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Standardization of Protocol for the Preparation of Linseed Beverage Using Lactic Acid Bacteria and Yeast

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A B S T R A C T

Linseed is one of the most important crops and it has exceptionally high content of Alpha-Linolenic acid, dietary fiber, high quality protein, phytoestrogens and minerals especially phosphorus, magnesium and calcium. To increase the uptake of linseed, value addition is attempted. Standardization of protocol was carried out based on pH, titrable acidity and sensory characteristics for the parameters - seed processing technique, substrate, sugar, inoculum, honey concentrations, incubation temperature and fermentation duration using *Lactobacillus acidophilus* and *Saccharomyces ellipsoideus*. Seed processing technique of roasting and powdering, substrate concentration of 10 % and 5 %, sugar concentration of 6.6 % and 10 % with sugar and honey in the ratio of 80:20 and 60:40 were selected for bacterial and yeast fermentation respectively. Incubation temperature of 30°C for 2 days with 8 % inoculum was found to be effective for the preparation of fermented beverage. Based on the experimental results standard protocol was constructed and fermented beverage is prepared using probiotic organisms *Lactobacillus acidophilus*, *Bacillus mesentericus*, *Saccharomyces ellipsoideus*, *Saccharomyces boulardii* and lactic acid bacterial isolate. Organoleptic evaluation recorded an overall acceptability of 6.7 to 7.5. Cost of the beverage ranged from rupees 66.05 - 33.16 due to the variations in amount of recovery.

Introduction

Linseed also known as "flax" is one of the oldest cultivated crops grown by man for food and fiber. Linseed, *Linum usitatissimum* L. belongs to family *Linaceae*. It is one of the most important industrial oilseed crops of the world and is cultivated over an area of 3.02 million hectares with a production of 2.57 million tonnes and productivity of 852 kg /ha. India ranks first in terms of area under linseed cultivation with 4.68 lakh ha and third in production with 1.68 lakh tonnes and 349 kg /ha productivity.

Linseed is rich in lipids, protein and dietary fiber. Linseed contains 41, 20, 28, 7.7 and 3.4 per cent of fat, protein, dietary fiber, moisture and ash respectively (Morris, 2003). It is the richest known vegetable source of Alfa-Linolenic acid (ALA) which is an omega-3-fatty acid, phytoestrogen lignans and dietary fibers (Giada, 2010). Alfa-Linolenic acid is beneficial for cardiovascular diseases and it is known to reduce blood lipids. Linseed contains high quality protein with amino acids like arginine, aspartic acid and glutamic acid.

Dietary fibers help to reduce constipation, to keep better bowel movement and as a hypo cholesterolemic agent. Lignans act as antioxidants, thereby decreasing the presence of free oxygen radical and it is also having anticancer property. These properties make it more favourable for food technologists to explore and develop nutraceutical foods.

Salminen *et al.,* (2007) formulated a fermented beverage from linseed. Defatted and crushed linseed was mixed with other cereals, supplements, fermented with probiotic organisms like *Lactobacillus* and *Bifidobacterium*, stabilized using pectin or gum and seasoned with fruits like berries to develop a beverage. Ground linseed based refreshments accompanied by a little lemon juice is commonly observed in Bolivia, Peru and Colombia. Raw or roasted linseed was mixed with water, a few cloves, cinnamon and nutmeg and boiled for 15 min. Then it is added with lemon juice and served after refrigeration. Because of its nutritional properties this is used as an energy drink in Bolivia.

Presence of Alpha-Linolenic acid, dietary fiber and lignans especially Secoisolariciresinol diglucoside (SDG) in linseed attract food technologists and exploration of its abilities is needed to be used in food processing sector. In this study an attempt was made to prepare a value added product from linseed by fermentation. Standardization of methodology was studied using *Lactobacillus acidophilus* and *Saccharomyces ellipsoideus* to open a way for large scale production.

Materials and Methods

Standardization of protocol to develop fermented product form linseed was studied. *Lactobacillus acidophilus* (MTCC-10307) obtained from MTCC, Chandigarh and *Saccharomyces ellipsoideus* (SY_2) (NCIM-3200) from NCIM, Pune were used as reference cultures. Observations like pH (Digital pH meter type MK-VI), titrable acidity (Srivastava and Kumar, 1993) and sensory evaluation were considered for standardization.

