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The effect of Potassium Sorbate on *Aspergillius Niger* in Gouda Processed Cheese

Nizar Issa Alrabadi

Associate Professor, Department of Food Science and Nutrition, Faculty of Agriculture, Jerash University, Jordan.

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ABSTRACT

This study investigates the effect of potassium sorbate on *Aspergillius niger* in Gouda processed cheese. Both microbiological and chemical analyses are performed. We first activate polyethylene films with potassium sorbate as an antifungal agent at different concentrations (0.0,1.0, 2.0, 3.0, 4.0, 5.0 and 6.0%). The minimum efficient concentration which is used in packaging Gouda processed cheese is found to be 2%. Thereafter, four treatments of cheese are executed on different samples. A control sample without coating, samples without coating and inoculated by *Aspergillius niger,* samples coated with polyethylene films that activated by potassium sorbate without inoculation and samples coated with the same films and inoculated by *Aspergillius niger*. The results indicate that that potassium sorbate is an effective antifungal packaging material of cheese against *Aspergillius niger*.
KEYWORDS: *Aspergillius niger, Potassium Sorbate, Gouda Processed Cheese, Microbiological Analysis, Chemical Analysis.*

INTRODUCTION

Potassium sorbate has been used as a well known preservative of food. It inhabits the growth of fungi in several products. The antimicrobial and preservative properties of sorbic acid were first discovered, both in Germany and in the United States, by E. Miller and C. M. Gooding in the late 1930s and early 1940s, respectively [1-3]. Sorbic acid became available commercially during the late 1940s and early 1950s when testing as a preservative agent increased. Currently, sorbic acid and its more water-soluble salts, especially potassium sorbate, are collectively known as sorbates and are used widely throughout the world as preservatives for various foods, as animal feeds, as pharmaceuticals and cosmetics, and in other industrial applications [4]. The extensive use of sorbates as preservatives is based on their ability to inhibit or delay growth of numerous microorganisms, including yeasts, molds and bacteria.

Pasteurized processed cheese products (PCPs) are cheese-based foods produced by comminuting, blending and melting one or more natural cheeses and optional ingredients into a smooth homogeneous blend with the aid of heat, mechanical shear and (usually) emulsifying salts (ES) [5]. Optional ingredients, which are determined by the product type, include water, dairy ingredients, emulsifying salts, flavors, colors, preservatives and condiments. This diary product is a vital source of protein, vitamins and minerals particularly calcium and phosphor which are essential components in most highly consumed foods. Therefore, extension shelf life of this diary food is very important.
Several researchers have focused on the incorporation of sorbic acid into food packaging materials. The effectiveness of sorbates against yeasts has been documented by many researchers [6 -10]. Numerous studies have also documented the effectiveness of sorbates against molds [7, 11-18].

Potassium sorbate is a considerable antimicrobial weak acid, owing to its desirable physiological properties, neutral flavor and its effectiveness against fungi (19-24]. This study investigates the effect of potassium sorbate on the growth of fungi, causing the main spoilage of Gouda cheese and thus to enhance the preservative effect and satisfy consumer demands. Section 2 discusses the materials and method, Section 3 reports the results of analysis, Section 4 concludes.

MATERIALS AND METHODS

Materials

Low density polyethylene (LDPE) which is 49 micron thick was used as a packaging material of Gouda processed cheese. The antifungal agent that we used in the study was Edible grade potassium sorbate (Pfizer). The potassium sorbate was widely used as an effective inhibitor against yeast and molds. The *Aspergillius niger* ATCC1015 was our target microorganism. Gouda cheese is obtained from the local diary market in Jordan. Moreover, butter was also purchased from same market. Emulsifying salts JOHA® SE (Germany) and NaCl salt (Edible grade) were used to prepare the Gouda processed cheese.

