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Process optimization for enzymatic hydrolysis of whey protein concentrate

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A study was under taken to optimize the enzymatic hydrolysis of whey protein concentrate for utilizing the resultant hydrolyzate in ragi based functional weaning food. Spray dried Whey protein concentrate (WPC) was reconstituted and heated to 80°C for 5 min. The reconstituted medium was inoculated separately with Neutrase, papain and trypsin enzymes at various enzyme to substrate ratio (E: S) and incubated for a period of 180 h at their respective optimum pH and temperature. The effect of enzyme on degree of hydrolysis was monitored at a regular interval of 30 minutes by measuring change in pH. From among the three enzymes tried for enzymatic hydrolysis, it was observed that Neutrase enzyme is superior with respect to the extent of hydrolysis obtained followed by trypsin and papain. Neutrase enzyme could able to give hydrolysis of 6.08 per cent degree of hydrolysis within 150 minutes of duration. From the study, it was found that reconstituting the spray dried WPC at 15 per cent protein level and adding Neutrase enzyme at concentration of 1:50 and incubation for a period of 150 min is optimum for obtaining the maximum degree of hydrolysis. Spray dried WPC reconstituted to 15 per cent protein level and hydrolysed using Neutrase enzyme at 1:50 ratio for a period of 150 min was further used in the formulation of weaning food.

Key Words : Process optimization, Enzymatic hydrolysis, Protein concentrate

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INTRODUCTION

Whey is considered as a way for health in light of the benefits that are imparted by whey proteins (Alok and Kanawajia, 2010). Whey is the largest by-product of the dairy industry. Whey proteins are also potential ingredients in a wide range of food applications due to their excellent nutritional and promising functional properties. Biological value of whey proteins is superior to most other proteins, whey proteins also have a high content of sulphur-containing amino acids, which support antioxidant functions (Sinha *et al.*, 2007). Presently, whey protein concentrate (WPC) constitutes very small proportions (10%) of protein utilization in food industry. More product formulation work is needed to move WPC into the general market place (Raju *et al.*, 2005). The functionalities of whey protein concentrate (WPC) could be further enhanced and nurtured by enzymatic hydrolysis enabling them to be used in wide array of food applications. Hydrolysis is known to improve nutritional and therapeutic characteristics due to release of bioactive peptides. These bioactive peptides represent potential health enhancing components for food and pharmaceutical applications. Hydrolysed whey proteins

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possess better digestibility and carry balanced essential amino acids which aid in high net protein utilization. Bioactive peptides which are released during whey protein hydrolysis are known to modulate absorption process in the gut. The enhancement of net water and electrolyte absorption by these peptides in small and large intestine is a major component of their antidiarrhoeal action. Whey protein hydrolysates could be used as potential ingredients in developing foods with special health claims. Hydrolysis and incorporation of WPC in the formulation of functional weaning foods are gaining popularity as it is not only upgrading the nutritional status of the product but also impart possible health benefits to the child.Protein hydrolysis could be accomplished by various proteolytic enzymes such as rennet, Neutrase, fungal proteinases, Flavourzyme which are called proteases. These enzymes catalyse the protein at a peptide linkage and produce smaller units or peptides (Khedar *et al.,* 1999). Proteolytic enzymes could be obtained from plants (papain and bromalein) animal (pepsin, trypsin and chymotrypsin) or microbes (Neutrase, Alcalase, Promase). Choice of the enzyme for protein hydrolysis depends mainly on the enzyme specificity, pH optimum and stability (Venkatesh, 1995). Mutilangi, (1988) used enzymes such as trypsin, alpha chymotrypsin, alkalase and Neutrase to produce hydrolysates from denatured whey protein by hydrolyzing it to 2.8, 4.3, 6.0 and 8.0 per cent degree of hydrolysis. There are several advantages of using protease to cause proteolysis instead of physical or chemical treatments (Reddy, 2000). Seenappa (2005) attained whey protein hydrolysis to an extent of 6.39 per cent in 150 min by using Neutrase enzyme at 1:25 enzyme to substrate ratio. Enzymatic hydrolysis of protein results in short chain peptides with characteristics amino acids composition and defined molecular size which are highly desired for specific food formulation (Clement *et al.,* 1999). There are several advantages of using proteases which cause proteolysis instead of physical or chemical treatment. Proteases from microbial sources are known to have broad specificity towards their substrates (Loffler, 1986). Whey protein hydrolysis with Neutrase enzyme was found to be slower as compared to casein hydrolysis (El-mayda *et al.,* 1986). Neutrase was found to be 5-16 times more active than enzymes such as pepsin, chymotrypsin or trypsin (Chen *et al.,* 1995). Enzymatic modification generally involves controlled proteolytic hydrolysis of protein to yield a mixture of peptides, which can improve desirable functionalities of proteins. Modification of protein refers to changes in conformational or structural features which subsequently alter the physico-chemical properties and thus, the functional properties (Reddy, 2000). The most important whey protein allergens are α-lactalbumin and β-lactoglobulin. Both these proteins are likely to provoke allergic responses. Shobha (2002) carried out *in vitro* digestion of WPC reconstitute to have 18 per cent whey protein at an enzyme to substrate ratio of 1:25. She observed 7 to 8 per cent degree of hydrolysis at pH 7 and temperature of 40°C for using it beverage preparation. Kusuma Rani (2006) reported that hydrolysis for a period of 180 minutes at 18 per cent whey protein concentration by using Neutrase enzyme at 1:25 enzyme to substrate ratio results in a maximum hydrolysis of 7.10 per cent, which was found to be optimum for weaning food formulation. Keeping above things in mind the study was under taken to optimize the enzymatic hydrolysis of whey protein concentrate for utilizing the resultant hydrolyzate in ragi based functional weaning food.

