ORIGINAL ARTICLE

Effects of Artificial Sweeteners on the Quality Parameters of Yogurt During Storage

Sabahat Yaqub¹, Hafiz Arbab Sakandar²,³, Nuzhat Huma¹, Faizan Ahmed Sadiq³, Qaiser Farid Khan⁵, Muhammad Imran², Abdur Rehman³, Rashida Perveen⁴, Ayesha Sameen¹

¹National Institute of Food Science and Technology, University of Agriculture, Faisalabad-Pakistan; ²Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 44000, Pakistan - E-mail: Hafizarbabsakandar@gmail.com; ³School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China; ⁴Department of Allied Health Sciences (HND Program) Superior University, Lahore, 54000, Pakistan; ⁵Sustainable Development Study Center (SDSC), Govt. College University, Lahore, Pakistan

Summary. Yoghurt is one of the famous nutritious fermented milk products which have myriad of positive health effects on human beings and curable against different intestinal diseases. This research was conducted to observe the effects of different artificial sweeteners on the quality parameters of yoghurt with relation to storage. Some people are allergic to natural sweeteners so artificial sweetener will be helpful for them. Physico-chemical, microbiology and various sensory evaluation tests were carried out with the interval of 7, 14, 21 and 28 days. It was determined from this study that addition of artificial sweeteners in yoghurt has shown much harmful effects on the yoghurt microorganisms and other physicochemical parameters from quality point of view. Significantly good results for acceptance were obtained when aspartame was added in yoghurt at level of 0.022 percent. In addition, growth of beneficial microorganisms in yoghurt was also improved as well as other sensory attributes were enhanced by the addition of aspartame.

Key words: Yoghurt/ artificial sweetener/ Storage

Introduction

Economy of south Asia especially Pakistan depends on live stock as it is agricultural country, so it plays a major role for countries prosperity and one of the fundamental stuff of livestock is milk. It is major part of human diet which provides minerals proteins and vitamins.

Lot of research has been conducted on milk and various modifications have been done to it according to the wishes of human beings. Plethora of products of milk is available in the world either fermented or non-fermented. Yoghurt is formed by several biochemical processes of fermentation by the microorganisms (1).

Dairy industry has been flourished by various fermented dairy products. Food products that have passed through the process of fermentation have fascinated the consumers because these products contain gigantic amount of minerals and vitamins and minute quantity of fat. Yoghurt is one of the major and basic fermented food products and is mostly accepted food by the consumers because it has high nutritive value (2).

Different types of yoghurt are available in market *i.e* set yoghurt, frozen yoghurt and stirred yoghurt. In India and Pakistan, the famous form of yoghurt is "*Dahi*". Fruit yoghurt is more known among the consumers due its various beneficial properties such as easy digestibility and therapeutic properties against various diseases as compare to the milk from which it is obtained (3).

Conventional method or commercial scale method is mostly used in Pakistan to prepare yoghurt or "Dahi". It is formed by using "Jaag" at home level. Dahi prepared by this method is susceptible to contamina-

tion. It is mostly bitter, week textured and has flavor which resembles with yeast. Syneresis is also a big problem with *Dahi* which endows it with unaccepted texture and appearance. Culture is used for the preparation of yoghurt at large scale (4).

Owing to beneficial health impacts and nutritional value yoghurt is famous milk product (5). Different types of additives are used in yoghurt to enhance its quality, nutritional quality value and health benefits for the consumers. Natural sweeteners are also one of the food additives which are used in yoghurt to improve its shelf life and taste. Natural sweeteners are defined as Sweeteners which are formed without using any chemical alteration during its processing. Several sweeteners are being used in yoghurt now a day while some of the others have been in use for centuries (Honey.com).

Mainly natural sweeteners are consisting of mono and disaccharides. These sweeteners provide sweet taste as well as nutritional value to product. Sweet taste fascinates some consumers to much. On the other hand, artificial sweeteners are products which have very low or no nutritional value at all. These compounds are artificially produced so that these have very high intensities of sweetness. Dental problems such as dental cavities and caloric value are limited using artificial sweeteners (6).

