Plant Archives Vol. 19 No. 1, 2019 pp. 870-874 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF THE ε-POLYLYSINE UTILIZATION ON THE PHYSIOCHEMICAL, MICROBIOLOGICAL AND RHEOLOGICAL PROPERTIES OF THE DRINKING YOGURT

Shaymaa Saady Lafta

Department of Food Science, Collage of Agricultural Engineering Science, University of Baghdad, Iraq.

Abstract

ε-Poly-L-lysine is a homopolymer linked by the peptide bond between the carboxylic and the epsilon amino group of adjacent lysine molecules. Post-acidification of drinking yogurt always occurs during cold storage causing short shelf life thus consumer unacceptability. The short storage life of particular drinking yogurt affects the consumption level as well. This study concentrated on the effect of preservatives on post-acidification of drinking yogurt. The drinking yogurt mixture was industry and inoculated with 2% starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. After the required inoculation; ε-polylysinewas added separately at different concentrations. Titratable acidity, pH and viscosity of drinkig yogurts during cold storage for 20 days were examined. Evaluation of sensory properties was done for the best concentrations of each preservative. The pH values and titratable acidity were significantly lower (<0.05) in drinking yogurts with added preservatives than those in blank. The results showed that å-polylysine used at 0.007% (w/v) respectively gave the best (optimum) inhibition of post-acidification during refrigerated storage for 20 days. The sensory evaluation revealed that 0.007% (w/v) ε-polylysine was the most preferred preservative to consumers with respect to taste, colour, mouth feel, appearance and overall acceptability.

Key words : ε-polylysine, drinking yogurt, rheological properties and Post-acidification.

Introduction

One of the oldest methods by human practiced is fermentation beings for the transformation of milk into products with an extended storage period or shelf-life. Titrable acidity of drinking yoghurt is an effect of lactic acidification obtained at the end of the incubation and post-acidification during storage (Shaymaa, 2014). Drinking yoghurt possesses many health benefits there exists a major problem in it manufacturing and during storage prior to consumption, called post-acidification. As bioactive ingredients, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* continue to produce lactic acid after production fermentation, making the yoghurt too sour. This phenomenon is not desirable. Post-acidification shortens yoghurt's shelf life result in an unacceptable taste (Beal *et al*., 1999).

In addition, adding of some preservatives to drinking yogurt is a method to improve the shelf life of the product by limiting the activity of starter cultures during storage period. There are many preservatives in the practice. Most available preservative in drinking yogurt industry is Potassium, Sodium scorbate. It is a chemical food preservative. In addition to chemical preservatives, there are some natural preservatives such as Nisin, Chitosan and Epsilon polylysine (Wu *et al*., 2011).

As natural preservative, ε-poly-L-lysine (ε-PL) is also a natural food preservative produced through natural fermentation using the bacteria *Streptomyces albulus*ssp. *Lysinopolymerus* under aerobic conditions. Polylysine consists as a small natural homopolymer of the essential amino acid, L-lysine. Its application includes cakes, rice, cheese and other dairy Industry foods and meats. ε- poly-L-lysine and its derivatives offer a wide range of unique applications such as food preservatives, emulsifying agent, dietary agent, biodegradable fibers, highly water absorbable hydrogels, drug carriers,

anticancer agent enhancer, biochip coatings (Shih *et al*., 2006). It is generally recognized as safe (GRAS) status for certain food applications (Food and Drug Administration, 2010).

Lot of researches have focused on how to improve the quality of the shelf life of drinking yogurt, especially the problem of post-acidification. But the effects are limited (Harbin Institute of Technology, 2011). Presently, potassium sorbate is commonly used to improve shelf life of fermented food items including yoghurt. But, the addition of natural preservatives to control post-acidification, such as ε-polylysine is of interest because of its biocompatibility, nontoxicity, improve quality and antimicrobial action., application ε-polylysine is used to improve the quality and extend shelf life of time-limited in dairy fermented products. ε-polylysine is used as functional dairy ingredients such as to improve the nutritional quality of yoghurt, encapsulation of pro-biotic bacteria etc. Nevertheless, little literature is available for the application of ε-polylysine in drinking yogurt. Further, their inhibitory effect on post-acidification of drinking yogurt fermented with combinations of starter culture bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) has been not investigated.