Corresponding Author: Nizar Issa Alrabadi, Associate Professor, Department of Food Science and Nutrition, Faculty of Agriculture, Jerash University, Jordan. Email: rabadinizar@yahoo.com

Methods

Procedures for antimicrobial LDPE films preparation

LDPE (2×2 cm) was soaked in potassium sorbate diluted in deionized water at concentrations of (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0) for 10 min. After the soaking; the films were air dried at laminar flow without U.V. lamp. Thereafter, the developed films were assayed for antimicrobial activity against the targeted microorganisms [25].

Antimicrobial activity assay of the developed LDPE

The potassium sorbate activated films were assayed for antifungal activity against the indicator strain *Aspergillius niger* .Individually samples (2×2cm) of the treated films were located on to the surface of sabourad dextrose agar for yeast and moulds according to [26] and the plates were incubated at room temperature up to 7 days [27]. Agar plates seeded with 25% of culture of *Aspergillius niger*. The treated face of the films were in contact with the agar, the plates were incubated at room temperature for 7 days and the hostile activity was evaluated by observing a clear zone of growth inhibition in the active LDPE films.

Cheese manufacture

Processed cheese is manufactured by adding water and emulsifying salt to shredded natural Gouda cheese, followed by heating the blend in a water bath up to 85 °c with a constant agitation until a homogenous mass is obtained. In addition to natural cheese, other ingredients like butter and non dairy products like salt may be included in the blend.

Microbial Analysis

Total microbial count was estimated. Plate count agar medium was prepared according to [26]. Plates were incubated at 32 °c for 48 hours. In addition yeast and moulds counts were estimated. Sabourad dextrose agar medium was prepared according to [26]. Plates were incubated at 21 °c for 7 days.

Chemical analysis

The chemical analysis included examining the following characteristics:

1. *Moisture and soluble nitrogen*

Determinations of moisture and soluble nitrogen contents were determined according to [28].

2. *Fat content*

Fat content was determined in cheese by the conventional Gerbers method as described in [29].

3. *Reduced suger:-*

The phenol-sulphuric acid method of [30] for reduced sugar.

4. *PH value*

The PH value of cheese was measured using a glass electrode PH meter Accumel® PH meter model 810 (fisher scientific) According to [29]. Both the microbial and chemical analyses are executed over 6 months.

The application

Antifungal activity of sorbate coated films during the storage of Gouda processed cheese

After cheese manufacture pouches of activated and non activated LDPE films were filled with Gouda processed cheese. Before sealed certain pouches of Gouda processed cheese were superficially spiked with 1.5ml suspension of *Aspergillius niger* ATCC1015 at 4.8x 105 cfu ml-1 /24 cm2.Whereas another pouches sealed without inoculation. All pouches were stored at room temperature 25 °c ± 2°c and humidity (49% + 2%). The treatments were as follows:

1: Control

2: Control+ *Aspergillius niger*. 4.8x 10^5 cfu ml^{-1} / 24 cm^2

3: sodium sorbate coated film

4: sodium sorbate coated film + *Aspergillius niger* 4.8x 10^5 cfu ml^{-1} /24 cm^2

The amount of Gouda processed cheese was calculated to give one container for each sample taking to analysis along the storage period sampling was carried out according to [28]. Microbial and chemical analyses were carried out for Gouda processed cheese at the day of manufacturing and with one month interval up to 6 months.

RESULTS AND DISCUSSION

Figure 1 shows the antifungal activity of potassium sorbate soaked LDPE at different concentrations against *Aspergillius niger*. The polyethylene food packaging materials were developed using different concentrations of potassium sorbate. The soaking procedures yielded vital results. Indeed, after immersion for 10 min. soaking of the polyethylene films into potassium sorbate solution, the films were always active against the *Aspergillius niger* in agar inhibition assays. The untreated films did not show any antifungal activity. Increasing the potassium sorbate above 2% concentration did not result in a parallel increase of the radius of the inhibition zone. Consequently, we used a concentration of 2% of potassium sorbate.

