



Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

FORMULATION OF STIRRED PROBIOTIC FRUIT YOGURT TO BOOST IMMUNITY

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Received – July 18, 2020; Revision – September 17, 2020; Accepted – December 08, 2020

Available Online March 25, 2021

DOI: [http://dx.doi.org/10.18006/2021.9\(Spl-1-GCSGD_2020\).S169.S175](http://dx.doi.org/10.18006/2021.9(Spl-1-GCSGD_2020).S169.S175)

KEYWORDS

Stirred yogurt

Probiotic drink

Passion fruit

ABSTRACT

The food we eat plays a key aspect in determining our overall health and immunity. Improving our immunity during the Covid-19 pandemic is challenging for all age groups. So this study focused on formulating a ready to drink called probiotic fruit yogurt from less utilized passion fruits (*Passiflora edulis*), as a good option to build resilience in the body against infections and also to help the planters of Thandikudi hills, Tamil Nadu to promote their harvest into a valuable product. Passion fruits were procured and handled in a very hygienic manner. The formulation of stirred fruit yogurts was carried out in three different ratios (10%, 15%, and 20% pulp). These samples were standardized by sensory evaluation (9 points hedonic scale) and physicochemical parameters (pH). Fruit yogurt made from 20% passion fruit pulp scored the highest value in the mean score (8.5±0.17) for sensory evaluation except for texture. The pH value of the passion fruit yogurt was 3.5 found and it was more acidic compared to the plain yogurt value of 3.7 because of the addition of fruit pulp which was balanced by the addition of sugar/stevia. The acceptability of the stirred probiotic fruit yogurt with 20% pulp was mainly because of the flavoring compounds of the yellow passion fruit (*P. edulis Sims f. flavicarpa* Deg).

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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1 Introduction

The immune system is the complex network of cells and protein molecules that protect us from disease by surveilling our body and responding to any foreign (non-self) substances they recognize as threats, mainly infectious microbes. Our immune system has synchronized along with assorted gut flora, not only to create a defense against pathogens but also to develop some tolerance for constructive microbes (Ley et al., 2006). The importance of this interaction explores that 70–80% of the body's immune cells are found in the human gut (Abbas et al., 2017).

Probiotics are live microbes, which provided adequate amounts of health benefits to the host. The confirmation of the impact of probiotics on human health is intensifying and driving the profitable development of functional foods termed as “Probiotic food” (Sanders, 2019). Probiotics showed therapeutic potential for diseases, including several immune response-related diseases, such as allergy, eczema, viral infection, and potentiating vaccination responses (Fang & Polk, 2011).

Yogurt is the milk product formed from the culturing of milk with bacterial cultures that contain the lactic acid-producing bacteria, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*. Furthermore, additional bacteria, such as strains of *Lactobacillus* and *Bifidobacterium* species, can be added for their health benefits (Hill et al., 2014).

Passion fruit (*Passiflora edulis Sims f. flavicarpa Deg*) grows in a perennial vine that is very vigorous in climbing. The woody vine yields round or ovoid fruits. The characteristics of the fruits are tough, smooth, and waxy with faint, fine white specks. Commonly, the rind is of two colors viz., dark purple and yellow. Fruit contains orange colored or pale pulpy juice with a large number of small, hard, pitted seeds which is either dark brown or black. The fruits are generally not used for table purpose (Sanders, 2009; Bakshi & Iqbal, 2019).

A combination of yogurt and fruit intake could provide high-quality protein, important fatty acids, a mixture of vitamins and minerals. This fruit yogurt bestows as both probiotic and prebiotic can to exert synergistic effects on health (Kailasapathy & Milchwissenschaft, 1998).

Improved probiotic viability was noted in fruit supplemented yogurt than plain yogurt due to fast consumption of phenolic compounds and organic acids such as citric acid by probiotic cultures. The inclusion of probiotic cultures and fruits in yogurt is recommended for enhancing the functional properties of traditional or home-made yogurt (Surajit, 2019). The current study was conducted to evaluate the sensory characteristics and physicochemical parameters of yogurt made from passion fruit.