Inoculum preparation method is given in figure 1**.** Population was determined by standard plate count method on MRS medium for lactic acid bacteria and Sabourd's agar medium for yeast SY_2 (Salminen *et al.*, 2008). Fermentation medium was prepared using required concentration of linseed, sugar, honey and inoculum. Samples were incubated at room temperature for fermentation until the standardization of temperature. Standardized parameters were considered for further experiments to standardize other parameters. Observations were taken at 2^{nd}, 3^{rd} and 4^{th} day of fermentation.

Parameters studied were as follows

1. Seed treatment – raw seed or roasted seed; inoculated or unioculated
2. Substrate concentration – 5, 10 and 15 %
3. Sugar concentration – 5, 6.6, 8 and 10 %
4. Inoculum concentration – 6, 8 and 10 %.
5. Honey concentration – sugar and honey in 100:0, 80:20, 60:40 and 50:50.
6. Incubation temperature–28, 30 and 32°C.
7. Fermentation duration – 2, 3 and 4 days.

Based on the experimental results standard protocol was constructed. Linseed fermented beverages using probiotic organisms *Lactobacillus acidophilus*, *Bacillus mesentericus*, *Saccharomyces ellipsoideus*, *Saccharomyces boulardii* and lactic acid bacterial isolate of linseed were prepared. Sensory evaluation was carried out for fermented linseed beverage by following 9 point hedonic scale by a panel of 17 judges. Cost and recovery of beverage was also calculated.

Results and Discussion

The protocol was developed for preparing fermented beverage of linseed considering parameters like seed processing; incubation temperature and duration; effective isolate selection and concentration of substrate, sugar, inoculum and honey. Biochemical characteristics including pH, titrable acidity and sensory characteristics like foam, flavor and taste were also considered for standardization.

Standardization for seed processing (Table 2) was tested and treatment (T_8) roasted, powdered with inoculation was selected for fermentation studies. This may be due to less amount of mucin compounds (form slimy sediment), easy availability of nutrients to microorganisms by powdering. Roasting process eliminates natural microbiota associated with linseed and there will be no competition for inoculum. Hence, in roasted treatment, the desired characteristics contributed by inoculum improve the quality of beverage.

Substrate concentrations (Table 3, 3A, 4 and 4A) of 10 per cent with 6.6 percent sugar concentration for lactic acid bacterial fermentation and 5 percent substrate with 10 per cent sugar concentration for yeast fermentation were preferred for beverage preparation. Biochemical characteristics like pH and titrable acidity in substrate concentrations 10 per cent and 15 per cent in lactic acid bacterial fermentation were on par with each other, thus 10 per cent concentration was selected for further studies. Substrate concentration of 5 per cent with 10 per cent sugar concentration yielded good results for fermentation of linseed using yeasts. This could be due to the availability of balanced concentration of carbohydrates (sugars) and substrate for fermentation by yeast.

Inoculum of 36-48 hrs old culture was used for fermentation. Population of inoculum was found to decrease after 48 hrs incubation (Table 1). This could be attributed to the fact that cultures might have entered stationary and death phase after 48 hrs incubation. Different inoculum levels (Table 5 and 5A) were added to substrate and results revealed that pH and titrable acidity increased with increase in inoculum levels. Inoculum concentration of 8 per cent and 10 per cent showed significant improvement in beverage properties, thus 8 per cent concentration was selected for fermented beverage preparation. Similar results were reported by Panesar *et al.*, (2010), for lactic acid production.

Drastic reduction in pH and more titrable acid production was observed by the supplementation of honey with fermentation medium. Similar results were earlier reported by Macedoi *et al.*, (2008), in 12 per cent nonfat dry milk containing 3 per cent of pasteurized honey fermented with *Lactobacillus* spp. and *Bifidobacterium* spp. Sugar and honey in the ratio of 8:2 and 6.5:3.5 for lactic acid bacterial and yeast fermentations were selected due to its favorable biochemical and sensory characteristics (Table 6 and 6A).

