilic groups with high % DH.

Functional properties

Solubility

Solubility of *Dagaa* protein hydrolysates at pH 3-10 is shown in Table 4. The alkali (NaOH) hydrolysate showed the highest solubility at pH 4. This could have been due to Na^{+} ions aiding solubility of the protein (salting in). As well as the fact that muscle proteins have a higher concentration of salt soluble protein (Cunningham and Froning, 1972). The different hydrolysates also presented differing solubility. EH and AH solubility profile as a function of pH showed the highest solubility of 32 % and 43.5 % at pH 5 and 7 respectively. Increase in the degree of hydrolysis increased the degree of solubility as is seen with AH. This is also true for Sardinella hydrolysates (Souissi *et al*., 2007). The improved solubility of AH (43 %) compared to EH (29 %) at pH 7 can be explained by the cleavage of proteins into smaller peptides that have increased solubility (Shahidi, 1994). The difference in solubility between AH and EH is due to differences in peptide lengths as well as the difference in hydrophobic/hydrophilic peptide ratios (Souissi *et al*., 2007). The lowest AH solubility was achieved at pH 5.0. This pH could be the pH near the *Dagaa* proteins PI according to Mazzaro-Manzano *et al*., (2012). AH solubility increased with the increase of pH and reached 43.5 % at pH 7.0. The hydrolysate with a higher degree of hydrolysis (AH), has lower molecular mass peptides (Souissi *et al*., 2007). Thus, better solubility than EH. This can be explained by the fact that hydrolysis exposes some of the hydrophobic groups to the surface. In addition, it converts some hydrophobic groups into hydrophilic ones by generating two-end carbonyl and amino groups, as reported by Kristinsson and Rasco (2000). Nitrogen solubility of the hydrolysate indicates potential applications in formulated food systems by providing attractive appearance and smooth mouth feel to the product, as suggested by Petersen (1981). The AH solubility profiles here are similar to those obtained by Santos *et al*., (2011) using flavourzyme on Bluewing Searobin (*Prionotus punctatus*).

Emulsification

The emulsifying capacity of the hydrolysates (AH and EH) was compared with undigested fresh *Dagaa* proteins (FD). The hydrolysates showed emulsifying ability. However, the stability of the emulsions was very low since they collapsed upon centrifugation. As shown in Table 5, the emulsifying capacity decreased and was deficient with

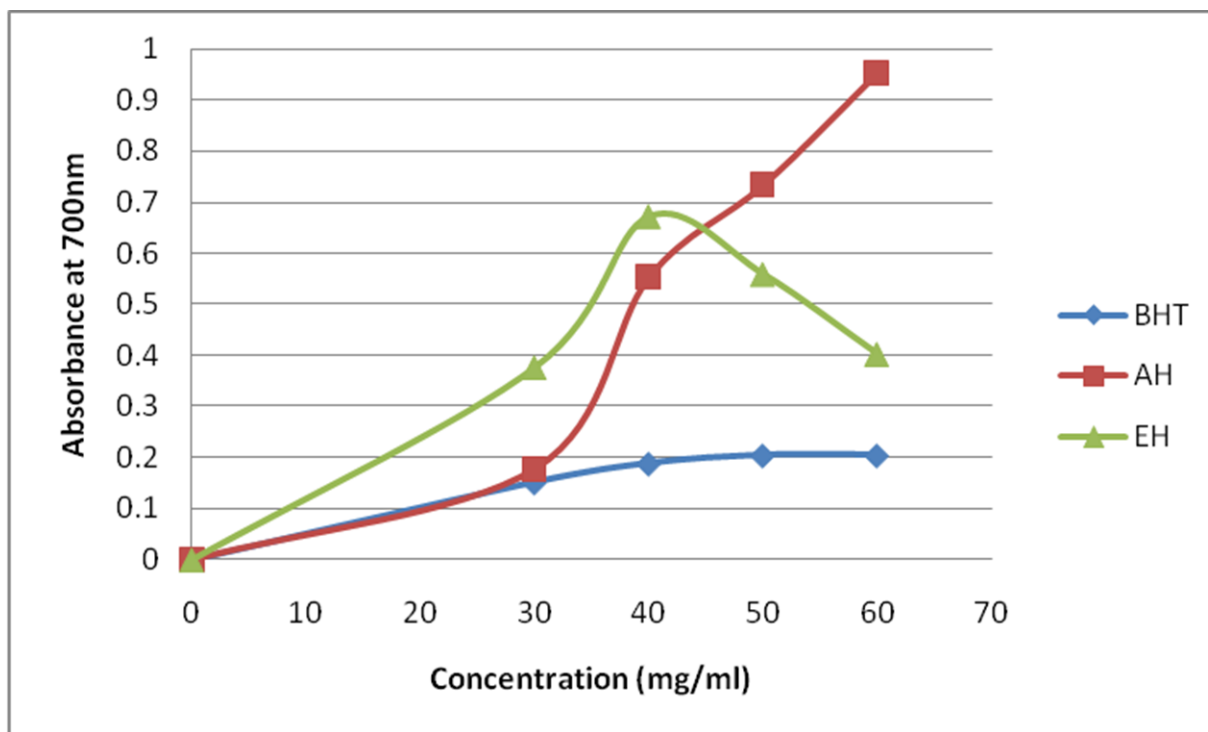

Figure 2. Ferric ion reducing antioxidant capacity of protein hydrolysates (AH andEH) and BHT.

