



# Asian Journal of **Biochemistry**

ISSN 1815-9923



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## Hypocholesterolemic Activity and Characterization of Protein Hydrolysates from Defatted Corn Protein

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### ABSTRACT

Enzymatic hydrolysis of plant protein is used as a source of bioactive peptides. In the present study, defatted corn protein was treated with Flavourzyme to produce hydrolysate with hypocholesterolemic activity for potential application in functional food. Defatted corn protein was prepared by an alkaline method and used as a substrate for enzyme hydrolysis. The derived hydrolysate was assessed for their hypocholesterolemic activity in different *in vitro* assay systems, including cholesterol micellar solubility inhibition and bile acids binding capacities. The bile acids used were: sodium glycocholate, sodium cholate and sodium deoxycholate. To know much about the defatted corn hydrolysate, the solubility, hydrophobicity and effect of amino acids content of hydrolysate on hypocholesterolemic activity were investigated. Hydrolysis with Flavourzyme for 90 min yielded a DH of 12.25%. The results showed that defatted corn hydrolysate had effective hypocholesterolemic activity. Hydrolysate had 67.42, 19.01, 9.99 and 86.90% of cholesterol micellar solubility inhibition, sodium glycocholate, sodium cholate and sodium deoxycholate, respectively. The amino acid analysis revealed that the predominant hydrophobic amino acids residue were Leucine, Valine, Alanine, Proline, Glycin and Phenylalanine. This result suggested that defatted corn protein hydrolysate could exhibit a hypocholesterolemic activity.

**Key words:** Defatted corn, hydrolysis, hydrolysate, hypocholesterolemic activity, hydrophobicity

### INTRODUCTION

A great deal of attention has appeared in recent literature regarding the assessment of hypocholesterolemic potential of peptides derived from various food sources (Park *et al.*, 2005; Zhong *et al.*, 2007; Megias *et al.*, 2009). *In vitro* studies using various chemical assays have been used to assess the potential of peptides to act as cholesterol-reducing agents in order to minimize absorption of cholesterol in the human body. Hypercholesterolemia is defined as an excessive level of cholesterol in the blood and this condition increases the risk of heart disease (Yoshie-Stark and Wasche, 2004). The reduction of cholesterol in micellar solution and binding of bile acids are used as an *in vitro* test of hypocholesterolemia (Zhong *et al.*, 2007).

Hydrolysis of protein with proteases has been reported to improve functional properties of food components. Solubility is one of the critical functional properties of protein use as a food ingredient because it greatly influences other properties, such as emulsification, gelation and

foaming (Idris *et al.*, 2003). Mannheim and Cheryan (1992) reported that solubility of the enzymatic hydrolysate of proteins from corn gluten meal was better than that of the unmodified proteins.

In recent times, there has been considerable attention on components in several plant foods including dietary peptides and functional protein hydrolysates due to their beneficial effects on metabolism. Among the plant sources, soybean is used widely to obtain protein hydrolysates. However, several researches have demonstrated and reported the preparation and functionality of corn hydrolysate (Lu *et al.*, 2000; Miyoshi *et al.*, 1990; Suh *et al.*, 2003; Yamaguchi *et al.*, 1996a,b; Yang *et al.*, 2007; Zheng *et al.*, 2006). Defatted corn, a byproduct of the corn oil industry, is nearly entirely used for animal feed. The limited utilization of this relatively low-cost ingredient warrants further research for exploiting its potential use in human foods. Apart from the biochemical structure such as high hydrophobicity, defatted corn is readily available relatively cheap making it potentially interesting raw material source for both food and non-food applications (Lu *et al.*, 2000). The effects of corn peptide on angiotensin-converting enzyme inhibition (Miyoshi *et al.*, 1990; Suh *et al.*, 2003; Yang *et al.*, 2007), alcohol metabolism (Yamaguchi *et al.*, 1996a, b) and antioxidant capacity (Zheng *et al.*, 2006) were reported. According to the special amino acid composition, in which there are large amounts of hydrophobic amino acids such as leucine, alanine and phenylalanine (Li *et al.*, 2008), defatted corn protein was thought to be a good resource to obtain hypocholesterolemic peptides. Moreover, Layman (2003) and Layman and Walker (2006) reported that, a high amount of branched chain amino acids in corn, especially of leucine play an important role in body weight metabolism. However, not a single research on hypocholesterolemic activity from defatted corn hydrolysate has been reported. In this study, Flavourzyme protease was used to produce hydrolysates and determine their hypocholesterolemic activity, in order to utilize defatted corn protein hydrolysates as a cholesterol-reducing agent for hypercholesterolemic patients.