These are obtained from chemical reactions of organic compounds and these may or may not be present in nature. These are most recent products and a lot of research is being conducted on its uses and enhancement (7).

Consumption of foods which have low caloric value and sweetness is increasing now a day. Intake of reducing sugars by the consumers is observed with keen interest in recent years. Developments in unconventional sugar free compounds are being carried out due to the saturation of markets with sweeteners (8). Tooth decay, obesity and other physical problems can be caused by the intake of high sucrose level. Those people who are conscious to health use sweeteners to avoid diseases. Alternative sweetened products also play a vital role in the life of the diabetic patients (9, 10). Now-a-days in market, food industry introducing several low-calorie sweetened products to replace sucrose in attempting to meet customer demands.

Market share of yogurts is majorly participated by low sugars (11).

Now a day, peoples are paying a lot of attention to their physical fitness and health by doing daily exercise, using fewer amounts of sugars, unsaturated fatty acids and salts. Improvements in daily eating habits and lifestyle are resulted due to the recent research. In contemporary times food science and technology goal is to Search related to good mitigate disease risks and buck up health of consumers (12).

Low caloric food had attracted the market nowa-days and a lot of attention is being paid on it because the diabetic patients who love to eat yoghurt but are not allowed to take sweet yoghurt due to presence of sucrose, low caloric yoghurt is highly recommended for them without having any health complications (9;10). Various artificial sweeteners can use but a parochial concentration is used i.e. acesulfame-K, saccharine, sucralose, cyclamate and Stevie (13). Various organoleptic attributes are owned by different sweeteners which depends on the intensity of sweetener (14; 15).

The present project was designed to evaluate the following objectives were to evaluate the impacts of artificial sweeteners on the quality parameters of yoghurt.

Material and Methods

The study was carried out in the Dairy, Food Microbiology and Bio-technology Lab, at national Institute of food Science and Technology (NIFSAT), University of Agriculture, Faisalabad. Raw milk was purchased from Dairy Farm in clean and pre-sterilized container and stored at 4°C.

Physicochemical Analysis of Milk

The raw milk was analyzed for different parameters such as fat, Total solids, pH, Acidity and Lactose according to the method described by AOAC (2000) (16).

Preparation of yoghurt

After mixing sweeteners with milk the solution was heated at 65 °C.

Homogenization

Mixture was homogenized at 1700 psi and its single stage homogenization.

Heating again

The single stage homogenized mixture was again heated at 85 °C for 35 minutes.

Cooling

Mixture was immediately cooled after heating in an ice bath.

Inoculation

The milk was inoculated with 2.0% starter culture i.e. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and mixed properly.

Packaging

Inoculated milk was poured in plastic cups of 300 mL volume and labeled.

Incubation

It was incubated at 34°C in incubator until pH of 4.2 was achieved.

Storage

The yoghurt was then cooled to a temperature of 6-7°C to check further fermentation and was subjected to physicochemical, microbiological and sensory evaluation.

Physicochemical analysis of yoghurt

Acidity and pH of yoghurt

The acidity and pH were determined by direct titration according to AOAC (2000) (16).

Syneresis

Syneresis of the yoghurt samples at different storage period was determined by following the method as described by Nafiseh et al. (2005) (17). Ten gram of artificial sweetened yogurt sample was placed on a filter paper resting on the top of a funnel. After 10 min of drainage in vacuum condition, the quantity of remained artificial sweetened yoghurt was weighted and syneresis was calculated.

Viscosity

Viscosity of the yogurt was determined by means of a Brookfield DV-E viscometer following the method as described by Aryana and McGrew (2007) (18). Apparent viscosity of yogurt was determined at 4-6 °C temperature. Spindle number 4 was with a rotation of 60 rpm reading was noted at 15 seconds. Viscometer reading was noted in centipoise (cP).

