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Effect of sorbate on yogurt fermentation and stability of sorbate

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تأثير السوربات على تخمر الياورت وثبات السوربات

أجريت هذه الدراسة لمعرفة تأثير السوربات على التخمر وخزن الياورت وفحص ثبات السوربات أثناء تسخين الخمائر وأثناء خزن المنتوج. وقد تم كذلك تقييم مستوى السوربات في بعض الصيانة من الياورت المأخوذ من السوق. وكانت مستويات السوربات في 42 عينة من الياورت المنكه والياورت الطبيعي مابين 0 و 453 ملغ في الكلغ. وقد لاحظنا أدنى مستوى في الياورت الطبيعي، أما المستويات المرتفعة فكانت في الياورت المنكه. وبينت هذه الدراسة أن نمو الباكتيريا اللبنية وإنتاج الحمض اللبني ثم خفضها بالسوربات أثناء التحضين والخزن. وهذا التخفيض أثناء التحضين يبين أن هذا الأخير يجب أن يكون لمدة أطول أو أن تضاف الخميرة بمستوى أكبر، وأما التخفيض الذي تم الحصول عليه أثناء الخزن فيوحي بأن الياورت تكون له مدة الصلاحية أطول. وكانت تحللات ضعيفة للسوربات بالتسخين تحت درجة 80 أو 100 لمدة 6 ساعات أو أثناء الخزن تحت 4 درجات أو حرارة عادية أو تحت 30 درجة على التوالي 42 و 30 و 10 أيام.

الكلمات المفتاحية : الياورت ـ السوربات ـ تحضين ـ تخزين ـ ثبات

Effet du sorbate sur la fermentation du yaourt et stabilité du sorbate

L'effet du sorbate sur la fermentation et le stockage du yaourt et la stabilité du sorbate durant le chauffage du mixe du yaourt et durant le stockage du yaourt ont été examinés. Les teneurs en sorbate de certains échantillons de yaourt commercial ont été déterminées. Dans 42 échantillons de yaourt aromatisés et non aromatisés, ces teneurs étaient comprises entre 0 et 453 ppm. Les niveaux les plus faibles ont été observés dans le yaourt nature, alors que les doses les plus élevées ont été observées dans le yaourt brasse aromatisé. Cette étude a montré que la croissance des bactéries lactiques et la production de l'acide ont été ralenties en présence du sorbate de potassium durant l'étuvage et le stockage. De telles réductions suggèrent d'augmenter la durée de l'étuvage du yaourt ou le taux de l'inoculum pour compenser l'effet du sorbate sur le levain. La dégradation du sorbate durant le chauffage du mixe du yaourt à 80 ou 100°C pendant 6 heures ou pendant le stockage du yaourt à 4°C, température ambiante ou 30°C pendant 42 j, 30j ou 10 jours respectivement était limité.

Mots clés: Yaourt - Sorbate - Étuvage - Stockage - Stabilité

Effect of sorbate on yogurt fermentation and stability of sorbate

This study was conducted to determine the effects of sorbate fermentation and storage of yogurt and to examine the stability of sorbate during yogurt mix heating of and during yogurt storage. The sorbate levels in some commercial yogurt samples were also determined. The results showed that sorbate content of 42 retail Moroccan flavored and unflavored yogurt samples were between 0 and 453 ppm. The lowest levels were found in plain yogurt samples, while the highest levels were found in stirred flavored yogurt. This study showed that lactic acid bacteria growth and acid production were reduced by potassium sorbate during incubation and storage. Such effect suggests that the yogurt incubation needs to be carried for longer time or the level of inoculation with of the starter culture should be increased. The sorbate losses in yogurt mix during heating at 80 or 100°C for up to 6 hrs or in yogurt during storage at 4°C, ambient temperature or 30°C for up to 42, 30 or 10 days, respectively, were limited.

Key words: Yogurt - Sorbate - Incubation - Storage - Stability

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INTRODUCTION

Sorbates are legally permitted preservatives in many countries and in a variety of food products (Ahlborg *et al.*, 1977). Sorbates are the leading preservatives in the food sector throughout the world due to their physiological inertness, their effectiveness even in the weakly acid pH range and their natural taste (Lück, 1990). The primary objective of using sorbates in some dairy products at 0.05-0.30% is to inhibit molds and yeasts that may spoil the product during the ripening period or in consumer packages during distribution (Sofos, 1989). In Morocco, sorbates are not allowed to be used on yogurt but they can be used in fruit preserves intended for use in the preparation of fruit-flavored fermented milks up to 1000 ppm (MAMVA & MSP, 1997). IDF (1992) reported in its general standard of identity of fermented milks that yogurt should not contains more than 50 ppm of sorbate, coming from flavoring components as a result of carry-over.