Methodology

Preparation of drinking yogurt

In the first step of preparation was 1:2 milk: waterthen 2% salt was add and the mixture was homogenization at15-20mpa at 55- 65°C and after that samplewas pasteurized at 80-85°C for 30 min. Inoculation was done with 2% Freeze Dried Direct Vat-Set (FD-DVS) starter cultures containing *Lactobacilus bulgaricus*s ub spp. *Delbruikii* and *Streptococcus salvarius* sub spp. *thermopiles* in 1:1 ratio. Starter culture was supplied by Delvo-Yog. Different concentrations of ε-polylysine (Lanzhou WeiRi Bioengineering Co., Ltd. China) was added to inoculated mixture separately (table 1). Then the mixture was transferred in to polyethylene cups and covered with the lids. After covering the cups, these were incubated in a walk-in incubation room. The pH was monitored by PH (Martini instruments) pH meter. Fermentation was stopped after 2.5 hours and then by rapidly cooled to 6^0C by placing in a refrigerator. Drinking Yoghurt products were subjected to sensory analysis after storage for 5 days in 6^0C.

Preparation of ε-Polylysine

ε-Polylysine samples were stored at 4^0C until use. Sample packets were removed from refrigerator prior to use. Cut lines of the top of the pouch were sterilized by using 70% ethyl alcohol and pouch was opened using flamed sterilized scissor. Preservative was measured and added using APX-200 model top loading balance (Qualitron (pvt) Ltd., Rajagiriya) according to concentrations and milk quantity used. Preservatives were added to inoculated milk separately using a sterilized measuring jar.

Preliminary study to select best concentration of ε-Polylysine

Titratable acidity, pH, firmness, total solids, yeast and mold count of drinking yogurt added different concentrations of ε-Polylysine was measured.

Shelf life evaluation

Drinking yogurt samples were tested for pH and titratable acidity. pH meter was used to measure the pH. Drinking yogurt samples were tested for 21 days in cold storage (4^0C). Three replicates were used for each treatment and results were evaluated using SAS soft ware package. Titratable acidity, pH and physical changes were monitored at room temperature (25^0C).

Rheological measurements

Rheological properties of the drinking yogurt samples after 5 day of storage at 4°C were determined in duplicate. This test include determination of viscosity according to Ostwald method by means of Ostwald viscometer (A.O.A.C, 2000). All measurements were carried out on 50 g of sample, which previously prepared by gently stirring in identical conditions. The apparent viscosity was calculated by following equation:

$$\frac{\text{Viscosity of known liquid Density of known liquid} \times \text{Time of it's dropped}}{\text{Viscosity of unknown liquid Density of unknown liquid} \times \text{Time of it's dropped}}$$

Sensory evaluation was carried out for appearance, texture, flavor and taste according to(Harper and Hall, 1969).

Microbiological analysis

Drinking yogurt samples were tested for coliforms, Yeast and Mold counts using standards microbiological methods.

Physical property analysis

Wheying off according to Shaymaa (2014) as follows: using cylinder volume 100 ml after 24 hr of storage for all the treatments at 4^oC. The syneresis was estimated as percentage of released whey over the initial gel size using following equation (Results of average of triplicate were considered):

$$\% \text{ Wheying off} = \frac{\text{Volume of supernatant}}{\text{Volume of yogurt sample}} \times 100$$

Determining of the acceptability of drinking yogurts added different concentration of preservatives

Preparation of Samples

Drinking yogurt samples were prepared according to the method described above.

Testing for sensory qualities

Sensory evaluation was done at MILCO Scan (pvt) Limited, Digana, Rajawella. The sensory characteristics were appearance, colour, texture, taste and overall acceptability.

Testing criteria

The seven point hedonic scale was used to evaluate the degree of preference for each attributes.

Serving samples

The samples were coded with three digit random numbers drawn from a random number table. Samples were served in random order. The panelists were provided with distilled water and they were asked to rinse their mouth after each testing.

