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Research Article

EFFECT OF ROASTING ON PREBIOTIC POTENTIAL OF SOYABEAN (*Glycine max*)

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ABSTRACT

The purpose of this study was to investigate the effect of roasting on prebiotic potential of Soyabean (*Glycine max*). Administration of prebiotic confers health benefits by modulating immune system. Soyabean pomace left after extraction of protein and lipid is good source of oligosaccharides and non-starch polysaccharides, which have ability to reach the colon, where it could get fermented and produce health beneficial products such as short chain fatty acids. The purpose of roasting was to minimize the antinutrient factors from raw soyabean. Soyabean was roasted and its ability to support the growth of *Lactobacillus acidophilus* was checked in reconstituted agar containing raw and roasted Soyabean as a sole carbon source. After incubation samples were tested for optical density, pH, % titratable acidity and antimicrobial effect against pathogen. Difference in raw and roasted soyabean was checked by subjecting the samples to SEM and X–ray diffraction. Increased optical density, lowering of pH and increase in titratable acidity in presence of roasted soyabean, confirmed the ability of roasted Soyabean to support the growth of *L. acidophilus*.

Keywords: Prebiotics, Soyabean *(Glycine max*), *Lactobacillus acidophilus,* roasting

INTRODUCTION

Gut is complex community where large variety of microorganisms harbors[1]. Microorganisms in the gut play an important role in maintaining wellbeing of human. Healthy microbial flora prevents proliferation of pathogens and boost immune system. Difference in microbial composition of healthy person and diseased person was reported[2]. Intestinal bowl diseased (IBD) person has decreased count of healthy gut flora when compared to healthy one. Restoration of gut flora with administration of prebiotic could enhance the therapeutic effects of drugs used to treat IBD. Prebiotic are indigestible substances which are having ability to reach the colon. Fermentation of indigestible substances in colon by resident bacteria produces short chain fatty acids (SCFAs)[3]. SCFAs mainly acetate, proprionate and butyrate. Butyrate produced by bacteria have positive effect on colonocytes and maintains proliferation of colonocytes[4]. Acid production resulted into lowering of pH. Acidic environment prevent proliferation of pathogenic microorganism. This would definitely help to treat intestinal diseases like IBD. Prophylactic role of prebiotic in prevention of IBD reported by researchers. Prevention of colitis by combination of fructooligosaccharide and inulin in HLA-B27 transgenic mice was reported by Hoentjen F[5.] Increased beneficial microbial count and increased concentration of butyrate showed attenuating effect on macroscopic and histological inflammation in HLA-B27 transgenic rats. Thus previous research described the attenuation of IBD by administration of prebiotic[6,7]

Soymilk and tofu prepared from Soyabean (*Glycine max*) highly consumed by people of Southeast Asia. Soymilk considered as one of nutritious health drink used widely as dairy milk alternative for those who shows lactose intolerant. Soya tofu, a protein rich paneer prepared form Soyabean. After extraction of essentials remaining pomace is used as in animal and poultry industry. Soyabean is rich source of protein, lipids, minerals and vitamin B. Soyabean also contains isoflavones, saponins, phytates, protease inhibitors, phenolic acids, lecithin, dietary fiber, phytosterols, and omega-3-fatty acids[8]. *In vitro* fermentability of Soyabean okara was already reported[9]. But protease inhibitors, lectins (hemagglutinins), and allergens (glycinin and β-conglycinin) are antinutritional factors present in raw soyabean, which needs to be eliminated. All these antinutritional factors are unstable to heat. Along with heat unstable antinutritional factors, some nutritional heat stable oligosaccharides, non starch polysaccharides, and phytates are also present in Soyabean which can confer health promoting effects. Thus from the literature it was found that roasting of raw Soybean could enhance the nutritional property of Soyabean. Although various studies have established the positive health benefits of prebiotics and Soyabean, it would be useful to understand the action of roasting on dietary fibre of Soyabean that contribute as significant sources of fiber and protein in a normal balanced diet. Thus the aim of present investigation was to evaluate the effect of roasting on prebiotic potential of Soyabean.