To our knowledge, most studies on the effect of sorbate on yogurt fermentation or storage were carried out under laboratory conditions rather than at the industrial scale. Hamdan *et al.* (1971) and Nakae *et al.* (1971) reported that sorbates retarded the growth of dairy lactic bacteria and decreased the rate of acid production during fermentation studies. It was also reported that sorbate prevented yeast fermentation and increased the shelf life of yogurt (Osborne & Pritchard, 1974; Jackson 1978; Yadav *et al.*, 1994).

While the stability of sorbate in yogurt is of practical importance to insure the maintenance of a sufficient amount not exceeding legislative limitations to prevent microbial proliferation, yet no work has been reported on storage and heat effects on this compound. Sorbate stability in model systems or some food products during their storage was reported by Bolin *et al.*, (1980), Vidyasagar & Arya, (1984), Gerschenson *et al.*, (1886), Guerrero *et al.*, (1990), and Leward (1990). This stability of sorbate is affected by various factors such as light, oxygen, pH, temperature, water activity and some food components (Arya, 1980; Bolin *et al.* 1980; Vidyasagar & Arya, 1984; Gerschenson *et al.* 1986).

The present study was undertaken to determine the levels of sorbate in yogurt products, to examine the effect of sorbate on yogurt fermentation, to evaluate the effect of sorbate on the shelf-life of yogurt and to investigate the stability of sorbate during yogurt processing and storage.

MATERIALS AND METHODS

1. Sorbate determination in milk products

Sorbate concentration was determined in retail yogurt samples. The samples included products from the three major processors of milk products in Morocco. The numbers of samples were 14 samples from each brand and included favored and unflavored samples and were analyzed in duplicate.

Sorbate concentrations in milk products were determined using a method based on the Provisional Standard of the International Dairy Federation (1987), with minor modifications. For the extraction of benzoate and hippurate, a sample of 2.0 g was transferred into 10 ml volumetric flask and 2.5 ml of 0.1 M sodium hydroxide solution were added to the flask. The sample was mixed, heated in water bath at about 70°C and cooled, the pH was adjusted to 8.0 by adding 0.1 M sulfuric acid solution, after, 2 ml of potassium hexacyanoferrate solution (106 g of potassium hexacyanoferrate in 1 liter) and 2 ml of zinc acetate solution (219 g of zinc acetate and 32 ml of acetic acid in 1 liter) were added, and finally methanol was used to complete the flask to 10 ml. The solution was transferred to 12 ml centrifuge tube and centrifuged for 10 minutes at 950 x g. The supernatant was filtered through a 0.45 μm membrane filter and used for HPLC analysis.

A mobile phase consisting of 10% methanol and 90% phosphate buffer solution (pH 6.7) was maintained at a flow rate of 1.0 ml/min through an endcapped octadecyl column (250x4.6 mm, i.d.) with 5μ particle size (IBM instruments Inc., Wallingford, CT).

Sorbates in milk product extracts were quantified using peak heights from sorbic acid standards of known concentrations, using a variable wavelength detector (LINEAR UVIS 204 Detector from Applied Biosystems). The wavelength used was 254 nm.

2. Sorbate effect on fermentation of laboratory made yogurt

Lactic acid bacteria used in this study, were isolated from a commercial yogurt. The isolation

was conducted by a successive incubations in sterilized milk samples at 45°C. These strains were grown and used as starter culture (ratio of about 1/1) of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Whole dried milk was reconstituted (10 %) and samples were fortified with 0.05, 0.1 and 0.2% of potassium sorbate, sterilized (120°C, 10 min), cooled to 45°C and inoculated with 2% of the starter culture obtained before. They were then homogenized, and 10.0 ml of each solution were transferred to 24 ml capped tubes. The tubes were held at 45°C for 10 hours.

To determine the effect of sorbate on yogurt fermentation, duplicate samples of fortified and non-fortified reconstituted milk samples were taken at every hour from each level of added sorbate and used for pH and lactic acid bacteria plate counts determinations.

3. Effect of sorbate yogurt fermentation of at industrial scale

The yogurt was produced using a whole milk in which the solids content was increased by addition of non-fat dry milk and sugar. The mix was then homogenized, heated (92°C, 3 min.) cooled to 45°C and inoculated with a culture of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The milk mix was then placed in consumer sized packages of 125 ml and incubated at 45°C.

