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## Hydrophilic and hydrophobic peptides of probiotic fermented whey

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**Abstract**

Fermented milk products have many health-promoting effects, such as improvement of lactose metabolism, reduction of serum cholesterol and reduction of cancer risk. The beneficial health effects have been attributed to the release of bioactive peptides during fermentation. In this regard, a study was envisaged to prepare whey from WPC enriched low fat milk and to identify the peptides in probiotic fermented whey employing *Lactobacillus acidophilus* and *Bifidobacterium longum*. The designates of fractions in nano filtered SW<sub>4</sub> whey revealed presence of 4 to 5 hydrophobic peptides and 1 to 2 hydrophilic peptides in the permeate. Similarly, the permeate of SW<sub>5</sub> revealed 5 to 6 peptide fractions of hydrophobic nature and 2 to 4 hydrophilic peptides. Thus, there is an increase in fractions with increasing WPC levels. The fermentation by probiotics are thus beneficial in forming peptides. Further research is required for assessing their bio activity.

**Keywords:** Probiotic whey, *Lactobacillus acidophilus*, *Bifidobacterium longum*, peptides

**Introduction**

Probiotics are lactic acid bacteria like *Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Bifidobacterium* species. These are used as starter cultures for the preparation of fermented milk, owing to their effectiveness in the prevention and treatment of diverse spectrum of gastrointestinal disorders. The health or disease conditions can be improved or cured by probiotic lactobacilli and bifidobacteria both in human beings and animals. (Renner, 1986) [8]. During fermentation process protein and other nutrients are broken down resulting in formation of bioactive peptides as end products. Biologically active peptides have been found to have specific activities such as anti-hypertensive, anti oxidative, anti-microbial, immunomodulatory, opioid or mineral binding activities. Milk borne bioactive peptides are regarded as highly prominent ingredients for health promoting functional foods or pharmaceutical preparations (Clare and Swaisgood, 2000) [2].

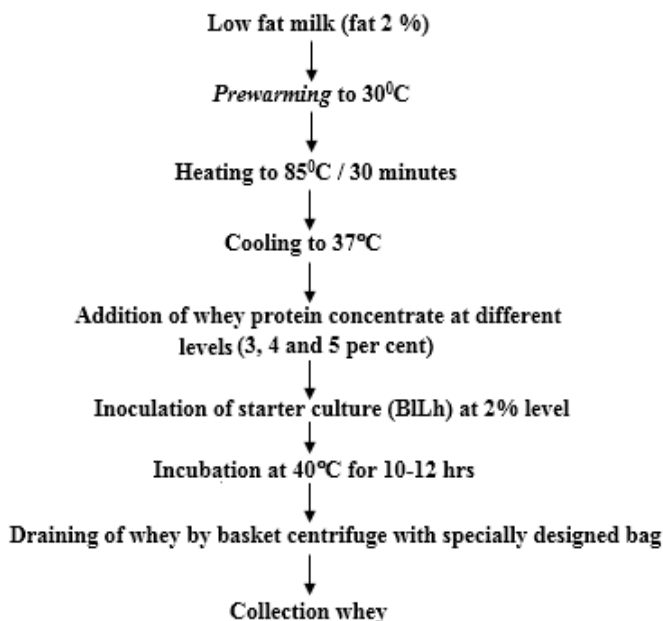
Whey proteins are very good source of essential amino acids especially sulphur containing amino acids. They are also very good growth promoters of the probiotic lactic cultures. Biological value, protein efficiency ratio, net protein utilization was high with whey protein compared to casein (Tamime *et al.*, 1995) [9].

**Materials and Methods****Preparation of probiotic fermented whey**

Preparation of probiotic fermented whey from low fat 2 per cent milk was heated to 85°C for 30 min and cooled to 37°C inoculated with 2 per cent single culture and 2 per cent combined culture separately.

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Low fat milk with 2 per cent fat, was preheated and filtered / clarified. The milk was pasteurized at 85 °C for 30 minutes and cooled to 37°C. Whey protein concentrate at different levels viz., 3, 4 and 5 per cent was added. Then it was inoculated with one per cent *Lactobacillus acidophilus* and one per cent *Bifidobacterium longum* and incubated at 40°C. When the curd has set firmly, it was broken and whey was drained by using basket centrifuge (1000-1200 rpm) using specially designed bag at the Department of Livestock Products Technology (Dairy Science), Madras Veterinary College, Chennai.

### Isolation of Bioactive Peptides

Two-step ultrafiltration process

Two-step ultra filtrations proposed by Gauthier *et al.* (2003) [3] was followed for isolating the bioactive peptides. The whey

permeate was first Ultrafiltered using a 30 KDa membrane in order to remove the enzyme and the non- hydrolyzed proteins. The retentate or reaction mixture was discarded whereas the Permeate so-called total hydrolysate (TH), was further fractionated using a 500 Da (Millipore) membrane (Philanto-Leppala *et al.* 1996) [7], giving a retentate composed of a mixture of polypeptides and a permeate composed of amino acids.