2 Materials and Methods

2.1 Sample collection

A fresh pack of standardized milk was collected for the preparation of a thick yogurt formation because the addition of fruit pulp would dilute the stirred yogurt. Fully ripened yellow passion fruit was collected from the planter of Thandikudi hills - a Village in Kodaikanal Block in Dindigul District of Tamil Nadu State in India. The fruits were washed well and cut into two halves. Then the pulp along with the seeds was scooped out.

2.2 Formulation of Fruit yogurt

Milk sample was collected and boiled; this was followed by the cool down to a temperature of 37- 41°C. Further, two spoons of the home-made yogurt culture were added and stir well. This mixture was covered and incubates in a warm place for 5-7 hours. Fruit pulp with seeds was added to the Yogurt and blended thoroughly till all the firm curd is broken down. The mixture was refrigerated at 0-5°C and this probiotic fruit yogurt can be used for 3 days of opening. The detailed procedure of probiotic fruit yogurt formulation is as below.

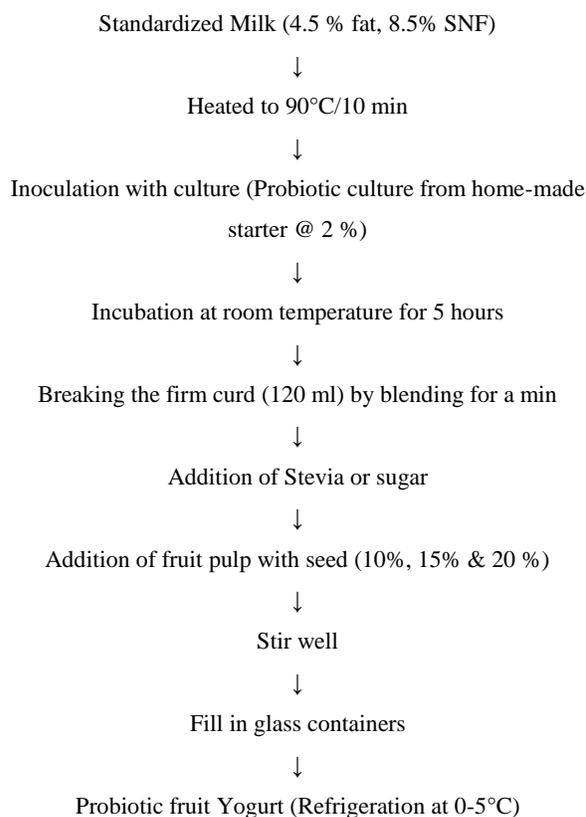


Table 1 Mean score of 9-point hedonic scale

Quality Parameters	10% pulp yogurt	15% Pulp Yogurt	20 % Pulp Yogurt
Color and appearance	8.5±0.11	8.6 ±0.19	8.8±0.15
Flavor	8.1±0.17	8.2 ±0.16	8.4±0.25
Consistency	8.4±0.20	8.3 ±0.10	8.0±0.23
Taste	7.8±0.21	8.1 ±0.17	8.3±0.21
Overall acceptability	8.2±0.19	8.1 ±0.19	8.5±0.17

Table 2 Nutrient Analysis per 8 ounces / 227 g

Nutrients	Fruit Yogurt (Present Research)	Commercial Yogurt (USDA, 2019)
Energy	126kcal	138 kcal
Fat	5.7g	7.38g
Carbohydrates	14.2g	10.6g
sugars	14.2g	10.6g
Protein	4.3g	7.88g
Sodium	140mg	104mg
Calcium	157mg	275mg

2.3 Sensory Evaluation

The formulated stirred probiotic fruit yogurt was tested organoleptically using a 9-point hedonic scale. The primary purpose for the widespread acceptance of the 9-point hedonic scale is that its categorical nature and limited choices make it easy for both study participants and researchers to use. Its simplicity further makes the 9-point hedonic scale suitable for use by a wide range of populations without extensive training. Therefore, the primary concern of a study is measuring hedonic differences among foods, beverages, and consumer products and predicting their acceptance, the 9-point hedonic scale has proven itself to be a simple and effective measuring device (Lim, 2011).

