

# Evaluation of Potassium sorbate and E-polylysine for their inhibitory activity on post-acidification of set yoghurt under cold storage for 20 days

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**Abstract-** Post-acidification of set yoghurt always occurs during cold storage causing short shelf life thus consumer unacceptability. The short storage life of particular yoghurt affects the consumption level as well. This study concentrated on the inhibitory effect of preservatives on post-acidification of yoghurt. The set yogurt mixture was produced and inoculated with 2% starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. After the required inoculation;  $\epsilon$ -polylysine (as natural preservative) and potassium sorbate (as chemical preservative) were added separately at different concentrations. Titratable acidity, pH and firmness of 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 yoghurts with added preservatives than those in blank yoghurt. The results showed that  $\epsilon$ -polylysine and potassium sorbate used at 0.005% (w/v) and 0.1% (w/v), respectively gave the best (optimum) inhibition of post-acidification during refrigerated storage for 20 days. The sensory evaluation revealed that 0.005% (w/v)  $\epsilon$ -polylysine was the most preferred preservative to consumers with respect to taste, colour, mouth feel, appearance and overall acceptability. Hence, these results support the conclusion that there is an inhibitory effect of potassium sorbate and  $\epsilon$ -polylysine on post-acidification of set yoghurt during cold storage while the later was most preferred by the panelists.

**Index Terms-** Post-acidification, Set yoghurt,  $\epsilon$ -polylysine, Potassium sorbate, Cold storage

## I. INTRODUCTION

Fermentation is one of the oldest methods practiced by human beings for the transformation of milk into products with an extended storage period or shelf-life. Titratable acidity of yoghurt is an effect of lactic acidification obtained at the end of the incubation and post-acidification during storage [2]. Although yoghurt possesses many health benefits there exists a major problem in yoghurt 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 by consumers.

Post-acidification is mainly affected by the choice of species, storage temperature and storage time [2]. Lan *et al.* (2011) mentioned that acidification of yogurt can be controlled by giving pasteurized or ultra-high pressure treatment to kill bacteria; rapid cooling after fermentation; add bacteriocins; adjust the leavening agents namely of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* ratio. Some researchers are trying to improve the genetic level of culture strains including alter cell membrane permeability, the use of genetic engineering and artificial mutation breeding techniques to control the after acidification [11].

In addition, adding of some preservatives to yoghurt is a method to improve the shelf life of the product by limiting the activity of survivors of starter cultures during storage period. There are many preservatives in the practice. Most available preservative in yoghurt industry is Potassium/ Sodium sorbate. It is a chemical food preservative. In addition to chemical preservatives, there are some natural preservatives such as Nisin, Epsilon polylysine and Chitosan.

Potassium or sodium salts of sorbic acid are more commonly used than the sorbic acid. Its antimycotic activity is released at low pH of  $<6.5$  [9]. Potassium Sorbate is a naturally occurring organic acid that has been used extensively as a fungistatic agent for foods. The antimicrobial action of potassium sorbate is due to its inhibitory influence on various enzymes in the microbial cell. The enzymes inhibited by potassium sorbate include the following: 1. Enzymes involved in carbohydrate metabolism such as enolase and lactate dehydrogenase. 2. Enzymes of the citric acid cycles such as malate dehydrogenase, isocitrate dehydrogenase, ketoglutarate dehydrogenase, succinate dehydrogenase, and fumerase. 3. Several enzymes containing SH group, and other enzymes such as catalase and peroxidase. Potassium sorbate is safe with regard to health and have the lowest allergenic potential of all food preservatives. It is generally recognized as safe (GRAS) for use in foods under regulations of us FDA.

As natural preservative,  $\epsilon$ - poly-L-lysine ( $\epsilon$ -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 foods and meats.  $\epsilon$ -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 [8]. It is Generally Recognized As Safe (GRAS) status for certain food applications [3].

A lot of researches have focused on how to improve the quality of the shelf life of yoghurt, especially the problem of post-acidification. But the effects are limited [5]. 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  $\epsilon$ -polylysine is of interest because of its biocompatibility, nontoxicity, and antimicrobial action. Recently, application  $\epsilon$ -polylysine is used to improve the quality and extend shelf life of time-limited foods including fermented foods.  $\epsilon$ -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  $\epsilon$ -polylysine in set yogurt. Further, their inhibitory effect on post-acidification of set yoghurt fermented with combinations of starter culture bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) has been not investigated.