## **MATERIALS AND METHODS**

**Materials:** Defatted corn meal was obtained from China Corn Oil Company Ltd., Shandong Province, P.R. China. Sodium taurocholate, sodium cholate and cholestyramine were purchased from International Laboratory USA (Nanjing, P.R. China). Sodium glycocholate and 1-anilino-8-naphthalene Sulfonate (ANS) were purchased from Sigma Chemical Co. (Shanghai, China) and Flavourzyme provided by Novo Nordisk (Bagsvaerd, Denmark). Total cholesterol assay kit was purchased from Zhejiang Dongou Bioengineering Co. Ltd., China. Macroporous adsorption resin DA201-C were from Jiangsu suqiung water treatment engineering group co. Ltd. (China). All the chemicals used were of analytical grade and purchased from Sinopharm Chemicals Reagent Company (SCRC), Shanghai, China.

## **Methods**

### **Preparation and hydrolysis of defatted corn protein**

**Preparation of defatted corn protein:** The defatted corn flour protein was prepared by the method of Kongo-Dia-Moukala and Zhang (2011) with some modifications. The defatted corn flour was mixed with distilled water at a ratio of 1:10 (v/w), incubated to pH 11.5 at 50°C for 2 h and adjusted to pH 4.5 to precipitate the proteins. The proteins precipitated were freeze-dried for further experiments analysis.

**Enzymatic hydrolysis of defatted corn protein:** Hydrolysis was executed through modification of the procedure previously reported by Zhang *et al.* (2009). The defatted corn protein obtained after freeze-dried was dispersed in distilled water at a concentration of 5% (w/v) before hydrolysis with Flavourzyme. Homogenization was carried out for 30 min at 50°C and pH adjustment to pH 6.5 with NaOH solution (0.5 M). After equilibrium reached the optimum condition, the reaction was initiated by adding 3% of Flavourzyme with continuous stirring. Hydrolysis was carried out at a constant pH value by continuous addition of 0.5 M NaOH solution for 90 min. The Degree of Hydrolysis (DH) of defatted corn protein was calculated using a pH-stat method (Adler-Nissen, 1986). The mixture was then heated at 75°C for 10 min to inactivate the enzyme and centrifuged by cold centrifuge (ZOPR-52D, Hitachi Koki Co, Japan) at 1100× g for 25 min. The supernatant was freeze-dried, packed in polyethylene bags and stored in the desiccator for further use.

**Proximate analysis:** The proximate analysis of defatted corn protein hydrolysate was determined according to AOAC (2005). The moisture content was determined by drying in an oven at 105°C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 550°C for 8-12 h. The crude protein was determined by the micro-Kjeldahl method and a Conversion factor of N×6.25 was used to quantify the crude protein content as previously reported (Kamara *et al.*, 2009). Fat was determined by Soxhlet extraction (James, 1995).

**In vitro cholesterol micellar solubility inhibition:** The *in vitro* cholesterol micellar solubility inhibition of the defatted corn hydrolysate was measured by the method of Bangoura *et al.* (2009) with some modifications. Micellar solution (1 mL) containing 10 mM sodium taurocholate, 0.4 mM cholesterol, 1 mM oleic acid, 132 mM NaCl, 15 mM sodium phosphate (pH 7.4) was prepared by sonication. Ten milligram of defatted corn hydrolysate sample was added to each tube containing one milliliter of micellar solution. The mixture was incubated at 37°C for 24 h and centrifuged at 14000 rpm for 30 min at 37°C. The supernatant fraction was collected for the determination of cholesterol content.

**In vitro bile acids binding capacity:** The *in vitro* bile acid binding was executed through the procedure previously reported by Kongo-Dia-Moukala *et al.* (2011).

