American-Eurasian J. Agric. & Environ. Sci., 13 (11): 1497-1502, 2013
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2013.13.11.81265

The Antifungal Effect of Potassium Sorbate on *Penicillium sp* in Labaneh

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Abstract: This study investigated the antifungal effect of polyethylene films coated with potassium sorbate on *Penicillium sp*. At beginning, we used different concentrations of potassium sorbate in coating the polyethylene films to determine the most effective concentration against *Penicillium sp*. The concentrations used were (0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 5.0, 5.5 and 6.0%) and 2.5% was the minimum that must be used in packaging Labaneh. Afterward, we tested the effectiveness of four diverse treatments of Labaneh samples. A control sample without coating, samples without coating and inoculated by *Penicillium sp,* samples coated with polyethylene films that activated by potassium sorbate without inoculation and samples coated with the same films and inoculated by *Penicillium sp*. The results of the microbial analysis which lasted for 30 days showed that potassium sorbate is an effective antifungal packaging material of Labaneh against *Penicillium sp*.

Keywords: Labaneh · Potassium Sorbate · Penicillium Sp · Antifungal Effect · Microbiological Analysis

INTRODUCTION

Potassium sorbate is a mould inhibitor used in organic livestock production. Sorbic acid was first discovered in the Mountain Ash Tree (*Sorbus aucuparia* or *Sorbus americana*). Today most potassium sorbate is made synthetically. Potassium sorbate is a naturally occurring unsaturated fatty acid and is completely safe with regard to health and has the lowest allergenic potential of all food preservatives. Many researchers have investigated the effectiveness of potassium sorbate in preserving food. Early Liewen and Marth [1] studied the inhibition of *Penicillia* and *Aspergilli* by potassium sorbate and found significant effects. Bullerman [2] demonstrated a general suppression of growth and a virtual elimination of patulin production with potassium sorbate at 0.10%. A lot of researchers have focused on the incorporation of sorbic acid and benzoic acid or their corresponding acid anhydrides into food packaging materials [3-7]. Yigit and Korukluoglu [8] found that potassium sorbate is a suitable preserving agent to inhibit growth of fungi in fermented products of pH near 4.5 regardless levels of NaCl. For products of slightly higher pH, the addition of potassium sorbate is suggested in combination with NaCl. They studied naturally fermented black olives. Heydaryinia *et al.* [9] compared the inhibitory effect of different concentrations of sodium benzoate and potassium sorbate on *Aspergillus niger* and *Penicillium notatum*. Moreover, they tested the probability of existing and synergistic effect in combining these two preservative together. They found that Potassium sorbate with all the concentrations had a better effect on *Aspergillus niger:* like benzoate, increasing concentrations of sorbate resulted in a higher preservative effect. Regarding the combinations, no synergistic effect has been observed and no combination in the prevention of growth was better than the 0.1% concentrations of the two preservatives. Palou *et al.* [10] have also reported that potassium sorbate, sodium benzoate and ammonium molybdate, among the wide range of chemicals tested, were superior for the control of post-harvest *Penicillium* decay of citrus fruit but the mixtures didn't significantly enhance the effectiveness of potassium sorbate or sodium benzoate alone. According to Valencia-Chamorro *et al.* [11] among all organic acid salts tested, potassium sorbate and sodium benzoate were the most effective salts in controlling both *Penicillium digitatum* and *Penicillium italicum* on citrus fruit, but the use of mixtures of parabens or organic acid didn't provide an additive or synergistic effect for mould inhibition

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when compared to the use of single chemicals. Saidi *et al.* [12] studied the effects of sorbate fermentation and storage of Moroccan yogurt and examined the stability of sorbate during yogurt mix heating and storage. They showed that lactic acid bacteria growth and acid production are reduced by potassium sorbate during incubation and storage. Guynot *et al*. [13] studied the effect of benzoate, propionate and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5-5.5). They found that potassium sorbate is the most effective in preventing fungal spoilage of this kind of products at the maximum concentration tested (0.3%).

Food safety and preservation is one of the most important topics that aim to enhance human health and wellness. This study focused on Labaneh as one of the traditional dairy products in Middle East and specifically in Jordan. It is a dried yoghurt made from different types of milk. Its acidity makes it a suitable environment for the growth of yeast and moulds. This study investigated the antifungal effect of potassium sorbate on *Penicillium sp* in Labaneh. In particular, it aimed to increase the shelf life of Labaneh using potassium sorbate as a preservative.