The objectives of this study were: (1) to study inhibitory effect of Epsilon polylysine on post-acidification of set yoghurt under refrigerated storage (3) to study inhibitory effect of potassium sorbate on post-acidification of set yoghurt under refrigerated storage (4) to select the best preservative from  $\epsilon$ -polylysine and potassium sorbate which control the post-acidification of yoghurt (5) to determine the best concentration of each preservative.

## II. METHODOLOGY

### 2.1 Preparation of plain set yogurt

Raw milk was warmed to 40 °C and fortified with 2% (w/v) skim milk powder and sugar 11% (w/v). Mixture was blended using electrically operated blender. Then mixture was homogenized at 200 psi for 5 minutes using FBF 015 type, 06071880, (Parma, Italy) homogenizer. Homogenized mixture was pasteurized to 95 °C and held for 8 minutes by P13HRB model, ser. no 3010062560 (Vulcan-Lava Ltd., Sweden) pasteurizer. It was cooled down to 42 °C. Inoculation was done with 2% Freeze Dried Direct Vat-Set (FD-DVS) starter cultures containing *Lactobacillus bulgaricus* sub spp. *delbruiikii* and *Streptococcus salvarius* sub spp. *thermophilus* in 1:1 ratio. Starter culture was supplied by Delvo-Yog. Different concentrations of  $\epsilon$ -polylysine (Zhengzhou Bainafu Bioengineering Co., Ltd.He’Nan, China) and potassium sorbate (Jayes, Denmark) were added to inoculated mixture separately (Table 2.1). 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 PH55, ser. no 1001367 (Martini instruments) pH meter. Fermentation was stopped after 2.5 hours and then by rapidly cooled to 6 °C by placing in a refrigerator. Yoghurt products were subjected to sensory analysis after storage for 4 days in 6 °C.

Table 2.1 Treatment preparation according to the preservatives and their concentrations.

Preservative	Treatment Number	Concentration % (w/v)
Control (without adding of preservatives)	0	0
Potassium sorbate	T 11	1) 0.050
	T12	2) 0.075
	T13	3) 0.100
$\epsilon$ -Polylysine	T21	1) 0.005
	T22	2) 0.015
	T23	3) 0.025

### 2.2 Preparation of preservatives

All preservative samples were stored at 4 °C 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. Preservatives from three types were 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.

### 2.3 Preliminary study to select best concentration of each preservative

Titratable acidity, pH, firmness, Total solids, Yeast and Mold count of each yoghurt added different concentrations of three preservatives were measured.

#### 2.3.1 Shelf life Evaluation

Yoghurt samples were tested for pH and titratable acidity. Model PH 55, 1001367 type pH meter was used to measure the pH. Yogurt samples were tested for 21 days in cold storage (4 °C). 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°C).

#### 2.3.2 Microbiological analysis

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

### 2.3.3 Physical property analysis

Firmness was measured by using digital penetrometer-G 10777 (Trifoly industries, Italy). Diameter of the nobe was 1.135cm.

## 2.4 Determining of the acceptability of yoghurts added different preservatives

### 2.4.1 Preparation of Samples

Yoghurt samples were prepared according to the method described above.

### 2.4.2 Testing for sensory qualities

Sensory evaluation was done at MILCO (pvt) Limited, Digana, Rajawella. The sensory characteristics were appearance, colour, texture, taste and overall acceptability. The panel consists of 30 untrained panelists.

### 2.4.3 Testing criteria

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

### 2.4.4 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.

### 2.4.5 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.

## III. RESULTS AND DISCUSSION

### 3.1 Acidification of yoghurt during cold storage

#### 3.1.1 Titratable Acidity (% lactic acid)

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

Titratable acidity of yoghurts with commonly available preservative in market; potassium sorbate was observed for 20 days comparing with a control (Table 3.1).

Table 3.1 Mean values and variation of titratable acidity of yoghurt samples incorporated with potassium sorbate (C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>K) for 20 days under 4 °C refrigeration.

