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RESEARCH ARTICLE

FERMENTATION POTENTIAL OF PREBIOTIC JUICE OBTAINED FROM NATURAL SOURCES.

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Abstract

"Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, which can improve host health is defined as prebiotics." Prebiotics are a source of food for probiotics to grow, multiply and survive in the gut. Prebiotics are fibres which cannot be absorbed or broken down by the body and therefore serve as a great food source for probiotics. Research shows that there are different types of prebiotics, in a similar manner as there are different types of probiotics. With prebiotics, the key differentiating factor is the length of the chemical chain – short chain; medium chain or long chain determines where in the gastrointestinal tract the prebiotic has its effect, and how the benefits may be felt by the host. In the present study Aloe Vera and germinated Alfalfa seeds were used as a source of prebiotic. Strains of Lactobacillus. acidophilus and Lactobacillus.plantarum were used for fermentation of juice made from aloe vera and germinated alfalfa seeds.From the result obtained it was observed that 5% of juice was effective in promoting the growth of L.acidophillus and Lactobacillus.plantarum. Whereas decreased acidity with the increasing concentration indicated that higher concentrations retard the growth of bacteria. Overall it was concluded that at a particular concentration of aloe vera could possibly be used and there is no major change on growth of bacteria by adding different amount of alfalfa seeds.

Introduction:-

"Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health"[5]. Some possible beneficial health effects of prebiotics are as follows:
Modulation of the colonic microflora
Enhancing resistance to pathogens

Reducing the risk of colon cancer, heart disease, obesity, diabetes and digestive tract disorders [6] Enhancing mineral bioavailability and adsorption
Lipid modulation[7,8,9,10,11,12,13]

Although any undigested food ingredient (like nondigestible carbohydrates, certain lipids as well as some peptides and proteins) that is selectively fermented by the beneficial bacteria of the gut may be a prebiotic candidate,

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nondigestible carbohydrates, are the most studied[9]. Prebiotics comprise disaccharides (such as lactolose and lactitol), oligosaccharides [such as fructooligosaccharides (FOSs) and transgalactooligosaccharides (TOSs)], soybean oligosaccharides (mainly trisaccharide raffinose and the tetrasaccharide stachyose), lactosucrose, xylooligosaccharides and polysaccharides (such as resistant starch) [14,15,16]. The most intensive studies have focused on FOSs and TOSs. FOSs such as inulin (*e.g*. Raftiline HP) and oligofructose (like Raftilose P95) are β-linked fructose monomers and can be found in plants (*e.g*. barley, wheat, asparagus, garlic, leek, onion, artichoke, chicory roots, banana, etc.). TOSs are β-linked galactose units synthesized from lactose via enzymatic transgalactosylation using β-galactosidase and are found in fermented products like yoghurts, as the result of bacterial activity on milk sugars[14,17]. Fructooligosaccharides are not degraded or absorbed in the stomach or in the small intestine and reach the colon (largely intact) where they are fermented by the gut bacteria (specially bifidobacteria and lactobacilli), to short-chain fatty acids (SCFA) (mainly acetate) and other 137 metabolites (*e.g*., lactate)[9,18,19,20].

Dairy based prebiotics:-

Dairy products appear to be an excellent mean for inventing nutritious foods. Such probiotic dairy foods beneficially affect the host by improving survival and implantation of live microbial dietary supplements in the gastrointestinal flora, by selectively stimulating the growth or activating the catabolism of one or a limited number of health-promoting bacteria in the intestinal tract, and by improving the gastrointestinal tract's microbial balance [1].

Non dairy prebiotics:-

Probiotic products available in the market today, are usually in the form of fermented milks and yoghurts; however, with an increase in the consumer vegetarianism, there is also a demand for the vegetarian probiotic products. And, owing to health considerations, from the perspective of cholesterol in dairy products, and economic reasons, alternative prebiotics for probiotics products need to be searched [2].Among this lactose intolerance is a major factor for the use of non dairy prebiotics.