Figure 1: Antifungal Activity of potassium sorbate soaked LDPE at different concentrations against *Aspergillius niger*.

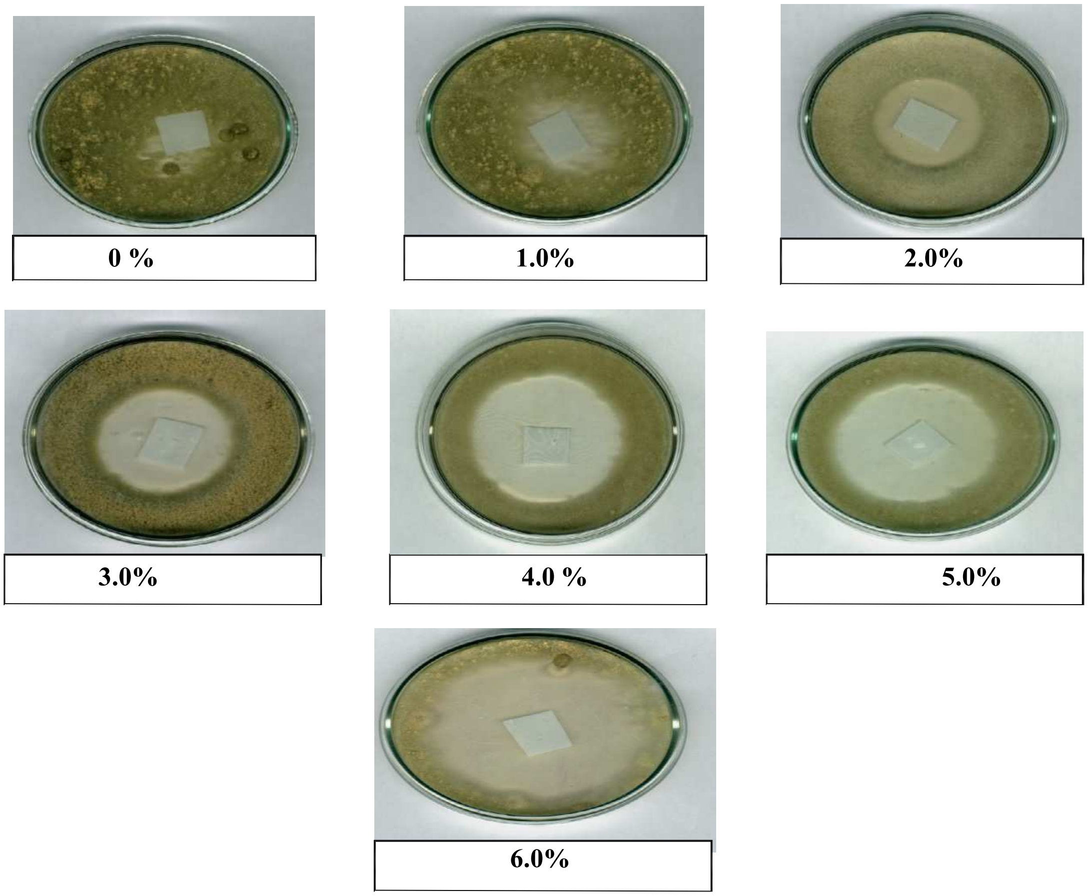

Table 1 displays the effect of potassium sorbate coated films on total bacterial count of Gouda processed cheese. The results indicate that potassium sorbate coated films had significantly reduced the increase in the total bacterial count in Gouda processed cheese after six months of storage at room temperature. The control sample showed an increase in the total bacterial count from $1.2*10^{1}$ cfu/g to $8.2*10^{4}$ cfu/g. On the other hand, the potassium sorbate coated film (treatment 3) showed only a slight increase in the total bacterial count from $2.2*10^{1}$ cfu/g to $6.1*10^{2}$ cfu/g after six months of storage. Moreover, the sodium sorbate coated films of Gouda cheese which were inoculated with *Aspergillius niger* (treatment 4) showed an increase in the total bacterial count from $2.0*10^{2}$ cfu/g only to $1.9*10^{3}$ cfu/g after six months of storage. The antimicrobial action of sorbate acid is based on the inhibition of various enzymes in the microbial cell. Enzymes of carbohydrate metabolism such as enolase and lactate dehydrogenase. Sorbic acid intervenes relatively powerfully, though not very specifically, in the citric acid cycle. In addition, sorbic acid forms covalent bonds with SH groups of enzymes by way of its own double bonds, the SH groups thereby being inactivated. The action of sorbate acid is directed primarily against yeast and molds. Bacteria are only partially inhibited, the catalase positive more so than catalase negative [2].

Table (1): Effect of potassium sorbate coated films on total bacterial count of Gouda processed cheese

Storage (month)	Treatments			
	1	2	3	4
Fresh	[illegible]	[illegible]	[illegible]	[illegible]
1	$1.5*10^{2}$	$2.0*10^{2}$	$3.4*10^{2}$	$2.3*10^{2}$
2	$2.9.7*10^{2}$	$3.7*10^{2}$	$3.4*10^{2}$	$2.6*10^{2}$
3	$4.8*10^{2}$	$3.9*10^{2}$	$5.1*10^{2}$	$3.8*10^{2}$
