

Production and keeping quality of yogurt from buffalo and cow milk-a traditional milk product of high health value

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Yogurt is an ancient fermented milk product reported before 5000 BC. It is not only nutritionally rich and easily digestible milk product, but also contains probiotic. However, majority of people are only concerned about flavor and other health benefits are neglected. Modification to this traditional milk product should be done without changing its natural properties, which was the focus of present work. The production of fruit yogurt using apple, pineapple, strawberry, grapes and pomegranates was carried. The bacterial flora present in yogurt which is potentially probiotic was also characterized. Further, the effect of storage period on yogurt was studied during 15 days. The results showed that in pomegranate yogurt, off flavors develop quite faster than in other types. The best flavor remained for longer time in case of apple yogurt. *Faluda* (a product of rose flower) was tried for the first time to enhance the flavor of yogurt in present study. Properties like pH, viscosity and color were also studied during this period. Decrease in viscosity and pH was observed leading to decrease in consistency and increase in off flavors. Fruit yogurt has double advantage. First in this case fruits are hydrolyzed and second hydrolysis of milk by probiotic is also a remedy for lactose intolerant people and also to treat various diseases.

Keywords: Yogurt, Production, Fruits, Keeping quality

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Yogurt is a fermented milk product containing a mixture of lactic acid bacteria. Yogurt is quite popular milk food product in Balkans, North Africa, Mongolia, Turkey, Iraq, Iran and Syria from ancient time as well as in many Asian countries. In India traditionally yogurt was prepared in earthen pots by adding sugar and small amount of starter culture which was kept overnight¹. Since Second World War, its popularity reached European countries and United States. New methods have been devised for yogurt production either by adding new fruits and/ flavor. Mixed bacterial cultures were used in ancient times as exact science behind yogurt production was unclear but by experience starter cultures were responsible for yogurt production was revealed fact. Single culture of lactic acid bacteria is also being used in recent productions^{2,3}.

No records are available, which state the exact origin of yogurt, but its existence is found in many civilizations. Milk production in Middle East region at ancient time was seasonal; because of scarcity of

milk; yogurt was ultimate choice. The subtropical climate and vast number of sources of contamination like air, the milking animal, feeding stuff and milkman were ultimate factors responsible for spoilage of milk. Nomadic people from Turkey devised a fermentation process where milk was initially dried by heating to concentrate and eradicate pathogens. Gradually lactic acid bacteria lead to souring milk which was called yogurt. The history also quotes treatment of diarrhea of Emperor Francis I of France where yogurt was used for treatment of diarrhea⁴.

The compound responsive to characteristic aroma in yogurt was found as acetaldehyde⁵. The probiotic present in yogurt helps in fighting harmful flora if their number is restored in the body⁶. The Greek meaning of probiotic is "on behalf of". Lilly and Stillwell introduced this concept which states the enhancement of growth of one microorganism by another⁷.

The reports on anticancer property of yogurt were reported way back in 1986. A comparative study of cheese and yogurt was carried in relation to cancer.

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Over 1000 cases were studied which relates to fat content in cheese was responsible for cancer whereas those consuming yogurt had negative response for cancer⁸. Later antitumor activity of yogurt was studied by Perdigon *et al.* (1998). The experiment was carried on mice by inducing tumor and subsequent inhibition of tumor by periodic feeding of yogurt. The mechanism suggested was immunomodulator activity and reduction of inflammatory immune response. It was unclear whether microbial flora is responsible for antitumor activity or the fermentation products of yogurt. Study these days is being carried on separation of individual components of yogurt and effect thereof as anticancer compounds⁹. Apart from replacing the harmful flora of gastrointestinal tracts yogurt also helps in enhancement of the immunity. The lactic acid bacterium present in yogurt binds to the luminal surface of intestine thereby competing with pathogenic bacteria and increases the intestinal barrier. This also enhances the mucosal IgA responses to harmful bacteria¹⁰.

Lactose in yogurt is easily digested than lactose in other milk products. This is because of beta galactosidase system present in lactic acid bacteria in yogurt. The study was carried by Martini *et al.* (1991)¹¹. Yogurt also provides ample amount of growth factors and minerals which includes vitamin B1, vitamin B2 and vitamin B12 along with Ca, P, Mg, Zn, folic acid and niacin. Yogurt is globally accepted food as nutrient dense. Furthermore, the hydrolyzed products in yogurt are an excellent source of shortfall nutrients¹².

