SYNOPSIS

STUDIES ON THE PREPARATION AND CHARACTERISATION OF PROTEIN HYDROLYSATES FROM GROUNDNUT AND SOYBEAN ISOLATES

Proteins are important in food processing and food product development, as they are responsible for various functional properties that influence consumer acceptability. Both animal and plant proteins are used commercially as functional ingredients. Plant proteins are the most abundant in the world. A number of vegetable proteins have been tried for incorporation in various food products as functional and nutritional ingredients.

Oilseed protein products are rapidly gaining importance in protein supplementation because of their unique functional properties. The intrinsic properties of proteins like the amino acid composition and conformation of the proteins, methods and conditions for their isolation, degree of purification and processing alterations are some of the important factors that influence the functional properties of food proteins. Various approaches like chemical modification, physical treatments and enzymatic modification have been tried to improve functional characteristics of proteins. Chemical modifications such as succinylation, acetylation and mild alkali hydrolysis have been reported to improve the functional properties. One of the major drawbacks of these approaches is the deterioration of the nutritional quality owing to the blocking or destruction of essential amino acids. One of the important ways to enhance the functional properties of oilseed proteins is enzymatic modification. Enzymatic modification occur under mild conditions retaining nutritional value and offer a convenient means for improving functional properties of proteins. By controlling the extent of hydrolysis it is possible to enhance various functional properties to develop new functional ingredients to fabricate new food analogs simulating traditional foods.

Protein hydrolysates find application in special foods such as those designed for children, old people, athletes and also in pharmaceutical preparations developed for convalescents and those who suffer from digestive disorders. Foods based on highly hydrolysed proteins are useful in controlling food allergies. The major ways of supplying tailored amount of amino acids are i) enzymatic protein hydrolysates and ii) a mixture of synthetic amino acids. They are preferred over synthetic amino acids at moderate cost because of availability on commercial scale and high quality of enzyme hydrolysed products. Enzymatic protein hydrolysates containing short chain peptides with defined amino acid composition and molecular size are preferred for specific formulations. These protein hydrolysates score over elemental diets in which the protein component consists exclusively of a mixture of free amino acids. The short chain peptides are absorbed preferentially over free amino acids in the gut. Protein hydrolysates offer as an alternative to intact proteins and elemental formula in the development of special formulations designed to provide nutritional support .

Soybean and groundnut are the most widely cultivated oilseeds all over the world. Dehulled soybean contains 17-18% oil and 25-35% protein depending on the variety. Similarly, dehulled groundnut depending on the variety contains 50-60% oil and 30-35% protein. Defatted soybean flour contains 50-55% protein of good nutritional quality. Soybean proteins are rich in lysine and deficient in methionine. The major intrinsic anti nutritional factor is trypsin inhibitor, which affects its utility. Groundnut proteins are deficient in lysine and methionine. The protein digestibility corrected amino acid score for soy protein and groundnut protein is 0.92 and 0.52 respectively. The protein ingredients such as defatted flour, protein concentrate and protein isolates from these sources have a good potential for preparing speciality foods under native and modified conditions. The protein hydrolysates obtained from these sources can effectively replace commercially available milk protein hydrolysates because

of their good nutritional quality.

In general, oilseed proteins offer resistance to enzymatic hydrolysis. Studies on hydrolysis of pure proteins would help in understanding of the various structural features of the protein both at surface and subunit levels. Though the resistance to hydrolysis of seed proteins by enzymes is well documented, work on the relative effectiveness of the major proteins with different proteolytic enzymes is less. Glycinin and conglycinin are the major fractions of soybeans and make up to 70% of the total proteins. Arachin and conarachin are the major fractions, which make nearly 80% of the total groundnut proteins. The individual roles of major protein fractions are important in order to understand the overall functional profile of the total proteins and protein ingredients. Glycinin is poorer in functional properties compared to conglycinin. This has been attributed to the compact structure of the high molecular protein fraction in which hydrophobic groups are buried inside. The functional properties of protein isolates and isolated fractions can be modified either by chemical means or enzymatic hydrolysis. Although sufficient information is available on the enzymatic modification of soybean or groundnut flour and its effect on functional properties, the information on the controlled hydrolysis of major globulins of oilseeds are limited.