Statistical analysis

The non parametric ranking procedure was used with Friedman Rank Sum test for evaluation of quality parameters. The data was analyzed with MINITAB soft ware package. The significant level of 0.05 was used for analysis.

Results and Discussion

Acidification of yoghurt during cold storage

Titratable Acidity (% lactic acid)

Measuring of titratable acidity (TA) was done for 20 days with 4 days interval. Samples with three different concentrations of preservative were used to measure TA.

Titratable acidity of drinking yogurts with commonly available preservative in market. Storage time and ε-Polylysine (ε-PL) had significant effect on lactic acid % of the drinking yogurt (<0.05). Table 2 shows the continuous increasement of the acidity of blank drinking yoghurt and controlling of acid production in drinking yogurt added ε-PL. (Tamime and Robinson,1999). found that polylysine electrostatically adsorb onto cell membrane and abnormal distribution of the cytoplasm (Shih *et al*., 2006). There were not significant different among three levels of ε-PL (<0.05). It means that even low concentrations of ε-PL can inhibit the activity of lactic acid bacteria. So, addition of 0.007% concentration of ε-PL is sufficient for the inhibiting of post production acidification. Poly-L-lysine has been shown to inhibit growth in a wide variety of organisms at concentrations of less than 21μg/mL (Yoshida and Nagasawa, 2003).

pH

The pH of the drinking yogurts was measured for 20 days with 4 days interval.

The pH values of graph considerably decreased from 0 day to 20 days. However, it was observed that pH values of the drinking yogurts added preservative were not changed dramatically. According to the data analyzed; the fig. 1 showed that after 5 days storage period of drinking yogurt, there was not remarkable decrease in the pH values. It was same for the yogurts added ε-PL (fig. 1).

The antimicrobial action of ε-polylysine is due to its inhibitory influence on various enzymes in the microbial cell. The pH of the drinking yogurt added ε-polylysine also controlled After 5 days of cold storage, pH of yogurt added ε-PL has controlled and there were not significant different among three levels (<0.05). The antimicrobial effect of polylysine requires slightly acidic conditions and in alkaline conditions greater amounts of ε- PL are needed as antimicrobial activity is lowered (Food and Drug Administration, 2010).

Viscosity property of drinking yoghurt during cold storage

The viscosity of all the samples studied were not significantly different (>0.05) in zero time, viscosity samples was increased in 10th day of post production and it has maintained until 20th day (table 2) (Harbin Institute of Technology, 2011).

Microbiological analysis of drinking yogurt samples with tested antimicrobials during cold storage at 4°C

All the samples added preservative was safe to consumers. There were not contaminations with Coliform and *Escherichia coli*. Drinking yogurts added preservative were free from Coliform and *Salmonella*. E-PL has an ability to inhibit the growth of Ecoli bacteria. Even though, ε-PL has good antifungal activity, moulds were present than standard level (Tamime and Robinson, 1999).

Comparison of best concentrations

There was best concentration level of preservative discussed above. Those best concentrations were selected by using especially: the variation of titratable

acidity, pH and texture of drinking yogurts 0.01% (w/v) of ε-polylysine were better than other concentrations of each preservative. GRAS (2010) mentioned that ε-polylysine can be used at levels ranging from 0.005%-0.06% (w/v) as dairy products additives (Food and Drug Administration, 2010).

ε-PL can be controlled the post-acidification of drinking yogurts. It means that, it can inhibit the activity of starter culture in post fermentation with small concentration for it. using of chemical preservative can be harmful. Hence, there is a trend to use natural preservatives instead of chemical preservatives. Natural preservatives are non toxic, biodegradable and biocompatible to human. Further; ε-PL has many benefits to human (A.O.A.C., 2000; Yoshida andNagasawa, 2003; Shih *et al*., 2006).

Whey separation

Syneresis is a measure of the quantity of separated whey from the the most important factors effecting on consumers acceptance. In general It is better that the amount of whey to be separated was few in the final product (Shaymaa, 2014). The results in table 3 indicates, the amount of separated whey is higher in control drinking yogurt treatmentwhile low concentration of ε-PL was prevented wheying off.