METHODOLOGY

Whey protein concentrate (WPC) (PROCON 3700 WPC 70) was procured from Mahaan Protein Ltd, New Delhi. Neutrase enzyme 0.8 L, was procured from Novozymes, South Asia Pvt Ltd. The enzymes Trypsin and Papain were obtained from Sisco Research Laboratories, Mumbai. The chemicals and reagents used were of analytical grade. For all analytical purpose freshly prepared reagents were used. In order to select an appropriate enzyme for enzymatic hydrolysis of WPC, spray dried WPC was reconstituted to have 12 per cent protein and heated to 80°C for 5 min. The reconstituted medium was inoculated separately with Neutrase, papain and trypsin enzymes at 1:25 enzyme to substrate ratio (E:S) and incubated for a period of 180 h at their respective optimum pH and temperature. The effect of enzyme on degree of hydrolysis was monitored at a regular interval of 30 minutes by measuring change in pH. An enzyme which resulted in a maximum hydrolysis with minimum time was used further in the investigation. Spray dried WPC-70 was reconstituted to 12, 15, 18 and 21 per cent total solids using potable water and adjusted to optimum pH. Neutrase enzyme was added to the reconstituted samples at various enzymes to substrate ratio (1:25, 1:50, 1:75 and 1:100). *In vitro* digestion was carried out for a period of 150 min in a water bath

maintained at 40 °C. Subsequently, the enzyme activity was terminated by heating the samples to 85°C for 10 min. The extent of hydrolysis was estimated. The samples which resulted in maximum hydrolysis with minimum enzyme concentration was selected for further studies. The pH of reconstituted samples was measured using digital pH meter (Elico make). Acidity was measured by titrating against 0.1N NaOH using phenolphthalein indicator and expressed in terms of per cent lactic acid as per the method described in IS:SP:18 (Part XI) 1981. Degree of hydrolysis was determined by the pH stat method as per the method of Adler-Nissen (1986). The degree of hydrolysis (DH) was commonly measured and monitored by the amount of base that is consumed to maintain pH during the process of hydrolysis. Per cent degree of hydrolysis was calculated by using the following formula.

$$\text{Degree of hydrolysis (DH)} = B \times N_b \times \frac{1}{\alpha} \times \frac{1}{MP} \times \frac{1}{h_{tot}} \times 100$$

where,

B : Base consumption in ml

N_b : Normality of the base

$1/\alpha$: Average degree of dissociation of the alpha amino group (it is a constant value depends on temperature and pH, the dissociation values for pH 7.0, 7.5, 8.0 and 8.5 are 3.0, 1.63, 1.20 and 1.06, respectively).

MP : Mass of protein in gram

h_{tot} : Total number of peptide bonds in the protein substrate (m.eq./g of protein h_{tot} for whey protein is 8.8).

For every 30 minutes, DH was calculated throughout 3 h of hydrolysis till the maximum hydrolysis was attained with minimum bitterness.

The results were analyzed statistically for test of significance by using statistical packages for social sciences (SPSS) version 8 software programme.