During pregnancy excessive use of drugs is avoided for treating infections around the fetus. A case study was carried on 32 women found positive for infection during first trimester. Treatment of infection was done by intra-vaginal application of yogurt. The result was replacement of pathogenic flora by lactic acid bacteria in yogurt¹³. Osteoporosis is common disease in women and old age men. A detail description has been given by Nieves (2005). Out of various Ca sources available like nuts, fish with bones, dark green vegetables, cereals, etc., milk products particularly yogurts has been found to be more important¹⁴. Treatment of diarrhea by yogurt has been previously described. Also effect of yogurt on constipation was also studied by Nakamura *et al.* (2001). Combined effect of yogurt supplemented with brewer's yeast cell wall on constipation in rats was studied. Constipation in rats was induced by loperamide¹⁵.

Yogurt has been consumed since a very long time. The discovery of yogurt dates before 5000 BC by Mesopotamians. The herdsman carried milk in bags made of stomach of cattle which naturally contained coagulating enzymes like chymosin. The warm climate observed during that period was responsible for proper fermentation, which was carried by the natural flora present in the bags made by stomach of cattle. Also yogurt can be stored for long periods of time. The health benefits of yogurt were revealed with passing time and with experience. Now, the clear health facts behind consumption are being revealed by studying individual components¹⁶.

The detailed health benefits mentioned above stresses the necessity of yogurt production and need for popularization of its production. In present work, popular Indian fruits have been added to yogurt. Beneficial flora in yogurt was also extracted during the study. Periodic studies on storage of yogurt at refrigerated conditions were also carried out.

Materials and methods

Collection of milk and heat treatment

Raw milk was collected from healthy cow and buffalo, followed by pasteurization (heated at 90-95°C for 5 minutes) to inactivate pathogenic microorganisms and lipase enzyme. Protocol followed includes homogenization of milk, pasteurization of milk followed by cooling to room temperature. After cooling to 42°C, milk was inoculated with already fermented milk as starter culture. Starter cultures were added to these milk samples and were incubated undisturbed to produce set yogurt. After incubation properties of yogurt were noted and were kept at refrigerated condition¹⁷.

Preparation of flavoring materials

Fruits selected were apple, grapes, pineapple, strawberry, and pomegranate. Fruits were selected on the basis of availability and abundance in local market. These were cut in cubes and placed in sucrose solution for 5 hrs followed by drying of excess moisture in oven at 40°C. Fruits were added before fermentation. Attempt was to study the breakdown of sugars in fruit and study its influence on pH, viscosity, appearance and flavor of yogurt.

Bacteriological analysis

Microbiological studies were carried after production of fruit yogurt. MRS (deMann, Rogosa

and Sharpe) agar; a supportive medium for lactic acid bacteria was used for this purpose. Cultures from various fruit yogurt were studied for morphological, biochemical properties and carbon sources utilization. Bergey's Manual of Systematic Bacteriology was used for identification of those cultures.

Periodic studies on properties of yogurt

After production of fruit yogurt; this was kept in refrigerated condition. One of the goals of study was to evaluate the quality of yogurt in refrigerated conditions. Flavor is most important criterion for selectivity of food products. However, other properties were also studied, i.e., pH, viscosity and color. Properties of yogurt were continuously monitored over a period of 15 days. Viscosity was measured by drop collapse test, pH was measured by pH meter whereas visual observations were noted for color. A panel of 10 young people was selected and maintained throughout the study to observe periodic changes in flavor of fruit yogurts during refrigerated conditions.

Results and discussion

Production of fruit yogurt

Tasty fruit yogurt was prepared from buffalo and cow milk incorporated with addition of apple, grapes, pineapple, strawberry, and pomegranate. Flavor is most important property of food products rather than its nutritive value. However, yogurt is food product which posses both. After production of yogurt, *faluda* was also added to enhance the flavor. Mouth -feel of *faluda* yogurt was one of the most appealing tastes. These fruit yogurt were analyzed further for lactic acid bacteria.

Studies on lactic acid bacteria in yogurt

Bacteria were obtained in pure form after cultivation on MRS agar. Colony morphology on MRS agar was initially studied followed by bacterial morphology and biochemical characteristics. About 20 bacteria were fully characterized. Genera like *Bifidobacterium*, *Streptococcus*, *Lactobacillus* and *Pediococcus* were found. Species of *Lactobacillus* dominated the lactic acid bacteria followed by *Pediococcus* and *Bifidobacterium* whereas only one species of *Streptococcus* was encountered (Table1). The identified bacteria were proven probiotic. Moreover, the acidity caused by lactose fermentation itself is a controlling factor for other pathogenic bacteria.