MATERIALS AND METHODS

Soyabean (*Glycine max*) was purchased from local market and authenticated by Agharkar Research Institute, Pune, India. *Lactobacillus acidophilus* (NCIM-5426) purchased from National Collection of Industrial Microorganisms, Pune. deMan Rogosa Sharpe agar, Nutrient agar and MacConkey agar other ingredients used to prepare media was purchased from Himedia, Mumbai, India. Other chemicals used of analytical grade.

Physicochemical investigation

Crude fibre

The sample was treated successively with boiling solutions of sulphuric acid and potassium hydroxide of specified concentrations. The residue was separated by filteration (w2), washed, dried, weighed and kept to form ash within a range of 475 or 500 °C. The loss of weight resulting from ash corresponds to the crude fibre present in the sample (w3).[10]

Percent crude fibre = [(W2-W1)-(W3-W1)/ Fresh weight of the sample] * 100

Sample was roasted in hot air oven at 121°C for 30 min. Sample was subjected to Scanning electron microscopy (SEM) and X-ray diffraction (XRD) to evaluate the effect of roasting.

Scanning Electron Microscopy (SEM)

The scanning electron microscopy (SEM) was performed with a scanning electron microscope (XL-30-ESEM, FEI, Holland), using a voltage of 15 kV. Samples were fixed with double-sided tape onto aluminium cylinders and coated with a layer of gold.

X-ray Diffraction

X-ray Diffraction patterns were obtained with an X-ray diffractometer Brucker D8 Advanced equipped with a copper anode X-ray tube. The diffractometer was operated at 80 mA and 5k, and the spectra were scanned over a diffraction angle (2θ) range of 10-80° at 6°/min.

Inoculum preparation and cultivation conditions

Reconstituted MRS broth was prepared by using Tryptone, meat extract, yeast extract, potassium phosphate, tween80, ammonium citrate, sodium acetate, magnesium sulphate, manganese sulphate[11] and finally soyabean was added at final concentration of (1-3%). The reconstituted MRS broths were sterilized at 121°C for 15min. The overnight (18h) culture was added to the media and incubated under microaerophillic condition for 48h. Samples were withdrawn after specific time interval. Removed samples were subjected to measure optical density at 600nm, pH, % titratable acidity, dry mass and antimicrobial activity against *S. aureus* and *E.coli*.

RESULT AND DISCUSSION

Physicochemical investigation of soyabean

Crude fibre of Soyabean was found to be 17.17%

Scanning Electron Microscopy

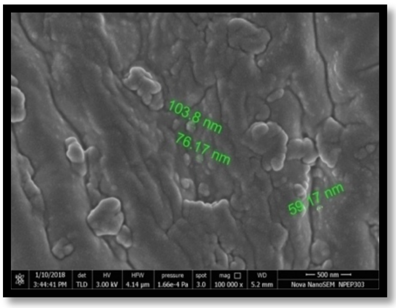


Figure 1: SEM of raw Soyabean

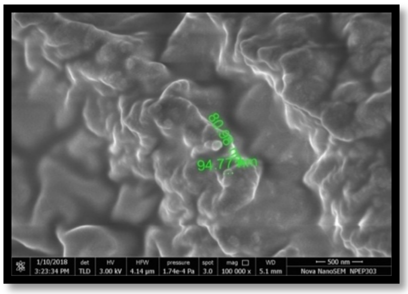


Figure 2: SEM of roasted Soyabean

Figure 1 and 2 shows SEM of raw and roasted Soyabean which determined shape and surface morphology of raw and roasted Soyabean. In raw soyabean the rounded outer surface are not swollen, after roasting these become slightly swollen as heat changes the structure.