2.4 Nutrient Content analysis

The nutrient analysis of the formulated 20% passion fruit pulp yogurt for the macronutrients and micronutrients followed the AOAC method 20th edition, 2016 (AOAC, 2016). The nutrients analyzed were Energy, Carbohydrate, Protein, Fat, Sodium, and Calcium.

2.5 Characterization of Microbial Culture

Overnight culture should be given for culture identification. One loopful of culture was taken and inoculated in peptone broth. Then after 24 hours, once confluent growth appeared, the culture was centrifuged. Taken in pellets and then sent to the biotechnology lab for identification (Dubey & Maheshwari, 2006). BLAST analysis is used for the identification of bacteria. This procedure helps to

identify the strain of the bacteria present in the formulated yogurt to prove it to be a probiotic drink.

3 Results

3.1 Sensory attributes

The formulated fruit yogurts with three different ratios of fruit pulp (10%, 15%, and 20%) were analyzed organoleptic evaluation and tabulated in table 1. The samples were tested for their sensory attributes by 25 Semi trained panel members. The data gathered was statistically analyzed using mean \pm standard deviation. The statistical analysis report revealed that the overall acceptability of 20 % passion fruit pulp incorporation was found to be highly acceptable. Whereas the overall acceptability of 10% pulp yogurt was found to be less acceptable and 20 % passion fruit pulp incorporation score high acceptability because of the good taste, excellent flavor, and better color.

Out of the three tested ratios of passion fruit pulp yogurt, the 20% pulp incorporated yogurt scored a maximum except for the consistency. To further scrutinize this finding the researcher planned to do a comparative test between the plain yogurt and 20% pulp fruit yogurt before standardizing the formulation. The results were analyzed statistically by mean and standard deviation which are graphically represented in Figure 1. The semi-trained panel members observed two aspects. One is the improved flavor from the aromatic compounds present in passion fruit pulp and the other one is the crunchy seeds that enhance the texture.

