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PRODUCTION AND EVALUATION OF YOGURT WITH WATERMELON (CITRULLUS LANATUS) JUICE

SAMADRITA SENGUPTA[1]
ANKITA CHAKRABORTY[2]
JAYATI BHOWAL[3]

[1,2,3] School of Community Science and Technology, Indian Institute of Engineering Science And Technology, Shibpur, (Formerly Bengal Engineering And Science University, Shibpur), Howrah, West Bengal, India

ABSTRACT

Fruity Yogurt was prepared by adding watermelon juice with milk. The physico-chemical, organoleptic and microbiological properties were analyzed to assay the quality of the Yogurts. Physicochemical properties of yoghurt samples including moisture, protein, fat, carbohydrate and ash were determined at first days of storage. Acidity, pH, syneresis, water holding capacity (WHC) and sensory quality of Yogurt samples were determined on 14 days of storage. There were significantly differences between Control Yogurt and Fruity Yogurt in the pH, moisture, ash, fat, protein, carbohydrate and Total solid content ($p \leq 0.05$). Highest values for WHC (56.69±0.02) and lowest values for syneresis (28.78±0.29) were belonged to Yogurt with watermelon juice. The Fruity Yogurt had higher acidity (1.18 ±0.01),viscosity (715±0.42)and lower Penetration Value (440±0.65) than Control Yogurt (CY). The results showed that utilization of watermelon juice affect the Yogurt characteristics, which reached at desirable count of Lactic acid bacteria. Sensory evaluation results showed that there were no significant differences ($p \leq 0.05$) among the Yogurt samples. The Fruity Yogurt had the highest overall acceptability (8.2± 0.46) scores compared to control Yogurt. The results of current study demonstrated that the addition of fruits to the Yogurt significantly improved the quality of Yogurt.

KEYWORDS: Yogurt, Watermelon juice, lactic acid bacteria.

INTRODUCTION

Yogurt is fermented milk and is consumed worldwide (Mansour, Khalifa and Hanafy, 1994) due to its therapeutic and nutritive values (Perdigon et al., 2002; Karagul, Wilson and White, 2004). This “biotechnological” food is rich source of proteins, carbohydrates, vitamins, fats, phosphorous and calcium. The uniqueness of Yogurt is attributed to lactic acid fermentation during its production which makes Yogurt easily digestible (Taracki, 2003) and increase the bioavailability of calcium in intestine (Singh and Muthukum, 2008). The health beneficial properties of live lactic acid bacteria in Yogurt includes curing of some intestinal disorders

(Shahani and Chandan, 1979) decreased risk of cancer, lowering blood cholesterol and improved digestion of lactose by mal digesters (Shahani and Chandan, 1979; Marona and Pedrigon, 2004; Hasannejad, et al., 2004).Fortification of Yogurt by addition of different fruit juices has been increasingly worldwide (Shukla, Jain and Sandhu 1987). Organoleptic evaluations of fruit juice fortified Yogurts have a marked preference nowadays (Barnes et al., 1991). Manufacture of Fruity Yogurts using different types of seasonal fruits is now very popular dairy product in India (Desai, Toro and Joshi, 1994), but very little study has been done in preparation and evaluation of watermelon juice incorporated Yogurt. The high water content (92%) of watermelon makes the juice an excellent diuretic. It is among the best dietary source of lycopene, an antioxidant whose protective effects work wonder on the human body due to its cancer fighting ability (Martha et al., 2013). Watermelon juice is an excellent source of Vitamin A and C, chlorophyll, Vitamin B1 and B6, potassium, iodine, magnesium, zinc and citrulline (Arteuro et al. 2012) but low in calories. Thus blending of Yogurt with watermelon juice would produce nutritionally balanced food. Watermelon is very popular and cheap seasonal fruit in India. A large amount of watermelon undergoes spoilage during its peak production time. Utilization of watermelon juice in production of fruity Yogurt prevents the huge wastage.

The objective of the present investigation was to develop a new type of fruity milk Yogurt by adding watermelon juice and to study sensory, physical, chemical and microbiological parameters of the Yogurt.

MATERIALS AND METHODS

All chemicals and dyes used in this study were of analytical grade, purchased from Merck, India. The bacteriological media were obtained from Himedia laboratories Pvt Ltd., India. Skim milk powder (Amul Spray Per 100g: Milk Fat 19g, Milk protein 22g, Carbohydrate 50 g, added Vitamins and Minerals), honey and watermelon were procured from local market of Kolkata in India. One of the Yogurt starter cultures Streptococcus thermophillus (MTCC 1938) was provided by Microbial Type Culture Collection and Gene Bank, Chandigarh. Lactobacillus The another starter culture was isolated in School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah, India.