OBSERVATIONS AND ASSESSMENT

Spray dried WPC was reconstituted and protein was enzymatically hydrolysed by 3 types of enzymes namely Neutrase, papain and trypsin. The effect of type of enzymes on extent of hydrolysis was monitored at an interval of 30 min for a period of 180 min. The extent of hydrolysis as affected by the type of enzyme and duration of incubation is presented in Table 1.

Amongst 3 enzymes, Neutrase was found to be significantly active as compared to papain and trypsin. With the increase in the incubation period from 30 to 150 min there was progressive and significant increase in the extent of hydrolysis obtained irrespective of type of enzyme used. The extent of hydrolysis obtained was found to be 6.08, 4.63 and 5.59 per cent for Neutrase, papain and trypsin enzyme, respectively after 150 min of reaction (Fig. 1). The extent of hydrolysis obtained was significantly higher with Neutrase followed by trypsin and papain. The results of the study revealed that Neutrase is better than other enzymes and hence, Neutrase was used for further studies. Spray dried WPC was

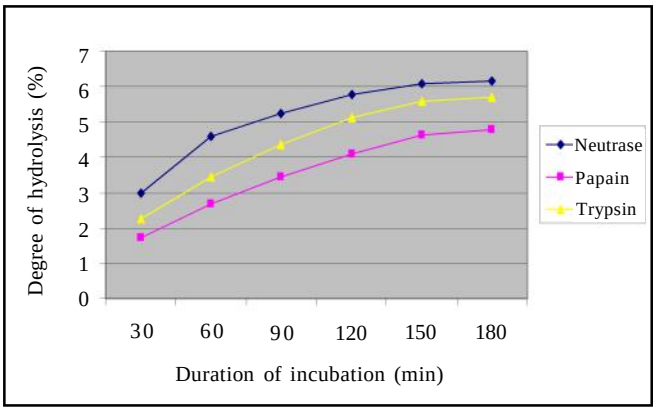

Fig. 1 : Effect of different type of enzymes on degree of hydrolysis of whey proteins

Table 1: Effect of different enzymes on degree of hydrolysis of whey proteins

Type of enzymes	Duration of hydrolysis (min)						Mean
	30	60	90	120	150	180	
	Extent of hydrolysis (%)						
Neutrase	2.99	4.58	5.23	5.78	6.08	6.15	5.135
Papain	1.74	2.67	3.45	4.09	4.63	4.78	3.560
Trypsin	2.27	3.45	4.36	5.13	5.59	5.71	4.418
		Enzymes				Duration	
F test		*				*	
C.D. (P=0.05)		0.180				0.299	

*All values are average of three trials *Enzyme to substrate ratio 1:25

reconstituted to 12, 15, 18 and 21 per cent protein level and added with Neutrase enzyme at various concentration (1:25, 1:50, 1:75 and 1:100) to elicit the combined effect of level of protein in the substrate and enzyme concentration on the extent of hydrolysis.

In an attempt to explore the benefit of enzymatic hydrolysis of whey proteins three enzymes namely Neutrase, papain and trypsin were used for hydrolysis. It is known that the amino acids when they are in their main chain carry lesser biological activity but when they are enzymatically hydrolysed they release certain peptides, some of these peptides demonstrated to be biologically very active and such peptides are designated as bioactive peptides (Mullally *et al.,* 1997 and Mercier *et al.,* 2004). Enzymatic hydrolysis of whey proteins aid in their application in children food formulation as it reduces the whey protein allergenicity besides adding on to many health benefits (Castro *et al.,* 1996).

From among the three enzymes tried for enzymatic hydrolysis, it was observed that Neutrase enzyme is superior with respect to the extent of hydrolysis obtained followed by trypsin and papain. Neutrase enzyme could able to give hydrolysis of 6.08 per cent degree of hydrolysis within 150 minutes of duration. The maximum hydrolysis that could be obtained by trypsin and papain were only 5.59 and 4.63 per cent. It is observed that Neutrase is more active than other two enzymes. Monti and Jost (1978) and El-mayda *et al.* (1986) reported that the Neutrase enzyme from *Bacillus subtilis* is more proteolytic as compared to many other proteolytic enzymes and also it produces shorter chain peptides. Further the results are in conformity with the reports of earlier workers (Schmidt and Poll, 1991 and Shobha, 2002). Ju *et al.* (1995) who studied the performance of Neutrase, trypsin and protease from *Bacillus licheniformis* and reported that the extent of hydrolysis obtained was highest in case of Neutrase followed by trypsin and protease from *Bacillus licheniformis* at all durations of incubation. The performance of papain in hydrolysing whey protein was studied by Lieske and Konard (1996 a and b), they observed a maximum hydrolysis of 4-5 per cent in a span of 120 min. Schmidth and Poll (1991) observed slower papain activity as compared to trypsin. The extent of hydrolysis obtained after 150 min of incubation was insignificant, higher with respect to Neutrase enzyme as compared to papain and trypsin. Hence, for further studies Neutrase enzyme was used.