X-ray Diffraction

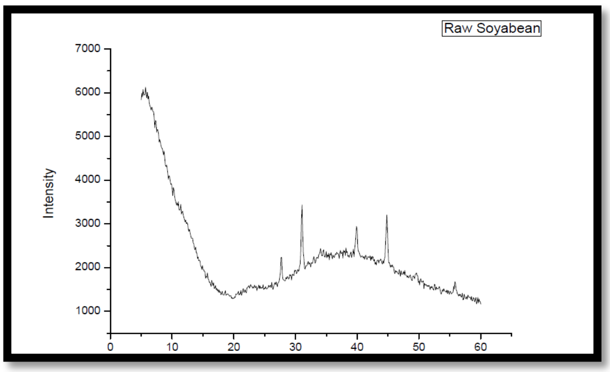


Figure 3: X- ray differactograms of raw soyabean

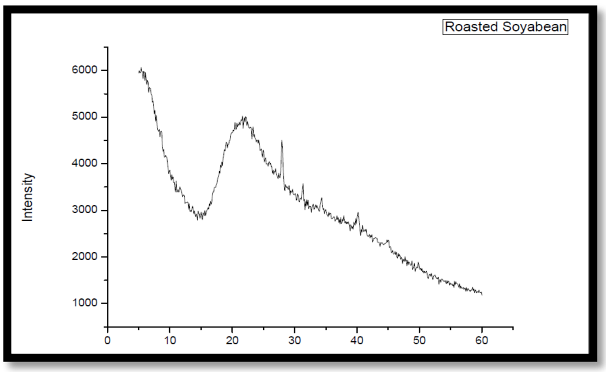
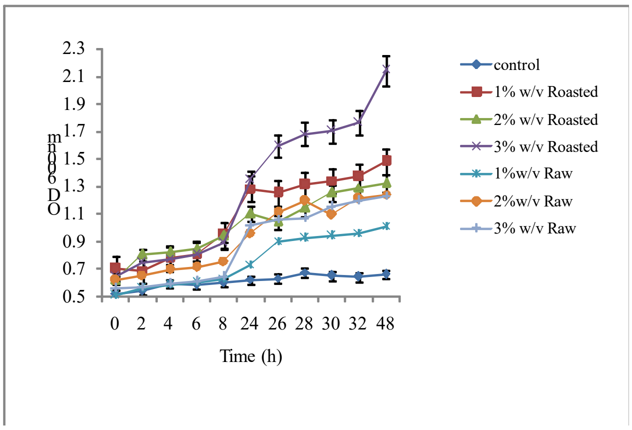


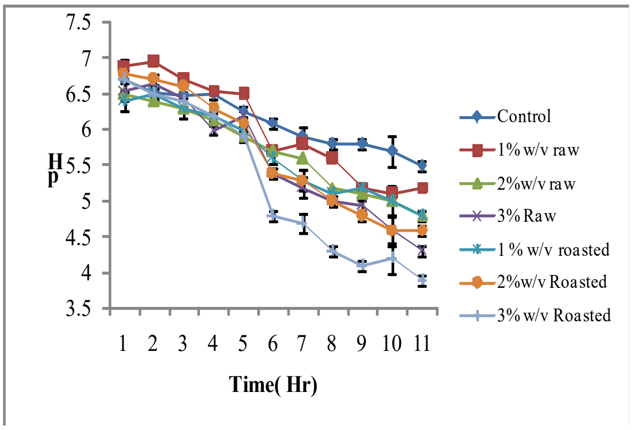
Figure 4: X- ray differactograms of roasted soyabean

Figure 3 and 4 shows the X-ray diffraction patterns of raw and roasted soyabean. X- ray diffraction was based on constructive interference of monochromatic X- rays and a crystalline sample. The difference in raw and roasted soyabean indicates the drastic change in distribution of the crystalline phases when the soyabean was exposed to thermal treatment.



*Error bars represent standard deviations of duplicates.