Table.1 Population density of inocula (cfu/ml)

Inoculum	**2nd day**	**3rd day**
Lactobacillus acidophilus	4.63×10^7 cfu/ml	1.46×10^7 cfu/ml
Saccharomyces ellipsoideus (SY_2)	16.1×10^5 cfu/ml	6.9×10^5 cfu/ml

Table.2 Standardization for seed processing for fermented beverage production

Treatments	pH	TA	Froth	Flavour	Taste
T_1 – Raw seed + un inoculated	5.54^{e}	0.05^{bc}	More	Bad	-
T_2 – Raw seed + inoculated	5.31^{d}	0.05^{bc}	More	Bad	-
T_3 – Raw seed, powdered + un inoculated	4.73^{b}	0.42^{a}	Very less	Bad	-
T_4 – Raw seed, powdered + inoculated	4.34^{a}	0.41^{a}	Very less	Not good	More sour taste
T_5 – Roasted seed + un inoculated	5.28^{d}	0.04^{c}	Very less	Not bad	Less sour taste
T_6 – Roasted seed + inoculated	4.95^{c}	0.05^{b}	Very less	Not bad	Less sour taste
T_7 – Roasted seed, powdered + un inoculated	5.26^{d}	0.07^{bc}	Very less	Good	Less sour taste
T_8 – Roasted seed, powdered + inoculated	4.73^{b}	0.13^{bc}	Very less	Grainy, good	Sour and good

Table.3 Sensory characteristics of beverages prepared with varying substrate concentration

Treatment details			Days of incubation								
	Organism	Substrate concentration	Foam			Flavour			Taste		
			2	3	4	2	3	4	2	3	4
T_1	***L. acidophilus***	**5 %**	No	No	No	Good grainy	Good	Nutty and grainy	More sweet	Sugary	Little sour and good
T_2	***L. acidophilus***	**10 %**	No	No	No	Grainy	Good	Slight fermented	Sugar and sour	Sour and sweet	Sour and good
T_3	***L. acidophilus***	**15 %**	No	No	No	Good	Good	Grainy and nutty	Sugary	Sugary and nutty	Slightly sour and sweet
T_4	**SY_2**	**5 %**	Yes	Yes	Yes	Alcoholic	Good, Alcoholic	Good	Not slimy	Little sour and good	Not good
T_5	**SY_2**	**10 %**	Yes	Yes	Yes	Alcoholic	Fermented	Not good as T_2	Little slimy and alcoholic	Gummy and not good	Not good, gummy
T_6	**SY_2**	**15 %**	Yes	Yes	Yes	Grainy, fermented	Fermented	Bad	More alcoholic and slimy	Too much slimy and not good	Not good, too much gummy

Table.3A Titrable acidity and pH of beverages prepared using different substrate concentrations

Treatment details			Days of incubation					
Treatment	Organism	Substrate concentration	pH			Titrable Acidity		
			2	3	4	2	3	4
T_1	*L. acidophilus*	5 %	4.60^{a}	4.56^{a}	4.52^{a}	0.06^{d}	0.08^{e}	0.13^{d}
T_2	*L. acidophilus*	10 %	4.64^{a}	4.51^{a}	4.21^{b}	0.10^{cd}	0.15^{e}	0.29^{c}
T_3	*L. acidophilus*	15 %	4.65^{a}	4.53^{a}	4.30^{b}	0.14^{c}	0.23^{d}	0.35^{bc}
T_4	SY_2	5 %	4.09^{c}	4.05^{c}	3.95^{d}	0.31^{b}	0.33^{c}	0.40^{b}
T_5	SY_2	10 %	4.09^{c}	4.08^{bc}	4.07^{cd}	0.50^{a}	0.50^{b}	0.60^{a}
T_6	SY_2	15 %	4.19^{b}	4.16^{b}	4.15^{bc}	0.54^{a}	0.60^{a}	0.62^{a}

Table.4 Sensory characteristics of beverages prepared using different sugar concentrations