The extent of hydrolysis increased with increase in the concentration of enzyme from 1:100 to 1:50. Further increase in the concentration of enzyme to 1:25 did not significantly improve the extent of hydrolysis. The extent of hydrolysis also increased with increase in the level of protein in the substrate upto 15 per cent thereafter the extent of increase was non-significant. At 1:50 enzyme to substrate ratio the hydrolysis obtained was 6.04, 6.39, 6.53 and 6.64 for substrate protein level of 12, 15, 18 and 21 per cent, respectively.

From the study it is evident that reconstituting the spray dried WPC at 15 per cent protein level and adding Neutrase enzyme at concentration of 1:50 and incubation for a period of 150 min is optimum for obtaining the maximum degree of hydrolysis. Spray dried WPC reconstituted to 15 per cent protein level and hydrolysed using Neutrase enzyme at 1:50 ratio for a period of 150 min was further used in the formulation of weaning food. From the results presented in Table 2, it is evident that with increasing in the concentration of enzymes from 1:100 to 1:50, there was significant increase in the extent of hydrolysis at all levels of protein concentration. Further

Table 2 : Effect of level of whey protein in substrate and enzyme to substrate ratio on degree of hydrolysis by neutrase

Level of protein in the substrate (%)	Enzyme to substrate ratio				Mean
	1:100	1:75	1:50	1:25	
	Extent of hydrolysis (%)				
12	5.08	5.68	6.04	6.19	5.747
15	5.33	5.97	6.39	6.52	6.052
18	5.42	6.08	6.53	6.67	6.175
21	5.48	6.15	6.64	6.76	6.257
F test	Protein level	E:S	Protein x E:S		
	*	*	NS		
C.D. (P=0.05)	0.198	0.314	---		

* All values are average of three trials NS= Non-significant

increase in the level of enzyme to 1:25 there was no significant improvement in the hydrolysis. When the protein content in the substrate increased from 12 to 15 per cent there was significant improvement in the extent of hydrolysis, thereafter there was no further significant increase in the extent of hydrolysis. The study demonstrated that reconstituting WPC or ultra filtering whey to have 15 per cent protein on dry matter basis and incubating with Neutrase enzyme at 1:50 enzyme to substrate ratio is the optimum conditions for obtaining maximum hydrolysis. Castro *et al.* (1996) reported that bitterness is observed in whey protein hydrolysates when degree of hydrolysis exceeds 12 per cent. However, it was observed that when enzyme to substrate ratio is less than 1:20 did not produce bitterness even after 200 min of incubation. From the result it is evident that reconstituting whey protein concentrate to 15 per cent protein and enzymatic hydrolysis with Neutrase at 1:50 E: S ratio for a period of 150 min is the better option to obtain a maximum hydrolysis of 6 per cent without noticeable bitterness. Amongst three enzymes used for enzymatic hydrolysis of whey proteins, Neutrase enzyme was found to be more effective as compare to trypsin and papain. The respective extent of hydrolysis obtained after 150 min of incubation was found to be 6.08, 4.63 and 5.59 per cent for Neutrsae papain and trypsin enzymes. Neutrase was found to be better in its performance and hence, used for further experiments. From among various combinations of Neutrase enzyme concentration and different levels of protein in the substrate tried, a protein level of 15 per cent in the substrate and enzyme to substrate ratio of 1:50 was found to be optimum for obtaining maximum hydrolysis of 6 per cent at duration of 150 min as compared to other levels of protein.

LITERATURE CITED

Adler-Nissen, J. (1986). *Enzymic hydrolysis of food proteins*. Elservier Applied Science Publishers, New York, U.S.A.

Alok Chatterjee and Kanawajia (2010). Whey proteins for health living. *Indian Dairy Man*, **62** (4):66-73.

Castro, S., Peyronel, D.V. and Cantera, A.M.B. (1996). Proteolysis of whey proteins by a bacillus subtilis enzyme preparation. *Internat. Dairy J.*, **6** : 285–294.

