

## Value addition to soybean whey through microbial and enzymatic intervention

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The wide range of functional properties of soy protein and its high nutritive value makes it base of a novel food platform. Various products are in the market like soy protein isolate, soy protein concentrate, tofu, soymilk which are well accepted by the consumers. Nevertheless, soybean processing operations generate a large proportion of liquid effluent termed as soybean whey. This yellowish liquid waste can be functionally and nutritionally valuable because of their nutrient composition. Discarded whey is not only accountable for pollution problem, but also represents an economic and nutritional penalty in this era. Till now, there are only few reports on effective use of soybean whey, for this reason the present article emphasis to summarize all the extensive research developed for its utilization, so that soybean whey can be well recognized as a potential feedstock both for the microbial and enzymatic intervention.

**Keywords:** Soybean whey, microbial and enzymatic intervention, value addition

### Introduction

Soybean whey, by product of the tofu processing industry is a greenish-yellow liquid that remains after curdling. This particular liquid waste is drained off in considerably large amounts, about 85-90% of the total soymilk<sup>1</sup>. Usually, whey has been disposed of as sewage or on unused land. Disposal of soybean whey not only results in to water pollution, but also represents an economic penalty against the process. The batch processing of soybean to soy protein isolate resulted one third part of processing in the form of whey which possess chemical oxygen demand (COD) of 25,000 mg/L<sup>2</sup>. Even high biological oxygen demand (BOD) of 13,730 ppm can be a major threat to the environment on waste disposal<sup>3</sup>. For this reason extensive research is required to develop a suitable technology and to promote utilization of such organic waste.

### Composition of Soy Whey

Soy whey is majorly composed of proteins which are found to be functionally and nutritionally valuable<sup>4</sup>. In general, it has been observed that with the type, amount of coagulant and processing method the whey protein content varies. Rackis *et al*<sup>5</sup> studied the quality characteristics of four types of soy whey,

generated during soybean processing (Fig. 1). Alcohol extraction for protein concentrate preparation from soybean meal resulted in least amount of whey protein fraction (2.2%), while acid leach and whey released from protein isolate prepared by the method of Circle *et al*<sup>6</sup> resulted in considerable amount of protein 15.2 and 15.4%, respectively. Highest amount of protein content (39.05%) was noticed in hot leach process. Such results confirm that though soy whey contain good amount of protein, the ratio of these components differ with processing method and from batch to batch. Therefore, it is essential to carry out proper biochemical characterization of whey so that a more experimental approach can be chosen for its utilization.

Soy whey apart from being rich in proteins<sup>7</sup> is also a good source of polyunsaturated fats, minerals and bioactive substances such as isoflavones, oligosaccharides etc (Table 1)<sup>8-9</sup>. Stachyose (6.4 g/L), raffinose (1.6 g/L), sucrose (11.3 g/L), fructose (1.1 g/L) and glucose (1.2 g/L) are present in significant amount<sup>10</sup>.

### Biotechnological Intervention

#### Microbial Intervention

Utilization of soy whey dates back to 1964, when a research group from Northern Regional Research Laboratory, U.S. Department of Agriculture, Illinois utilized this particular byproduct for growing

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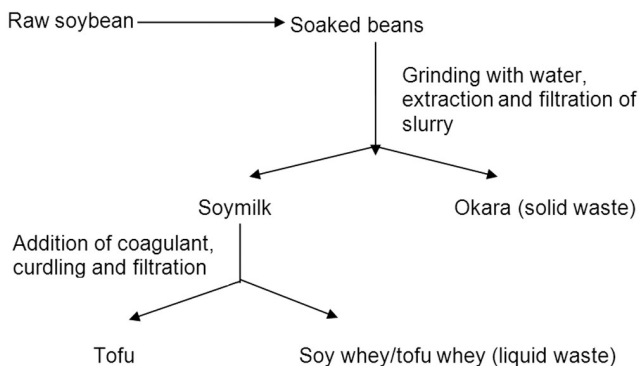


Fig. 1 — Waste generated during tofu preparation.