Treatment	Storage period (days)				
	0	5	10	15	20
Control	0.75±0.01 <sup>a</sup>	0.90±0.01 <sup>a</sup>	0.95±0.01 <sup>a</sup>	1.06±0.02 <sup>a</sup>	1.26±0.01 <sup>a</sup>
T11	0.62±0.01 <sup>bc</sup>	0.75±0.01 <sup>bc</sup>	0.79±0.01 <sup>b</sup>	0.80±0.01 <sup>b</sup>	0.80±0.01 <sup>b</sup>
T12	0.63±0.02 <sup>b</sup>	0.76±0.01 <sup>c</sup>	0.78±0.01 <sup>c</sup>	0.79±0.01 <sup>bc</sup>	0.79±0.01 <sup>bc</sup>
T13	0.60±0.01 <sup>c</sup>	0.72±0.01 <sup>d</sup>	0.75±0.00 <sup>d</sup>	0.78±0.01 <sup>d</sup>	0.78±0.01 <sup>d</sup>

\*All values in the same column with different superscripts are significantly different at (p<0.05). control; no (C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>K) added, T11; contains 0.05 g 100 g-1, T12; contains 0.075 g 100 g-1, tT13; contains 0.10 g 100 g-1

According to the data showing in Table 3.1, titratable acidity of yoghurt increases for three weeks. Different superscripts of mean values shows significantly difference of the acidity of each preservative (<0.05). SLS 735; part 2 (1989) has mentioned that acceptable acidity range is 0.8-1.25%. The control which not added potassium sorbate was reached to 1.26% acidity. So it has only 20 days shelf life and other C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>K added yogurts have more than 20 days shelf life. Acidity of control was significantly different (<0.05) from other. According to the data analyzed, storage period and treatment with the potassium sorbate are significantly affected to the titratable acidity. Moreover, it reveals that interaction of storage period \* treatment has significant effect to acidification of yoghurt.

Titratable acidity of yogurts has increased over the time and acidity of yoghurt which not added C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>K increased drastically than others. Hence, this experiment has shown the inhibitory activity of potassium sorbate on post fermentation. In other words, it merely inhibits the activity of starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). Tamime and Robinson, (1999) has mentioned that there is an antimycotic activity of potassium sorbate [9].

0.1% potassium sorbate added yoghurt got lowest acidification rate. So it has low post –acidification. It means, 0.1% sorbate added yogurt has longer shelf life than others (0.05% and 0.075% sorbate added yoghurts). Hamdan *et al.* (1971) has reported that sorbates retarded the growth of commercial yoghurt cultures and decreased the rate of acid production during fermentation study conducted in laboratory [4].

ε- Poly-L-Lysine is the other preservative which used for controlling of post- acidification in this experiment.

Table 3.2 Mean values and variation of titratable acidity of yoghurt samples incorporated with  $\epsilon$ - Poly-L-Lysine for 20 days under 4 ° C.

Treatment	Storage period (days)				
	0	5	10	15	20
Control	0.75±0.01 <sup>a</sup>	0.90±0.01 <sup>a</sup>	0.95±0.01 <sup>a</sup>	1.06±0.01 <sup>a</sup>	1.26±0.01 <sup>a</sup>
T21	0.63±0.01 <sup>b</sup>	0.76±0.01 <sup>b</sup>	0.75±0.01 <sup>b</sup>	0.80±0.02 <sup>b</sup>	0.81±0.01 <sup>b</sup>
T22	0.68±0.02 <sup>c</sup>	0.74±0.00 <sup>b</sup>	0.77±0.01 <sup>c</sup>	0.79±0.01 <sup>b</sup>	0.79±0.01 <sup>b</sup>
T23	0.69±0.01 <sup>c</sup>	0.71±0.01 <sup>d</sup>	0.75±0.01 <sup>b</sup>	0.79±0.01 <sup>b</sup>	0.79±0.01 <sup>b</sup>

\*All values in the same column with different superscripts are significantly different at (p<0.05).