**Determination of solubility:** Solubility was determined according to the procedure of Kamara *et al.* (2010) with slight modifications. Twenty milligrams of defatted corn hydrolysate were dispersed in twenty milliliter deionized water, adjusted to pH 2, 4, 6, 8, 10 and 12 with 0.1 N HCL or 0.1 N NaOH. The suspensions were magnetically stirred at ambient temperature for 30 min and centrifuged at 12100×g for 10 min. Protein content were determined by the Kjeldahl method (Amadou *et al.*, 2010). Solubility was calculated according to Eq. 1:

$$\text{Solubility (\%)} = \left( \frac{\text{Protein content in supernatant}}{\text{Protein content in sample}} \right) \times 100 \quad (1)$$

**Determination of hydrophobicity (H<sub>0</sub>):** Values of H<sub>0</sub> were determined by the hydrophobicity fluorescence probe using 1-anilino-8-naphthalene Sulfonate (ANS) according to the method reported by Kato and Nakai (1980).

**Determination of amino acids composition:** The amino acids composition was determined according to procedure previously reported by Kongo-Dia-Moukala *et al.* (2011).

**Statistical analysis:** All analyses were carried out in triplicates and data were presented as Mean±SD. Analysis of variance (ANOVA) was carried out using SAS package (SAS Institute, Cary, NC). Comparisons between means were done using a Duncan's multiple-range test with a probability of  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Proximate analysis:** Defatted corn protein was hydrolyzed with Flavourzyme and the entire mixture was freeze dried to produce protein hydrolysate. The Degree of Hydrolysis (DH) of Flavourzyme treated hydrolysates was 12.25% (Fig. 1). The results revealed that hydrolysates obtained from the supernatants contained more soluble nitrogen compounds as compared to the original material.

The proximate analysis of the defatted corn protein hydrolysate showed that the hydrolysate had high protein content (66.69%) and may be used as potential source of proteins for food applications (Table 1). The high protein content resulted from the activities of protein solubilization during hydrolysis, the removal of insoluble undigested non-protein substances and the partial removal of lipid after hydrolysis (Benjakul and Morrissey, 1997). The ash and lipid contents of the Flavourzyme hydrolysate were 8.01 and 0.21%, respectively. The low lipid content in the defatted corn hydrolysate might significantly increase stability towards lipid oxidation which may also enhance product stability (Foh *et al.*, 2011a). These results corroborated with those reported by Foh *et al.* (2011b).

**In vitro cholesterol micellar solubility inhibition:** The *in vitro* cholesterol micellar solubility inhibition of compound serves as a significant indicator of its potential hypocholesterolemic activity. This method depends on the reduction of cholesterol in the micellar solubility solution. Defatted corn

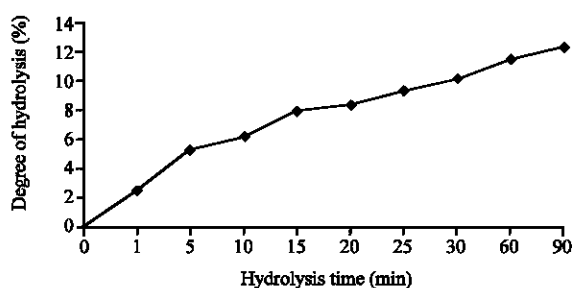


Fig. 1: Hydrolysis curves of defatted corn protein with flavourzyme protease

Table 1: Proximate composition of defatted corn hydrolysate (g/100 g, dry matter basis)

Composition (%)	Defatted corn hydrolysate
Moisture	8.14±0.26
Ash	8.01±0.83
Crude fat	0.21±0.12
Crude protein (N×6.25)	66.69±0.33
Carbohydrate and others (by difference)	16.95

Values are Means±SD of three determinations

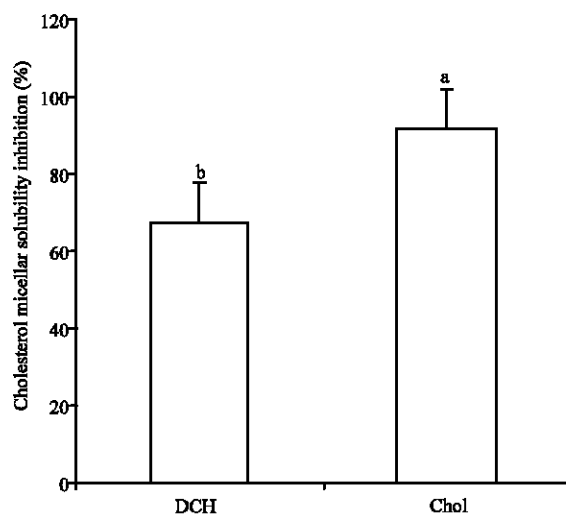


Fig. 2: Cholesterol micellar solubility inhibition of defatted corn hydrolysate, DCH: Defatted corn hydrolysate and Cholestyramine (Chol) was used as a positive control. Different letters indicate significant differences ( $p < 0.05$ )