Periodic studies on properties of yogurt

The enzymes at prolonged storage might have been continued to function leading to off flavors. Yogurt after production was kept at refrigerated condition, i.e., 5°C. At prolonged storage freshness of yogurt was decreased to a considerable level. It was also noted that off flavors developed in yogurt flavored with pineapple within first 5 days. Some natural compounds in pineapple or their conversion might have been responsible for off flavors. Decrease in viscosity was observed as the storage period was increased. The decrease in consistency affects appearance of yogurt and also taste. Flavor is the most important attribute when food products are considered for commercial purpose. Flavor is also affected by prolonged storage. Color of yogurt was influenced towards end of fifth day, which might have been by dissolution of fruit components in yogurt. With increasing storage period there was decrease in pH. Some enzymes might have continued to be functional at lower temperature (Table 2).

Initial viscosity was high in fruit yogurt made from buffalo milk. There was also decrease in pH with increase in storage. Viscosity of most fruit yogurt was decreased during storage. Color appeared to various fruit yogurt after fifth day. Light yellow color was developed in yogurt prepared from apple and pineapple. Greenish and pink color developed respectively in grapes and strawberry. Light pink color was developed in pomegranate yogurt towards the end of fifteenth day (Table 3). Further addition of *faluda* (a combination of rose and jelly frequently added in ice creams especially in India) to yogurt also had attractive taste.

To increase the medicinal importance, yogurt is being fortified with herbal extracts. Effect of *Aloe vera* on probiotic was studied by Panesar & Shinde (2012), who reported the total viable count of probiotic was satisfactory. The population of probiotic, i.e. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was above 10⁹CFU/ml during prolonged storage period¹⁸.

Similar studies using herbal extracts had been carried against *Helicobacter pylori* the causative agent of several deadly diseases, viz. chronic gastritis, peptic ulceration and gastric cancer. Yogurt was modified by addition of cinnamon and licorice which inhibited the growth of *H. pylori* but did not have any influence on *Lactobacillus* spp during refrigerated storage¹⁹.

Table 1—Morphological properties and Organic substrates utilization by various isolates

Source of Milk	Colour	Size	Shape	Margin	Elevation	Opacity	Consistenc	Gram	Shape	Endospore Motility	Catalase	Oxidase	Indole	MR	VP	Citrate	Lactose	Maltose	Mannitol	Fructose	Sucrose	Dextrose	
Cow	Colorless	0.5 mm	Circular	Entire	Convex	Opaque	Sticky	+	Long rod	NE NM	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp</i>
Cow & buffalo	Cream	0.2 mm	Irregular	Undulate	Raised	Opaque	Smooth	+	Club	NE NM	-	-	-	+	+	-	+	+	+	+	+	+	<i>Bifidobacterium sp</i> (5)
Cow	Cream	0.2 mm	Circular	Entire	Convex	Opaque	Smooth	+	Long rod	NE NM	-	-	-	-	+	-	+	+	+	+	+	+	<i>Lactobacillus sp</i>
Buffalo	Pale yellow	0.6 mm	Spindle	Entire	Concave	Opaque	Rough	+	Cocci	NE NM	-	-	-	-	+	-	+	+	+	+	+	+	<i>Streptococcus sp</i>
Cow	Colorless	0.5 mm	Round	Entire	Entire	Opaque	Smooth	+	Rod	NE NM	-	-	-	-	+	-	+	+	+	+	+	+	<i>Lactobacillus sp</i>
Cow	Colorless	0.5 mm	Circular	Undulate	Raised	Opaque	Rough	+	Rod	NE NM	-	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus sp</i>
Buffalo	Cream	0.4 mm	Round	Undulate	Raised	Transparent	Smooth	+	Long rod	NE NM	-	-	-	-	+	+	+	+	+	+	+	+	<i>Lactobacillus sp</i>
Buffalo	Cream	0.3 mm	Round	Entire	Convex	Opaque	Smooth	+	Long rod	NE NM	-	-	-	+	+	-	+	+	+	+	+	+	<i>Lactobacillus sp</i> (3)
Cow & buffalo	Colorless	0.3 mm	Circular	Entire	Convex	Translucent	Sticky	+	Cocci	NE NM	-	-	-	-	+	-	+	+	+	+	+	+	<i>Pediococcus Spp.</i> (6)

*Number in bracket following identified bacteria indicates frequency

Effect of ginseng plant on probiotic was recently studied by Hekmat *et al.* (2013). Ginseng is a perennial plant found mostly in Northern America, Korea and China. When consumed the plant extract is not absorbed by the intestine because of its higher molecular weight. The probiotic are believed to degrade ginsenosides into several smaller products which can enter human circulation system. Beside antimicrobial and antioxidant properties; ginseng extract is also shown to enhance the immunity. The

probiotic was not inhibited by ginseng extract which further recommends the incorporation of ginseng in yogurt and other milk products²⁰.