To determine the effect of sorbate on yogurt fermentation process, samples of consumer sized packages were fortified just before the fermentation with potassium sorbate at 0,1% level and then incubated in the same condition as the non-fortified samples. Duplicate samples of sorbate fortified and non-fortified samples were taken at random at the beginning of the fermentation and every 30 min until the end of the process (3 hours). The samples were immediately placed in the cooling tunnel (-10°C) and then transported to the laboratory for analysis.

4. Sorbate effect on storage of laboratory made yogurt

Yogurt samples obtained under the same conditions described above in laboratory made yogurt were used in this study. The fermentation was however stopped by placing samples in the freezer for about 15 minutes when their pH was about 4,5. After 4 hours of storage under refrigeration they were stored under either of the following conditions: 30 days at 4°C, 10 days at ambient temperature (18-21°C), 5 days at 30°C. Samples were taken at random in duplicate at various intervals and were used for pH determination and lactic acid bacteria cell counts.

5 Sorbate Effect on storage of industrial made yogurt

Samples fortified with 0.1% potassium sorbate and non-fortified of consumer-sized packages obtained at the end of the fermentation were immediately cooled in the cooling tunnel, transported to laboratory and stored for 24 hours in a refrigerator. They were stored under either of the following conditions: 42 days at 4°C, 30 days at ambient temperature and 3 days at 30°C. Samples were taken at random in duplicate at various intervals and were used for pH determination and lactic acid bacteria and yeast plate counts (APHA, 1992).

6. Sorbate stability during storage of industrial made yogurt

To determine the effect of storage on sorbate in industrial yogurt, fortified with potassium sorbate (0.1%) samples were stored under the same conditions as in the study of the effect of sorbate on industrial yogurt. Duplicate samples were taken every 6, 5 and 2 days from yogurt stored at 4°C, ambient temperature and 30°C, respectively. The samples were used for pH determinations.

7. Heat effect on sorbate in yogurt mix

To study the heat effect on sorbate in yogurt mix, industrial made yogurt mix not incubated was fortified with 1% potassium sorbate and frozen until application of heat treatment. Samples of 2.0 g of fortified yogurt mix were transferred to glass test tubes (16x125 mm), capped and placed in covered water baths at either 80 or 100°C for up to 6 hrs. Duplicates tubes were sampled every 2 hours and sorbate levels were measured.

8. Analytical determinations

Measurement of pH were done with a Tacussel Model PNH 130T. The pHmeter was standardized with two buffer solutions before making determinations.

Titrable acidity expressed as % of lactic acid was measured using 9 g sample, which was titrated with sodium hydroxide 0.1N (APHA, 1978).

Bacterial cell counts were estimated by plating dilutions of the yogurt sample on MRS. All plates were incubated at 37°C and colonies were counted after 72 hours.

9.Statistical analysis

Analyses of variance were used to determine the significance of the effects of sorbate on yogurt fermentation and storage conditions. For sorbate degradation, retention was determined by comparing amounts in the initial unheated samples to those found after heat treatment. All statistical analyses used a 5% level of significance.

RESULTS AND DISCUSSION

1. Sorbate levels in yogurt products

The number of samples used, the averages and the ranges of sorbic acid levels found in yogurt samples of the three main brands are given in table 1. The samples used, included flavored and unflavored yogurt samples.

Table 1. Average sorbic acid levels in yogurt products

Yogurt products	Average sorbate in mg/kg	Number of samples	Sorbate range in mg/kg
Yogurt brand A	74	14	0 - 239
Yogurt brand B	29	14	0 - 113
Yogurt brand C	372	14	246 - 453

An analysis of variance of the effect of yogurt brand showed that the difference between the levels of sorbic acid were different at the 5% level. The averages sorbic acid levels given in table 1 were statistically different ($p<0.05$) when the Benferoni test was used. The lowest levels of sorbate were found in natural yogurt samples with an average of 85 ppm and a range of 0-262 ppm, while the highest levels were found in stirred flavored yogurt with an average of 191 ppm and a range of 1-452 ppm.

The presence of these levels of sorbate in retail Moroccan yogurt is not allowed, since in Morocco, sorbates can not be used in yogurt but they can be used in fruit preserves indented for use in the preparation of fruit-flavored fermented milks up to 1000 ppm (MAMVA & MSP, 1997).

IDF (1992) reported in its general standard of identity of fermented milks that yogurt should not contains more than 50 ppm of sorbate, coming from flavoring components as a result of carry-over. This means that of the three manufacturers only one have an average sorbate less than this limit.

The sorbate levels found in this study agree with those found by Raissouni *et al.* (1991), in natural yogurt of 200 ppm. They found however that stirred-flavored yogurt did not contains any sorbate.