Isolation of Lactobacillus spp

Lactobacillus was isolated from locally purchased Yogurts by de Mann Rogosa Sharpe (MRS) (14) agar media. Approximately 1 gm of curd sample was mixed with 9 ml of sterile

phosphate buffer saline (PBS), homogenized gently, serially diluted 10 fold in PBS and Pour plated aseptically on MRS agar media. Plates were incubated at 37^{o}C for 48 hrs in anaerobic condition. Colonies differ in morphology, pigmentation; shape and size was sub cultured in MRS broth. Each individual subculture sample was examined by Gram staining and catalase production activity. Only Gram positive and catalase negative samples were then purified by streak plating using the same medium. After several subcultures, finally the single colony of lactobacillus was isolated by observing their colony morphology and some biochemical tests (Gram staining, catalase, endospore and motility test). Isolated colonies with typical characteristics of Lactobacillus were maintained at 4^{o}C in MRS broth until used in Yogurt development.

Identification of Lactobacillus spp

Identification of the isolated bacteria as Lactobacillus species was performed according to their morphological, cultural, physiological and biochemical characteristics by the procedures as described in Bergey's Manual of Systematic Bacteriology (De Man, Rogosa and Sharpe, 1960). The tests carried out were Gram reaction, motility test, production of catalase, endospore test, milk coagulation activities, nitrate reduction test, urease test, H_2S production, starch hydrolysis test, sugar fermentation profile, production of ammonia from arginine, 0.4% phenol tolerance test and NaCl tolerance (4%) assay.

Preparation of Watermelon Juice

Fresh watermelon (Citrullus lanatus) collected from local market was washed with clean water and the skin was separated with the help of knife aseptically. The seeds were removed and watermelon pieces were steam blanched for 5 minutes. The juice was obtained by blending by blender and then filtering with clean cheese cloth. The juice was kept in plastic containers and stored at freezing temperature until preparation of Yogurt.

Yogurt Making

The milk (fresh cow milk) was pasteurized at 60°C for 30min. Milk Powder (Amul Spray) was used to prepare reconstituted milk with 16% (w/v) total solids. Milk was sterilized at 121°C for 5 min and cooled to 42–45ºC. After cooling, CMC was added to both the fresh cow milk and dried powder (Amul Spray) milk at 0.755 (v/v). Water melon Juice was then added to only dried powder (Amul Spray) milk at 15% (v/v). After addition of Watermelon Juice to reconstituted dried powder (Amul Spray) milk, the mixture were homogenized in a

homogenizer (REMI MOTORS-RQ-122) prior to inoculation with starter culture until fruit juice got mixed intimately with the reconstituted dried powder (Amul Spray) milk. Milk mixtures were aseptically inoculated with 2% of mixed strain starter (Lactobacillus delbrueckii subsp. bulgaricus**,** and Streptococcus thermophillus, 2:1 v/v). Inoculated milk was then poured into 100 ml sterile transparent plastic cups with lids and incubated at 37°C for 6 hours. The plastic cups were pre-washed with boiled water before use. Fresh milk inoculated Yogurt and dried powder milk inoculated Yogurt is termed as Control Yogurt and Watermelon Juice Yogurt fermented with Watermelon Juice. And Both Control and Watermelon juice added Yogurts obtained were stored at 4°C for further analysis.

Proximate Composition Analysis

All the Yogurt samples were analyzed in triplicate for protein, fat, ash, moisture, carbohydrate and total solid content by using the method of AOAC (AOAC.2005).

Physical Properties

a. Whey Drainage

Whey Drainage was removed from the Yogurt, using a syringe within 24 h after the Yogurt fermentation had completed. The relative amount of whey drained off (in ml per 100 ml of initial sample) was calculated as the whey Drainage (Fiszman, Lluch and Salvador, 1999).

b. Syneresis

An amount of 20 g of the Yogurt was spread in a thin layer to cover the surface of the What man No 1 filter paper. The Yogurt was filtered under vacuum for 10 min. The liquid that passed through the filter paper was collected and recorded. The Percentage of Syneresis (PS) was calculated as the weight of the liquid divided by the weight of the initial sample multiplied by 100 (Wu, Hulbert and Mount 2001).

c. Water-Holding Capacity

A 10-g sample was centrifuged at 3,000 rpm for 60 min at 10°C. The supernatant was removed within 10 min and the wet weight of the pellet was recorded. The Water Holding Capacity was expressed as percentage of pellet weight relative to the original weight of Yogurt (Parnell-Clunies et al., 1986)

d. Viscosity Measurement

Apparent viscosity was determined by using a RV Brookfield viscometer (Stoughton, USA) on 100 mL Yogurt samples at room temperature. Samples were stirred for 40 sec before

measurement. Readings were converted to centipoise units. Viscosity values were measured at 10 rpm with spindle (Gassem and Frank. 1991).

e. Titratable Acidity

The acid content of Yogurt samples was determined according to AOAC technique (AOAC 2005). Twenty grams of well homogenized sample was placed in a beaker and was titrated against 0.1N NaOH with phenolphthalein as indicator. Titratable acidity was expressed as g equivalent lactic acid/100g.

f. pH determination

The pH of the Yogurt samples were measured directly using pH meter (Hanna pH meter No. 211). The Yogurt samples were stirred with a small amount of distilled water before pH measurement.