4	$7.2*10^{2}$	$5.0*10^{2}$	$5.3*10^{2}$	$4.5*10^{2}$
5	$6.4*10^{4}$	$3.2*10^{3}$	$5.7*10^{2}$	$1.0*10^{3}$
6	$8.2*10^{4}$	$3.4*10^{3}$	$6.1*10^{2}$	$1.9*10^{3}$

1: Control

2: Control+ *Aspergillius niger*. $4.8x\ 10^{5}$ cfu ml^{-1} / 24 cm^{2}

3: *Potassium sorbate* coated film

4: *Potassium sorbate* coated film + *Aspergillius niger* $4.8x\ 10^{5}$ cfu ml^{-1} / 24 cm^{2}

Table 2 displays the effect of potassium sorbate coated films on yeast and molds count of Gouda processed cheese. The results indicate that potassium sorbate coated films had considerably reduced the increase in the yeast and molds count in Gouda processed cheese after six months of storage at room temperature. The sample which was inoculated with *Aspergillius niger* (treatment 2) showed an increase in the total bacterial count from $2.0*10^2$cfu/g to $2.4*10^5$ cfu/g. On the other hand, the potassium sorbate coated films of Gouda cheese which were inoculated with *Aspergillius niger* (treatment 4) showed a slight increase in the yeast and molds count from $2.3*10^2$ cfu/g only to $3.5*10^2$ cfu/g after six months of storage. Yeast and molds were not detected at all in treatment 3 which was coated with potassium sorbate films. These results of microbial analysis are consistent with who found significant effect of potassium sorbate as an antifungal agent in different types of food [19-24, 31]. The growth of most yeasts can be inhibited by 0.01 to 0.2% sorbate. Many factors, (for example, pH; water activity; the presence of sodium chloride, sucrose, and/or organic acids; temperature; species and strain of yeast; and the amount of oxygen present) can affect the inhibitory concentration of sorbate [4]. Sorbate effectively inhibits the growth of molds, including mycotoxin-producing types, in culture media and in food products [32-33]. The minimum inhibitory concentration of sorbate for most molds ranges from 0.001 to 0.1% and is influenced by pH, species, strains and other factors [34].