Storage time and  $\epsilon$ - Polylysine ( $\epsilon$ -PL) had significant effect on lactic acid % of the yoghurt (<0.05) (Table 3.2). Table 3.2 shows the continuous increasement of the acidity of blank yoghurt and controlling of acid production in yoghurt added  $\epsilon$ -PL. Shih *et al*, (2004) found that polylysine electrostatically adsorb onto cell membrane and abnormal distribution of the cytoplasm [8]. There were not significant different among three levels of  $\epsilon$ -PL (<0.05). It means that even low concentrations of  $\epsilon$ -PL can inhibit the activity of lactic acid bacteria. So, addition of 0.005% concentration of  $\epsilon$ -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 100  $\mu$ g/mL [12].

### 3.1.2 pH

The pH of the set yoghurts was measured for 20 days with 4 days interval.

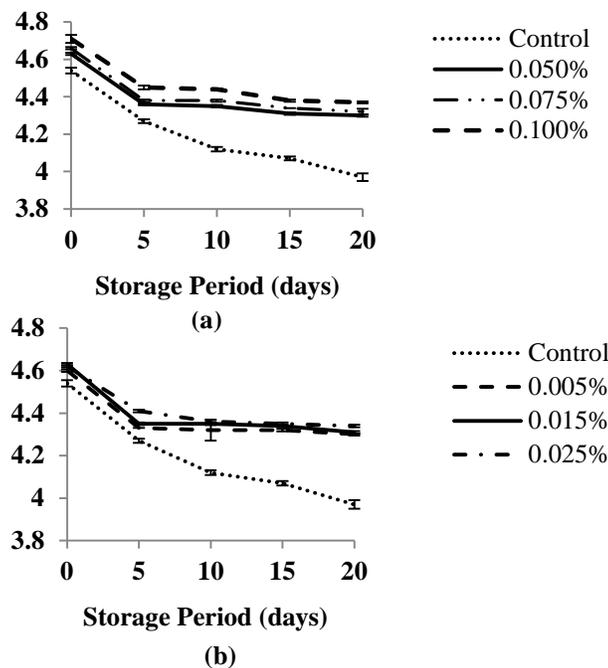


Figure 3.1 Variation of pH of yoghurt samples added with different antimicrobial agents during cold storage for 20 days at 4 ° C (a) yoghurt added with C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>K (b) yoghurt added with  $\epsilon$ -polylysine, Control; no preservative added

The pH values of both graphs considerably decreased from 0 day to 20 days. However, it was observed that pH values of the yoghurts added two preservatives were not changed dramatically. According to the data analyzed; there was significant effect of potassium sorbate and  $\epsilon$ -PL on pH of set yogurt (<0.05). Figure (a) showed that after 5 days storage period of yogurt, there was not remarkable decrease in the pH values. It was same for the yogurts added  $\epsilon$ -PL (Figure b).

Even though there was not significantly different of pH of the yoghurt added potassium sorbate at 20<sup>th</sup> day of post production; yogurt 0.01% showed the highest pH (Figure a). Controlling of pH by potassium sorbate could be due to inhibiting of the activity of starter culture. The antimicrobial action of sorbic acid is due to its inhibitory influence on various enzymes in the microbial cell.

The pH of the yoghurt added  $\epsilon$ -polylysine also controlled (Figure b). After 5 days of cold storage, pH of yogurt added  $\epsilon$ -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  $\epsilon$ - PL are needed as antimicrobial activity is lowered [3].

### 3.2 Textural property of set yoghurt during cold storage

The firmness of all the samples studied were not significantly different ( $>0.05$ ). Firmness of samples was increased in 10<sup>th</sup> day of post production and it has maintained until 20<sup>th</sup> day (Figure 3.2). Tamime and Robinson (1999) has mentioned that acid milk gelation in yoghurt happens due to association of casein micelles with one another via ca-phosphate bridges [9].