### Designation of fractions of peptides by RP-HPLC

10-50µg of lyophilized whey permeate was diluted with PBS to 40 ml, and subjected to reverse phase HPLC. The peptides were eluted by a linear gradient from 100 per cent solvent A to 40 per cent solvent B over 60 minutes at a flow rate of 1.0ml/minutes, and absorbance was detected at 215 nm (first-HPLC run). Six different combinations of probiotic culture using three single probiotic cultures viz. *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus helveticus* (Bl, La, Lh) at 2 per cent level and three combined cultures viz. (*Bifidobacterium longum* with *Lactobacillus acidophilus*), (*Bifidobacterium longum* with *Lactobacillus helveticus*) and (*Lactobacillus acidophilus* with *Lactobacillus helveticus*) (BILa, BILh, LaLh) at 2 per cent (1:1 ratio) were used. The optimum acidity of 0.9 to 1 per cent was reached by La, Bl, BILa cultures in 12 hours.

### Results

#### Peptide Fractions of Whey Obtained from WPC Added Probiotic Whey

Preliminary trial on PAGE was done to identify the peptide fractions in whey. The filtrate obtained by ultrafiltration was then subjected to nanofiltration at a cut off of 40 kDa and 500 Daltons. The permeate of SW<sub>4</sub> and SW<sub>5</sub> whey were used to run RP-HPLC. The graph obtained is presented as Figure 1 & 2. Addition of WPC increased the total solids content as well as yield of chakka and this was supported by Jayaprakash (1998) [4]. Further, it is observed that, addition of WPC increased the formation of peptides during fermentation.