Microbiological Analysis

Microbiological analysis of prepared Yogurt samples included determination of total viable count, total coliform count, total yeast and mold count and Lactobacillus count in freshly prepared and in 7 and 14 days of cold storage at 4^{o}C according to the "Standard Methods for Examination of Dairy Products" by American Public Health Association (APHA,1989).

Ten grams of Yogurt samples were homogenized using vortex (Type NM 110, Ankara, Turkey) stirrer with 90 ml sterile peptone water to obtain a 10^{-1} dilution. Further tenfold serial dilution was made using the same diluents till a dilution of 10^{-6} was obtained. Aliquot of (0.1 ml) suitable dilution was spread plated in triplicates onto prepared, sterile and dried Petri dishes of suitable media for the enumeration of different organisms. The total number of viable microbes per gram of Yogurts was obtained by multiplying the number of colony forming units (CFU) on the plate with respective dilution factor and then was converted into logarithmic form. Plate count Agar (PCA) was used for total viable count enumeration. MRS agar was used for assaying the starter culture Lactobacillus. Potato dextrose agar (PDA) was used for determining yeasts and molds. Experiments were conducted in triplicate. Total coli form was enumerated by Most Probable Number (MPN) method.

Sensory Evaluation of Yogurt Sample

The Yogurt samples were kept at 4^{0}C until evaluation. 20 members were chosen from the department of School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah, India. They were assisted in developing a consensus evaluation for flavour attributes for fortified Yogurts. Evaluation was done at Nine

Point Hedonic Scale. The quality properties that were evaluated were color, firmness, smoothness, taste, sweetness, sourness, flavor and overall acceptance. The information contained on the sensory performance was indicated as9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike, 2=dislike very much, 1=dislike extremely.

Statistical Analysis

Statistical analysis was performed by using one way analysis of variance (ANOVA) and the means were compared across groups by Tukey test. All analyses were carried out in triplicate. Results were analyzed with the OriginPro 8 and the significant differences were determined at ($p \leq 0.05$).

RESULTS AND DISCUSSION

The results of the proximate composition of the Yogurt samples were shown in **Table 1**. The protein content of Yogurt fermented with watermelon juice was significantly higher ($p \leq 0.05$) than that of the CY. WJY was significantly low ($p \leq 0.05$) in fat content (3.40 ± 0.02%) compared with fresh cow milk Yogurt (3.67 ± 0.02%). There was no significant difference ($p \leq 0.05$) in the ash content of CY and WJY. Moisture was highest in CY and subsequently the total solid was least in this same sample. The total solid could be improved by adding milk powder to the milk before fermentation. Increasing the total solid causes an increase in the nutritive value of the product. The total solid determined was significantly high ($p \leq 0.05$) in WJY. This could be attributed to the fact that processing of Yogurt involved reduction in fat content and increasing the total solid. CY was significantly low ($p \leq 0.05$) in carbohydrate content compared to WJY sample.

Table1. Proximate Composition of Yogurt Samples (%)

Sample	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Total solid (g/100 g)
CY	3.04 ± 0.09^{b}	3.67 ± 0.02^{a}	0.63 ± 0.04^{a}	88.19 ± 0.56^{a}	4.47 ± 0.09^{b}	11.81 ± 0.05^{b}
WJY	3.21 ± 0.08^{a}	3.40 ± 0.02^{b}	0.55 ± 0.01^{b}	87.08 ± 0.46^{b}	5.76 ± 0.01^{a}	12.92 ± 0.56^{a}

Results are expressed as mean ± SD (n=3). Mean Values having different superscript letter in columns are significantly different ($p \leq 0.05$).CY- Control Yogurt; WJY- Watermelon Juice Yogurt.

After fermentation, WJY showed a similar pH reading compared to CY. In terms of lactic acid percentage (TTA), it showed that WJY had significantly different acid lactic percentage compared to CY. It also suggested that addition of watermelon juice into Yogurt may change the acid lactic percentage in Yogurt. CY showed a significantly higher PS ($p \leq 0.05$), as compared to WJY. This increase in PS was probably due to the decreasing water holding capacity that led to more release of whey. Whey Drainage refers to the appearance of whey on the gel structure (Lucey J, 2001).