Hardness

Hardness of yogurt gel was measured in the fermenting container by texture profile analysis by using method of (Adachi et al. 2003) (19) with a texture analyzer. Hardness of yogurt gel was measured inside in the fermenting container by texture profile analysis with a rheometer using a cylinder plunger (16 mm), a compression rate of 5 mm/sec and 75% (22 mm) deformation at 10 °C.

Microbiological analysis

Total Plate count

Plate count agar was prepared according to the method of David and Fankhauser (2005) (20) by autoclaving at 121 °C for 15 minutes and CFU was measured

according to the method as recommended by Cappuccino et al. (1996) (21).

Statistical analysis

Data obtained was subjected to statistical analysis using two factorial Completely Randomized Designs (CRD) and ANOVA techniques as described by Steel et al. (1996) (22) to evaluate the quality and acceptability of yoghurt.

Treatment plane is shown in Table 1 given below. This treatment plan was adopted for present research.

Results and Discussions

This research is designed to check the effects of "Artificial sweeteners" on the quality characteristics of yogurt with passage of time. First of all milk was taken from dairy form of Faisalabad Agriculture University and was pasteurised. Homogenization was done along with addition of artificial sweeteners and again pasteurization was carried out. It was cooled to 43°C and culture was inoculated and incubated for 3-4 hours in 300 ml cups. When yoghurt was prepared it was stored at 4-6 °C. various physicochemical, sensory and test were carried out with the interval of seven days up to 28th day of storage.

Three dimensional network of milk protein is responsible for the formation of texture in milk and gel is formed due to acid formation by culture. Fermentation causes development of hydrostatic bonds between casein and whey protein which bestow structure to yoghurt. Aggregation of casein micelle and calcium phosphate solubilization is started at 5.3 pH and by lowering pH below 5 it forms harder, firm gel and complex interaction between casein micelles. It will be maximum at 4.6 pH which is isoelectric point of casein.

Table 1. Treatment Plan

Sweetener	% of weight
Sucrose (Control)	4.000
Saccharine	0.013
Acesulfame-K	0.02
Aspartame	0.022
	Sucrose (Control) Saccharine Acesulfame-K

pН

Fermented dairy products are perishable and the pH of these products decreases as time passes. In yoghurt, acidity increases due to production of lactic acid by bacteria.

Results are highly significant with respect to artificial sweetener and storage time.

Yoghurt Consumer's acceptance depends on the pH of yoghurt. It is not accepted by the consumer if it is very low because it causes more sourness in yoghurt. It is vivid from the Graph. 1 that T_{\circ} has maximum decrease in pH while T_{2} has minimum decrease in pH with storage.

Acidity

Overall production production of lactic acid in percentage is called acidity of yoghurt. Production of lactic acid in milk and milk products is due to conversion of lactose into lactic acid by bacteria.it is increases as the storage time accelerated.

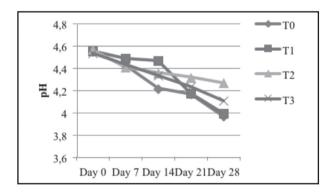


Figure 1. Effect on pH

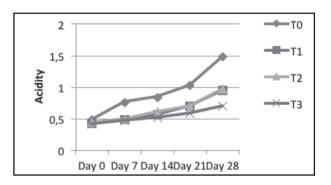


Figure 2. Effect on acidity

Graph 2 depicts results related to acidity of yoghurt with storage. Acidity of yoghurt increased with time. Maximum alternations in acidity were observed in case of T_0 treatment and T_3 showed minimum change with time. However it is deducted from the research that maximum resistance was observed in T_3 with passage of time. It revealed that activity of microbes was under control during storage. It was resulted that all treatments had highest at 28^{th} day of storage while minimum at 0 day of storage.

Syneresis

Exclusion of whey from yoghurt which appears on the surface is called syneresis. It is resulted by the shrinkage of yoghurt gel and whey is separated from the milk which is not fascinated by the consumers. The syneresis is measured as volume per ml of the whey water separated from the curd. Wheying off or syneresis is the major problem during the storage of yoghurt.