In this work, protein hydrolysates from soybean and groundnut were prepared using different proteolytic enzymes starting with different materials like defatted meal, protein isolates and purified fractions. With the use of different enzymes and by varying the experimental parameters like E/S ratio, pH, temperature and combinations of proteolytic enzymes, it was possible to tailor the functional characteristics of various protein hydrolysates. Attempt has also been made to correlate various functional characteristics with some of the biophysical and biochemical parameters.

CHAPTER 1: INTRODUCTION

The introduction reviews the information regarding the physico-chemical characteristics, nutritional, and functional properties of soybean and groundnut proteins. This also includes the review of literature on enzymatic hydrolysis of food proteins, methods of isolation and physico-chemical characteristics of the purified high molecular weight protein fractions of soybean and groundnut (glycinin and arachin).

CHAPTER 2: MATERIALS AND METHODS

This presents details regarding the materials and experimental methods used in the investigation.

CHAPTER 3: RESULTS AND DISCUSSION

This has the results followed by the relevant discussion, which are given in the following Sections.

PART – A: STUDIES ON SOYBEAN PROTEINS

SECTION 1: ENZYMATIC HYDROLYSIS OF SOYBEAN FLOUR

The maximum degree of hydrolysis (DH) obtained with papain, alcalase and fungal protease enzymes were 18.6%, 29.6% and 32.4%, respectively. A comparison of the DH values obtained with different proteases showed that fungal protease was more effective among the proteases used for the hydrolysis of defatted soy flour (DSF). The overall effectiveness of different proteases in getting higher DH was in the order fungal protease > alcalase > papain.

Enzymatic modification of DSF to low DH (4-6%) resulted in remarkable increase in emulsification capacity (EC) and marginal increase in foaming capacity (FC). The extent of improvement in EC followed by limited proteolysis was almost same for different proteolytic enzyme modified flours. The fat absorption capacity (FAC) and water absorption capacity (WAC) of enzyme hydrolysed DSF was higher than that of intact DSF. Extensive hydrolysis impaired the overall functionality of DSF. The trypsin inhibitor activities of low DH and high

DH enzyme modified freeze dried DSF were similar to control, suggesting that trypsin inhibitors were resistant to enzymatic attack. The nitrogen content of spray-dried protein hydrolysate obtained with papain, alcalase and fungal protease was almost same (9-9.2 %). The nitrogen content of DSF increased to 11.5% after acid wash. The protein hydrolysate prepared by hydrolysis of wet protein isolate obtained by alkali extraction followed by iso-electric precipitation had higher nitrogen content (14.5%).Amino acid composition of protein hydrolysates showed that the nutritional quality of protein was retained after enzymatic hydrolysis. The bitterness of protein hydrolysates was in the order papain< alcalase=fungal protease. The spray dried protein hydrolysate of DSF obtained by different proteolytic enzymes showed inactivation of lipoxygenase and urease activity. The trypsin inhibitor activity of DSF hydrolysate was in the range 20-22 TIU/mg sample. The lower trypsin inhibitor activity of spray dried hydrolysates compared to DSF may be due to application of heat in spray drying process. The protein hydrolysate was soluble over a wide range of pH (2.0-11.0); at iso-electric pH, the solubility was >98%.