protein hydrolysate reduced the cholesterol in the micellar by 67.42% (Fig. 2). Cholestyramine was used as a positive control and reduced cholesterol by 91.13%. Defatted corn protein hydrolysate showed a significant ( $p < 0.05$ ) lower cholesterol micellar solubility inhibition than cholestyramine. This result is in good agreement with those obtained by other researchers. Zhong *et al.* (2007) reported the cholesterol micellar solubility inhibition of soy Alcalase protein hydrolyzed and found that cholesterol was reduced by 48.6%. Also casein tryptic hydrolysate reduced the cholesterol micellar solubility by 20% (Nagaoka *et al.*, 2001). Therefore, this result suggests that hydrophobic amino acids contain in defatted corn protein hydrolysate favors its immersion in the lipid micelles.

***In vitro* bile acids binding capacities:** Sodium glycocholate, sodium cholate and sodium deoxycholate were used to test the *in vitro* bile acid binding capacity of the defatted corn protein hydrolysate. Sodium glycocholate was bound by defatted corn protein hydrolysate to the degree of 19.01% and cholestyramine a positive control had 72.77% of glycocholate binding capacity (Fig. 3a). Defatted corn protein hydrolysate showed a significant ( $p < 0.05$ ) lower glycocholate binding capacity. Figure 3b displays that defatted corn protein hydrolysate bound sodium cholate by 9.99% and cholestyramine by 85.78%. Similar to glycocholate, a significant ( $p < 0.05$ ) difference was observe between cholestyramine and defatted corn protein hydrolysate. Sodium deoxycholate was bound to a degree of 86.90 and 99.15% by defatted corn protein hydrolysate and cholestyramine respectively (Fig. 3c). Obviously cholestyramine bound all the bile acids highly as expected and defatted corn protein hydrolysate bound sodium deoxycholate strongly via hydrophobic reactions (Higaki *et al.*, 2006). Kern *et al.* (1978) proposed that less sodium cholate, a trihydroxy bile acid was bound than dihydroxy bile acid because hydrophobic interactions are involved with binding. Series of studies have been done on the hypocholesterolemic effects of proteins, most of which supported the hypothesis that a peptide with high bile acid binding capacity could inhibit the reabsorption of bile acid in the ileum and decrease the blood cholesterol level (Iwami *et al.*, 1986).

**Solubility:** Solubility profile of defatted corn protein hydrolysate at different pH (2, 4, 5, 8, 10 and 12) was presented in Fig. 4. The solubility was nearly linear to pH suggesting that the pH has