Puttemans *et al.* (1985), found that sorbate levels in Belgian yogurt with fruit were below the maximum level allowed (150 ppm) in most samples and only two samples have higher levels of sorbate, while yogurt samples with fruits aroma did not contain any sorbate. Stijve & Hischenhuber (1984) analyzed 30 samples from various origins and found that most samples of fruit-flavored yogurt contained sorbic acid levels between 44 and 98 ppm.

In the US, sorbates are considered GRAS (Sofos, 1989). They may be used in more than seventy food products having standards of identity and their use may be requested in any food product that allow preservatives (Branen *et al.*, 1990).

Fondu *et al.* (1984) reported that sorbates are used in france up to 2000 ppm in yogurt with fruit, in Spain up to 600 ppm in all fermented milk products and in Germany up to 1500 ppm in fruit yogurt and nut yogurt.

2. Sorbate effect on industrial yogurt fermentation

Table 2 shows industrial made yogurt results obtained on the effects of 0.1% potassium sorbate on the lactic acid bacteria growth and acid production during 3 hours of incubation at 45°C. All samples treated with sorbate had higher pH values and lower bacterial counts than samples without added sorbate, after any given time of incubation.

An analysis of variance of the effect of sorbate on pH decrease showed that the difference between the pH of the yogurt samples without sorbate and yogurt samples with sorbate was significant at 5% level. This means that the addition of sorbate to the yogurt mix decreased the rate of pH reduction during the incubation. This slowing in pH reduction was due to the effect of sorbate on lactic acid bacteria growth as shown in the table 2.

Table 2. Sorbate effect on acidity and lactic acid bacteria changes during incubation at 45°C of industrial made yogurt

Incubation time in hours	0% sorbate pH	0% sorbate %Acid	0% sorbate log (cfu/ml)	0.1% sorbate pH	0.1% sorbate %acid	0.1% sorbate log (cfu/ml)
0	6.1	0.29	6.1	6.1	0.30	6.1
0.5	5.9	0.51	6.5	5.9	0.38	6.2
1	5.5	0.62	7.8	5.7	0.52	7.3
1.5	4.8	0.80	8.5	5.2	0.64	8.1
2	4.7	0.94	9.2	5.0	0.84	8.8
2.5	4.7	0.97	9.7	5.0	0.87	9.1
3	4.5	1.07	9.9	4.8	0.90	9.6

Each value is an average of duplicate samples.
% Acid: titrable acidity(%)

Sellars & Babel (1985) pointed out that yogurt having a pH of 4.0 is acceptable to consumer and to attain this pH, cooling should be started when the pH is 4.3 to 4.4. The incubation time needed to reach the pH of 4.4 was about 3 hours for samples without sorbate and over 3 hours for samples with added sorbate. These results suggest that the addition of sorbate retarded growth of lactic acid bacteria, thereby delaying acid production. Thus, the slow decrease in pH and lactic bacteria growth during incubation, suggest that the yogurt incubation need to be carried for longer time or the level of inoculation with the starter culture should be increased.

This study is in agreement with the finding of Hamdan *et al.* (1971) who reported that sorbates retarded the growth of commercial yogurt cultures and decreased the rate of acid production during fermentation study conducted in laboratory. Nakae *et al.* (1971) reported also that sorbate inhibited acid production of lactic acid bacteria strains obtained from commercial fermented milk products.

3. Sorbate effect on laboratory made yogurt fermentation

Table 3 shows the effect of the three levels of potassium sorbate (0.05, 0.1 and 0.2 %) on lactic acid bacteria growth and acid production in laboratory made yogurt during 10 hours of incubation at 45°C. Samples treated with either level of sorbate had higher pH values than those without added sorbate.

These results suggest that acid production and bacterial growth were retarded but not inhibited by the addition of sorbate and increasing the amount of added sorbate also tended to increase this effect of redardation in yogurt samples.

Table 3. Sorbate effect on pH and lactic acid bacteria changes during incubation at 45°C of laboratory made yogurt

Incubation time in hrs	pH 1	pH 2	pH 3	pH 4	Log(cfu/ml) 1	Log(cfu/ml) 2	Log(cfu/ml) 3	Log(cfu/ml) 4
0	6.6	6.6	6.6	6.6	2.9	3.0	3.0	2.9
1	6.4	6.4	6.5	6.5	3.3	3.2	3.3	3.2
2	5.7	5.7	6.1	6.4	4.4	4.5	3.5	3.6
3	5.1	5.1	5.9	6.2	6.0	5.1	4.1	4.0
4	4.5	4.5	5.4	5.7	8.3	7.4	4.9	4.4
5	4.3	4.3	5.1	5.3	10.5	9.2	6.7	4.9
6	4.2	4.2	4.9	5.2	11.7	11.1	9.3	7.2
7	4.1	4.1	4.7	4.9	11.0	11.5	10.7	8.6
8	4.1	4.1	4.5	4.7	10.4	11.0	10.9	9.6
9	4.0	4.0	4.3	4.5	9.1	10.1	11.4	10.3
10	4.0	4.0	4.2	4.4	8.0	9.3	11.3	10.8

1: samples without sorbate added; 2: samples with 0.05% sorbate
3: samples with 0.10% sorbate; 4: samples with 0.20% sorbate
Each value is an average of duplicate samples.