SECTION 2: ENZYMATIC MODIFICATION OF SOY PROTEIN ISOLATE

The DH of soy protein isolate obtained with papain, alcalase and fungal protease was 7.5%, 9.5% and 18.9% respectively. The effectiveness of proteolytic enzymes for hydrolysis of soy protein isolate (SPI) was lower compared to DSF. The overall effectiveness of proteases towards hydrolysis of SPI was in the order fungal protease>alcalase>papain. Comparison of the hydrolysis curves of freeze dried and spray dried SPI showed their susceptibility towards proteolytic enzymes was almost same. The solubility of SPI followed the typical U-shape pattern. The minimum solubility was found at pH 4.5 (iso-electric pH). A low DH of 3-5% obtained with papain, alcalase and fungal protease increased the solubility of modified SPI up to 29-35% at pH 4.5 and 97-98% at pH 7.0. Extensive hydrolysis of SPI increased the solubility at pH 4.5 up to 49-54% with different proteolytic enzymes. Limited hydrolysis of

SPI with different proteolytic enzymes increased the EC. Among the proteases papain modified SPI showed more EC compared to alcalase and fungal protease modified SPI. The FC of low DH modified SPI was higher compared to unmodified SPI. Fungal protease modified SPI showed higher FC compared to papain and alcalase modified SPI. Extensive hydrolysis of SPI with proteolytic enzymes brought drastic reduction in EC. Although the FC of SPI extensively hydrolysed using papain, alcalase and fungal protease was higher than intact SPI, the FC values were lower than the corresponding low DH modified SPI. The maximum DH obtained with SPI with proteolytic enzymes was lower than that of DSF. SPI with limited proteolysis showed remarkable increase in FC but the increase was marginal with DSF.

SECTION 3: CONTROLLED ENZYMATIC HYDROLYSIS OF GLYCININ

Glycinin, the major protein fraction of soybean when hydrolysed with different proteolytic enzymes showed that papain had the least effect followed by alcalase and fungal protease. The enzymatic hydrolysis followed typical Michaelis-Menten pattern. The affinity of glycinin to proteolytic enzymes was in the order fungal protease > alcalase > papain as shown by the K_m values. The SDS-gel electrophoretic pattern of glycinin showed bands corresponding to acidic (30-33kD) and basic subunits (29-22kD). The pattern observed for enzymatically-modified glycinin suggested the preferential cleavage of acidic subunits compared to basic subunits. The hydrolysis of isolated acidic and basic subunits of glycinin with fungal protease showed that basic subunits were less susceptible. A maximum DH of 26% was obtained with acidic subunits at the end of 4h hydrolysis compared to 9-10% DH with basic subunits. The K_m values for acidic and basic subunits for fungal protease correlated well with cleavage susceptibility. Glycinin possess poor functional properties; enzymatic modification with proteolytic enzymes improved a few of the functional characteristics. Papain with limited proteolysis increased the FC almost three fold with good foam stability. There was no difference in the EC of low DH modified glycinin samples. The FAC of papain, alcalase, and

fungal protease modified glycinin decreased compared to unmodified glycinin. Limited proteolysis of glycinin did not bring significant differences in the WAC. The molecular sieve chromatography on Sepharose-6B gel showed single peak for glycinin. In the case of modified glycinin it could be resolved in to two peaks. The first peak had same V_e/V_o as that of glycinin but the second peak had higher V_e/V_o. This indicated that even low DH degraded glycinin into low molecular weight peptides. The electrophoresis pattern of gel filtration chromatographic peaks suggested that peak1 and peak 2 did not correspond to native glycinin; acidic subunits were readily hydrolysed compared to basic subunits. Peak 2 did not give bands on the 10% gel suggesting that peak 2 was extensively hydrolysed and the molecular weight of the resulting peptides were low. A low DH of 4-5% resulted in drastic reduction in mean residue ellipticity of modified glycinin for all the enzymes tested. However, both glycinin and modified glycinin exhibited characteristic near UV CD peaks at 263, 275, 283 and 291nm. This suggests that even a low DH disrupts the tertiary structure of glycinin. The EC and FC of glycinin were lesser than that of SPI. The poor functionality of glycinin may be due to the closely packed conformation of glycinin in which the hydrophobic groups are buried inside. The enhancement in functionality by limited proteolysis could be due to the exposure of hydrophobic groups.