It is evident from the Graph 3 that T_1 showed maximum increase in syneresis with storage and T_0 showed minimum increase in syneresis with respect to other treatments but at 28^{th} day all treatments revealed maximum syneresis and minimum at 0 day of storage.

Viscosity

Consumers acceptance of yoghurt is also depend on viscosity of yoghurt. Thickness of yoghurt has direct relation with viscosity.

The results for viscosity of yoghurt samples are given in Graph 4. T_0 showed maximum viscosity and it gradually increased with passage of time while T_1 , T_2

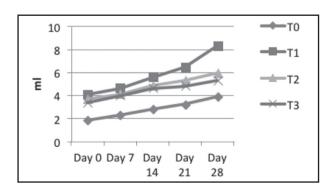


Figure 3. Effect on Syneresis

and T₃ have almost same viscosity and little bit increase was observed with the passage of time.

Hardness

The main objective in the production of yoghurt was to maintain uniform texture and particularly hardness among different units, processing dates and shelf life. It is observed from the results that the hardness of yoghurt samples were highly significant with respect to the storage time and artificial sweeteners interaction.

The mean values for hardness of yoghurt samples are given in Graph 5. Maximum increase in hardness was observed in T_3 while minimum was seen in T_1 . This shows that results are highly significant.

Microbiological analysis

Total Plate count

It was observed that the bacterial count was decreased with passage of time. This was due to production of

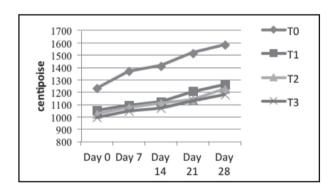


Figure 4. Effect on Viscosity

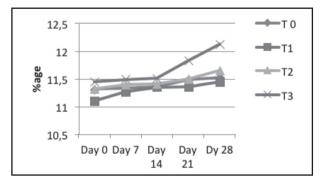


Figure 3. Effects on Hardness

lactic acid which made unfavourable conditions for bacterial growth.

However, total plate counts for all the samples decreased with the time. Decrease in total plate count from 0 to 28 days for control (sucrose) was 23.92x10⁷ to 9.59x10⁷. Sample with saccharine decreased TPC from 23.28x10⁷ to 9.86x10⁷ and for yoghurt treated with acesulfame-K was 23.68x10⁷ to 10.11x10⁷. Aspartame treated sample decreased TPC during storage from 23.79x10⁷ to 10.89x10⁷. It is evident from above data that sample with aspartame showed best results among other treatments for microbial analysis.

It is resulted from analysis that sample with sucrose had increased growth of lactic acid bacteria while treatments with artificial sweeteners have detrimental effects on micro biota.

Conclusion

It is crux from the contemporary research that the addition of artificial sweeteners in yoghurt has revealed much detrimental effects on the yoghurt micro biota and other physicochemical parameters from quality point of view. Best physicochemical, sensory and microbiological properties were observed with the addition of 0.022 percent of aspartame. Furthermore, by adding of 0.022 percent aspartame in yogurt the growth of beneficial microorganisms was improved, and it also contributed to the enhancement of sensory attributes i.e. taste, colour flavour, appearance, and texture.

References

- Almena, M., K. McEvoy, B. Yon and A. Howard. 2005. Sensory description of plain yoghurt made from milk of different origins. Dairy Science 88: 266-267.
- 2. Hernandez, K., I. Harte and M. Phillips. 2008. Survival of Lactobacillus acidophilus and Bifidobacterium animalis ssp. lactis in stirred fruit yoghurts. LWT Food Science and Technology 41: 1317–1322.
- 3. Meydani, S.N., Ha, W.-K. 2000. Immunologic effects of yoghurt. Am. J. Clin. Nutr.,71: 861-872.
- 4. Mahmood, M., Abbas, N. and Gilani AH. 2008. Quality of Stirred Buffalo Milk Yogurt Blended With Apple and Banana Fruits. Pak. J. Agri. Sci., Vol. 45(2).