Table 1 — Physico-chemical characterization of soy whey<sup>28</sup>

Dry matter (DM)	89.7 ± 0.8
Sucrose (% w/w DM)	21
Raffinose (% w/w DM)	3.7
Stachyose (% w/w DM)	16.3
Fructose (% w/w DM)	2.6
Glucose (% w/w DM)	5.4
Protein (% w/w DM)	17.1 ± 0.88
Mineral (mg/g DM)	112

mushrooms<sup>11</sup>. Among eight fungi assessed for mycelial growth only two showed faster growth while one fungus *Agaricus campestris* failed to survive. *Tricholoma nudum* in presence of ammonium acetate and *Boletus indecisus* showed the highest rate of growth in the form of sphere. By the use of concentrated soy whey up to 50% as a medium, the rate of mycelial growth of both the fungi, *T. nudum* and *B. indecisus* were doubled. The work confirmed that the whey nitrogen can be converted to mycelial protein more efficiently.

An attempt to convert the soy whey into nutritious beverage through microbial fermentation was done by Pinthong in 1986<sup>12</sup>. The author initially treated soy whey with microbes (*Leuconostoc mesenteroides* B35 and *Saccharomyces cerevisiae* 3051) to get rid of flatulence creating oligosaccharides and beany flavour. Fermentation of soya whey by *L. mesenteroides* B35 resulted in sweet fruity while *Saccharomyces cerevisiae* 3051 fermented soy whey had soy sauce odour. This study was the first report on utilization of soy whey as food. Khare *et al*<sup>13</sup> inspected that the popularization of soy milk and tofu among consumers will lead to rapid increase in the soy whey disposal problem. Hence, the research group utilized soy whey as a suitable substrate for the citric acid production. Immobilization of *Aspergillus*

*niger* in agarose beads were used for this purpose. Initial trials of experiment resulted in only 5.25 g/L of citric acid due to low sugar content. Therefore, soy whey supplemented with 10% sucrose was used as medium to harvest the citric acid. After 10<sup>th</sup> day of incubation maximum citric acid yields of 15.5 g/L and 19 g/L with free and immobilized cells, respectively, were recorded. Repeated batch studies, where whey was replaced after 48 h resulted in an enhanced yield (up to 27 g/L) when immobilized cells were used. It was concluded from the experimentation that like milk whey, soy whey is also a nutritive medium for viable production of citric acid. Concoction of soy whey with wheat bran and okara (solid waste product generated in the production of soymilk) was evaluated as substrate for  $\alpha$ -galactosidase production by Song and Chang<sup>14</sup>. One of the major reasons for the water pollution is the disposal of soy whey. Hence, this major work focussed on its utilization in an economically feasible way with cost reduction during waste treatment. The crude enzymes produced by *Aspergillus awamori* NRRL 4869 exhibited the highest activity on soy whey supplemented substrates.

Soy whey is well composed of indigestible oligosaccharides raffinose and stachyose, in combination with other degradable sugar like sucrose, glucose and fructose<sup>15</sup>. Only few studies have been carried out till date for the utilization of soy whey for probiotic growth. Nguyen *et al*<sup>16</sup> evaluated soy whey as a growth medium for a *Lactobacillus paracasei* ssp. *paracasei*. Their results demonstrated that relatively high viable counts of these lactobacilli could be obtained in glucose buffered tofu whey in comparison to cow milk. Studies on recovery of magnesium and protein from soy whey using a two step process are gaining importance. But the major issue still residing is its disposal because whey permeate contains almost the total fermentable sugars and isoflavones. Ounis *et al*<sup>17</sup> overcame this issue by using electro membrane processed whey (45% and 54% of the recovered initial protein and mineral contents, respectively) as a growth medium for *Lactobacillus plantarum* LB17. The study suggested that demineralized whey is a potent low cost substrate for the *Lactobacillus* strain because more than 60% of oligosaccharides such as stachyose, raffinose and sucrose were metabolized by the bacteria.

A study conducted by Fung and coworker<sup>10</sup> strongly recommended that the fermented whey could be developed into a functional beverage. After

analyzing microbial growth, pH and titratable acidity it has been observed that lactic acid production was 10.84 mg/mL while acetic acid was 7.23 mg/mL. Strains used were *Lactobacillus acidophilus* FTCC 0291, *L. casei* FTCC 0442, *L. fermentum* FTD 13, and *Bifidobacterium bifidum* BB12, *L. acidophilus* ATCC 4962. Out of these five strains, *L. acidophilus* FTCC 0291 showed increased utilization of oligosaccharides and reducing sugars. Fung and co-worker in 2010<sup>18</sup> further studied the proteolytic activity of *L. acidophilus* FTCC 0291 for bioactive peptide synthesis during probiotic fermentation of soy whey. It was the first report that showed soy whey conversion to bioactive beverage.