An analysis of variance indicated that sorbate had a significant effect ($p<0.05$) on pH decrease. The average pH of yogurt without sorbate was statistically different from the average pH of yogurt with 0.1 and 0.2% sorbate and was not different from the average pH of yogurt with 0.05% sorbate, when the Dunnett test was used. This means that the addition of sorbate to the yogurt mix decreased the rate of pH reduction during the incubation. The maximum bacterial load was reached between 6 and 7 hours for yogurt without and with 0.05% sorbate, while this maximum was reached after 9 hours for samples with 0.1% sorbate and 10 hours for samples with 0.2% sorbate.

The incubation time needed to reach the pH of 4.4 which is considered as the end of the incubation (Sellars & Babel, 1985) was about 4 h for samples without sorbate and about 5, 8 and 10 h for samples with 0.05, 0.1 and 0.2% sorbate, respectively.

This study is in agreement with the finding of Hamdan *et al.* (1971) who reported that sorbates retarded the growth of commercial yogurt cultures and decreased the rate of acid production during fermentation studies conducted in laboratory. Nakae *et al.* (1971) reported also that sorbate inhibited acid production of lactic acid bacteria strains obtained from commercial fermented milk products.

4. Sorbate effect on storage of industrial made yogurt

Data of the effect of 0.1% potassium sorbate on acid production and lactic acid bacteria reduction

during storage of industrial made yogurt at 4°C, room temperature (18-21°C) and 30°C are shown in tables 4, 5 and 6. Yogurt storage at these temperatures was studied to simulate most of the Moroccan distribution conditions.

In general, the samples treated with sorbate showed retarded acid production and low reduction in numbers of lactic acid bacteria during storage at all temperatures, compared to samples without sorbate added; and on the last day of each storage conditions pH values were highest in controls and lowest in samples containing 0.1% sorbate.

ANOVA of the effect of sorbate on pH decrease showed that the pH of yogurt with and without sorbate, stored under the three conditions are significantly different at 5 % level.

Sellars & Babel (1985) pointed out that yogurt which has a pH of 4.0 is very acceptable to the consumer. In this study, the pH of yogurt samples were under 4 after about 24, 6 and 1 days of storage of yogurt with no sorbate at 4°C, room temperature and 30°C, respectively, while these pH values were still over 4 after 42, 30 and 2 days of storage of yogurt with sorbate under the respective conditions outlined before. Thus, the decrease in pH that normally occurs during storage of yogurt was slowed, allowing a longer storage periods.

Table 4. Sorbate effect on acidity and lactic acid bacteria changes in industrial made yogurt during storage at 4°C

Storage time in days	..... 0% sorbate ..... pH	% acid	log (cfu/ml)	..... 0.1% sorbate ..... pH	% acid	log (cfu/ml)
0	4.6	0.97	9.9	4.8	0.92	9.6
3	4.4	1.23	-	4.7	0.87	-
6	4.2	1.36	8.2	4.6	0.95	9.0
9	4.1	1.38	-	4.5	1.01	-
12	4.1	1.39	7.7	4.5	1.01	8.8
15	4.1	1.40	-	4.5	1.05	-
18	4.1	1.43	6.5	4.5	1.09	8.1
21	4.0	1.47	-	4.5	1.10	-
24	4.0	1.48	5.7	4.5	1.13	7.8
27	4.0	1.48	-	4.4	1.14	-
30	3.9	1.51	5.3	4.4	1.15	7.7
33	3.9	1.51	-	4.4	1.15	-
36	3.8	1.59	4.5	4.3	1.21	7.2
39	3.8	1.61	-	4.3	1.28	-
42	3.7	1.61	3.3	4.2	1.29	6.6

%acid: titrable acidity (%)

Table 5. Sorbate effect on acidity and lactic acid bacteria changes in industrial made yogurt during storage at ambiant temperature