The whey Drainage appeared only CY. The whey Drainage indicated weakness of the gel network; reducing the Water Holding Capacity and increasing syneresis. Viscosity and Penetration data indicated in **Table 2** showed that WJY had higher Viscosity 715±0.42 Cp whereas CY had significantly lower value (710±0.49) Cp ($p \leq 0.05$) presumably due to the higher percentage of moisture. Both CY and WJY exhibited nearly similar penetration value range between 445±0.56 mm/10^{th} mm and 440±0.65mm/10^{th} mm respectively.

Table 2. Physical Properties of the Yogurts

Parameters	CY	WJY
pH	4.05± 0.03[a]	4.03± 0.04[b]
TTA (%)	1.08± 0.01[b]	1.18± 0.01[a]
Whey Drainage (%)	0.21±0.00[a]	0.00±0.00[b]
Water Holding Capacity (%)	50.21±0.03[b]	56.69±0.02[a]
PS (%)	36.54±0.04[a]	28.78±0.29[b]
Brookfield Viscosity in Centipoises (Cp) at 25° C	710±0.49[b]	715±0.42[a]
Penetration at 25 ° C(1/10^{th} mm)	445±0.56[a]	440±0.65[b]

Results are expressed as mean ± SD (n=3). Mean Values having different superscript letter in rows are significantly different ($p \leq 0.05$). CY- Control Yogurt; WJY- Watermelon Juice Yogurt; TTA- Titratable acidity; PS- Percentage of Syneresis.

The total viable count (TVC), Coliform count, Yeast and mold count and Lactobacillus count of Control Yogurt and Yogurt with watermelon juice at zero time, 7 days and 14 days of cold storage at 4^0C were presented in **Table 3**. Results revealed that the microbiological analysis exhibited approximately the same behavior of both the Yogurts. There were no notable differences in term of total microbial load among the control and watermelon supplemented Yogurts. No coliform and no yeast and mold were detected in any of the Yogurt samples

prepared in this study. Lactobacillus species decreases during storage. This result had similarity with that of earlier report (Canganella et al., 1998).

Table 3. Microbial Analysis of Yogurt during storage

Microbial Properties	CY			WJY		
	0 Day	7 Days	14 Days	0 Day	7 Days	14 Days
Total Viable Count (TVC) (Log cfu/g)	3.18×10^6	3.34×10^6	3.60×10^6	3.24×10^6	3.26×10^6	3.61×10^6
Coliform (MPN/g)	0.00	0.00	0.00	0.00	0.00	0.00
Yeast & Mold (cfu/g)	0.00	0.00	0.00	0.00	0.00	0.00
Lactobacillius spp.	7.6×10^6	6.8×10^5	5.4×10^4	7.4×10^6	6.5×10^5	5.5×10^4

Results are expressed as mean ± SD (n=3). CY- Control Yogurt; WJY- Watermelon Juice Yogurt

Sensory Evaluation of the Yogurts

The result of the sensory evaluation of the Yogurt samples was shown in **Table 4**. The WJY was significantly different (P≤ 0.05) in terms of sourness from CY. There was no significant difference (p≤ 0.05) in the flavor of both the Yogurt samples. There was no significant difference in the aftertaste. In overall acceptability, CY was accepted by the pane list but the new Yogurt fortified with water melon juice was most preferred.

Table 4. Sensory evaluation of the Yogurts

Sample	Color	Firmness	Smoothness	Taste	Sweetness	Sourness	Flavor	Overall Acceptance
CY	8.0± 0.21^b	7.2± 0.25^b	8.2± 0.32^b	8.5±0. 27^a	5.2± 0.34^b	6.8± 0.49	7.8± 0.36^a	7.9± 0.29^b
WJY	8.1± 0.34^a	7.4± 0.15^a	8.6± 0.45^a	8.5± 0.78^a	5.6± 0.28^a	5.2± 0.98^b	7.8± 0.38^a	8.2± 0.46^a

Results are expressed as mean ± SD (n=3). Mean Values having different superscript letter in columns are significantly different (p≤0.05) Mean Values having same superscript letter in columns are significantly different (p≤0.05)..CY- Control Yogurt; WJY- Watermelon Juice Yogurt.

CONCLUSION

New type of fortified fruity Yogurt with acceptable physicochemical, organoleptic and microbiological qualities was produced. This new product will help Yogurt industries to enrich the commercial Yogurt and also help to utilize the seasonal fruit. Fruit juice addition may increase the acceptability of Yogurt. Using watermelon, commercial Yogurt could be enriched. Further work is needed to improve texture of watermelon Yogurt possibly by using different stabilizers.

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