Treatment details			Days of incubation								
	Organism	Sugar concentration	Foam			Flavour			Taste		
			2	3	4	2	3	4	2	3	4
T_1	*L. acidophilus*	5 %	No	No	No	Slightly grain	Good	Grainy	More sweet and slightly sour	Sour taste	Slightly sour
T_2	*L. acidophilus*	6.6 %	No	No	No	More grainy	Sour	Sour	Little sweet and sour	Sour taste	Slight sweet and more sour
T_3	*L. acidophilus*	8 %	No	No	No	grainy and light sour	Sour and grainy	Sour	sweet and little sour	Sour and sweet	Sour
T_4	*L. acidophilus*	10 %	No	No	No	Grainy	Fermented	Slight fermented	Sugar and sour	Bitter	Sour and good
T_5	SY_2	5 %	Yes	Yes	Yes	Fermented	Off flavour	Off flavour	Sour and sweet	Bitter	Bitter
T_6	SY_2	6.6 %	Yes	Yes	Yes	Little off-flavour	Off flavour	Off flavour	bitter	Bitter	Bitter
T_7	SY_2	8 %	Yes	Yes	Yes	Little off-flavour	Off flavour	Off flavour	bitter	Little sour and good	Bitter
T_8	SY_2	10 %	Yes	Yes	Yes	Alcoholic	Alcoholic	Good	Not slimy	Sweet and little sour	good

Table.4A Titrable acidity and pH of beverages prepared with varying sugar concentrations

Treatment details			Days of incubation					
Treatment	Organism	Sugar concentration	pH			Titrable Acidity		
			2	3	4	2	3	4
T_1	*L. acidophilus*	5 %	4.34^{ab}	4.11^{f}	4.01^{b}	0.13^{d}	0.22^{bc}	0.27^{b}
T_2	*L. acidophilus*	6.6 %	4.36^{bc}	4.13^{e}	3.99^{b}	0.14^{cd}	0.23^{bc}	0.27^{b}
T_3	*L. acidophilus*	8 %	4.41^{c}	4.10^{f}	3.98^{b}	0.11^{cd}	0.22^{c}	0.24^{b}
T_4	*L. acidophilus*	10 %	4.64^{a}	4.51^{a}	4.21^{a}	0.10^{bc}	0.15^{d}	0.29^{b}
T_5	SY_2	5 %	4.50^{a}	4.23^{b}	3.98^{b}	0.28^{a}	0.29^{a}	0.31^{b}
T_6	SY_2	6.6 %	4.46^{a}	4.22^{c}	4.00^{b}	0.26^{ab}	0.28^{ab}	0.28^{b}
T_7	SY_2	8 %	4.43^{a}	4.19^{d}	3.99^{b}	0.24^{ab}	0.28^{ab}	0.29^{b}
T_8	SY_2	10 %	4.09^{c}	4.05^{g}	3.95^{b}	0.31^{a}	0.33^{a}	0.40^{a}

Table.5 Sensory characteristics of beverages prepared using different inoculum concentrations

Treatment details			Days of incubation								
	Organism	Inoculum concentration	Foam			Flavour			Taste		
			2	3	4	2	3	4	2	3	4
T_1	*L. acidophilus*	6 %	No	No	No	Grainy	Good grainy	Grainy and a little sour	Sweet	Sour and sweet	Sour and sweet
T_2	*L. acidophilus*	8 %	No	No	No	Good	Good	Sour and Grainy	Sour than T_1	Sour as same as T_3	More sour
T_3	*L. acidophilus*	10 %	No	No	No	Grainy	Little sour	Sour	Sour than T_1	Sour and good	More sour
T_4	SY_2	6 %	Yes	Yes	Yes	fermented	fermented	More fermented	Sweet and a little bitter	Little bitter	Bitter
T_5	SY_2	8 %	Yes	Yes	yes	Fermented	Fermented	More fermented	Sour and good	Sour and good	Bitter
T_6	SY_2	10 %	Yes	yes	Yes	Fermented	More fermented	Little off-flavour	Sour and bitter	Sour and bitter	Bitter

Table.5A Titrable acidity and pH of beverages prepared using different inoculum concentrations

Treatment details			Days of incubation					
Treatment	Organism	Inoculum concentration	pH			Titrable Acidity		
			2	3	4	2	3	4
T_1	*L. acidophilus*	6 %	4.62^{a}	4.03	3.97^{a}	0.15^{b}	0.22^{c}	0.27^{d}
T_2	*L. acidophilus*	8 %	4.43^{b}	4.08	3.97^{a}	0.14^{b}	0.21^{c}	0.26^{d}
T_3	*L. acidophilus*	10 %	4.25^{d}	4.00	3.95^{a}	0.15^{b}	0.20^{c}	0.29^{cd}
T_4	SY_2	6 %	4.38^{bc}	4.01	3.73^{b}	0.23^{a}	0.29^{b}	0.37^{bc}
T_5	SY_2	8 %	4.34^{c}	3.99	3.70^{b}	0.23^{a}	0.32^{ab}	0.46^{a}
T_6	SY_2	10 %	4.30^{cd}	3.93	3.71^{b}	0.23^{a}	0.35^{a}	0.44^{ab}