Aloe Vera and Alfalfa:-

Aloe Vera pulp due to its composition is likely to promote the growth of probiotics [3]. Aloe vera contains most of its carbohydrates in the form of mannose polymers, vitamin A, Vitamin B1, B6 and vitamin C[4] and hence could serve as good medium for cultivating probiotics. Both aloe vera and alfalfa is highly nutritive easy digestible and economic also. So we made an effort through present investigation to observe the fermentation potential of juice obtained from Aloe vera and alfa alfa seed mix by L.acidophilus and Lactobacillus.plantarum. Juice obtained from Aloe vera and alfalfa seeds could possibly be exploited as a potential combinational remedy for reducing the risk of colon cancer, heart disease, obesity, diabetes and digestive tract disorders.

Material and methods:-

The study was divided into six phases:

1. First phase was procurement of strains of L.acidophilus and Lactobacillus.plantarum was procured from IMTECH Chandigarh and aloe vera and alfalfa seeds were procured from local vendor.
2. Second Phase - Germination of alfalfa seeds.
3. Third phase - Preparation of different concentration of aloe vera and alfalfa seeds juice. Total volume of juice was 100 ml.
4. Phase four - Activation of strains. Strains of Lactobacillus Acidophilus and Lactobacillus plantarum were activated in 100 ml MRS broth. MRS broth was prepared by diluting 5.5g of MRS broth in 100ml of water. Then MRS broth was autoclaved at 121˚C for 15 min and when it gets cool it is inoculated with strains and incubated at 37˚C for 24 hour in BOD incubator. After 24 hour activated strains were kept in refrigerator.
5. Phase five - Inoculation, incubation and chemical assay of the samples.

Concentration of samples prepared:

Sample no	Aloe Vera Volume (ml)	MRS Broth Volume (ml)
A (acidophilus)	5	95
	25	75
	50	50
B(acidophilus)	5	95
	25	75
	50	50

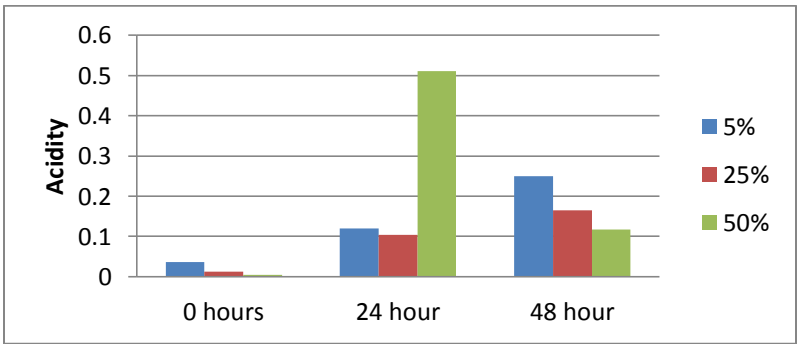
C(acidophilus)	5 25 50	95 75 50
AP(plantarum)	5 25 50	95 75 50
BP(plantarum)	5 25 50	95 75 50
CP(plantarum)	5 25 50	95 75 50

Inoculation of samples with strains of Lactobacillus.acidophilus and Lactobacillus.plantarum. Inoculated samples were kept in BOD incubator for 0, 24 and 48 hours.

Chemical assay - Acidity of each sample was measured using pH meter at different hours. Last phase was statistical analysis. Mean standard deviation and ANOVA was used for the analysis.

Results and Discussion:-

In the present study "Fermentation Potential of Prebiotic Juice Obtained from Natural Sources" different concentrations of aloe vera i.e. 5%, 25%and 50% with different amount of alfalfa seeds ie 15g, 30g and 45 g were mixed to get prebiotic juice. Their acidity is evaluated at 0 hour, 24 hour and 48 hours.



