3. Hydrolysis of whey protein from dairy and low molecular weight protein from sesame seeds

In the recent years, dietary proteins are known to have a wide range of nutritional, functional and biological properties. Many of these properties are attributed to physiologically active peptides encrypted in protein molecules. Some of the proteins from both animals and plants are important sources of bioactive peptides (Miyoshi et al., 1995; Yano et al., 1996; Gobbetti et al., 1997). Growing interest has been shown in recent years for bioactive peptides derived from dietary proteins. Such peptides have been found to exert various bioactivities both in vitro and in vivo. They may have protective functions, regulation of digestion, nutrient uptake and metabolic or physiological role in the biological system. Milk proteins are one of the important sources from animals containing potential bioactive peptides (Smacchi and Gobbetti, 2000). Whey proteins from milk traditionally was defined as a byproduct of dairy industries with little or no commercial value. This view has changed radically with increasing number of technical and nutritional evaluation of the whey (Clare and Swaisgood, 2000; FitzGerald and Meisel, 2000; Korhonen, 2002; Walzem et al., 2002).

Bioactive peptides are inactive when they are present within the sequence of the parent protein and can be released by enzymatic hydrolysis with digestive enzymes or by fermentation. In case of milk proteins, bioactive peptides can be released by the action of proteolytic enzymes obtained from
various microorganisms (Foegeding et al., 2002; Silvestre, 1997). The enzymatic hydrolysis has been one of the most common ways to produce bioactive peptides. Hydrolysate peptide composition and consequently their properties are dependent on protein and enzyme used, as well as, on the conditions of hydrolysis (such as temperature, pH, enzyme to substrate ratio and reaction time etc). There are limited reports on the whey protein hydrolysates having different biological activities. Many casein-derived ACE inhibitory peptides have also been reported to have the bioactive peptides (Karaki et al., 1990; Sekiya et al., 1992; Yamamoto et al., 1994; Nakamura et al., 1995; Maeno et al., 1996; Chiba and Yoshikawa, 1991).

Some of the proteins from plant sources are also rich source of bioactive peptides (Miyoshi et al, 1995; Yano et al, 1996). From plant source, oil seeds mainly the sesame proteins are rich in sulphur containing amino acids and lysine. There are reports of proteolytic activity in the sesame protein mainly β-globulin (Tasneem and Prakash, 1989; Rajendran and Prakash, 1988). Compared to α-globulin, the protein β-globulin has better degree of proteolytic hydrolysis (Tasneem and Prakash, 1992). Therefore, β-globulin was selected for hydrolysis to produce the hydrolysates with different bioactivities. In the present chapter hydrolysis of whey protein concentrate and α-lactalbumin from bovine milk and β-globulin from sesame seed was carried out to obtain hydrolysates rich in peptides.
The enzymatic hydrolysis of whey protein concentrate, α-lactalbumin from bovine milk and β-globulin were carried out with enzymes of narrow and broad specificities in single and double enzyme combinations. The enzymes fungal protease, pancreatin and subtilisin were used for the study. The enzymatic hydrolysis of whey protein concentrate and α-lactalbumin was carried out with fungal protease and pancreatin enzymes and the hydrolysis of β-globulin was done with subtilisin enzymes. The degree of hydrolysis thus obtained for the various hydrolysates is given in Table 8. From the table it is clear that the whey protein hydrolysate is having a better degree of hydrolysis compared with the other hydrolysates. The β-globulin hydrolysate was having least degree of hydrolysis ($9 \pm 2\%$). This is due to the presence of strong disulfide bonds in the protein molecule (Tai et al., 1999). The better degree of hydrolysis obtained for the whey protein concentrate is due to the broad specificity of enzymes used for the hydrolysis.

The hydrolysates thus obtained were assessed for various biological activities. The antioxidant potential of the WPC hydrolysates was checked by DPPH radical scavenging activity using ascorbic acid as standard antioxidant. The antioxidant activity of the hydrolysate was obtained by determining the IC$_{50}$ values. The IC$_{50}$ is the concentration of hydrolysate at which the 50% radical scavenging activity is achieved. A high radical scavenging activity was observed in the whey hydrolysates in a concentration dependent manner, the IC$_{50}$ values were found to be $0.110 \pm 0.01$ mg/ml and
for ascorbic acid it was found to be $0.191 \pm 0.01$ mg/ml as mentioned in Table 9. The WPC hydrolysate was also checked for their total antioxidant potential and it was found to be $5900 \pm 210$ μmol/g of ascorbic acid equivalents. Whey hydrolysates obtained above was checked for angiotensin converting enzyme inhibitory activity also. The $IC_{50}$ value of the whey hydrolysates against ACE was $600 \pm 35$ μg/ml versus $215 \pm 15$ μg/ml for ramipril, which is a known standard ACE inhibitor. The antibacterial activity of the whey hydrolysate was estimated against the microbes namely *Escherichia coli*, *Listeria monocytogenes* and *Bacillus cereus* and the inhibitory activity of the hydrolysate were found to be insignificant.