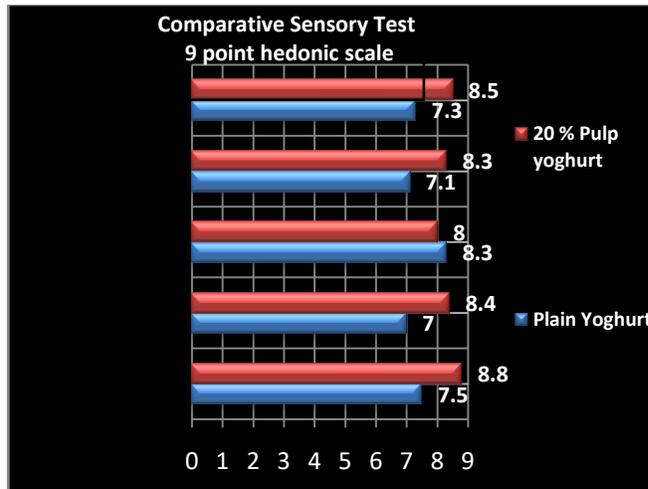


Figure 1 Comparative test graphical representation

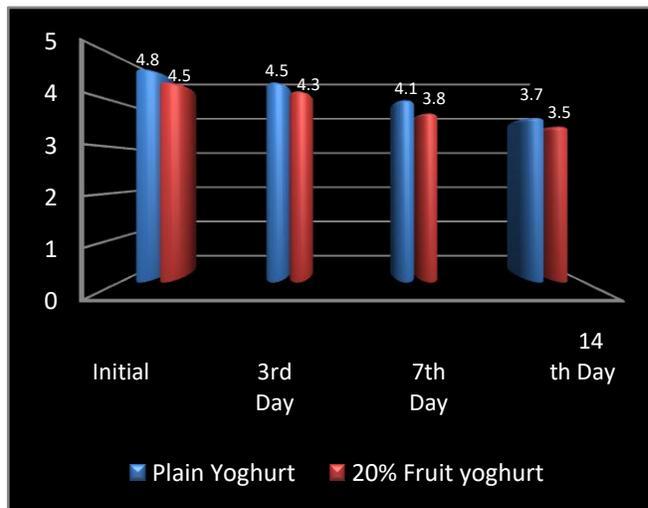


Figure 2 pH value of yogurts

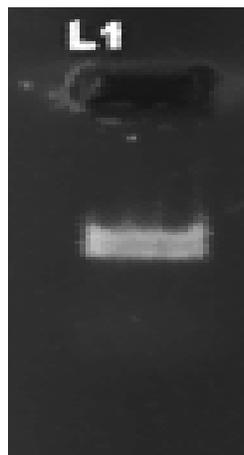


Figure 3 PCR products of 16s rRNA gene. (L1 – AM1)

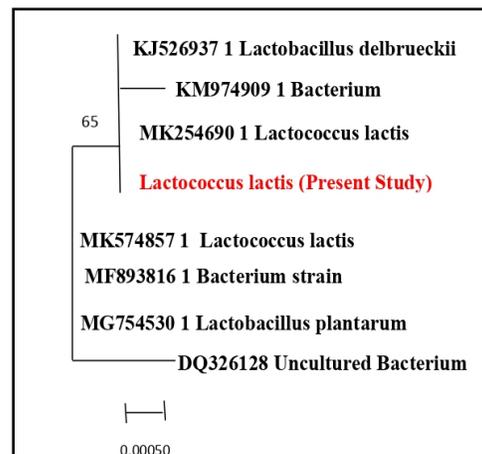


Figure 4 Phylogenetic tree

3.2 Changes in pH during storage

The researcher compared the pH score of the plain yogurt and 20% fruit yogurt to find out the physicochemical property. The stirred yogurts were refrigerated after fermentation and fruit pulp incorporation. The value was recorded by immersing the cathode of the pH meter into the sample. The pH value is higher in the formulated product when compared to the plain yogurt. The addition of fruit pulp also alters the pH and acidity of yogurt. Passion fruit pulp inclusion elevates the acidity due to the acidic character of the pulp. The result is graphically represented in figure 2. The pH reaches the minimum value of 3.5 for fruit yogurt on the 14th day.

3.3 Nutrient Content

The nutrient analysis of the formulated 20% passion fruit pulp yogurt was analyzed for the macronutrients and micronutrients based on AOAC method 20th edition, 2016. The values obtained were tabulated below in Table 2.

The content of calories, fat, and protein was a little lower compared to the commercial yogurt. Due to the addition of fruit pulp, there was an increase in the carbohydrates, and salt content of the stirred probiotic fruit yogurt.

3.4 Microbial identification of bacteria report

The prepared culture was sent for molecular identification of bacteria. Based on the higher percentage similarity against the reference species using BLAST analysis reported that the species that fermented the formulated fruit yogurt was *Lactococcus lactis*. Polymerase chain reactions were performed in the following temperature and timing conditions programmed in Applied Biosystems, thermal cyclers.

Initial denaturation at 94 °C for 5 min., number of cycles – 35, denaturation process carried out at 94 °C for 30 sec, annealing took place at 56 °C for 30 sec, extension passed at 72 °C for 1min and final extension process completed at 72 °C for 10 minutes for amplification.

The amplified products were tested on 1.5% agarose gel electrophoresis and the molecular weight was checked using a molecular weight marker (100bp ladder). The amplified sequences belong to 16S rRNA figured in figure 3.

The amplified sequences belong to 16S rRNA were confirmed by similarity index built in the NCBI's BLAST program. Based on the higher percentage similarity against the reference species, the species utilized in this study were assigned as *L. lactis* species refer to figure 4.

4 Discussions

The 20% passion fruit pulp incorporated stirred yogurt scored a maximum in color and appearance, flavor, taste, and overall acceptability except for the consistency. The pH reaches the maximum value of 3.5 for 20% fruit yogurt on the 14th day which is more acidic compared to plain yogurt. The nutrient content of the standardized fruit yogurt reveals that the 100 gram provides 126 kcal of energy, 14.2 g carbohydrates, 4.3 g of protein, 5.7g of fat, and 157 mg of calcium. The microbial identification of bacteria report confirms the species that fermented the formulated fruit yogurt was *L. lactis*.