- 5. Wood, B.J.B. 1992. The lactic acid bacteria in health and disease. London: Elsevier Appl. Sci. 151-339.
- Pınar S. K. 2015. Assessment of nutritional status of elderly individuals: a Mini-Nutritional Assessment. Progress in Nutrition 17: 284-291.
- 7. Artificial Sweeteners: Calorie-Free, Risk-Free? | Jigsaw Health." Magnesium Supplements | Jigsaw Health. Web.01Mar.2010.http://www.jigsawhealth.com/resources/artificial-sweetner.
- 8. Pinheiro, M.V. S, M. N. Oliveira, A. L. B. Penna and A. Y. Tamime. 2005. The effect of different sweeteners in low-calorie yogurts A review. Int. J. Dairy Tech., 58: 193-194.
- Castro, A. P. and L. J. Franco. 2002. Characterization of the consumption of alternative sweeteners and dietetic products for diabetics. Arq Bras. Endocrinol. Metabol. 46: 280-287.
- Cardello, H. B., M. Da Silva and M. H. Damasio. 1999.
 Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. Plant Foods Hum. Nutr., 54: 119-130.
- 11. Reis, C. R. 2007. Yogurt "light" strawberry flavor: equivalence of sweetness, characterization and sensory impact of packaging on consumer buying intention. Thesis (Ph.D. in Food Science and Technology), Federal University of Vicosa, Vicosa, Brazil.
- 12. Matsubara, S. (2001) Functional foods: A trend that opens up prospects for dairy products. Dairy Ind., 610-18.
- 13. Brazil. 1995. Adopting the use of sucralose as a sweetener for food function and diet drinks. Ministry of Health Secretariat of Health Surveillance. Ordinance No 318 of 24 November 1995.
- 14. Cardoso, J. P., J. R. Battochio and H. B. Cardello. 2004. Equivalence of sweetness of a sweetener and the sweetening power as a function of the temperature of consumption embeds prepared teammate with the soluble powder. Tecnol. Chem. Alim., 24: 448-452.
- Schiffman, S. S., E. A. Sattely-Miller, B. G. Graham, J. L. Bennett, B. J. Booth, N. Desai and I. E. Bishay. 2000. Effect of temperature, pH and ions on sweet taste. Physiol. Behav., 68: 469-481.
- AOAC. 2000. Association of Official Analytical Chemists. Official methods of analysis. 17th edition published by AOAC. Po box 540. Benjamin Franklin station, Washington, DC., USA.
- 17. Nafiseh, V., M.M. Tehrani and F. Shahidi. 2008. Optimizing of fruit yoghurt formulation and evaluating its quality during storage. American Eurasian J. Agriculture and Environmental Sci. 3: 922-927.
- 18. Aryana, KJ and McGrew, P (2007). Quality attributes of yogurt with Lactobacillus casei and various prebiotics. LWT Food Sci. Technol., 40: 1808-1814.
- Adachi, M., J. Kanamori, T. Masuda, K. Yagasaki, K. Kitamura, B. Mikami and S. Utsumi. 2003. Crystal structure of soybean globulin. Glycinin homohexamer. PNAS. 100: 7395-7400.

- 20. David, B. and Fankhauser 2005. Pour plate technique for bacterial enumeration. Unvi. Of Cincinnati Clermont College, Batavia OH 45103, pp: 1-3.
- 21. Cappuccino, J.G. and N. Sherman, 1996.Microbiology a Laboratory Manual, pp. 137–49. The Benjamin/Cummings Pub. Co. Inc. New York, USA.
- 22. Steel, R., J. Torrie and D. Dickey, 1996. Principles and Procedures of Statistics: A biometrical Approach, 3rd Ed. Mc Graw Hill Book Co., New York.

Correspondence:

Hafiz Arbab Sakandar

Department of Microbiology Quaid-i-Azam University, Islamabad, 44000, Pakistan

E-mail: Hafizarbabsakandar@gmail.com