However herbal extracts added in yogurt affects flavor of yogurt. Despite medical benefits of such yogurts flavor is considerably lost. Undoubtedly plain yogurt has large number of health benefits. Plain yogurt can be made more attractive and appealing milk product by addition of various fruits to it.

Table 2—Change of various properties during storage of yogurt prepared from cow milk

Storage period	Fruits Selected	pH	Viscosity	Flavor	Color
1 st Day	Apple	5.5	Medium	Creamy and sweet	Colorless
	Pineapple	5.0	Low	Sweet	Light yellow
	Grapes	5.5	High	Sweet	Colorless
	Strawberry	5.0	Medium	Strawberry flavor	Light pink
	Pomegranate	5.5	Medium	Sweet	Colorless
5 th Day	Apple	5.4	Medium	Appealing apple flavor	Colorless
	Pineapple	5.0	Low	Slightly bitter	Light yellow
	Grapes	5.5	Medium	Sweet and slightly citrus	Slightly Greenish
	Strawberry	4.4	High	Sweet strawberry flavor	Light Pink
	Pomegranate	5.3	High	Sweet	Colorless
10 th Day	Apple	5.3	High	Sweet & apple flavor	Light yellow
	Pineapple	4.0	Low	Bitter & rancid	Light yellow
	Grapes	5.0	Low	Not much sweet	Greenish
	Strawberry	4.0	High	Sweet & tasty	Pink
	Pomegranate	5.3	High	No pomegranate flavor	White
15 th Day	Apple	5.0	Low	Sweet	Light yellow
	Pineapple	4.0	Low	Bitter	Off white
	Grapes	5.0	Low	Not much sweet	Greenish
	Strawberry	4.0	Medium	Sour	Pink
	Pomegranate	5.0	Medium	Sweet	Light pink

Table 3—Change of various properties during storage of Yogurt prepared from buffalo milk

Storage period	Fruits Selected	pH	Viscosity	Flavor	Color
1 st Day	Apple	5.0	High	Sweet & creamy	Colorless
	Pineapple	4.5	Low	Sweet	Light yellow
	Grapes	4.4	High	Sweet	Colorless
	Strawberry	5.0	High	Sweet strawberry	Light pink
	Pomegranate	5.5	High	Sweet	Colorless
5 th Day	Apple	4.7	Medium	Sweet apple	Colorless
	Pineapple	4.5	Low	Slightly bitter & rancid	Light yellow
	Grapes	4.4	Medium	Low in sweet	Greenish white
	Strawberry	4.5	High	Sweet strawberry	Pink
	Pomegranate	5.5	High	Sweet	White
10 th Day	Apple	4.5	Medium	Sweet & apple flavor	Light yellow
	Pineapple	4.4	Low	Bitter & rancid	Light yellow
	Grapes	4.0	Low	Not much sweet	Light green
	Strawberry	4.5	High	Sweet strawberry	Light pink
	Pomegranate	5.0	Medium	Pomegranate not mixed	White
15 th Day	Apple	4.0	Low	Sweet	Light yellow
	Pineapple	4.0	Low	Bitter	Light yellow
	Grapes	4.0	Low	Not much sweet	Greenish
	Strawberry	4.5	Medium	Sour	Light pink
	Pomegranate	5.0	High	Sweet	Light pink

Conclusion

Flavors are focus of food products. Moderate flavored milk product like plain yogurts can be made tastier by adding fruits which already possesses high nutrient values. Likewise dual benefits of flavor as well as nutrients can be gained. Keeping this in view various fruit yogurts were prepared and stored at refrigerated conditions where it was found that prolonged storage leads to off flavors. Pineapple yogurts tend to produce off flavors within five days. Mouth feel values of apple yogurts were higher towards the end of fifteenth day also which suggests apple yogurts are the most preferable followed by grapes, pomegranate and strawberry yogurts. Moreover the bacteriological studies carried reveal the presence of probiotic. This traditional milk product of high health value is being replaced by high calorie milk products like *paneer*, cheese and cold milks. Hence popularization of yogurts with fruit can bring a change towards a healthy life style.

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