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RESEARCH PAPER

Development of Yoghurt with Bioactive Molecules

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ABSTRACT

Bioactive yoghurt an innovative functional dairy food refers to the incorporation of bioactive components from *Aloe vera* to promote better health. The process parameters like incubation temperature, percentage of total solids, starter culture and bioactive component were optimized based on the incubation time and quality of the developed bioactive yoghurt. The optimized process parameters were Incubation time, Incubation temperature 4 hours (43°C), Total Solids (23 %), *Aloe vera* (1%) and (2%) Starter cultures (*L.lactis : L.acidophilus*). Quality parameters such as hardness, firmness, cohesiveness, consistency and the index of viscosity of bioactive yoghurt were found to be 10.5 g, 69.97 g, -17.29 g, 366.29 g mm, -5.89 g mm, respectively. pH and the titratable acidity were found to be 4.59 and 0.96 %, respectively. The microbial analysis revealed that the bioactive yoghurt had a shelf life of 12 days under refrigerated condition (8±2°C) with overall acceptability score of 7.

Keywords: Bioactive, yoghurt, starter culture, incubation

Yoghurt is an innovative functional product, produced by the lactic acid fermentation of milk by addition of a starter cultures containing *Streptococcus* and *Lactobacillus species* (Akpan *et al.*, 2007). McKinley, (2005) have reported that yoghurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B), thiamin (vitamin B), and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value. Yoghurt, particularly a low-fat variety providing an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient-dense food (Kavita, 2005).

Bioactive food components have multiple metabolic activities allowing for beneficial effects in several diseases and target tissues. Bioactive food components are usually found in multiple forms such as glycosylated, esterified, thiolyated, or hydroxylated. However, probiotics, conjugated linolenic acid, long-chain omega-3 polyunsaturated fatty acid, and bioactive peptides are most commonly found in animal products such as milk, fermented milk products (Karaca *et al.*, 1995).

In *Aloe vera*, about 200 active compounds have been recorded including vitamins, amino acids, minerals, enzymes, polysaccharides, fatty acids and more. The most potent polysaccharide in *Aloe vera* is acetylated mannose or acemannan, which is used in European AIDS treatment. Fatty Acids, like gamma-linoleic acid, reduce inflammation, allergic reactions, blood platelet aggregation and improve wound healing. Polysaccharide, phenolic and chromone compounds are the core components of *Aloe vera* having anti-viral, anti-bacterial and immune boosting powers (Wollowski *et al.*, 2001). Large polysaccharide molecules from *Aloe vera* have been shown to produce Tumour Necrosis Factor (TNF) in the body. Gibberlin, sterols and the amino acids (phenylalanine and tryptophan) are also involved in anti-inflammatory and wound healing processes. Lignins can help detoxify the blood and intestine by binding chemically to fats (Katz, 2006). Many yoghurts fortified with fruits (strawberry, mango, coco) were available commercially in the markets. So, there exists an idea to fortify yoghurt with therapeutic values. Hence, a study with the incorporation of bioactive components from *Aloe vera* in yoghurt was undertaken with the objectives of developing a functional dairy product named 'Bioactive Yoghurt' and to optimize the various process parameters like incubation temperature, total solids percentage, starter cultures and its percentage and percentage of bioactive component and evaluating the quality parameters such as pH, titratable acidity, microbial load, texture profile analysis and sensory evaluation of the developed product.

Table 1: Methods to determine the Quality parameters

Parameters	Method
pH	pH meter (Century Instruments Ltd., India)
Microbial load	Total Plate count method
Texture profile analysis (TPA)	Texture Analyzer
Titratable Acidity (TA)	AOAC, 1997
Organoleptic Evaluation	9 – point hedonic scale (Peryam and Pilgrims, 1957).
Statistical Analysis	ANOVA using SPSS 15.0

MATERIALS AND METHODS

Yoghurt is made with a variety of ingredients including milk (Hatsun pvt ltd.), skimmed milk powder (Hatsun pvt ltd.), bacterial cultures (*L. delbrueckii, L. lactis and L. acidophilus)* and *Aloe vera* extract were prepared freshly in microbiology lab, Tamilnadu Agricultural University. The process of preparing starter culture and yoghurt have been explained in the Fig. 1 (US patent, 2008). The process parameters like temperature (41 and 43°C), total solids (21, 23 and 25 %), starter cultures (*L.delbrueckii, L.lactis, L.acidophilus*), percentage of starter cultures (2 and 2.5 %) and percentage of bioactive component (0.5, 1 and 1.5 %) were changed and observed in different trials. The quality of bioactive yoghurt is assessed by the following tests mentioned in the (Table 1).