PART- B: STUDIES ON GROUNDNUT PROTEINS

SECTION 4: ENZYMATIC MODIFICATION OF GROUNDNUT PROTEIN ISOLATE

Groundnut protein isolate (GPI) was more susceptible to hydrolysis with papain, alcalase and fungal protease compared to SPI. The maximum DH of GPI obtained with papain, alcalase and fungal protease was 18.6%, 17.4% and 26.6% respectively. The comparatively high affinity of GPI for proteolytic enzymes may be because groundnut proteins are less hydrophobic compared to soybean proteins. The effectiveness of different enzymes was in the order fungal protease > papain > alcalase. The solubility curve of GPI followed U-shaped pattern with the minimum at pH 4.5. The low DH modified GPI with different proteolytic

enzymes at isoelectric pH showed solubility of 27-28%. However, high DH increased the solubility up to 47-55% at pH 4.5.The EC and FC of GPI increased after limited proteolysis. The effectiveness of different proteolytic enzymes in enhancing the EC was almost same. However, alcalase and fungal protease enzymes were more effective compared to papain in enhancing the FC by limited proteolysis. Extensive hydrolysis of GPI resulted in a remarkable reduction of EC. Although the FC of high DH modified GPI with different enzymes were higher than unmodified GPI, the values were lower than that of low DH modified GPI.

SECTION 5: ENZYMATIC HYDROLYSIS OF ARACHIN AND ITS EFFECT ON FUNCTIONAL PROPERTIES

Arachin, the major protein fraction of groundnut purified by ammonium sulfate precipitation eluted as a single peak with V_e/V_o =1.5. Arachin with a low DH of 3-5% showed two peaks, the first peak at V_e/V_o similar to arachin and second peak with a V_e/V_o =1.8 This suggested that even at low DH arachin degraded into low molecular weight peptides. The K_m values for arachin with different proteases were 0.83-0.931%. The K_m values were lower when compared to the values obtained with glycinin as substrate. Arachin was the preferred substrate over glycinin for the proteolytic enzymes used. The maximum DH of arachin obtained with papain, alcalase and fungal protease was 23.6%, 18.6% and 25.5% respectively. The effectiveness of these enzymes was in the order fungal protease > papain > alcalase. These results obtained with arachin were comparable to that of GPI. This suggests that the proteolytic enzymes have got equal effectiveness for GPI and arachin. The solubility of modified arachin to low DH (3-5%) was 14-16%. Extensive hydrolysis increased the solubility up to 55-60%. Hydrolysis increased the solubility of arachin considerably. Limited proteolysis increased the EC of arachin. Among the different enzymes papain was more effective in enhancing the EC. The FC of alcalase-modified arachin was remarkably high (two fold). Papain and alcalase modified arachin showed marginal increase in FC. Excess hydrolysis impaired the functionality of arachin except solubility, The SDS-PAGE pattern of arachin was similar to

those already reported in the literature. Papain, alcalase and fungal protease degraded high molecular weight subunits. The pattern for alcalase and fungal protease was similar. The action of papain on arachin subunits was different. The low molecular weight subunit disappeared only after hydrolysis for 1h with different enzymes. The EC of arachin was higher than that of glycinin. There was considerable difference in the FC between the two fractions. The overall effect of proteases in enhancing the functionality differed considerably. Comparison of the DH obtained with flour, isolate and purified fractions of groundnut and soybean showed that the different proteases acted differently in getting high DH and in changing functional characteristics. DSF gave high DH with proteolytic enzymes compared to SPI. The effect of proteases on GPI and arachin was similar. In general, the study has indicated that groundnut and soybean proteins are resistant to hydrolysis by proteolytic enzymes. The bitterness of hydrolysate was more with soy proteins than groundnut proteins. The affinity of proteases towards groundnut proteins was more than that of soybean proteins. Thus by using appropriate proteolytic enzymes under specified conditions the functional characteristics of seed proteins can be tailored to meet our requirements.

CHAPTER 4: SUMMARY AND CONCLUSIONS

This has the general summary of the investigation focusing on the important findings of the investigation. The references are arranged in alphabetical order.

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