$\alpha$-Lactalbumin is the second most prevalent whey protein. Enzymatic hydrolysis of $\alpha$-lactalbumin with double enzyme combination namely fungal protease and pancreatin was carried out. The $\alpha$-lactalbumin hydrolysate thus obtained was checked for antioxidant activity by DPPH radical scavenging action using ascorbic acid as standard. A high radical scavenging activity was observed for the $\alpha$-lactalbumin hydrolysate in a concentration dependent manner and the $IC_{50}$ values was found to be $0.18 \pm 0.02$ mg/ml and for ascorbic acid it was found to be $0.191 \pm 0.01$ mg/ml (Table 10). The total antioxidant activity was found to be $3100 \pm 120$ μmol/g ascorbic acid equivalents. The $\alpha$-lactalbumin hydrolysate obtained in the above experiments was also checked for angiotensin converting enzyme inhibitory activity. The $IC_{50}$ value of the $\alpha$-lactalbumin hydrolysate against ACE was
found to be $410 \pm 30 \mu g/ml$ compared to $215 \pm 15 \mu g/ml$ for ramipril. 

$\alpha$-Lactalbumin hydrolysate was checked for the inhibitory activity against *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes*. The pattern of inhibition by the $\alpha$-lactalbumin hydrolysate against the different microbes namely *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes* is shown in Fig. 28-30 respectively. From the figures it can be seen that the hydrolysate thus obtained was having better anti-microbial activity against the above microbes.

The whey protein concentrate and $\alpha$-lactalbumin protein hydrolysates were shown to have lower ACE inhibitory effect compared to the synthetic antihypertensive ramipril ($IC_{50} = 215 \pm 15 \mu g/ml$). This does not negate against the application of the above protein-derived hydrolysates in the treatment/prevention of hypertension. It is to be expected that milk protein-derived ACE inhibitory peptides obtained from food source, unlike ramipril, would have no undesirable side effects. As a consequence, whey protein derived ACE inhibitory peptides/hydrolysates may find application as a nutraceutical in various physiologically functional foods. The antioxidant activity shown by the whey protein derived hydrolysates is much better compared to the ascorbic acid. Therefore, these hydrolysates can play a role as a potential and natural (food derived) antioxidants. These hydrolysates can be used in various foodstuffs and can be further utilized to prevent and cure various physiological diseases.
There are reports of sesame protein having proteolytic activity (Rajendran and Prakash, 1988). Studies on the hydrolysates obtained from this protein are limited. The protein was hydrolyzed by using protease namely subtilisin from *Bacillus subtilis*. Enzymatic preparation obtained from *Bacillus subtilis* contains two kinds of proteases (Millet, 1970), which have different catalytic mechanisms and therefore could be of interest in this process. Moreover, this is an adequate enzymatic preparation for food technology purposes, as well as being simpler and less expensive since two enzymes are contained in the same extract. The enzyme to substrate ratio was maintained at 1:20 (w/w) ratio. The details are explained under materials and methods section and the degree of hydrolysis was found to be 9 ± 2%. The hydrolysates thus obtained were checked for various bioactivities *in vitro*.

Antioxidant potential of the β-globulin hydrolysate was investigated by DPPH radical scavenging activity using ascorbic acid as standard. A high radical scavenging activity was observed in the β-globulin hydrolysate in a concentration dependent manner, the IC$_{50}$ values were found to be 0.20 ± 0.02 mg/ml (Table 11). The total antioxidant activity was found to be 1600 ± 80 μmol/g tocopherol equivalents. The β-globulin hydrolysates obtained above were also checked for angiotensin converting enzyme inhibitory activity. The IC$_{50}$ value of the β-globulin hydrolysate against ACE was 380 ± 25 μg/ml compared to 215 ± 15 μg/ml for ramipril. The antibacterial activity of the
β-globulin hydrolysate against the microbes namely *Escherichia coli*, *Listeria monocytogenes* and *Bacillus cereus* were found to have very marginal effect.

In summarizing the results of this chapter, the proteins WPC, α-La, β-globulin hydrolysates had lower ACE inhibitory potencies than the synthetic antihypertensive Ramipril (IC$_{50}$ = 215 ± 15 μg/ml) and does not negate against the application of the above protein-derived hydrolysates in the treatment/prevention of hypertension. It is to be expected that the protein-derived ACE inhibitory peptide rich hydrolysates obtained from food source, unlike Ramipril, would have no undesirable side effects. The protein derived ACE inhibitory peptides/hydrolysates may find application as a nutraceutical in various ‘physiologically functional foods’. The antioxidant activity shown by these protein-derived hydrolysates is much better compared to the standard antioxidant. So the obtained hydrolysates can play a role as potential food derived antioxidant. These hydrolysates can be used in various foodstuffs or can be further purified to know the peptide in the fraction, which is showing the bioactivity and can be used for the development of peptides in pharma and other applications.