*p<0.005

Table 4.1:- Acidity of L.acidophilus fermented juice using 15gm alfalfa seeds

Time	Concentration of Aloe Vera			p value
	5%	25%	50%	
0 hour*	0.04 ± 0.001^{bc}	0.02 ± 0.03^{bc}	0.01 ± 0.01^{bc}	0.01
24 hour*	0.121 ± 0.006^{ac}	0.10 ± 0.016^{ac}	0.51 ± 0.057^{ac}	0.01
48 hour*	0.254 ± 0.010^{ab}	0.16 ± 0.100^{ab}	0.11 ± 0.07^{ab}	0.02

Anova($p<0.05$) mean value with same superscripts are significantly different as tested by ANOVA POST HOC test
Table 4.1 shows that acidity decreases with increase in concentration of juices. But at 24 hours of incubation at 50% concentration of juice, acidity was highest but shows a dip at 48 hours of incubation i.e. from 0.51 to 0.11. Whereas with increase in incubation time acidity of juices at 5% and 25% increases. This shows statistically significant result between the concentration of juices and time of incubation with addition of 15 g alfalfa seeds.

Table - 4.2:- Acidity of L.acidophilus fermented juice using 30gm alfalfa seeds

Time	Concentration of aloe vera			p- value
	5%	25%	50%	
0 hour*	0.03±0.010	0.10±0.01	0.05±0.10	0.01
24 hour	0.12±0.064	0.10±0.01	0.81±0.01	0.284

48 hour*	0.26±0.002	0.11±0.05	0.12±0.010	0.02

* p<0.005

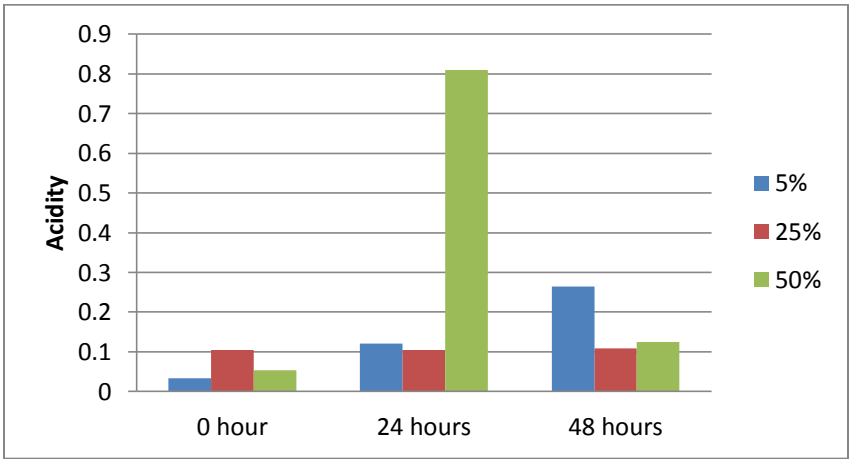


Table 4.2 shows that at 24 hour of incubation, in 50% of juice concentration acidity was highest which shows a dip at 48 hours of incubation time i.e. from 0.81 to 0.12. Whereas with increase in incubation time acidity of juices in varying concentration i.e. 5% and 25% increases. This gives statistically significant result between the concentration of juices and time of incubation with addition of 30 g alfalfa seeds.

Table 4.3:- Acidity of L.acidophilus fermented juice using 45 gm alfalfa seeds

Time	Concentration of juices			p- value
	5%	25%	50%	
0 hour*	0.04±0.001	0.06±0.059	0.04±0.01	0.02
24 h0ur*	0.11±0.068	0.10±0.01	0.07±0.010	0.01
48 hour*	0.24±0.010	0.21±0.010	0.17±0.01	0.02