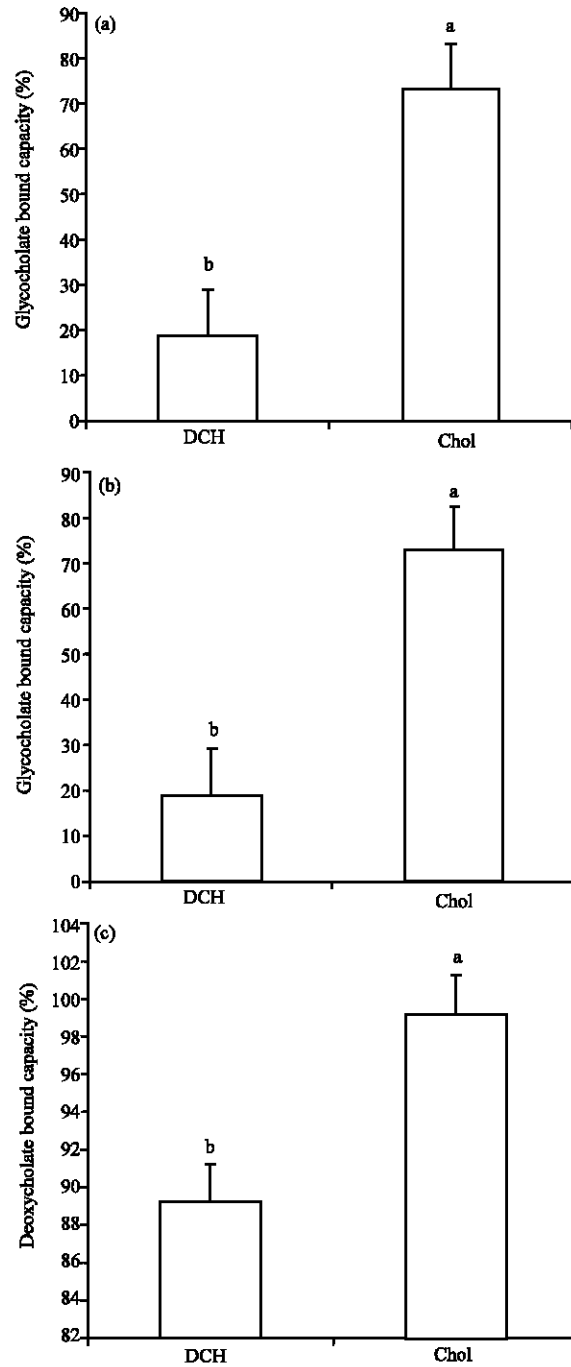


Fig. 3 (a-c): (a) Sodium glycocholate, (b) Sodium cholate and (c) Sodium deoxycholate binding by peptides from defatted corn hydrolysate of defatted corn protein, DCH: Defatted Corn Hydrolysate and Cholestyramine (Chol) was used as a positive control. Different letters indicate significant differences ( $p < 0.05$ )

minimal impact on the solubility of the protein material. Notwithstanding the linear curve, it could be observed that between pH 4.0 and 4.5 (near the isoelectric point), the net charge of the original

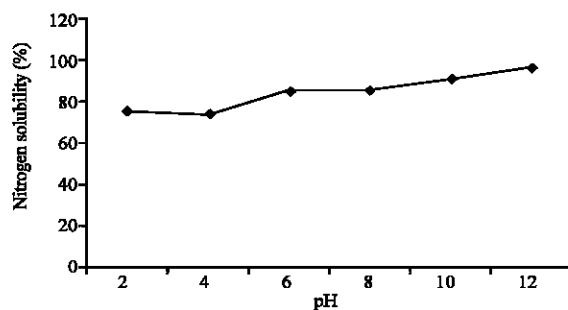


Fig. 4: Nitrogen solubility of defatted corn hydrolysate

proteins were minimized. Consequently, more protein-protein interactions and fewer protein-water interactions occurred resulting in minimal solubility at the isoelectric point. Similar finding has been reported by many authors (Adler-Nissen, 1976; Chobert *et al.*, 1988). Defatted corn protein hydrolysate had a minimum solubility value of 73.56% at pH 4.0 with a flat curve over a wide pH range. The minimum solubility of defatted corn protein hydrolysate was achieved at pH 4.0 because the defatted corn protein had an isoelectric point of 4.0-4.5 and the lowest solubility is known to occur at isoelectric point (Kanu *et al.*, 2007). The increase in solubility at isoelectric point as well as at other pH values could be attributed to the hydrolysis (Kain *et al.*, 2009). This is not surprising because the solubility at the isoelectric point of proteins increases with hydrolysis. This suggests that, the increased protein solubility could be due to smaller molecular peptides produced by actions of Flavourzyme protease. Unfolding of protein molecule due to hydrolysis was also of the reason for its improved solubility (Bandyopadhyay *et al.*, 2008). This trend in solubility is in agreement with previous reports (Clemente *et al.*, 1999; Aluko and Monu, 2003).

**Hydrophobicity ( $H_0$ ):** Hydrophobicity ( $H_0$ ) value is an indicator of the number of hydrophobic groups on the surface of a protein in contact with the polar aqueous environment (Wang *et al.*, 2006). The surface hydrophobicity, is an index of the protein's capacity for intermolecular interaction and hence its functionality. Changes in surface hydrophobicity as result of proteolysis; influences the functional properties especially the interfacial properties of the hydrolysates.  $H_0$  value of defatted corn protein hydrolysate was 138.71. These results supported the finding of Foh *et al.* (2011a) who reported that,  $H_0$  value of hydrolysate from Nile tilapia was 168.01. These results in combination with those of protein solubility indicate that defatted corn hydrolysate had a high proportion of soluble and hydrophobic peptides which could confer surface properties by exposure of hydrophobic group (Galazka *et al.*, 1999).