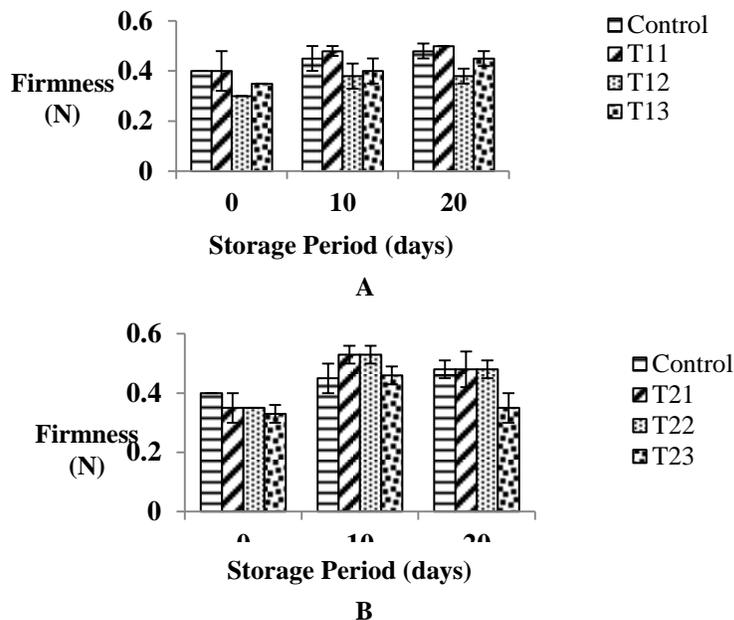


Figure 3.2 Variation of firmness during cold storage of yoghurt added with potassium sorbate (A), polylysine (B)

Further, high heat treatment of the milk base leads to firmer gels and faster gelation. In addition, gel firmness continues to increase with time; during the cold storage period of the product. Firmness of yoghurts added  $\epsilon$ - PL was higher than the yoghurt added potassium sorbate. Shih *et al*, (2006) has mentioned that  $\epsilon$ - PL is biodegradable fibre.

### 3.3 Microbiological analysis of set yoghurt 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*. Yoghurts added preservatives were free from Coliform and *Salmonella*. E-PL has an ability to inhibit the growth of Ecoli bacteria. Even though,  $\epsilon$ -PL has good antifungal activity, moulds were present than standard level. Tamime and Robinson (1999) have mentioned that potassium sorbate does not reduce actual number of yeast and moulds in the product, but merely inhibit their activity [9].

### 3.4 Comparison of best concentrations of three preservatives

There was best concentration level of each preservative discussed above. Those best concentrations were selected by using especially: the variation of titratable acidity, pH and texture of set yoghurts. 0.1% (w/v) of potassium sorbate and 0.005% (w/v) of  $\epsilon$ -polylysine were better than other concentrations of each preservative. GRAS, (2010) mentioned that  $\epsilon$ -polylysine can be used at levels ranging from 0.005%-0.06% (w/v) as food additives [3].

Sorbate concentrations in milk products were determined using a method based on the Provisional Standard of the International Dairy Federation (1987), with minor modifications. All the samples were controlled the acid development of the set yogurts. Figure 3.3 showed that lactic acid% of set yoghurts does not changed. But the acidity of yoghurts added K-sorbate was lower than others.

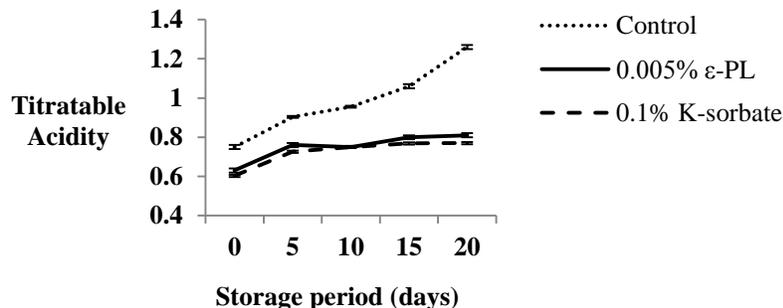


Figure3.3 Variation of titratable acidity of set yoghurt with best concentrations of the two preservatives at 4 °C

Eventhough, the inhibiting mechanism of both preservatives are different to each other, all preservatives can be controlled the post-acidification of yoghurts. Among two optimum concentrations in this experiment; potassium sorbate got higher concentration when comparing with polylysine. It means that, it needs higher concentration (0.1% w/v) to inhibit the activity of starter culture in post fermentation. In contrast;  $\epsilon$ -PL needs small concentration for inhibition.

K-sorbate is commonly available chemical preservative for set yogurt. But 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;  $\epsilon$ -PL has many benefits to human [1], [8].

Firmness during cold storage ranged from 0.35N to 0.45N and 0.35N to 0.51N for milk preserved by K-sorbate and  $\epsilon$ -PL. It was in blank yoghurt was from 0.4N to 0.48N. So there was no deviation of texture of two preservatives.