The human gut is populated with as many as 100 trillion cells, whose collective genome, the microbiome, is a reflection of evolutionary selection pressures acting at the level of the host and the level of the microbial cell (Ley et al., 2006). The stirred yogurt fermented with *S. thermophilus* St-Body 1 at 37°C for 24 hours resulted in the highest score at each sensory evaluation category (Ho-Jin et al., 2005)

The higher the casein fraction and/or the fat level, the less hiatus in the network were observed. It is also evident that the addition of whey proteins strengthens the firmness properties of low-fat yogurts when compared to characteristics of full-fat yogurt (Sonne et al., 2011). On the other hand, increasing the level of whey proteins by modification of the whey protein: casein ratio in the milk base used for yogurt production led to a major increase in granular texture perception (Kailasapathy & Milchwissenschaft, 1998).

Nutritional facts of commercial yogurt given by the U.S. Department of Agriculture were, Calories: 138kcal, Protein: 7.88 g, Fat: 7.38g, Carbohydrates: 10.6g, Sugars: 10.6g, Calcium: 275mg, and Sodium: 104mg for plain yogurt (USDA, 2019). The content of calories, fat protein, and calcium were a little lower compared to the commercial yogurt. Due to the addition of fruit pulp, there was a mild increase in the carbohydrates, sugar, and sodium content of the stirred probiotic fruit yogurt.

Synergised health benefits may be exerted when yogurt and fruits are eaten together which provides potential prebiotic and their effects (Fernandez & Marette, 2017). The yogurts were flavored with 0.1% (wt/wt) strawberry flavoring containing 17 odorous compounds mixed with propylene glycol. The concentrations of the aroma compounds ranged from 1.01 to 32.53 mg/kg of yogurt (Saint-Eve et al., 2006). The pH values arrived by the researcher for stirred fruit yogurt agree with the findings of Tesfaye & Selvakumar (2015). The protective effects against the influenza virus were mostly derived from the cell components of *L. lactis* subsp. *cremoris* FC and its metabolites, such as exopolysaccharides. Many studies have shown that *L. lactis* can

support barrier function in terms of improved mucus, production of antimicrobial peptides, and secretion of soluble immunoglobulin IgA (Martín et al., 2014)

Conclusion

The research was conducted to formulate stirred fruit yogurt which has a probiotic effect that is very important for gut health that is a good sign to boost one's immune system. Fruit yogurt made from 20% passion fruit pulp scored the highest value in the mean score (8.5 ± 0.17) for sensory evaluation except for texture and the pH range of the fruit yogurt (3.5) is more acidic compared to the plain yogurt (3.7) because of the addition of fruit pulp which is balanced by the addition of sugar/stevia. The acceptability of the stirred probiotic fruit yogurt with 20% pulp is mainly because of the flavoring compounds and the crunchy seeds of the yellow passion fruit. The BLAST analysis reveals that the species identified in the formulated fruit yogurt was *L. lactis*.

The shelf-life study to find out the growth of other microorganisms is under process. This research aims to be a corkscrew for the planters to convert their perishable produce into a valuable ready to drink convenient food with an extended shelf life and yields more profit for their produce, which also makes it available all through the year.

Recommendations and suggestion

Many locally available fruits can be converted in this way without the addition of class II preservatives thereby making them consumable for all age groups and any health conditions. Further, this study can be extended by incorporating passion fruit peel powder which is prebiotic into the stirred probiotic fruit yogurt. It can be analyzed for physicochemical characteristics and symbiotic effect.

Acknowledgments

The researcher acknowledges her heartfelt gratitude to Mr. N. Jeyasekaran, Mr. A.K. Jeya Prakash, Mr. R. Mahesh Narayanan, and Mr. G. Deepan, the Planters of Pattiveeranpatti for contributing ripened yellow Passion fruits for the study whenever needed which helped the researcher to finish the research on time.

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