Figure 5: Effect of roasting of soyabean on growth of *L. acidophilus* in reconstituted MRS Broth



*Error bars represent standard deviations of duplicates

Figure 6: Changes in pH by *L. acidophilus* in presence of raw and roasted Soyabean

Effect of Soyabean (*Glycine max)* on growth of *L. acidophilus*

Optical density (OD) measurement of bacterial cultures is a common technique use in microbiology. Researchers have primarily relied on spectrophotometers to make these measurements, however the measurement is actually based on the amount of light scattered by the culture rather than the amount of light absorbed. The increase in the cell size and cell mass during the development of an organism is termed as growth. Bacteria is unicellular (single cell) organism. When the bacteria reach a certain size, it divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. The dynamics of the bacterial growth was studied by plotting the cell growth (OD at 600nm) versus the incubation time.[12]

The increase in the cell mass of the organism was measured by using the spectrophotometer. The spectrophotometer measures the turbidity or optical density which was the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture was directly related to the number of microorganism present, either viable or dead cells, and was a convenient and rapid method of measuring cell growth rate of an organism.[12]

The optical densities of raw and roasted Soyabean containing *L. acidophilus* are represented in Figure 5. The optical densities of the *L. acidophilus* containing roasted Soyabean to be slightly higher than optical densities of raw Soyabean and control.

Changes in pH of the media by *Lactobacillus acidophilus*

Figure 6 indicates that reduction in pH of reconstituted broth containing Soyabean was distinct compared to control (without prebiotic). The reduction in pH was from 6-6.5 (t=0) to 4 - 3.5 after 48 h of incubation. *Lactobacilli* belong to the lactic acid bacteria that produce lactic acid and derive energy from the fermentation process. pH of the medium is very important for optimum growth of the microorganism. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH. Figure 6 indicates that reduction in pH of reconstituted broth containing Soyabean was distinct compared to control (without prebiotic). End product of fermentation process is short chain fatty acids (SCFAs)[13]. The production of lactic acid and the resulting low pH is important for preventing non indigenous organisms in the intestine.

The change in pH of the *L. acidophilus* containing raw and roasted Soyabean is represented in figure 6. The changes of pH of the *L. acidophilus* containing roasted soyabean to be lower than the raw Soyabean and the control.

Effect of prebiotic raw soyabean on Lactic acid production (% titratable acidity) by *Lactobacillus acidophilus* and without prebiotic (Control)

Table 1: Effect of raw soyabean on lactic acid production (% titratable acidity) by *L. acidophilus*

Time (h)	Control (without prebiotic)	1%w/v Raw Soyabean	2%w/v Raw Soyabean	3%w/v Raw Soyabean
0	0.02±0.05	0.10±0.06	0.05±0.05	0.05±0.5
2	0.05±0.05	0.12±0.03	0.15±0.03	0.15±0.03
4	0.13±0.03	0.15±0.03	0.2±0.02	0.2±0.02
6	0.2±0.02	0.21±0.01	0.28±0.01	0.26±0.01
8	0.22±0.01	0.28±0.01	0.33±0.03	0.29±0.02
24	0.29±0.03	0.40±0.06	0.44±0.01	0.42±0.01
26	0.30±0.01	0.56±0.02	0.48±0.01	0.52±0.07
28	0.34±0.01	0.62±0.05	0.53±0.07	0.62±0.04
30	0.38±0.06	0.74±0.04	0.67±0.06	0.72±0.02
48	0.43±0.02	0.90±0.02	0.94±0.06	1.05±0.02

*All values are expressed as Mean ± SD, n=2

Table 2: Effect of roasted Soyabean on Lactic acid production (% titratable acidity) by *L. acidophilus* and without prebiotic