**Amino acid composition:** The defatted corn protein hydrolysate was subjected to amino acid composition analysis to determine the possible effect of the amino acid on hypocholesterolemic activity. Table 2 shows the amino acid composition of defatted corn protein hydrolysate. It is clear that the defatted corn protein hydrolysate contains all the essential amino acids in good proportion (28.06 g/100 g hydrolysate). Some hydrophobic amino acids, such as Glycine, Isoleucine, Leucine, Alanine, Lysine and Proline were reported to contribute to the hypocholesterolemic activity. Kwon *et al.* (2002) isolated a tetrapeptide with the amino acid sequence (Leu-Pro-Tyr-Pro) from soy glycinin. They reported that the hypocholesterolemic effect of this peptide was related to a leucine residue at the N terminus. It was found that the predominant amino acids amongst the



Table 2: Amino acid composition (g 100 g<sup>-1</sup>) of defatted corn hydrolysate

Amino acid	Amount
Aspartic acid	5.13
Glutamic acid	8.22
Serine	4.26
Histidine	2.80
Glycin	4.13
Threonine	3.63
Arginine	1.44
Alanine	5.15
Tyrosine	3.48
Cysteine	0.24
Valine	5.40
Methionine	0.64
Phenylalanine	4.12
Isoleucine	3.63
Leucine	6.12
Lysine	1.13
Proline	5.10
Tryptophan	1.72

hydrophobic amino acids in defatted corn protein hydrolysate were Leucine, Valine, Alanine, Proline, Glycin and Phenylalanine. This result indicated that defatted corn protein hydrolysate could exhibit a hypocholesterolemic activity.

## CONCLUSION

The results in present study show that Flavourzyme is a good protease for the production of defatted corn protein hydrolysate with hypocholesterolemic activity. The amino acid analysis revealed the presence of high amount of hydrophobic amino acids having a significant influence in hypocholesterolemic activity. Moreover, defatted corn protein hydrolysate had high surface hydrophobicity with increase in solubility. Furthermore, the results also indicate that defatted corn is a good source for hydrolysates with higher hypocholesterolemic activity which can compete with peptides and protein powders currently available in the market.

The defatted corn peptides prepared with Flavourzyme thus might be utilized to develop physiologically functional food with hypocholesterolemic activity.

## ACKNOWLEDGMENTS

The work reported in this study is a continuation of the on-going research supported by the financial assistance of Chinese Scholarship Council.

## REFERENCES

- AOAC, 2005. Official Methods of Analysis of the Association of Official Analytical Chemist. 18th Edn., Horwitz William Publication, Washington, DC., USA.
- Adler-Nissen, J., 1976. Enzymatic hydrolysis of proteins for increased solubility. *J. Agric. Food Chem.*, 24: 1090-1093.
- Adler-Nissen, J., 1986. *Enzymic Hydrolysis of Food Proteins*. Elsevier Applied Science Publishers, London, pp: 116-124.