Preparation of Starter culture	Preparation of yoghurt
Preparation of MRS broth	Raw milk (2 l)
↓	↓
Sterilization of broth (15 psi for 30 min in autoclave)	Modifying the Total solids of milk (addition of 21, 23 and 25 % of skimmed milk powder)
↓	↓
Cooling (35°C)	Pasteurization (63°C for 30 minutes)
↓	↓
Inoculation of culture (*L.lactis* & *L.acidophilus*)	Cooling (38°C)
↓	↓
Incubation (12 hours)	Addition of bacterial culture (one loop of specific cultures, *L.delbrueckii, L.lactis* and *L.acidophilus*)
↓	↓
Fresh starter	Addition of bioactive component (0.5, 1 and 1.5 %)
	↓
	Incubation (41 and 43°C)
	↓
	Refrigeration (4°C)

Fig. 1: Process flow chart

Table 2: Trials with homogenous starter cultures to optimize process parameters

Starter cultures	Incubation temperature (°C)	Starter culture (%)	Total Solids (%)	Incubation time (h)
L. delbruekki	41	2	21	10.0
			23	9.5
			25	9.0
		2.5	21	10.0
			23	9.0
			25	8.5
	43	2	21	10.0
			23	9.0
			25	9.0
		2.5	21	9.0
			23	8.5
			25	8.0
L. lactis	41	2	21	8.5
			23	7.5
			25	7.0
		2.5	21	7.0
			23	7.0
			25	6.5
	43	2	21	7.5
			23	7.0
			25	7.0
		2.5	21	7.0
			23	6.5
			25	6.0

RESULTS AND DISCUSSION

Optimization of Process parameter

The data on the trials with homogenous starter cultures (*L.delbrueckii and L.lactis,*) are presented in the Table 2. These homogeneous cultures took nearly 9.5 to 8.5 hrs and 7.5 to 7 hours to form yoghurt at different treatment combinations. The homogenous starter culture took longer time to form yoghurt due to the slow action of cultures in producing lactic acid than others. By increasing the starter culture percentage to 2.5 %, the yoghurt formed had sour taste and flavor due to increase in acidity (high lactic acid production). So, from these trials it was confirmed that the maximum starter culture percentage would be 2 %. The incubation temperature of 41 and 43°C took a minimum time of 8.5 and 8 hrs in case of *L.delbrueckii* culture whereas in *L.lactis* culture it required a minimum incubation time of 6.5 and 6 hrs at the incubation temperature of 41 and 43°C. From this it was inferred that *L. lactis* formed yoghurt 2 hrs earlier than *L.delbrueckii* culture. Chandan (1999) have reported similar results for the production of yoghurt with lactobacillus cultures (*L.delbreuckii and L. bulgaricus*) which took an incubation time of 8.5 and 9 hrs at 42°C for yoghurt formation.

Table 3: Trials with heterogenous starter cultures to optimize process parameters

Starter cultures	Incubation temperature (°C)	Starter culture (%)	Total Solids (%)	Incubation time (h)
L.lactis : L.acidophilus	41	2	21	6.0
			23	5.5
			25	5.0
	43	2	21	4.5
			23	4.0
			25	4.0

Table 4: Heterogeneous starter cultures and *Aloe vera* concentration to optimize process parameters

Starter cultures	Incubation temperature (°C)	Starter culture (%)	Total Solids (%)	Aloe vera concentration (%)	Effect
L. lactis: L.acidophilus	43	2	23	0.5	no change
				1	good
				1.5	masked yogurt flavor

Table 3 showed that the trials with heterogeneous starter cultures (*L.lactis and L.acidophilus*) in the ratio of 1:1 took only four hours to form yoghurt at 43 °C and maximum of 6 hours at 41 °C with 2 % starter culture (Champagne and Gardener, 2005). This is due to the reduction in incubation time which leads to faster tendency of *L.acidophilus* to convert into lactic acid. The trials revealed that the optimum incubation temperature for yoghurt formation is 43°C. The total solids play a major role in the formation of yoghurt by providing a good texture. The result show that the total solid percentage of 21 % produced yoghurt with soggy texture. At 23 % total solids yoghurt had good texture (semi gel) compared to harder yoghurt texture with 25 % total solids. From these trials, the required total solids percentage for the formation of yoghurt was confirmed as 23 %. These values are in accordance with the results of Salvador and Fiszman (2004) with total solids of 20 % in buffalo milk for yoghurt formation.

The bioactive yoghurt was prepared with heterogeneous starter cultures and with different concentrations of *Aloe vera* (0.5, 1 and 1.5 %). From the Table 4 it can be observed that the bioactive yoghurt at 0. 5% *Aloe vera* concentration was similar to plain yoghurt and with 1 % concentration of *Aloe vera* it was found to be better in *Aloe vera* taste. The yoghurt prepared with *Aloe vera* (1.5 %), it totally masked the taste and flavor of yoghurt exhibiting a bitter taste. Chauhan *et al.,* (2007) reported that *Aloe vera* extract of more than 2 % recorded lowest scores of sensory evaluation.