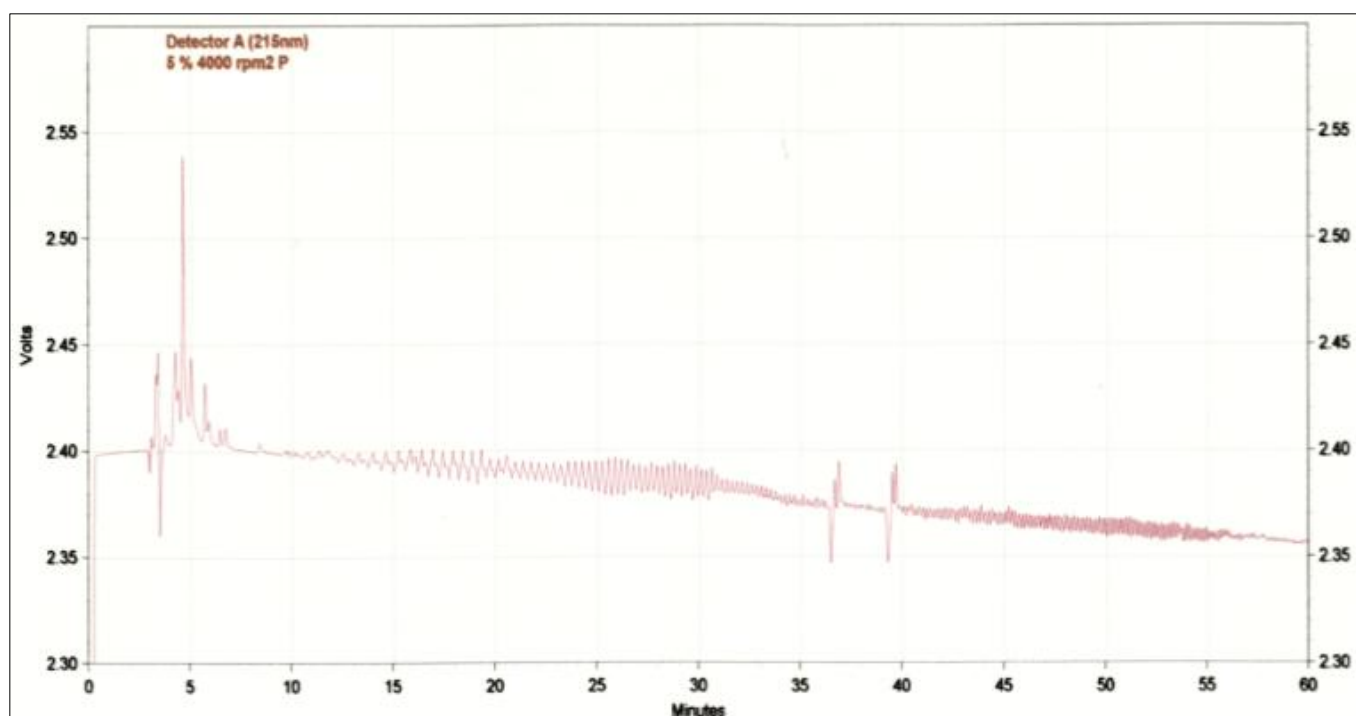


Fig 1

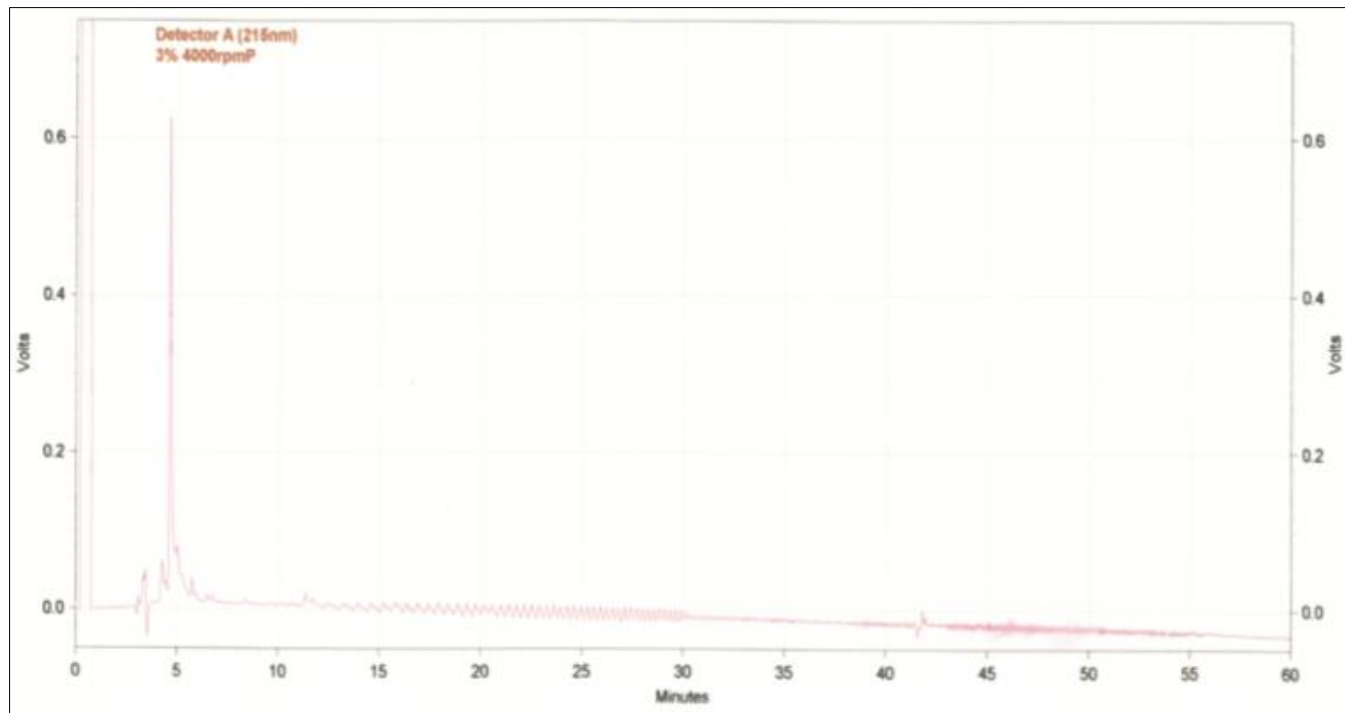


Fig 2

### Discussion

The end product of fermentation, “peptides” were isolated from the fermented milk by ultrafiltration followed by nanofiltration to less than 30 KDA. By reverse phase high performance liquid chromatography (Yamamoto *et al.*, 1999) [6] revealed the peptide peaks in whey from 4 per cent and 5 per cent level of substitution of WPC designate.

Nakamura *et al.* (1995) [10] reported that bioactive peptides were released from caseins and whey proteins. During fermentation of milk with various cultures. The two ACE – inhibitory peptides (Val-pro-pro and Ile-pro-pro) in milk that was fermented with a starter culture composed of *L. helveticus*.

Preliminary trial on PAGE showed the presence of peptide bands in whey (Takano, 2002). The designates of fractions in nanofiltered SW<sub>4</sub> whey revealed presence of 4 to 5 hydrophobic peptides and 1 to 2 hydrophilic peptides in the permeate. Similarly, the permeate of SW<sub>5</sub> revealed 5 to 6 peptide fractions of hydrophobic nature and 2 to 4 hydrophilic peptides. Thus, there is an increase in fractions with increasing WPC levels. Marshall and Tamime (1997) [5] observed that the peptides were formed and amino acids degraded from milk proteins during fermentation. The fermentation by probiotics are thus beneficial in forming peptides. Further research is required for assessing their bio activity.

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