- Aluko, R.E. and E. Monu, 2003. Functional and bioactive properties of quinoa seed protein hydrolysates. *J. Food Sci.*, 68: 1254-1258.
- Amadou, I., T. Amza, M.B.K. Foh, M.T. Kamara and G.W. Le, 2010. Influence of *Lactobacillus plantarum* Lp6 fermentation on the functional properties of soybean protein meal. *Emir. J. Food Agric.*, 22: 456-465.
- Bandyopadhyay, K., G. Misra and S. Ghosh, 2008. Preparation and characterization of protein hydrolysates from Indian defatted rice bran meal. *J. Oleo Sci.*, 57: 47-52.
- Bangoura, M., X. Wenshui and Z. Jiali, 2009. *In vitro* binding capacity of cholesterol and bile salts by partially depolymerized chitosans. *Am. J. Food Technol.*, 4: 126-135.
- Benjakul, S. and T. Morrissey, 1997. Protein hydrolysates from pacific whiting solid wastes. *J. Agric. Food Chem.*, 45: 3423-3430.
- Chobert, J.M., C. Bertrand-Harb and M.G. Nicolas, 1988. Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem.*, 36: 883-886.
- Clemente, A., J. Vioque, R. Sanchez-Vioque, J. Pedroche, J. Bautista and F. Millan, 1999. Protein quality of chickpea (*Cicer arietinum* L.) protein hydrolysates. *Food Chem.*, 67: 269-274.
- Foh, M.B.K., I. Amadou, M.T. Kamara, B.M. Foh and W. Xia, 2011a. Effect of enzymatic hydrolysis on the nutritional and functional properties of Nile tilapia (*Oreochromis niloticus*) proteins. *Am. J. Biochem. Mol. Biol.*, 1: 54-67.
- Foh, M.B.K., M.T. Kamara, I. Amadou, B.M. Foh and X. Wenshui, 2011b. Chemical and physicochemical properties of tilapia (*Oreochromis niloticus*) fish protein hydrolysate and concentrate. *Int. J. Botany*, 6: 21-36.
- Galazka, V.B., E. Dickinson and D.A. Ledward, 1999. Emulsifying behavior of 11S globulin *Vicia faba* in mixtures with sulphated polysaccharides: Comparison of thermal and high-pressure treatments. *Food Hydro.*, 13: 425-435.
- Higaki, N., K. Sato, H. Suda, T. Suzuka and T. Komori *et al.*, 2006. Evidence for the existence of a soybean resistant protein that captures bile acid and stimulates its fecal excretion. *Biosci. Biotechnol. Biochem.*, 70: 2844-2852.
- Idris, W.H., E.E. Babiker and A.H. El-Tinay, 2003. Fractionation, solubility and functional properties of wheat bran proteins as influenced by pH and/or salt concentration. *Nahrung*, 47: 425-429.
- Iwami, K., K. Sakakibara and F. Ibuki, 1986. Involvement of post-digestion hydrophobic peptides in plasma cholesterol-lowering effect of dietary plant proteins. *Agric. Biol. Chem.*, 50: 1217-1222.
- James, C.S., 1995. *Analytical Chemistry of Foods*. 1st Edn., Blackie Academic and Professional, London, ISBN-9: 0-7514-0196-X.
- Kain, R.J., Z. Chenc, T.S. Sonda and J.C. Abu-Kpawoh, 2009. Study on the effects of enzymatic hydrolysis on the physical, functional and chemical properties of peanut protein isolates extracted from defatted heat pressed peanut meal flour (*Arachis hypogaea* L.). *Pak. J. Nutr.*, 8: 818-825.
- Kamara, M.T., Z. Huiming, Z. Kexue, I. Amadou and F. Tarawalie, 2009. Comparative study of chemical composition and physicochemical properties of two varieties of defatted foxtail millet flour grown in China. *Am. J. Food Technol.*, 4: 255-267.
- Kamara, M.T., I. Amadou, F. Tarawalie and Z. Huiming, 2010. Effect of enzymatic hydrolysis on the functional properties of foxtail millet (*Setaria italica* L.) proteins. *Int. J. Food Sci. Technol.*, 45: 1175-1183.