Table.6 Sensory characteristics of beverages prepared using different prebiotic (honey) concentrations

Treatment details			Days of incubation					
	Inoculum	Sugar: Honey	Foam		Flavour		Taste	
			2	3	2	3	2	3
T_1	***L. acidophilus***	**100:0**	No	No	Sweet	Sweet and grainy	More sweet	Little sweet
T_2	***L. acidophilus***	**80:20**	No	No	Good, grainy	Good, grainy	Sour and good	Sour and a little sweet
T_3	***L. acidophilus***	**60:40**	No	No	Grainy, good	Good	Sweet and sour (not sour than T_2 and T_4)	More sour
T_4	***L. acidophilus***	**50:50**	No	No	Good, grainy	Little fermented	More sour	More sour
T_5	**SY_2**	**100:0**	Yes	Yes	Good, fermented	Good	Sweet and good	Little bitter
T_6	**SY_2**	**35:65**	Yes	Yes	Grainy, fermented	Good, fermented	Sweet	Sour and good
T_7	**SY_2**	**50:50**	Yes	Yes	Good (better than T_8)	Grainy	Sour and good	Sour and good
T_8	**SY_2**	**65:35**	Yes	Yes	Grainy, fermented	Fermented	Sour and a little sweet	More sour

Table.6A Titrable acidity and pH of beverages prepared using different prebiotic (honey) concentrations

Treatment details			Days of incubation			
Treatment	Inoculum	Sugar: Honey	pH		Titrable Acidity	
			2	3	2	3
T_1	***L. acidophilus***	**100:0**	4.52^{a}	4.08^{a}	0.13^{e}	0.41^{e}
T_2	***L. acidophilus***	**80:20**	4.30^{cd}	3.98^{a}	0.28^{b}	0.49^{de}
T_3	***L. acidophilus***	**60:40**	4.20^{bc}	3.91^{b}	0.20^{d}	0.71^{bcd}
T_4	***L. acidophilus***	**50:50**	4.20^{bc}	3.71^{c}	0.23^{cd}	0.73^{bcd}
T_5	**SY_2**	**100:0**	4.27^{b}	3.66^{c}	0.26^{bc}	0.55^{cde}
T_6	**SY_2**	**35:65**	4.20^{bc}	3.62^{c}	0.38^{a}	0.74^{bc}
T_7	**SY_2**	**50:50**	3.99^{d}	3.47^{d}	0.28^{b}	0.88^{ab}
T_8	**SY_2**	**65:35**	4.20^{bc}	3.50^{d}	0.29^{b}	1.11^{a}

Table.7 Sensory characteristics of beverages incubated at different temperatures

Treatment details			Days of incubation					
	Inoculum	Incubation temperature	Foam		Flavour		Taste	
			2	3	2	3	2	3
T_1	***L. acidophilus***	**28°C**	No	No	Good grainy smell	Grainy and fermented smell	Sour	More sour
T_2	***L. acidophilus***	**30°C**	No	No	Grainy and fermented smell	fermented smell, good	Sweet, a little sour	Sour
T_3	***L. acidophilus***	**32°C**	No	No	Grainy and fermented smell	fermented smell, good	Sweet and sour	Sour
T_4	**SY_2**	**28°C**	Yes	Yes	Fermented smell	Fermented smell	More sour and good	Sour and a little bitter
T_5	**SY_2**	**30°C**	Yes	Yes	Little fermented smell	Little fermented smell	Sweet and good	Sour and good
T_6	**SY_2**	**32°C**	Yes	Yes	Fermented smell	fermented smell	Sour and sweet	Sour and good

Table.7A Titrable acidity and pH of beverages incubated at different temperatures