* p<0.05

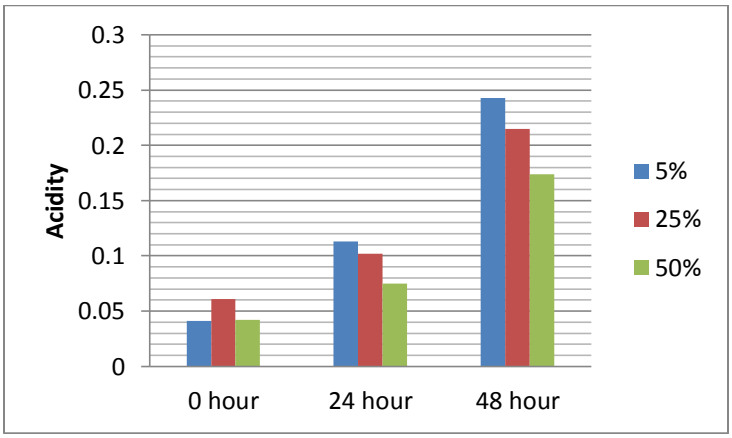


Table 4.3 shows that with increase in concentration of juices the acidity decreases. Whereas with increase in incubation time, acidity of juices of varying concentration i.e. 5%, 25% and 50% increases. This gives statistically significant result between the concentration of juices and time of incubation with addition of 45 g alfalfa seeds.

Table 4.4:- Acidity of L.plantarum fermented juice using 15g alfalfa seeds.

Time	Concentration of juices			p-value
	5%	25%	50%	
0 hour*	0.09±0.01^{ba}	0.057±0.010^{b}	0.02±0.105^{bc}	0.02
24 hour*	0.17±0.001^{a}	0.13±0.015^{a}	0.118±0.01^{a}	0.01
48 hour*	0.19±0.010^{a}	0.16±0.010^{a}	0.13±0.010^{a}	0.01

* p<0.005

Anova(p<0.05) mean value with same superscripts are significantly different as tested by ANOVA POST HOC test Table 4.4 shows that with increase in concentration of juices the acidity decreases. Whereas with increase in incubation time acidity of juices of varying concentration i.e. 5% ,25% and 50% increases. .

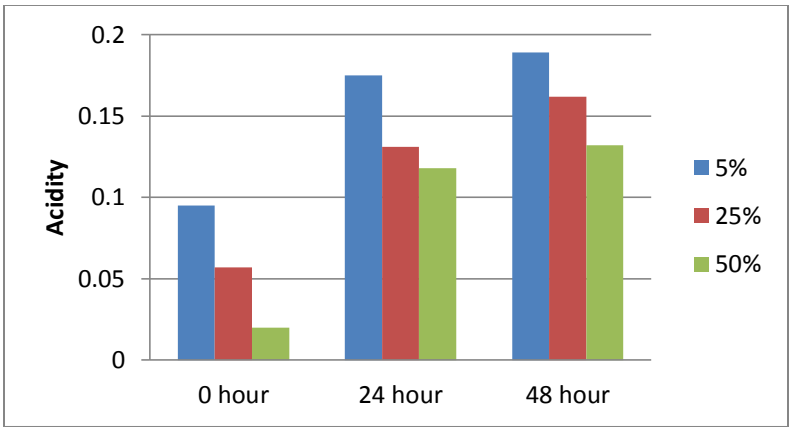


Table 4.5:- Acidity of L.Plantarum fermented juice using 30g alfalfa seeds.

Time	Concentration of juice			p-value
	5%	25%	50%	
0 hour*	0.12±0.01^{bc}	0.01±0.071^{bc}	0.15±0.01^{bc}	0.01
24 hour*	0.16±0.010^{ac}	0.12±0.01^{ac}	0.06±0.01^{ca}	0.01
48 hour*	0.17±0.01^{ab}	0.15±0.01^{ab}	0.13±0.01^{ab}	0.02

* p<0.005

Anova(p<0.05) mean value with same superscripts are significantly different as tested by ANOVA POST HOC test Table 4.5 shows that with increase in concentration of juices the acidity decreases. Whereas with increase in incubation time acidity of juices of varying concentration i.e. 5%, 25% and 50% increases.