Storage time in days	..... 0% sorbate ..... pH	% acid	log (cfu/ml)	..... 0.1% sorbate ..... pH	% acid	log (cfu/ml)
0	4.6	0.97	9.9	4.8	0.81	9.6
2	4.1	1.38	7.8	4.6	0.86	8.4
4	4.1	1.70	6.1	4.5	1.07	7.9
6	4.0	1.85	5.6	4.5	1.14	7.2
8	4.0	1.86	4.9	4.4	1.17	5.8
10	3.8	1.85	3.9	4.4	1.20	5.4
12	3.8	1.88	-	4.3	1.23	-
14	3.8	1.92	-	4.3	1.25	-
16	3.8	1.94	-	4.3	1.28	-
18	3.8	1.94	-	4.3	1.30	-
20	3.8	1.95	-	4.2	1.29	-
22	3.8	1.96	-	4.2	1.31	-
24	3.7	1.98	-	4.2	1.31	-
26	3.7	1.98	-	4.1	1.42	-
28	3.6	2.04	-	4.1	1.47	
30	2.5	2.21	-	4.0	1.43	

% acid: titrable acidity (%)

Table 6. Sorbate effect on acidity and lactic acid bacteria changes in industrial made yogurt during storage at 30°C

Storage time in days	..... 0% sorbate ..... pH	% acid	log (cfu/ml)	..... 0.1% sorbate ..... pH	% acid	log (cfu/ml)
0	4.6	1.07	9.9	4.7	1.05	9.2
1	3.9	1.75	7.9	4.1	1.59	8.6
2	3.8	2.10	5.8	3.9	1.95	6.6
3	3.7	2.42	5.1	3.8	2.31	5.6
4	3.6	2.44	4.3	3.7	2.32	4.6

% acid: titrable acidity (%)

The counts of viable bacteria decreased from the initial level of greater than 10^9 to less than 10^7cfu/ml after 36, 6 and 2 days of storage of yogurt with sorbate at 4, room temperature(18-21°C) and 30°C, respectively, while this reduction of bacteria was reached after only 12, 2 and 1 day of storage of yogurt without sorbate under the respective conditions outlined before.

IDF (1992) outlined in its general standard of identity of fermented milks that the minimum counts of characteristic microorganisms are 10^7 cfu/g at the time of sale. If we consider this level as being the end of product's shelf-life, the addition of 0.1% sorbate had increased the shelf-life of yogurt by about three times at either temperature.

The results of this study are in agreement to Hamdan *et al.* (1971) finding, that the pH of laboratory made yogurt with 0.07 and 0.1% potassium sorbate were still over 4 after 4 weeks of storage, while the pH of yogurt were under 4 after 2 weeks of storage at 4°C. Yadav *et al.* (1994) reported that potassium sorbate (0.03%) increased shelf-life of laboratory made yogurt by up to 15 days at 7°C.

The yeasts monitoring during yogurt storage under the conditions outlined before showed that 0.1% sorbate inhibited yeasts growth during the periods of study, which were 42, 30 and 4 days at 4°C, ambient temperature and 30°C, respectively, while yeasts were detected in yogurt without sorbate only at the end of storage periods at 4 and ambient temperature. The yeast counts were 38 and 2.4 10^2 cfu/ml after 39 and 42 days of storage at 4°C. These counts were 64 and 3.4 10^4 cfu/ml after 22 and 30 days of storage at room temperature. The yeasts were not detected in yogurt without sorbate stored at 30°C during 4 days.

5. Sorbate effect on storage of laboratory made yogurt

Data of the effect of 0.1% potassium sorbate on acid production and lactic acid bacteria growth during storage of industrial made yogurt at 4°C, room temperature(18-21°C) and 30°C are shown in tables 7, 8 and 9 respectively. The results obtained under this study are comparable to those of the sorbate effect on storage of industrial yogurt.

ANOVA of the effect of sorbate level on pH decrease showed that the pH of yogurt with and without sorbate, stored under the three conditions are or not significantly different at 5 % level.

Table 7. Sorbate effect on pH and lactic acid bacteria changes during laboratory yogurt storage at 4°C

Storage in days	0% sorbate pH	0% sorbate Log (cfu/ml)	0.05% sorbate pH	0.05% sorbate Log (cfu/ml)	0.1% sorbate pH	0.1% sorbate Log (cfu/ml)
1	4.5	11.00	4.5	11.84	4.5	11.70
3	4.3	10.84	4.3	11.95	4.4	11.95
5	4.2	10.00	4.1	12.18	4.2	12.36
10	4.0	8.00	4.0	10.45	4.1	10.48
15	3.9	7.00	3.9	9.18	4.0	9.87
20	3.9	5.20	3.9	8.30	3.9	9.30
25	3.8	4.48	3.9	7.46	3.9	8.68
30	3.8	0.48	3.8	5.48	3.8	7.08