- Kanu, P.J., K. Zhu, M.Z. Hui, H. Qian, K.J. Baby and K. Zhu, 2007. Sesame protein 11: Functional properties of sesame (*Sesamum indicum* L.) protein isolate as influenced by pH, temperature, time and ratio of flour to water during its production. *Asian J. Biochem.*, 2: 289-301.
- Kato, A. and S. Nakai, 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta*, 624: 13-20.
- Kern, F., H.J. Birkner and V.S. Ostrower, 1978. Binding of bile acids by dietary fiber. *Am. J. Clin. Nutr.*, 31: 175-179.
- Kongo-Dia-Moukala, J.U. and H. Zhang, 2011. Defatted corn protein extraction: Optimization by response surface methodology and functional properties. *Am. J. Food Technol.*, (In Press).
- Kongo-Dia-Moukala, J.U., H. Zhang and P.C. Irakoze, 2011. *In vitro* binding capacity of bile acids by defatted corn protein hydrolysate. *Int. J. Mol. Sci.*, 12: 1066-1080.
- Kwon, D.Y., S.W. Oh, J.S. Lee, H.J. Yang and S.H. Lee *et al.*, 2002. Amino acid substitution of hypocholesterolemic peptide originated from glycinin hydrolyzate. *Food Sci. Biotechnol.*, 11: 55-61.
- Layman, D.K. and D.A. Walker, 2006. Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J. Nutr.*, 136: 319S-323S.
- Layman, D.K., 2003. The role of leucine in weight loss diets and glucose homeostasis. *J. Nutr.*, 133: 261S-267S.
- Li, X.X., L.J. Han and L.J. Chen, 2008. *In vitro* antioxidant activity of protein hydrolysates prepared from corn gluten meal. *J. Sci. Food Agric.*, 88: 1660-1666.
- Lu, X.X., X.H. Chen and J.Z. Tang, 2000. Studies on the functional property of enzymatic modified corn protein. *Food Sci.*, 21: 13-15.
- Mannheim, A. and M. Cheryan, 1992. Enzyme-modified proteins from corn gluten meal: Preparation and functional properties. *J. Am. Oil Chem. Soc.*, 69: 1163-1169.
- Megias, C., J. Pedroche, M.M. Yust, M. Alaiz, J. Giron-Calle, F. Millan and J. Vioque, 2009. Sunflower protein hydrolysates reduce cholesterol micellar solubility. *Plant Food Hum. Nutr.*, 64: 86-93.
- Miyoshi, S., H. Ishikawa, T. Kaneko, F. Fukui, H. Tanaka and S. Maruyama, 1990. Structures and activity of angiotensin-converting enzyme inhibitors in an a-zein hydrolysate. *Agric. Biol. Chem.*, 55: 1313-1318.
- Nagaoka, S., Y. Futamura, K. Miwa, T. Awano and K. Yamauchi *et al.*, 2001. Identification of novel hypocholesterolemic peptides derived from bovine milk beta-lactoglobulin. *Biochem. Biophys. Res. Commun.*, 281: 11-17.
- Park, V.V., M.S. Koo, T.D. Kasymova and D.Y. Kwon, 2005. Isolation and identification of peptides from soy 11S-globulin with hypocholesterolemic activity. *Chem. Nat. Comp.*, 41: 710-714.
- Suh, H.J., J.H. Whang, D.B. Suh, S.H. Bae and D.O. Noh, 2003. Preparation of angiotensin I converting enzyme inhibitory from corn gluten. *Process Biochem.*, 38: 1239-1244.
- Wang, J., M. Zhao, X. Yang and Y. Jiang, 2006. Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. *J. Cereal Sci.*, 44: 93-100.
- Yamaguchi, M., F. Nishikiori, M. Ito and Y. Furukawa, 1996a. Effect of corn peptide on alcohol metabolism and plasma free amino acid concentrations in healthy men. *Eur. J. Clin. Nutr.*, 53: 682-688.
- Yamaguchi, M., M. Takada, O. Nozaki, M. Ito and Y. Furukawa, 1996b. Preparation of corn peptide from corn gluten meal and its administration effect on alcohol metabolism in stroke-prone spontaneously hypertensive rats. *J. Nutr. Sci. Vitaminol.*, 42: 219-231.

- Yang, Y., G. Tao, P. Liu and J. Liu, 2007. Peptide with angiotensin I-converting enzyme inhibitory activity from hydrolyzed corn gluten meal. *J. Agric. Food Chem.*, 55: 7891-7895.
- Yoshie-Stark, Y. and A. Wasche, 2004. *In vitro* binding of bile acids by lupin protein isolates and their hydrolysates. *Food Chem.*, 88: 179-184.
- Zhang, J., H. Zhang, L. Wang, X. Guo, X. Wang and H. Yao, 2009. Antioxidant activities of the rice endosperm protein hydrolysate: Identification of the active peptide. *Eur. Food Res. Technol.*, 229: 709-719.
- Zheng, X.Q., L.T. Li, X.L. Liu, X.J. Wang, J. Lin and D. Li, 2006. Production of hydrolysate with antioxidative activity by enzymatic hydrolysis of extruded corn gluten. *Applied Microbiol. Biotechnol.*, 73: 763-770.
- Zhong, F., J. Liu, J. Ma and C.F. Shoemaker, 2007. Preparation of hypocholesterol peptides from soy protein and their hypocholesterolemic effect in mice. *Food Res. Int.*, 40: 661-667.