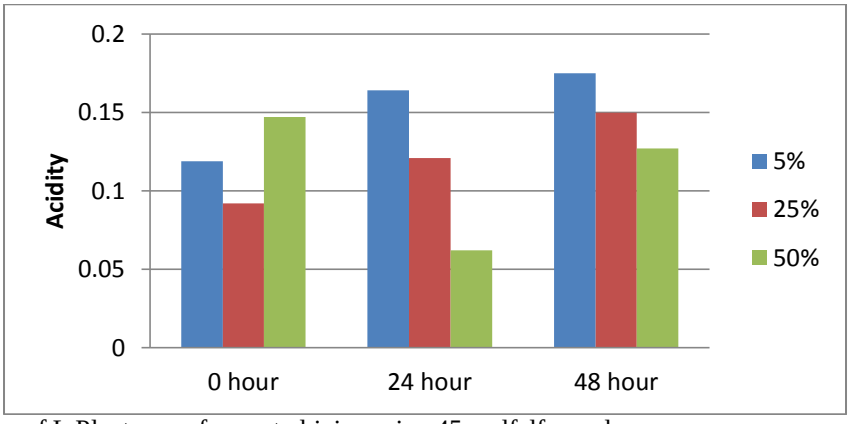
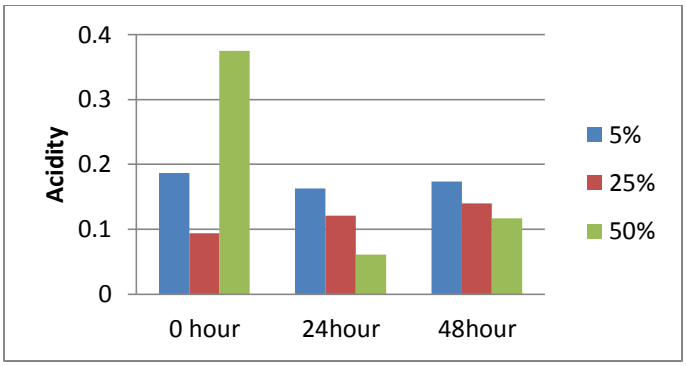


Table 4.6:- Acidity of L.Plantarum fermented juice using 45 g alfalfa seeds.

Time	Concentration of juices			
	5%	25%	50%	p –value
0 hour	0.18±0.001	0.09±0.01^{bc}	0.37±0.01^{bc}	0.422
24 hour*	0.16±0.01	0.12±0.038^{ac}	0.06±0.06^{ac}	0.01
48 hour*	0.17±0.01	0.14±0.01^{ab}	0.11±0.01^{ab}	0.02

* p<0.005

Anova(p<0.05) mean value with same superscripts are significantly different as tested by ANOVA POST HOC test Table 4.6 shows that with increase in concentration of juices the acidity decreases. Whereas with increase in incubation time acidity of juices with varying concentration i.e. 5% , 25% and 50% increases.



Conclusion:-

The study concluded that by increasing concentration of aloe vera acidity decreases due to increase in the production of some antimicrobial metabolites (short-chain organic acids) that could inhibit the bacterial. 5% was appropriate for the growth of L.acidophilus and L.plantarum. From the results obtained, it could be clearly advocated that Aloe Vera could promote the growth of L.acidophilus and L.plantarum at particular concentrations, and hence, could be used as a prebiotic for preparation of synbiotic therapeutic products. There is no major change in the growth of L.acidophilus by adding different concentration of alfalfa seeds because nutrients uptake by bacteria was more from aloe vera compare to alfalfa seeds. However, more in-vitro as well as in vivo studies such as strain specific effect, effect of purified components etc. followed by animal trials are pre-requisites before authenticating and endorsing this kind of combinational therapy and their health claims.

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