Treatment details			Days of incubation			
Treatment	Organism	Incubation temperature	pH		Titrable Acidity	
			2	3	2	3
T_1	***L.acidophilus***	**28°C**	4.04^{b}	3.98^{cd}	0.34^{a}	0.55^{a}
T_2	***L.acidophilus***	**30°C**	4.20^{a}	4.10^{b}	0.10^{d}	0.51^{b}
T_3	***L.acidophilus***	**32°C**	4.11^{ab}	4.13^{a}	0.14^{cd}	0.26^{e}
T_4	**SY_2**	**28°C**	4.03^{b}	3.92^{d}	0.19^{bc}	0.38^{c}
T_5	**SY_2**	**30°C**	4.15^{a}	4.02^{bc}	0.17^{bc}	0.34^{d}
T_6	**SY_2**	**32°C**	3.93^{c}	3.82^{e}	0.24^{b}	0.27^{e}

Table.8 Standardization of optimum fermentation duration for the preparation of linseed beverage

Treatment details			pH	Titrable acidity	Foam	Flavour	Taste
	Inoculum	Duration (Days)					
T_1	*L. acidophilus*	2	4.43[a]	0.18[d]	No	Grainy and fermented	Sour and little sweet
T_2	SY_2	2	4.32[b]	0.19[d]	Yes	Fermented	Little sweet and sour
T_3	*L. acidophilus*	3	3.90[c]	0.25[c]	No	Fermented, good	Sour and good
T_4	SY_2	3	3.92[c]	0.24[c]	Yes	Fermented	Sour and good
T_5	*L. acidophilus*	4	3.76[d]	0.54[a]	No	More fermented	More sour
T_6	SY_2	4	3.68[e]	0.43[b]	Yes	More fermented	More sour and good

Table.9 Mean scores of organoleptic evaluation of the fermented linseed beverage prepared using lactic acid bacteria and yeasts

Treatments	Appearance	Colour	Texture	Taste	Aroma	Astringent	Mouth feel	overall acceptability
T_1 (*Lactobacillus acidophilus*)	7.1 ± 0.70	7.0 ± 0.71	7.3 ± 1.05	7.5 ± 1.12	7.8 ± 0.66	6.8 ± 1.19	6.9 ± 1.54	7.5 ± 0.87
T_2 (*Bacillus mesentericus*)	7.1 ± 0.78	7.1 ± 0.83	7.1 ± 0.90	7.1 ± 0.66	6.8 ± 1.19	6.6 ± 0.78	6.9 ± 1.05	6.8 ± 0.95
T_3 (Lactic acid bacterial isolate)	6.9 ± 0.99	6.8 ± 1.20	6.9 ± 1.41	6.8 ± 1.42	6.8 ± 1.47	6.5 ± 1.18	7.0 ± 1.22	7.0 ± 1.12
T_4 (*Saccharomyces ellipsoideus*)	6.9 ± 1.22	7.1 ± 1.32	6.6 ± 1.33	6.5 ± 0.94	6.5 ± 1.62	6.9 ± 1.36	6.6 ± 1.37	6.8 ± 1.20
T_5 (*Saccharomyces boulardii*)	6.9 ± 1.09	7.1 ± 1.03	7.1 ± 1.11	6.4 ± 1.28	6.4 ± 1.33	6.4 ± 1.23	6.4 ± 1.58	6.7 ± 1.26

Table.10 Amount of recovery and cost of linseed fermented beverage using probiotic yeasts and lactic acid bacteria

Treatment details		Amount of beverage recovered / 1000 ml (ml)	Cost of production of beverage/ 1000 ml (rupees)
Treatment	Inoculum		
T_1	*Lactobacillus acidophilus*	588.89	51.45
T_2	*Bacillus mesentericus*	564.45	53.67
T_3	Isolate LAB-3	415.54	72.91
T_4	*Saccharomyces ellipsoideus*	684.45	34.00
T_5	*Saccharomyces boulardii*	704.44	33.04

Note – cost of ingredients linseed (1000 g) – Rs. 200, Sugar (1000 g) – Rs. 40, Honey (1000 ml) - Rs. 270, Inoculum (100 ml = 10 g linseed + 3 g sugar) - Rs. 2.12