Table (2): Effect of potassium sorbate coated films on yeast and molds count of Gouda processed cheese

Storage (month)	Treatments			
	1	2	3	4
fresh	N.D	$2.0*10^2$	N.D	$2.3*10^2$
1	N.D	$2.3*10^2$	N.D	N.D
2	N.D	$3.2*10^2$	N.D	N.D
3	N.D	$3.9*10^2$	N.D	N.D
4	N.D	$4.6*10^2$	N.D	$1.0*10^2$
5	N.D	$1.4*10^5$	N.D	$1.9*10^2$
6	N.D	$2.4*10^5$	N.D	$3.5*10^2$

1: Control

2: Control+ *Aspergillius niger*. 4.8x 10^5 cfu ml^{-1} / 24 cm^2

3: *Potassium sorbate* coated film

4: *Potassium sorbate* coated film + *Aspergillius niger* 4.8x 10^5 cfu ml^{-1} / 24 cm^2

N.D: not detected in 0.1g of sample

Table 3 shows the effect of potassium sorbate coated films on the chemical characteristics of Gouda processed cheese. The results indicate a minor effect of potassium sorbate coated films on the chemical characteristics of Gouda processed cheese. The chemical composition of Gouda processed cheese is related to its microbial content, since the coated films results in a good chemical composition and slight changes which may be due to the lost moisture, compared to the control sample. For example, the fat in treatment 1 (the control sample) increased from 43.28 to 44.92. Similarly, the fat increased in the potassium sorbate coated films of Gouda cheese which were inoculated with *Aspergillius niger* (treatment 4) from 43.28 to 45.21. Thus, no significant differences were noted between the two treatments. The values of the soluble nitrogen, pH and reduced sugar were also comparable among the four treatments.

Table (3): Effect of potassium sorbate coated films on chemical characteristics of Gouda processed cheese.

Storage (month)	Treatments																			
	1					2					3					4				
	DM	F/DM	S.N/DM	pH	R.S/	DM	F/DM	S.N/DM	pH	R.S/DM	DM	F/DM	S.N/	pH	R.S/DM	DM	F/DM	S.N/DM	pH	R.S/DM
fresh	47.50	43.2	0.81	5.40	1.4	47.50	43.2	0.81	5.40	1.40	47.50	43.2	0.8	5.40	1.40	47.5	43.28	0.81	5.40	1.40
1	47.73	43.5	0.83	5.11	1.2	47.74	43.5	0.83	5.14	1.22	47.78	43.6	0.8	5.42	1.17	47.7	43.58	0.82	5.32	1.26
2	47.99	43.8	0.84	5.23	0.9	48.00	43.9	0.83	5.14	0.86	48.02	43.9	0.8	5.30	0.88	48.0	43.93	0.84	5.35	0.84
3	48.88	44.0	0.89	5.17	0.8	49.00	44.0	0.90	5.27	0.87	49.03	44.1	0.9	5.38	0.76	49.0	44.18	0.86	5.33	0.52
4	50.52	44.6	0.95	5.11	0.7	50.50	44.6	0.91	5.17	0.69	50.36	44.6	0.9	5.39	0.60	50.0	44.23	0.92	5.29	0.49
5	51.29	44.7	0.95	5.13	0.6	51.32	44.7	0.91	5.28	0.55	51.80	44.8	0.9	5.19	0.51	51.9	44.48	0.95	5.25	0.44
6	51.92	44.9	0.88	5.14	0.4	52.09	45.0	0.88	5.70	0.40	52.04	45.5	0.8	5.21	0.46	52.1	45.21	0.71	5.28	0.41

DM denotes dry matter, F denotes fat, S.N denotes soluble nitrogen , pH denotes the pH value and R.S denotes reduced sugar.

CONCLUSION

This study investigated the effect of potassium sorbate as an antifungal agent on *Aspergillius niger* in Gouda processed cheese. We performed a microbial and chemical analysis. The results of the both analyses showed the effectiveness of potassium sorbate as a packaging material in preserving Gouda processed cheese against *Aspergillius niger*. Thus, the level of 2% of potassium sorbate reduced the population of the indicator strain on processed cheese products. Overall, the application of antimicrobial agents to packaging materials could be useful to prevent the growth of microorganisms on the product surface and hence may lead to an extension of the shelf-life and/or improved microbial safety of the product.

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