Time (h)	Control (without prebiotic)	1%w/v Roasted Soyabean	2%w/v Roasted Soyabean	3%w/v Roasted Soyabean
0	0.02±0.05	0.13±0.06	0.15±0.03	0.15±0.03
2	0.05±0.05	0.20±0.09	0.26±0.01	0.23±0.02
4	0.13±0.03	0.3±0.07	0.35±0.00	0.28±0.01
6	0.2±0.02	0.31±0.06	0.38±0.02	0.32±0.04
8	0.22±0.01	0.40±0.06	0.405±0.06	0.39±0.04
24	0.29±0.03	0.50±0.02	0.51±0.03	0.55±0.03
26	0.30±0.01	0.67±0.06	0.71±0.00	0.67±0.07
28	0.34±0.01	0.69±0.02	0.79±0.02	0.68±0.02
30	0.38±0.06	0.71±0.08	0.87±0.03	0.91±0.05
48	0.43±0.02	1.13±0.02	1.14±0.03	1.39±0.05

*All values are expressed as Mean ± SD, n=2

Titratable acidity (TA) refers to the total concentration of free protons and undissociated acids in a solution that can react with a strong base and be neutralized. On the basis of result obtained, the selected Soyabean showed very interesting potential prebiotic property. *L. acidophilus* produces lactic acid by anaerobic glycolysis of glycogen, the main carbon source for the *Lactobacilli* in the intestine. *Lactobacilli* might be using raw and roasted Soyabean as a carbon source. [14]

The % titratable acidity of the *L. acidophilus* containing raw and roasted Soyabean are represented in table 1 and 2 respectively. The % titratable acidity of the all *L. acidophilus* containing roasted Soyabean to be slightly higher than the titratable acidity of the raw Soyabean.

Antimicrobial activity of *Lactobacillus acidophilus* in presence of prebiotic Soyabean against *E. coli*

In the present investigation, Soyabean showed maximum antimicrobial activity against pathogenic Gram –ve *E. coli* microorganism which were compared with positive control. The zone of inhibition was observed against *E. coli.* This result showed that the prebiotic i.e., Soyabean stimulate the growth of *L. acidophilus* and suppress the growth of pathogenic microorganism.

Table 3: Antimicrobial activity of *L. acidophilus* containing prebiotic raw and roasted Soyabean against *E. coli.*

Strain	Incubation time(h)	Inhibition zone(mm)		
		L. acidophillus (without prebiotic)	Reconstituted MRS broth with raw soyabean	Reconstituted MRS broth with roasted soyabean
E.coli	Control	-	-	-
	T_{24}	6.6±0.30	9.3±0.28	15.6±0.14

The selected *L. acidophilus* was found to inhibit growth of *E.coli*. Significant increases in the inhibition of *E. coli* was observed in presence of raw and roasted Soyabean compared to control (without prebiotic). Higher zone of inhibition was observed in presence of prebiotic compared to control (without prebiotic)[15]. Production of metabolic by-products such as fatty acids, hydrogen peroxide, hydrogen and hydroxyl ions, and ammonia, as well as bacteriocins and bacteriocin-like substances are responsible to antimicrobial activity of *Lactobacilli.* Bacteriocins are proteinaceous, bactericidal substances synthesized by bacteria, which usually have a narrow spectrum of activity, inhibiting strains of the same or closely related species. Bacteriocins appear to be capable of displacing or suppressing the growth of other bacteria.

Protection of large intestine from pathogens could help to modulate health of large intestine. Production of antimicrobial substances by *Lactobacilli* along with prebiotic could prevent colonization by pathogenic microorganisms by reducing the numbers of viable pathogens or by affecting their metabolism, such as by neutralizing toxins.[16]

CONCLUSION

Increase in optical density of *L. acidophilus* was observed in the presence of roasted Soyabean (*Glycine max*) in the reconstituted MRS broth medium compared to raw and control. The increase in optical density, dry mass, lactic acid production and pH lowering effect observed after 48 h incubation in reconstituted MRS broth compared to control(without prebiotic). The increase in zone of inhibition in presence of roasted one indicated that Soyabean had a great prebiotic potential compared to raw when roasted. From results of all parameters, it can be concluded that the roasted Soyabean had great prebiotic potential and can promote the growth of *Lactobacillus acidophilus* and inhibit the replication of pathogenic bacteria.

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