Nisin production by using crude soy whey as medium was attempted by Mitra *et al*<sup>2</sup>. Biomass yield of 2.18 g/L and nisin yield of 619 mg/L was obtained from 200 mL soy whey. Further supplementation of the soy whey with micronutrients and sucrose led to 26% and 8% increase in biomass yields, respectively. Such studies confirmed the possibility of adding value to a waste stream by harvesting bioactive component through microbial growth. Kurniasari *et al*<sup>19</sup> evaluated the fermented soy whey broth as a replacement for Kecutan, a soymilk coagulant by using *Pediococcus acidilactici* F11 strain. The research group, supplemented whey with coconut water as carbon source at concentration of 25% and 50%. Fermented soy whey with coconut water, met the required condition of soymilk coagulant. A 25% supplementation of coconut water resulted in 38.59 mg/L of lactic acid in fermented whey. The study demonstrated that the fermented soy whey is an alternative coagulant which can be affordable and applicable in tofu industry. Singh *et al*<sup>20</sup> studied probiotic fermentation of soy whey by using mixed culture of *L. acidophilus*, *L. rhamnosus*, *B. longum*, *B. bifidum* and *Sachharomyces boulardii*. The optimum fermentation conditions were: incubation time 9.64 h; heating temperature 45.1°C; inoculum volume 7.96%; incubation temperature 34.56°C and heating time 5 min reflected in better radical scavenging, reducing power and metal chelation in comparison to the unfermented whey. The research group proposed that it might be due to antioxidant peptides and phenolics produced during fermentation which could react with free radicals to stabilize and terminate radical chain reactions. Researcher<sup>21</sup> explored whey as a liquid culture media for *Mycobacterium* strain. Soy whey supplemented with additives such as

albumin, dextrose, catalase, glycerol, potassium sulphate, magnesium citrate and sodium glutamate appeared to be cost-effective and economical substitute for Middlebrook 7H9 media in resource-limited settings.

Roopashri and Varadaraj<sup>22</sup> reported that *L. plantarum* culturing in soy whey broth supplemented with peptone, yeast extract, galactose and requisite salts resulted in substantial  $\alpha$ -D-galactosidase production and antibacterial activities. The group also explored potential of soy whey growth media for an appreciable phytase production using culture of *S. cerevisiae* MTCC 5421. Such elaborated study on soy whey, by taking into the account, phytate and oligosaccharide content opens a new avenue for enzyme production. Suwanposri *et al*<sup>23</sup> used soy whey for bio-cellulose production by *Komagataeibacter* sp. PAP1. The use of whey increased bio-cellulose production 3.6 fold compared to standard Hestrin-Schramm (HS) medium. Such results demonstrate that soy whey can be used as an alternative low-cost substrate for bio-cellulose production on commercial scale.

Enrichment of antioxidant phenolics through fermentation is well known technique. Xiao *et al*<sup>24</sup> used *L. plantarum* B1-6 for enriching soy whey with phenolics mainly isoflavone glycones. Fermented soybean whey showed better radical scavenging activity, ferric reducing antioxidant power and exhibited greater protection against oxidative DNA damage. Such study is in agreement with the other studies which suggest that fermented soy whey can be a better alternative as beverages in terms of nutrition and health. Effective use of soy whey as growth medium have been explored by researchers but still, it is a challenge to use whey at commercial scale because most of the studies has been done on small or laboratory scale for research purposes only. Nevertheless, by the right approach, soy whey could easily be incorporated into industry production lines.

#### Enzymatic Intervention

These studies are associated with the soy whey hydrolysate formation considering the functional and immunological properties assessment of the waste protein. Penas *et al*<sup>7</sup> studied the effect of high-pressure treatment on the hydrolysis of soy whey proteins by using digestive enzymes (trypsin, chymotrypsin and pepsin). The comparative studies were carried out at an atmospheric pressure (0.1 MPa

for 30 min at 37°C) and at high pressure condition (100 and 200 MPa for 15 min at 37°C). At high pressure conditions of 100 MPa, enhanced hydrolysis was observed. Hydrolysate contained 5 peptides lower than 14 kDa after hydrolysis by pancreatic enzymes (chymotrypsin and trypsin) and 11 peptides by pepsin under high pressure treatment. In 2006, Penas and coworker<sup>25</sup> studied the soy whey hydrolysis with alcalase, neutrase, corolase 7089 and PN-L by varying high pressure treatment from 100 MPa for a period of 15 min. Further increase in pressures higher than 300 MPa caused changes in the solubility of the proteins and consequently to precipitation. The result showed that 200 and 300 MPa proved to be efficient pressure parameters. Soy proteins have reported for some sensitive allergic responses in gastrointestinal, cutaneous, and respiratory symptoms. Therefore, Penas and coworker<sup>25</sup> further extended their work for assessing the residual immunoreactivity of soy whey hydrolysates after enzymatic (alcalase, neutrase and corolase PN-L) hydrolysis combined with high pressure. Negligible allergenicity was observed in the hydrolysates obtained by alcalase and corolase PN-L while with neutrase high pressure treatment at 300 MPa showed effective means of reducing antigenicity. The studies also showed that the high pressure treated whey have less immunochemical response to anti-Gly monoclonal antibodies.

