Amino acid analysis of soybean based synbiotic product by derivatization free high performance liquid chromatography

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INTRODUCTION

Emerging health consciousness during pandemic led the ways towards various trends in the societies all over the world. Probiotics had been important of them, but Synbiotics is a step ahead which combines probiotic and prebiotic. Probiotics are defined as selected, viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their effects in the intestinal tract. Some selected strains of Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus and Saccharomyces have been promoted in food products because of their reputed health benefits (Sharma et al., 2012). Prebiotic oligosaccharides are Non-Digestible Oligosaccharides (NDO) and low calorific compounds stimulating the growth and development of gastro-intestinal microflora described as probiotic bacteria. Dietary carbohydrates that show prebiotic ability include fructans, fructooligosaccharides (FOS) and inulin, galactooligosaccharides (GOS), polydextrose, resistant starch, soyoligosaccharides, xylooligosaccharides, isomaltooligosaccharides, and lactulose (Patel and Goyal, 2012). The positive influence of prebiotic substances, in intestinal flora has been tested in several studies, where the utilization of probiotic species in combination with prebiotic substances provides a combined effect called “synbiotic”.

Soybean (Glycine max) currently constitutes an important source of good quality vegetable protein and is considered as interesting alternative to the consumption of animal proteins. Soybeans are mainly composed of protein but also contain good amounts of carbs and fat. Soy consumption provides many health benefits due to the presence of beneficial compounds such as isoflavones. Interest in soy-based products has been steadily increasing and new products are being developed.

Beet root (Beta vulgaris) is being exploited as potent prebiotic in the recent past due to high Fructooligosaccharide percentage. Fructooligosaccharide are complex and slow carbohydrates are considered a functional food ingredient as it influences physiological and biochemical processes in humans. This results in better health and a reduction in the risk of many diseases. FOS is increasingly included in food products and infant formulas, as their prebiotic effect stimulates the growth of non-pathogenic gut micro flora. Consumption of FOS increases the faecal bolus and the frequency of deposits.

In our research, Combination of Soybean and beet root extract in 5:2 ratio is being used as liquid medium to conduct fermentation using novel strains of Lcasei, B.subtilis and S.blaurdii. Further, amino acid content is being examined using derivatization free RP-HPLC (Qualitative only).

2. MATERIAL AND METHODS

2.1. Mother culture preparation

The experiments starts with successful isolation of microorganisms are done by the biochemical analysis and each strain is cultures individually. The Lactobacillus casei and Bacillus subtilis strain is cultured in the MRS (peptone 10g/l, yeast extract 5g/l, beef extract 10g/l, sodium acetate anhydrous 5g/l, ammonium citrate tribasic 2g/l, KHPO4 2g/l, MgSO4.7H2O broth at 37°C for 20 h. The strain Saccharomyces blaurdii is cultured in the SD (Dextrose 40 g/l, peptone 10g/l) broth at 35°C for 18 hours. The Cells then harvested and resusppended in the sterilized physiological saline solution and adjusted to 10^6 CFU/ml.
2.2. Liquid medium preparation
The Soybean is cleaned and soaked in the water overnight. Dehull soybeans (remove the seed coat this can decrease the beany flavour at the final Product) and Discard the soaking water. Pre-treat the soybean with the sodium bicarbonate. Grinding of soybean with water in 1:1 ratio. Strain the soy milk and cooked at 110°C for 2-3 min, this will remove volatile beany flavour. By filtration remove the soy milk from soya pulp.
Beetroot (local market) is peeled and grounded with the grinding machine. Coarse particle content was separated gravimetrically by centrifuging beetroot Juice at 1400rpm for 20 minutes. Juice was electrically heated at a moderate temperature of 50°C. The beetroot juice is concentrated at 100°C.
Then, soy and beet extracts are combined 5:2 ratio.

2.3. Fermentation
The substrate is inoculated with 10⁶ CFU/ml Lactobacillus casei, Bacillus subtilis and Saccharomyces blaurdii in the Fermentor and allows fermenting for 37°C for 48 hour aerobically. Further, Analysis of amino acid in fermented liquid using derivatization free RP-HPLC is carried out.

2.4. RP-HPLC method development
System of Thermo Scientific UHPLC-Ultimate 3000 is used for analysis with C-18 (250mm×4.6mm×5µ) column primarily for RP-HPLC of sample. DAD (Diode Array Detector) is used for detection at 262nm.

2.4.1. Material
Standard amino acids; HPLC-grade acetone, ACN (Acetonitrile), Water; sodium acetate and others were of high purity availed by Sigma Aldrich.

2.4.2. Standard Solution
Standard solutions of free amino acids have been prepared with distilled water at 0.03M concentration and each stored in refrigerator.

2.4.3. Chromatography solvent system
Gradient system of following three solvents
Solvent A: 0.05M sodium acetate, pH 7.2
Solvent B: 0.1M sodium acetate: ACN (22:21), pH 7.2
Solvent C: ACN
Column Temperature: 50°C

3. RESULT AND DISCUSSION
3.1. Microbial count
Observation and monitoring of process of fermentation shown remarkable increase in the microbial count from 24 to 36 hr and then entered in stationary phase. As shown in Fig1.1, At 48 h of fermentation the viable count of L.casei was 1.74×10⁶, B.subtilis was 1.63×10⁶ and S.blaurdii was 1.41×10⁶. Later on, viable count decreased slowly.
Figure 1.1: growth curve of probiotic strains L.casei, B.subtilis and S.blaurdii respectively on various fermentation time.

3.2. PH

Figure 1.2 shows the pH valued obtained at various fermentation times. Analysis can ensure maximum activity of microbes approximately around 48 h of fermentation.

Figure 1.2: pH values at different fermentation time
3.3 Amino acid analysis

Figure1.4 and Table 1.1 infer that fermentation process leads to increase in amino acid content of the substrate in each observable amino acid as Tryptophan, Leucine, Isoleucine, Lysine, Valine, Cysteine, Glutamic acid, Glycine and Serine. The highest percentage of increase in concentration of amino acid is observed in Glutamic acid, Leucine, Isoleucine and Lysine.

![Retention time vs. mAU at 262nm](image)

Figure 1.3: RP-HPLC chromatogram showing peaks of Glu (Glutamic acid), Ser (serine), Gly (Glycine), Val (Valine), Ile (Isoleucine), Trp (tryptophan), Cys (cysteine), Lys (lysine), and Leu (Leucine) on running the sample for 50 min after pre-treatment of filtration by 4.5µ micro syringe filters.

<table>
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<tr>
<th>Ret. Time (min)</th>
<th>Peak Name</th>
<th>mAU (262nm)</th>
<th>Rel. Area (%)</th>
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<tr>
<td>3.46</td>
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<td>6.97</td>
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<td>Serine (Ser)</td>
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4. CONCLUSION

Based on the results of present study, It can be concluded that soy and beet root can serve as a medium of fermentation with novel probiotic strains of microorganisms. This research has made a rational that L.casei, B.subtilis and S.blaurdii shows exponential
growth on soy and beet extract with promising cell viable count for 48 h of fermentation. Future prospects include, Production of synbiotics from fermented liquid has potential and also functional food can be prepared by processing it as beverage, synbiotic tofu, synbiotic protein powder, supplements, etc. Products will show high amino acid content which directly enhances immunity as well as can be source of protein with synbiotic traits for lactose intolerant, Gluten intolerant and Vegan population. Health and food inclusion can be achieved using this research on practical grounds.

REFERENCES

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