Table 8. Sorbate effect on pH and lactic acid bacteria changes during laboratory yogurt storage at ambiant temperature

Storage in days	0% sorbate pH	0% sorbate Log (cfu/ml)	0.05% sorbate pH	0.05% sorbate Log (cfu/ml)	0.1% sorbate pH	0.1% sorbate Log (cfu/ml)
1	4.5	4.48	4.5	4.48	4.5	12.32
2	4.0	4.00	4.3	4.28	4.2	12.08
3	3.8	3.82	4.2	4.16	4.1	9.70
4	3.8	3.82	4.0	4.00	4.0	8.11
5	3.8	3.81	3.9	3.93	3.9	6.50
6	3.8	3.81	3.9	3.91	3.9	5.90
10	3.8	3.80	3.9	3.88	3.8	5.48

Table 9. Sorbate effect on pH and lactic acid bacteria changes during laboratory yogurt storage at 30°C

Storage in days	0% sorbate pH	0% sorbate Log (cfu/ml)	0.05% sorbate pH	0.05% sorbate Log (cfu/ml)	0.1% sorbate pH	0.1% sorbate Log (cfu/ml)
1	4.5	11.84	6.5	11.48	4.5	11.85
2	3.9	7.95	4.2	11.23	5.2	11.32
3	3.8	5.70	4.1	8.15	4.0	8.64
4	3.8	3.48	3.9	7.66	3.9	7.20
5	3.8	2.70	3.9	5.45	3.8	6.30

In this study, the pH of yogurt samples were under 4.0 after about 10, 2 and 1 days of storage of yogurt with no sorbate at 4°C, room temperature and 30°C, respectively, while these pH were still over 4 after 10, 4 and 3 days of storage of yogurt with 0.05 and 0.1% sorbate under the respective conditions outlined before.

The counts of viable bacteria decreased from the initial level of greater than 10^{11} to less than 10^7cfu/ml after 25, 4 and 2 days of storage of yogurt with sorbate at 4°C, room temperature (18-21°C) and 30°C, respectively, while this reduction of bacteria was reached after only 15, 2 and 1 day of storage of yogurt without sorbate under the respective conditions outlined before.

The yeasts monitoring during yogurt storage under the conditions outlined before showed that sorbate inhibited yeasts growth during the periods of study, which were 30, 10 and 5 days at 4°C, ambient temperature and 30°C, respectively, while yeasts were detected in yogurt without sorbate only at the end of storage periods. The yeast counts were about 24 cfu/ml at 4°C and between 2.4 10^2 and 4.2 10^2 cfu/ml at ambient temperature and 30°C. These results suggest also that sorbate inhibited yeast growth and are in agreement with the reports of Osborne &

Pritchard, 1974; Sofos & Busta, 1980, Jackson, 1978 and Lim & Lee, 1992. Suriyarachchi & Fleet (1981), found however that the growth of yeasts in yoghurt was not stopped by the addition of 100 ppm of sorbic acid.

6. Heat effect on sorbate in yogurt mix

When fortified yogurt mix samples were held at 80 and 100°C for up to 6 hrs, the losses of sorbate were 2 and 3% respectively. These losses are not enough to generate kinetic analysis and the degradation of sorbate is not significant enough under normal heat processing. These results suggest that yogurt mix pasteurization will not affect the level of sorbate in yogurt.

7. Sorbate stability during industrial yogurt storage

When yogurt samples were stored at 4°C for up to 42 days or at ambient temperature for up to 30 days or at 30°C for up to 10 days, the losses were only 1, 2 and 1% respectively. These losses were very limited and suggest that sorbate degradation is not significant, when yogurt shelf-life is taken into consideration.

Sorbate stability in dairy products during storage or heat treatment was not reported previously but similar results were observed on the relative stability of sorbate in model systems or some food products during their storage by Bolin *et al.* (1980), Vidyasagar & Arya, (1984), Guerrero *et al.* (1990), and Leward (1990). Their data show that sorbate losses were at most about 15% after a month of storage at 45°C. Gerschenson *et al.* (1886) reported however, that these losses reached about 30% after one month on the same conditions in a glucose model system. The stability of sorbate is affected by various factors such as light, oxygen, pH, temperature, water activity and some food components (Arya, 1980; Bolin *et al.*, 1980; Vidyasagar & Arya, 1984; Gerschenson *et al.*, 1986; Sofos, 1989). The degradation of sorbate in model systems followed first-order kinetics (Guerrero *et al.*, 1990; Gerschenson *et al.*, 1986) with energies of activation between 7 and 32 Kcal/ mole.

Since sorbates have been established as among the safest food additives (Sofos, 1989) and since they are effective in preserving and increasing the shelf-life of yogurt products, their use should be considered in these products specially in countries where refrigeration system and use are not common.