Recently, Matemu *et al*<sup>26</sup> studied functional properties of whey hydrolyzed by Protease M 'Amano' G, and resulting peptide mixtures were acylated with esterified fatty acids. Improved emulsion properties was noticed in peptide mixture as hydrolysate had a good balance of both hydrophilic and hydrophobic amino groups which is highly essential during protein oil interaction. The study provided a new means to utilize the soy whey hydrolysate as a novel emulsifier in food processing. Apart from the functional properties, protein hydrolysates also exert positive health benefits via bioactive peptides. Until now, the bioactive peptide production and characterization from soy whey hydrolysates has not been investigated systematically. Singh and Banerjee<sup>27</sup> converted this proteinaceous waste into bioactive peptide enriched hydrolysate. Soy whey protein was enzymatically treated with the *Aspergillus awamori* Nakazawa protease. Remarkable increase in the radical scavenging activity upto 70% was noticed at the optimized condition.

A new dimension to valorization of soy whey as a medium to harvest value added biochemical has been given by Corzo-Martinez *et al*<sup>28</sup> where the group used soy whey for the efficient synthesis of lactosucrose, a potential prebiotic oligosaccharide. The enzyme levansucrase SacB from *Bacillus subtilis* CECT 39 was used which transfructosylate lactose present in cheese whey permeate by using sucrose, raffinose and stachyose present in soy whey, as suitable donors of fructosyl moieties. Within a short reaction time, 80.1 g L<sup>-1</sup> of lactosucrose was obtained at 37°C. Such findings of enzymatic intervention open a new avenue to valorize this by products as renewable substrates for harvesting value added products.

### Conclusion

From the foregoing account, it is confirmed that the soy whey belongs to a group of cheap, sustainable feedstock which have a potential to be used in various segments of industry. Its application will greatly facilitate in alleviating the dependence upon expensive media and feedstock for microbial propagation and at the same time opens up a new avenue of generating the biofunctional compounds.

### Reference

- 1 Shurtleff W & Aoyagi A, 2000 Tofu & soymilk production, The book of tofu, volume II, A Craft and Technical Manual, Soyfoods Center.
- 2 Mitra D, Pometto A L III, Khanal S K, Karki B, Brehm-Stecher B F *et al*, Value-added production of nisin from soy whey, *Appl Biochem Biotechnol*, 162 (2010) 1819–1833.
- 3 Monajjemi M, Nurul Aminin A L, Ilkhani A R & Mollaamin F, Nano study of antioxidant activities of fermented soy whey prepared with lactic acid bacteria and kefir, *Afr J Microbiol Res*, 6 (2012) 426–430.
- 4 Rackis J J, Anderson R L, Sesame H A, Smith A K & Van Etton C H, Amino acids in soybean hulls and oil meal fractions, *J Agric Food Chem*, 9 (1961) 409–412.
- 5 Rackis J J, Honig D H, Sessa D J & Cavins J F, Soybean whey proteins—recovery and amino acid analysis, *J Food Sci*, 36 (1971) 10–13.
- 6 Circle S J, Julian P L & Whitney R W, Process for isolating soya protein, U.S. Patent (1959) 2, 881, 159.
- 7 Peñas E, Préstamo G & Gomez R, High pressure and the enzymatic hydrolysis of soybean whey proteins, *Food Chem* 85, 641–648.
- 8 Bazinet L, Ippersiel D & Lamarche F, Recovery of magnesium and protein from soy tofu whey by electro-dialytic configurations, *J Chem Tech Biotechnol*, 74 (1999) 663–668.
- 9 Sobral P A & Wagner J R, Relationship between the composition and antitryptic activity of soy and tofu wheys and thermal behavior of their isolated protein, *Informacion Tecnologica*, 20 (2009) 65–74.