Fig.1 Standardized protocol for the preparation of fermented linseed beverage

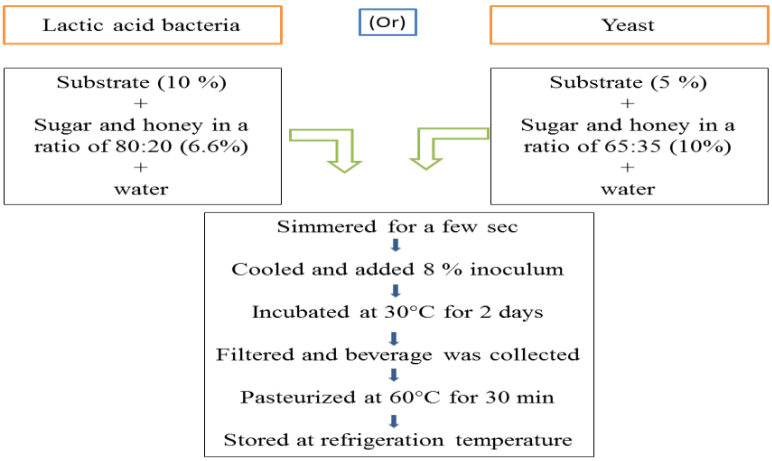
Preparation of fermented linseed beverage

Mother culture - Mass production of Lactic acid bacteria and yeasts inocula was done using MRS and yeast extract peptone dextrose broth and incubated at room temperature.

Starter culture - Roasted linseed powder (10 %), Sugar (3 %) and water was mixed, heated at 60°C for 20 min, cooled, filtered using muslin cloth and autoclaved at 121°C, 15 psi for 20 min. Starter medium was inoculated with 10 % mother culture and incubated at room temperature for 36 to 48 hrs and stored at 4°C.

Seed processing - Linseed was roasted at 60°C for 10-15 min, cooled and powdered using mixer.

Beverage preparation



Incubation temperature 28°C, 30°C and 32°C were tested for fermentation (Table 7 and 7A). Incubation at 32°C yielded significant reduction in pH of 4.11 and 3.93, more titrable acid production 0.142 per cent and 0.239 per cent and favorable sensory characters for both bacteria and yeast fermentations respectively. Favorable pH (4.43 and 4.32), Titrable acidity (0.178 % and 0.192 %) and sensory characteristics including good and fermented flavour with sour and little sweet taste was obtained on 2nd day of lactic acid bacterial and yeast fermentation. So incubation at 32°C for 2 days was selected for beverage preparation (Table 8).

Fermentation reported to reduce the pH and increase the titrable acidity of the sample. During standardization studies, some fermented linseed beverages with same pH reported to contain different amount of titrable acid. Titrable acidity is a simple measure of the related amount of acid anions in the sample and pH is a negative logarithm of concentration of free hydrogen ions. There is no direct relationship between titrable acidity and pH, although generally the pH decreases as the titrable acidity increases and *vice-versa*. The exact relationship differs from sample to sample and depends on various factors like buffering capacity of acid produced (Jolicoeur, 2011).

Foaming was observed in linseed beverages fermented using yeast and this could be due to the capacity of yeasts in the conversion of sugars into acid, alcohol and carbon dioxide. Absence of foam in lactic acid fermented beverage is due to the homo-fermentative nature of *Lactobacillus acidophilus* used in the investigation. The experiments were conducted

at room temperature before standardizing incubation temperature. Variation in biochemical and sensory characteristics of fermented beverages with same ingredients during standardization studies may be due to the differences in the activity and carbohydrate utilization ability of yeasts and lactic acid bacteria at varying room temperature.

Standardized protocol for the preparation of linseed beverage is explained in figure 1. Fermented beverages were prepared using probiotic organisms. Sensory evaluation (Table 9) recorded a mean score of 6.0 out of 9.0 (liked slightly) to 7.0 out of 9.0 (liked moderately). Beverage recovery after fermentation was studied.

Beverage recovery (Table 10) was more in beverages fermented using yeasts compared to that of lactic acid bacteria and cost of the beverage prepared using lactic acid bacteria was more. Amount of water retained is directly related to the substrate concentration and lactic acid bacterial fermentation involves more amount of substrate than yeasts fermentation. So amount of beverage recovery is less and cost of the beverage is more in lactic acid bacterial fermentation.

In conclusion the growing demand for food with nutritional and sensory quality as well as functional significance calls upon research to develop new products with consumer acceptance. A nutritionally rich beverage from linseed by fermentation was prepared as value addition. The developed protocol can be used for large scale production and further industrialization.

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