REFERENCES

Ahlborg UG, Dick J & Eriksson HB (1977) Data on preservatives in food. V*ar-Foeda* 29 (2): 41-96

APHA "American Public Health Association" (1978) Standard Methods for the Examination of Dairy Products. 14th. Washington DC

APHA "American Public Health Association" (1992) Compendium of Methods for the Microbial Examination of Foods. 3th ed. Am. Publ. Health Assoc. Washington DC

Arya SS (1980) Stability of sorbic acid in aquous solutions. *J Agric Food Chem* 28: 1246-1249

Bolin HR, King Jr.AD & Stafford AE (1980) Sorbic acid loss from high moisture prunes. *J Food Sci* 45: 1434-1435

Fondu M, van Gindertael-Zegers, De Beyl H, Bronkers G, Stein A & Carton P (1984) Food Additives Tables, updated edition. Ed. Elsevier, Amsterdam

Gerschenson LN, Alzamora SM & Chirife J (1986) Stability of sorbic acid in Model Food Systems of reduced water activity: Sugar solutions. *J Food Sci* 51: 1028-1031

Guerrero S, Alzamora SM & Gerschenson LN (1990) Stability of sorbic acid in aquous solutions of sodium chloride. *Lebensm. Wissen. Technol.* 23:271-273

Hamdan IY, Deane DD & Kunsman JE (1971) Effect of potassium sorbate on yogurt cultures. *J Milk Food Technol* 34: 307-311

International Dairy Federation (1987) Determination of benzoic and sorbic acid content in milk, dried milk, yogurt and other fermented milks. International Provisional Standard, 139. Brussels, Belgium

International Dairy Federation (1992) General satandard of identity of fermented milks. International Standard, 163. Brussels, Belgium

Jackson AC (1978) Short shelf-life milk products. *J Soc Dairy Technol* 31(2): 80-90

Leward DA (1990) Stability of sorbic acid in intermediate moisture systems. *Food Addit Contam* 7: 677-683

Lim YS & Lee SH (1992) Effect of potassium sorbate on the growth of yogurt starter and contaminant yeast. *J Korean Soc Food Nutr* 21: 534-538

Lück E (1990) Food Applications of sorbic acid and its salts. *Food Addit Contam* 7: 711-715

MAMVA & MSP (1997) Circulaire conjointe, N°001/97 du Ministère de l'Agriculture et de la Mise en Valeur Agricole et du Ministère de la Santé Publique relative à l'utilisation des additifs alimentaires

Nakae T, Kataoka K & Fujikawa K (1971) Metabolic activities of dairy lactic acid bacteria in the presence of preservatives. *J Food Hyg Soc Japan* 12: 105-110

Osborne RJW & Pritchard EW (1974) Preservation of fruit yoghurt by preservatives and by storage at low temperatures. *XIX International Dairy Congress 1E*, 809-810

Puttemans ML, Branders C, Dryon L & Massart DL (1985) Extraction of organic acids by ion-pair formation with tri-n-octylamine. Determination of sorbic acid, benzoic acid, and saccharin in yogurt. *J Asso Off Anal Chem* 68: 80-82

Raissouni B, Ouedrhiri M & Bougriane J (1991) Étude de deux additifs par spectrophotomètrie UV: l'acide benzoique (E210) et l'acide sorbique(E200) dans les boissons gazeuses et quelques produits laitiers commercialisés au Maroc. *Actes Inst Agron Vét (Maroc)* 11(1): 27-33

Sellars RL & Babel FJ (1985) Cultures for the manufacture of dairy products. CHR. Hansen's Labo., Inc. Milwaukee. Wisconsin

Sofos JN (1989) Sorbates food preservatives. CRC Press, Inc., Boca Raton, Florida

Sofos JN & Busta FF (1981) Antimicrobial Activity of sorbate. *J Food Prot* 44: 614-622

Stijve T & Hischenhuber C (1984) High performance liquid chromatographic determination of low levels of benzoic acid and sorbic acid in yoghurts. *Deutsche Lebensm Rundsch* 80: 81-84

Suriyarachchi VR & Fleet GH (1981) Occurence and growth of yeasts in yogurt. *App Environ Microbiol* 42: 574-579

Vidyasagar K & Arya SS (1984) Degradation of sorbic acid in fruits squaches and fish paste. *J Food Techn* 19: 447-454

Yadav VB, Jha YK, Garg SK & Mital BK (1994) Effect of supplementation and additives on sensory characteristics and biochemical changes of yogurt during storage. *Australian J Dairy Technol* 49: 33-38