Chen, S.A., Fornes, H.M. and Pearse, S.W.(1995). Thermostability and functional properties of hydrolysed whey protein concentrate and whey protein isolates. Ph.D. Thesis, Novonordix, Biochem. Inc,77, perry, chapel, church, R.D., franklin, N.C., **27**: 525-0576.

Clement, A., Vioque, J., Sanchez Vioque, R., Pedroche, J., Bautista, J. and Milan, F. (1999). Protein quality of chick pea protein hydrolysates. *Food Chem.*, **67**: 269-274.

El-mayda, E., Paquet, D., Ramet, J.P. and Linden, G. (1986). Proteolytic activity of bacillus subtilis Neutral protease preparation upon caseins and whey proteins of cow's milk. *J. Dairy Sci.*, **69** (2) : 305-310.

IS: (SP: 18) (1981). ISI Handbook of Food Analysis, Part XI: Dairy Products, Indian Standards Institution, New Delhi, India.

Ju, Z. Y., Otte, J., Madsen, J. S. and Qvist, K. B. (1995). E!ects of limited proteolysis on gelation and gel properties of whey protein isolate. *J. Dairy Sci.*, **78** : 2119-2128.

Khedar, E.E., Abou Shlove, Z.I. and Awad, S. (1999). Hydrolysis of cows and buffaloes whey proteins by commercial proteolytic enzyme as effected by sodium and calcium chlorides. *Alexandria J.Agril. Res.*, **44** : 129-150.

Kusuma Rani, G.S. (2006). Formulation of functional weaning food utilizing whey protein concentrate and probiotics. Thesis, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka (India) .

Lieske, B. and Konrad, G. (1996a). Physico-chemical and functional properties of whey proteins as affected by limited papain proteolysis and selective ultrafltration. *Internat. Dairy J.*, **6** :13–31.

Lieske, B. and Konrad, G. (1996b). Interrelation between pH and availability of alpha-lactalbumin and beta-lactoglobulin for proteolysis by papain. *Internat. Dairy J.*, **6** : 359–370.

Loffler, A. (1986). Proteolytic enzymes: Sources and applications. *Food Technol.*, **40** : 63-70.

Mercier, Amélie, Gauthier, Sylvie, F. and Fliss, Ismai (2004). Immunomodulating effects of whey proteins and their enzymatic digests. *Internat. Dairy J.*, **14** (3) : 175-183.

Monti, J.C. and Jost, R. (1978). Enzymatic solubilization of heat-denatured cheese whey protein. *J. Dairy Sci.*, **61** (9) : 1233-1237·

Mullally, M. M., Meisel, H. and Gerald, R. J. Fitz (1997). Identification of novel angiotensin-I converting enzyme inhibitory peptide corresponding to tryptic fragment of bovine beta lactoglobulin. *FEBS Lett.*, **402**:99.

Mutilangi,W.A.M. (1988). Enzymatic hydrolysis of heat denatured whey proteins and properties of hydrolysates, *Dissertation Abstracts International*, **B49** (4): 964.

Raju, P.N., Rao, L.H. and Devi, N.L. (2005). Whey protein and their uses in food industry. *Indian Food Industry.*,**24**:19-27.

Reddy, K.V.N. (2000). Effect of milk protein modification on physical chemical and sensory characteristics of cheddar cheese. M.Sc. Thesis, University of Agricultural Sciences, Bengaluru, Karnataka (India).

Schmidt, D. G. and Poll, J. K. (1991). Enzymatic hydrolysis of cc-lactalbumin and /Ilactoglobulin in buffer solutions by proteolytic enzymes. *Neth. Milk Dairy J.*, **45** : 225-240.

Seenappa (2005). Enzymatic modification of whey components in the formulation of weaning food for milk allergeic children. M Sc. Thesis, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka (India).

Shobha, K. P. (2002). Process optimization for production of WPC hydrolysates and its applications in ready to use beverages, M.Sc, Thesis, University of Agricultural Sciences, Bangalore, Karnataka (India).

Sinha, R., Radha, C., Prakash, J. and Kaul, P. (2007). Whey protein hydrolysate functional properties, nutritional quality and utilization in beverage formation. *Food Chem.*, **101**: 1484-1491.

Venkatesh, S.R. (1995). Enhancement of functional properties of milk proteins by modification using Neutrase. M.Sc. Thesis, University of Agricultural Sciences, Bangalore, Karnataka (India).

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