- 10 Fung W Y & Liong M T, Evaluation of proteolytic and ACE-inhibitory activity of *Lactobacillus acidophilus* in soy whey growth medium via response surface methodology, *LWT Food Sci Technol*, 43 (2010) 563–567.
- 11 Falanghe H, Smith A K & Rackis J J, Production of fungal mycelial protein in submerged culture of soybean whey, *Appl Microbiol*, 12 (1964) 330–334.
- 12 Pinthong R, Degradation of undesirable components in soybean whey, Progress Report of USAID / P STC Program (1986).
- 13 Khare S K, Jha K & Ghandhi A P, Use of agarose-entrapped *Aspergillus niger* cells for the production of citric acid from soy whey, *Appl Microbiol Biotechnol* 5 (1994) 571–573.
- 14 Song D & Chang S K C, Enzymatic degradation of oligosaccharides in Pinto bean flour, *J Agric Food Chem*, 54 (2006) 1296–1301.
- 15 Tenorio MD, Espinosa-Martos I, Préstamo G & Rupérez P, Soybean whey enhance mineral balance and caecal fermentation in rats, *Eur J Nutr*, 49 (2010) 155–163.
- 16 Nguyen T L, Champagne C P, Lee B H & Goulet J, Growth of *Lactobacillus paracasei* ssp. *paracasei* on tofu whey, *Int J Food Microbiol*, 89 (2003) 67–75.
- 17 Ounis W B, Champagne C P, Makhouloufa J & Bazineta L, Utilization of tofu whey pre-treated by electromembrane process as a growth medium for *Lactobacillus plantarum* LB17, *Desalination*, 229 (2008) 192–203.
- 18 Fung W Y, Woo Y P & Liong M T, Optimization of growth of *Lactobacillus acidophilus* FTCC 0291 and evaluation of growth characteristics in soy whey medium: a response surface methodology approach, *J Agric Food Chem*, 56 (2008) 7910–7918.
- 19 Kurniasari N, Sidar A, Rahayu E S, Utami T, The growth of *Pediococcus acidilactici* f-11 in tofu whey, proceeding of the 3<sup>rd</sup> international Conference of Indonesian Society for lactic acid bacteria (3rdic-islab): better life with lactic acid bacteria: Exploring Novel Functions of Lactic Acid Bacteria, (2011)
- 20 Singh A, Adak S & Banerjee R, Non dairy probiotic beverage as potent functional food. In *Advances in Industrial Biotechnology*. I.K International Publishing House Pvt. Ltd., New Delhi, (2012), 219–232.
- 21 Masangkey R F, The performance of tofu-whey as a liquid medium in the propagation of *Mycobacterium tuberculosis* strain H37Rv, *Int J Mycobacteriol*, 1 (2012) 45–50.
- 22 Roopashri A N & Varadaraj M C, Soy whey based medium for optimized phytase activity in *Saccharomyces cerevisiae* MTCC 5421 and  $\alpha$ -D-galactosidase and antibacterial activities in *Lactobacillus plantarum* MTCC 5422 by response surface methodology, *J Food Sci Technol*, 51 (2014) 519–526.
- 23 Suwanposri A, Yukphan P, Yamada Y & Ochaikul D, Statistical optimisation of culture conditions for biocellulose production by *Komagataeibacter* sp. PAPI using soya bean whey, *Maejo Int J Sci Technol*, 8 (2014) 1–14.
- 24 Xiao Y, Wang L, Rui X, Li W, Chen X, Jiang M & Dong M, Enhancement of the antioxidant capacity of soy whey by fermentation with *Lactobacillus plantarum* B1-6, *J Funct Food*, 12 (2015) 33–44.
- 25 Peñas E, Préstamo G, Polo F & Gomez R, Enzymatic proteolysis, under high pressure of soybean whey: Analysis of peptides and the allergen Gly ml in the hydrolysates, *Food Chem*, 99 (2006) 569–573.
- 26 Matemu A O, Kayahara H, Murasawa H, Katayama S & Nakamura S, Improved emulsifying properties of soy proteins by acylation with saturated fatty acids, *Food Chem*, 124 (2011) 596–602.
- 27 Singh A, Banerjee R (2013) Peptide enriched functional food adjunct from soy whey, a statistical optimization study. *Food Sci Biotechnol*, 22:65–71
- 28 Corzo-Martinez M, Luscher A, de las Rivas B, Muñoz R, Moreno F J, Valorization of cheese and tofu whey through enzymatic synthesis of lactosucrose, *PLoS ONE*, 10 (2015) e0139035.