### 3.5 Sensory Analysis

Measuring of sensory quality characteristics of yoghurt is important to get an idea about consumer acceptance of new product. Harper *et al*, (1991) mentioned that sensory quality of dairy products, including yogurt is vital since the best ingredients make the best final product and quality drives consumer acceptance [6].

#### 3.5.1 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 the yoghurt added 0.005% (w/v)  $\epsilon$ -polylysine. So it can be said that  $\epsilon$ -polylysine gives better taste and less sour taste. Yoghurt which did not added any preservatives (blank yoghurt) gave bad taste due to sourness. According to ideas of panelists; yoghurt with K-sorbate gave bitter taste. Tribby, (2009) found that potassium sorbate can cause off flavors of yoghurts [10]. Maki *et al*, (2012) has mentioned that  $\epsilon$ -PL has sweetness, astringency, harshness or other characteristic taste [7].

#### 3.5.2 Colour

Colour is the first attribute a consumer perceives in food. There was significant difference in colour of yogurt with preservatives and blank yoghurt ( $<0.05$ ). According to the sum of ranks, most preferred colour exhibited in yogurt added  $\epsilon$ -PL. Colour of the product is affected by its physical and chemical composition [9]. Colour of the yoghurt added preservatives may be varied due to changes of chemical composition of yoghurts.

#### 3.5.3 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; yoghurts added  $\epsilon$ -PL got most preferred aroma. Yoghurt added K-sorbate gave off odor. Lactic acid bacteria can decompose sorbic acid and gives a geranium like off odor.

#### 3.5.4 Mouth feel

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

#### 3.5.5 Appearance

Among three samples, yogurt added  $\epsilon$ -PL got better appearance than others. Whey separation could be seen in other yoghurts due to low pH.

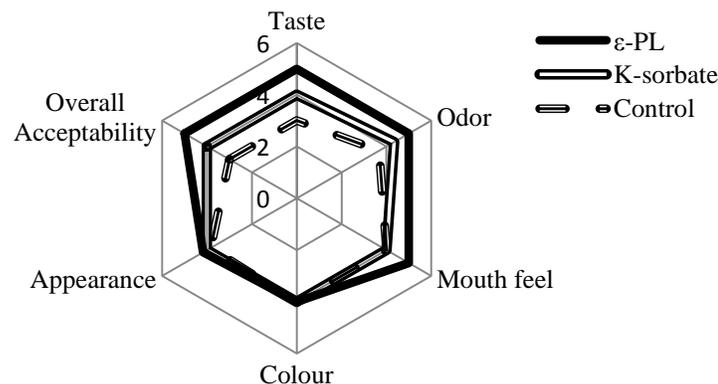


Figure 3.4 Spider-web diagram of sensory differences between selected yoghurt added with best concentrations of three preservatives samples

This web diagram showed that yogurt with  $\epsilon$ -PL gave better taste, odor, mouth feel and appearance than others.

#### 3.5.6 Overall acceptability

According to the results; yoghurt with 0.005% (W/V)  $\epsilon$ -PL gave highest acceptability than others. Some bacteria, yeast and mould decompose fat and oxidation. Therefore, it makes unfavorable odor, colour and appearance. The control yoghurt got lower ranks for overall acceptability.

#### IV. CONCLUSION

This study has shown that post-acidification of set yoghurt during cold storage can be controlled by adding potassium sorbate and  $\epsilon$ -polylysine. Those preservatives can successfully inhibit the activity of lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) in post fermentation of yoghurt. Potassium sorbate 0.1% (w/v) and  $\epsilon$ -polylysine 0.005% (w/v) are the best concentrations which inhibits the post-fermentation of lactic acid bacteria. Moreover, the addition of polylysine and potassium sorbate does not remarkably change acid gel formation and the texture of set yoghurt. When these are incorporated the preserved yoghurt has more than 21 days shelf life. Yoghurt added with  $\epsilon$ -polylysine 0.005% (w/v) concentration gives desirable sensory characteristics. Finally,  $\epsilon$ -polylysine is a good natural preservatives which can control the post-acidification of set yoghurt during cold storage compared when the chemical preservative, Potassium sorbate.

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