Table 5. The emulsification of corn oil by protein hydrolysates (AH and EH) and fresh Dagaa

SET	Emulsification
EH	0.0
AH	0.0
Fresh Dagaa	1.0

Hydrolysis. The trend in Table 5 has also been reported for Blue wing Searobin (*Prionotus punctatus*) (Santos *et al*., 2011). The decrease in emulsifying activity of the extensively hydrolysed hydrolysate is essentially due to the reduction of hydrophobicity of the hydrolysate and to changes in peptide size during hydrolysis (Souissi *et al*., 2007). The emulsifying capacity of undigested *Dagaa* was higher than that of the hydrolysates because of longer peptide chain length. Due to its high degree of hydrolysis, the hydrolysates mainly consisted of short peptides and only a limited concentration of surface-active large peptides. A direct relation between surface activity and peptide length was reported by Josti *et al*., (1977*).* The smaller peptides often have reduced emulsifying properties. Indeed, a peptide is required to have a minimum length of about 20 residues to possess good emulsifying and interfacial properties (Lee *et al.,* 1987). Similar observations were reported by Quaglia and Orban (1990) on the emulsifying capacity of Alcalase-hydrolysed sardine protein. The data showed a 1.5 to 2-fold reduction in the emulsifying capacity of the hydrolysates by increasing DH from 5 to 20 %. Other studies have shown a 2-fold reduction in emulsifying capacity by increasing DH from 6 -10 % (Souissi *et al*., 2007).

The emulsifying properties of hydrolysate compounds were probably impaired by the high solubility level and high % DH as has been previously reported by Santos *et al*., (2011); Thiansakul *et al*., (2007) and Wasswa *et al*., (2007). The emulsifying properties could also have been affected by hydrolysis conditions. Wachirattanapongmetee *et al*., (2009) suggests that extended hydrolysis at low (<0.5 %) ES ratios led to improved emulsifying ability, however extended hydrolysis time at higher ES ratios as done for AH led to reduced emulsifying capacity. Similarly the relatively high concentration of the hydrolysates 26.9 mg/ml and 13.2 mg/ml for AH and EH respectively, led to a decrease in emulsifying capacity due to increased protein to protein interactions and subsequent decrease in protein concentration at the oil-water interface (Huidobro *et al*., 1998 and Lawal, 2004).

Table 6. Fat absorption by protein hydrolysates (AH andEH) and fresh Dagaa

SET	fat absorption (ml g^{-1})
EH	18.3
AH	3.5
FD	3.6

Table 7. Whip ability (foaming properties) of protein hydrolysates **(**AH and EH) and fresh *Dagaa* (FD) at pH 4, 6 and 7

SET	EH	AH	FD
pH 4	1	1	0.2
pH 6	1	1	0.1
pH 7	1	1	1
Leakage (pH 7)	100 %	100 %	100 %

Fat absorption

Fat absorption of *Dagaa* protein hydrolysates (Table 6.) showed a negative correlation between the fat absorption and % DH. EH exhibited better fat absorption (18.3 ml/g protein) than undigested fish proteins (3.6 ml/g protein) and AH (3.5 ml/g protein). EH at 45 % DH had the highest fat absorption capacity, while AH had the lowest fat absorption capacity. These results are similar to those obtained by Limam *et al*., (2008) on Shrimp hydrolysates. These results could be explained by the fact that hydrolysis can liberate some peptides from the native protein, which would enhance the flexibility of the peptides of EH. However, the extensive hydrolysis in AH breaks many peptide bonds, thus contributing to the decrease of the oil binding properties as shown in AH. This is also true for previous findings by Santos *et al*., (2011) and Kristinsson (1998) who reported that the fat absorption of salmon protein hydrolysate decreased with increasing DH. The relative decrease in fat absorption with increase in % DH is due to physical entrapment of the oil (Galvez and Berge, 2013). However, Souissi et al., (2007) did not find any correlation between fat absorption and % DH in Sardinella hydrolysates. The *Dagaa* hydrolysate presented good oil absorption properties and may be used for such applications.

Foaming properties / Whippability and leakage

The foaming properties of the hydrolysates and the undigested fish proteins were determined by measuring their Whippability at pH values of 4.0, 6.0, and 7.0. As shown in Table 7, AH and EH had higher foam ability, than the undigested protein at pH 4 and 6. At pH 7, both hydrolysates (AH and EH) and the undigested *Dagaa* had similar foam stability of 1 with 100 % leakage. Poor foaming properties of the hydrolysates can be explained by the small size of peptides, which would hinder the formation of a stable film around the gas bubbles, and also by the apparition of hydrophilic peptides during extensive hydrolysis (Souissi *et al*., 2007). This is in line with previous findings that a good cohesiveness of films is only reached with high molecular mass peptides or partially hydrolysed proteins (Bombara *et al*., 1994). It has previously be reported that foaming capacity is improved upon limited proteolysis and decreases with increasing % DH and also with increasing pH values (Souissi *et al*., 2007).

CONCLUSION

The high protein recovery and high % DH using Alcalase on *Dagaa* validates the use of *Dagaa* for protein hydrolysate production. The potent antioxidant activities of the crude Alcalase hydrolysate (AH) at relatively low concentrations indicate minimal purification requirements. Moreover, it warrants further basic research to characterize the peptides that are responsible for these antioxidant properties, as well as applied research to investigate applications of the FPH as food, pharmaceutical and nutraceutical ingredients. *Dagaa* Alcalase hydrolysate is a potential natural substitute for synthetic commercial antioxidants which have been shown to have diverse human health effects. In addition, the processing parameters and the enzyme type can be varied to produce peptides with other bioactive properties.

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