From these results various treatment combinations the process parameters were optimized as the incubation time of 4 hours with 23 % total solids, 2% heterogeneous starter cultures (*L.lactis : L.acidophilus*) and 1 % *Aloe vera* concentration at the incubation temperature of 43°C (Fig. 2).

Quality Evaluation

For these optimized trials, the physicochemical changes in bioactive yoghurt were compared with that of plain yoghurt (control) and commercial yoghurt in terms of pH, titratable acidity, microbial load, Texture profile analysis, cutting strength and subjective evaluation and their results were presented and discussed here.

The microbial count, pH and titratable acidity of the yoghurt samples are shown in the Table 5. The lowest bacterial population was found to be 11 $\times 10^7$ cells/ml in plain yoghurt whereas the bioactive and commercial yoghurt had the population of 14 $\times 10^7$

cells/ml. In the case of fungi, it was observed that, the population was high in commercial yoghurt (19 $\times 10^4$ cfu/ml) and low in bioactive yoghurt (15 $\times 10^4$ cfu/ml).

Preparation of Bioactive yoghurt
Raw milk (2 l)
↓
Modifying the Total solids of milk (23 % i.e. 208 g of skimmed milk powder)
↓
Pasteurization (63°C for 30 minutes)
↓
Cooling (38°C)
↓
Addition of bacterial culture (L.lactis : L.acidophilus, 1:1)
↓
Addition of bioactive component (10 % i.e. 20 g of *Aloe vera*)
↓
Incubation (43°C for 4 hours)
↓
Refrigeration (4°C)

Fig. 2: Optimized process flowchart for bioactive yoghurt

The Lactobacillus population was observed maximum in bioactive yoghurt with the count of 32×10^8 cells/ml while the commercial yoghurt recorded the minimum value of 26 $\times 10^8$ cells/ml. But it was noted that, there was no yeast growth in all the yoghurt samples because, yeast grows mainly in the fruit juices where sucrose or sugar content is high (Yuceer *et al.,* 2001). But in curd the yeast population is there. In case of yoghurt, the sucrose level is low, which being unsuitable for the yeast population to growmay have been the reason behind this. The microbial analysis of bioactive yoghurt revealed that the storage period was found to be 12 days under refrigerated condition. The pH of the bioactive yoghurt was found to be 4.59 whereas plain and commercial yoghurt had a value of 4.56 and 4.52, respectively. The titratable acidity of commercial and plain yoghurt was found to be 0.95 and 0.94 respectively but the bioactive yoghurt recorded the lowest acidity value of 0.96, which could be due to the addition of *Aloe vera* extract which neutralized the acidity.

In yoghurt samples, cohesiveness, consistency, firmness, index of viscosity were determined using texture profile analysis curve. Table 6 showed the texture profile analysis values of the yoghurt samples. For the bioactive yoghurt sample, the firmness was found to be 69.97 g and for commercial and plain yoghurt it was 72.41 and 61.03 g respectively. The maximum cohesiveness was found to be -29.62 g in commercial yoghurt whereas the bioactive and plain yoghurt recorded the values of – 17.29 and – 4.82 g. The highest consistency value of 366.29 g mm was

Table 5: Comparison of quality parameters of yogurt samples

Yogurt samples	Microbial load				pH	TA
	Bacteria (10^{-7} cells/ml)	Fungi (10^{-4} cfu/ml)	Lactobacillus (10^{-8} cells/ml)	Yeast (10^{-2}cells/ml)		
Plain	13	18	27	nil	4.52	0.94
Bioactive	14	15	32	nil	4.59	0.96
Commercial	14	19	26	nil	4.56	0.95