Sensory analysis

Measuring of sensory quality characteristics of drinking yogurt is important to get an idea about consumer acceptance of new product (Harbin Institute of Technology, 2011) mentioned that sensory quality of dairy products, including drinking yogurt is vital since the best ingredients make the best final product and quality drives consumer acceptance (Harper and Hall, 1976).

Taste

Data analyzed has shown that there was a significant different at least between two samples (<0.05). Highest median value and highest sum of ranks were obtained for the taste in thedrinking yogurt added 0.007% (w/v) ε-polylysine. So it can be said that ε-polylysine gives better taste and less sour taste. Drinking yogurt which did not added any preservatives (blank drinking yogurt) gave bad taste due to sourness. It has mentioned that ε-PL has sweetness, astringency, harshness or other characteristic taste (Shaymaa, 2014).

Colour

Colour is the first attribute a consumer perceives in food. There was significant difference in colour of drinking yogurt with preservative and blank drinking yogurt (<0.05). According to the sum of ranks, most

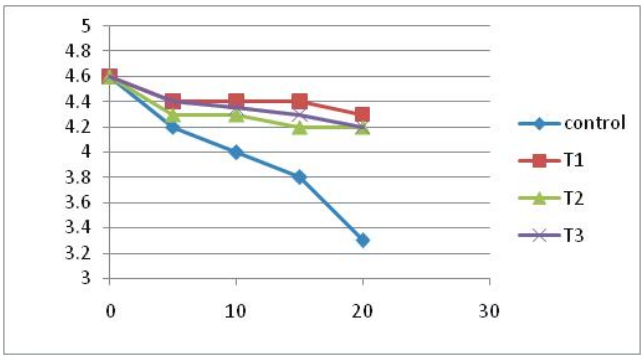

Fig. 1 : Variation of pH of drinking yoghurt samples added with different concentration of with ε-polylysine during cold storage for 20 days at 4°C, Control; no preservative added.

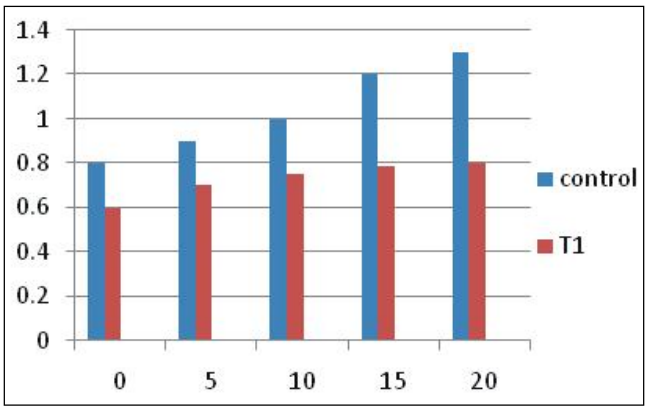

Fig. 2 : Variation of titratable acidity of drinking yogurt with best concentration of ε-polylysine at 4°.

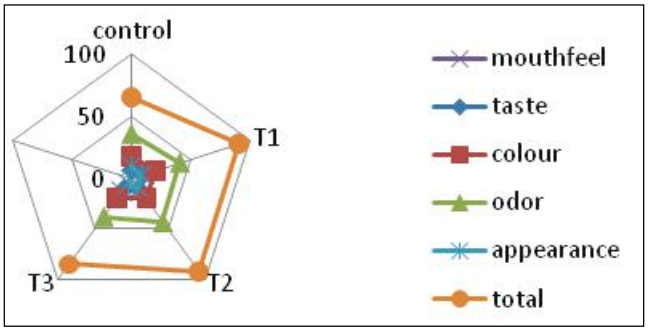

Fig. 3 : Spider-web diagram of sensory differences between selected yoghurt added with best and control treatment.

preferred colour exhibited in drinking yogurt added ε-PL. Colour of the product is affected by its physical and chemical composition (Tamime and Robinson, 1999). Colour of the yoghurt added preservative may be varied due to changes of chemical composition of drinking yogurt.

Odor

Due to the adjusted probability value was lower than 0.05, there was significant different between aroma of samples. According to the median value and sum of ranks;

Table 1 : Treatment preparation of ε-polylysine preservatives and their concentrations.