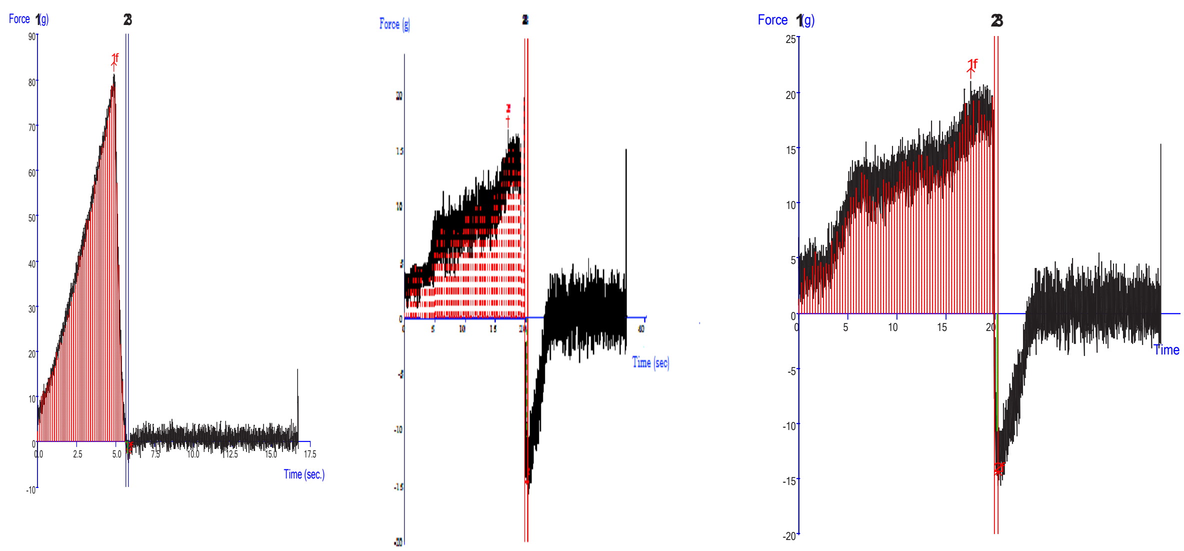
Table 6: Texture profile analysis of yogurt samples

Yogurt samples	Firmness (g)	Cohesiveness (g)	Consistency (g mm)	Viscosity (g mm)
Plain	61.03	-4.81	213.56	-0.2
Bioactive	69.97	-17.29	366.29	-5.89
Commercial	72.41	-29.62	358.83	-11.64

found in bioactive yoghurt and with a lowest value of 213.56 g mm in plain yoghurt. The commercial yoghurt attained the maximum viscosity value of – 11.64 g mm followed by bioactive yoghurt with a value of -5.89 g mm and plain yoghurt with 0.2 g mm viscosity. These values are in accordance with the results of Meullenet *et al.*, (1997).

The Fig. 3 shows the cutting strength of yoghurt samples. The maximum distance the probe moved gives the cutting strength and for bioactive yoghurt it was found to be 10.50 g whereas for commercial and plain yoghurt it was 20.92 g and 6.0 g respectively. For the bioactive yoghurt, the hardness, firmness, cohesiveness, consistency and the index of viscosity were recorded as10.5 g, 69.97 g, -17.29 g, 366.29 g mm and -5.89 g mm respectively.

The yoghurt samples (plain, bioactive and commercial) were analyzed organoleptically (Fig.



Plain yogurt **Bioactive yogurt** **Commercial yogurt**

Fig. 3: Comparison of cutting strength of yogurt samples

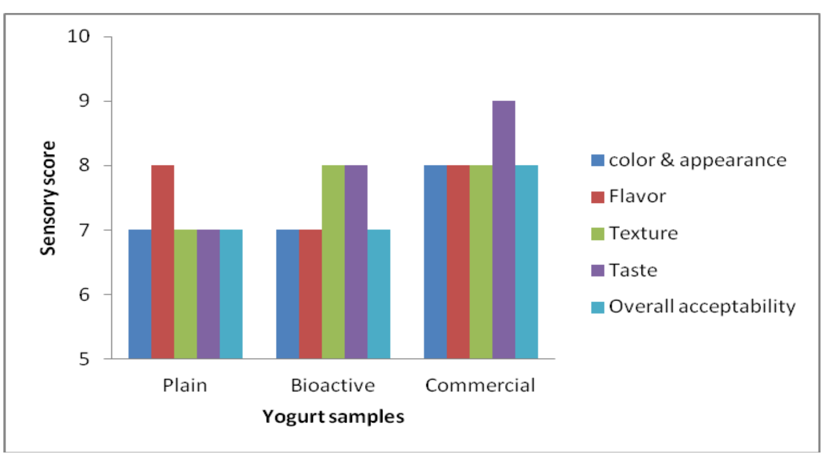


Fig. 4: Sensory evaluation of yogurt samples

4). The results showed that the taste, flavor, color and texture of bioactive yoghurt were similar to plain yoghurt. The overall acceptability of plain and bioactive yoghurt were found to be 7 (moderately liked) whereas the commercial yoghurt recorded 8 (like very much). The lower acceptability of bioactive yoghurt was might be due to the inherent flavor of *Aloe vera* incorporated in the yoghurt sample which masked the natural flavor and taste of yoghurt. These values are at par with the results of Kroger (1976).

CONCLUSION

Functional foods present great promise for future developments in human nutrition. Yoghurts are certainly common among the most promising products in this field. Yoghurt has many functional and nutraceutical values. In such way, Bioactive Yoghurt, a new approach to still enhance the therapeutic values of yoghurt.

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