Preservative	Treatment number	Concentration % (w/v)
Control	T0	0
ε-Polylysine	T1	0.007
	T2	0.014
	T3	0.021

Table 2 : The viscosity of all the samples during cold storage.

Concentration	Period storage days				LSD
	5	10	15	20	
Control	99.6	110.9	115.9	120.8	23.26 *
0.007	255.7	277.8	345.2	378.6	34.14*
0.014	277.9	299.3	330.7	399.4	31.3*
0.021	336.7	348.9	388.9	421.5	35.5*
LSD	38.4*	22.7*	21.6*	26.9*	…..

Table 3 : The effect of ε-PL addition on Whey separation (ml) for drinking yogurt.

Concentration	Period storage days			
	5	10	15	20
Control	20	35	40	47
0.007	0	0	8	12

drinking yogurts added ε-PL got most preferred aroma. Drinking yogurt without added ε-PL gave off odor.

Mouth feel

There was a significant difference at least between samples (<0.05). According to the results, drinking yogurt added ε-PL gave better mouth feel than other. Firmer or thicker samples will take longer to dissolve in the mouth. Sour taste positively correlated with tooth etch mouth feel. So blank drinking yogurt got bad mouth feel with respect to othertreatment.

Appearance

Among three samples, drinking yogurt added ε-PL got better appearance than control. Whey separation could be seen in control drinking yoghurts due to low pH.

Conclusion

This study has shown that post-acidification of drinking yogurt during cold storage can be controlled by adding ε-polylysine. These preservative can successfully inhibit the activity of lactic acid bacteria (*Streptococcus thermophillus* and *Lactobacillus bulgaricus*) in post fermentation of drinking yogurt, ε-polylysine 0.007% (w/v) is the best concentrations which inhibits the post-fermentation of lactic acid bacteria. Moreover, the addition of polylysine does not remarkably change acid gel formation of drinking yogurt. When this is incorporated the preserved drinking yogurt has more than 20 days shelf life. Drinking yogurt added with ε-polylysine 0.007% (w/v) concentration gives desirable sensory characteristics. Finally, ε-polylysine is a good natural preservative which can control the post-acidification of drinking yogurt during cold storage, physio chemical properties, sensory evaluation and rheological properties of it.

References

A. O. A. C. (2000). Association of official analytical chemists.

Beal, C., J. Skokanova, E. Latrille, N. Martin and G. Corrieu (1999). Combined effects of culture conditions and storage t ime on acidification and viscosity of stirred yogurt. *Journal of Dairy Science*, **82(4)** : 673-681.

Food and Drug Administration (2010). *Epsilon-polylysine GRAS notification*. Retrieved from http://www.accessdata.fda.gov.

Harbin Institute of Technology (2011). *Post-acidification*. Retrieved from http://2011.igem.org/Team;HIT-Harbin/Project.

Harper, W. J. and C. W. Hall (1976). *Dairy technology and engineering*. AVI Publishing Co., Inc

Shaymaa, S. L. (2014). Effect of microbial Transglutaminase on physiochemical properties of yogurt. *J. Food Industries & Nutr. Sci.*, **1** : 47-55.

Shih, I. L., M. H. Shen and Y. T. Van (2006). Microbial synthesis of poly (å-lysine) and its various applications. *Bioresource technology*, **97(9)** : 1148-1159.

Tamime, A. Y. and R. K. Robinson (1999). *Yoghurt: science and technology*. Woodhead Publishing.

Nelson, J. A. and G. M. Trout (1964). *Judging Dairy Products*. The Olsen Publishing Co. Milwakee, Wis. 53212. U.S.A.

Wu, L., X. Chen, M. Yang, Y. Y. Shao, W. C. Bao, N. Liu and H. P. Zhang (2011). Research Progress on Affecting Factors and Mechanisms of Yogurt Post-acidification. *Food Science*, **1** : 059.

Yoshida, T. and T. Nagasawa (2003). E-Poly-L-lysine: microbial production, biodegradation and application potential. *Applied Microbiology and Biotechnology*, **62(1)** : 21-26.