MATERIALS AND METHODS

Isolation and Purification of Moulds: Samples of Labaneh and yoghurt were used to isolate moulds. The samples that had a visible fungal growth were selected. With sterilized loopful, a swab was transferred from mouldy sample to sterilized distilled water, mixed and cultivated onto Potato dextrose agar medium according to Difco's Manual [14]. To inhibit the bacterial growth, as recommended by Cook [15], a mixture of chloramphenicol and chlortetracycline was added to molten agar medium immediately before pouring into Petri dishes. Specifically, two ml of 0.1 % of chloramphenicol and chlortetracycline per 100 ml of Potato dextrose agar medium were used. Plates were incubated at 25 C^{o} up to 7 days. Separate colonies were re-suspended into sterilized Potato dextrose broth medium. To purify each colony, small quantity of each suspension was punctured into Potato dextrose agar medium, fortified with the mixture of antibiotics by spotting with the aid of specific needle to obtain single colony. The previous step was repeated several times to get a purified isolate.

Identification of Isolated Strains: Each purified isolate was identified using standard strain of *Penicillium sp*. A comparison was made between isolates and the standards according to colony characteristics (color, rate of growth, basal mycelium and surface mycelium), conidiophores characteristics (length, diameter and wall character) and spores characteristics (color, diameter and its shape) following [16, 17].

Preparation of Antifungal Polyethylene Films: Polyethylene films (2×2 cm) were soaked with potassium sorbate diluted in deionized water at concentrations of (0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 5.0, 5.5 and 6.0%) for 10 min. After soaking, the films were air dried under sterilized conditions. The developed polyethylene activated films were assayed for antifungal activity against the indicator strain *Penicillium sp*. Individual samples (2×2cm) of the treated films were located on the surface of Potato dextrose agar media. Agar plates were seeded with 25% of culture of *Penicillium sp*. The treated films were in connection with agar. The plates were incubated at room temperature up to 7 days. We estimated the activity of potassium sorbate treated films by observing growth inhabitation as a clear zone around the films.

Manufacturing Labaneh: Labaneh was made by heating cow's milk to 85°C for 20 min, cooling to 40°C, inoculating with 2% starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* in equal proportions) and holding for 4 h until a pH 4.6 was attained. The resulting yogurt was placed in cloth bags and left to drain by gravity at 6°C overnight. The obtained Labaneh was divided into four samples. The first was a control sample (without coating), the second was without coating and inoculated by *Penicillium sp*, the third was coated with polyethylene films that activated by potassium sorbate without inoculation and the fourth samples coated with the same films and inoculated by *Penicillium sp*. All samples were put in pouches at fridge temperature for one month during which microbial analysis was performed. We used 2.5% concentration of potassium sorbate in packaging Labaneh because that concentration was the minimum that showed inhibitory effect against *Penicillium sp*.

Microbiological Analysis: The microbial count of each sample was estimated using different media, each to detect certain type of bacteria. The total bacterial counts were enumerated on (NA) nutrient agar (Difco), plates were incubated for 48h at 32°C. For counting the total coliform bacteria, the VRBA (Violet red bile agar) medium (Difco) was used [18]. Plates were incubated at 37°C for

48h. The Baird-Parker agar BPA [19] was used to enumerate the total staphylococci; plates were incubated at 37°C for 48h. Nutrient agar was used for detecting spore former bacteria as described by Difco's Manual [14]; the plates were incubated for 72h at 32°C. Finally, potato dextrose agar was used to detect yeast and moulds.

RESULTS AND DISCUSSION

Antifungal Activity of Potassium Sorbate Coated Polyethylene Films Against the Indicator Strain *Penicillium Sp:* The study showed interesting results; indeed, 10 minutes after soaking the polyethylene films into potassium sorbate solution at different concentrations, the films always showed activity against the indicator strain in agar inhibition assays. In all the cases untreated films did not show any antimicrobial activity. Increasing the potassium sorbate concentration by more than 2.5% did not result in a large increase of the radius of the inhibition zone, only slightly larger, which could not be observed between the close concentrations as shown in Figure 1. To verify the efficacy of the developed films, Labaneh was manufactured and packaged with the activated film with a concentration of 2.5% of potassium sorbate.

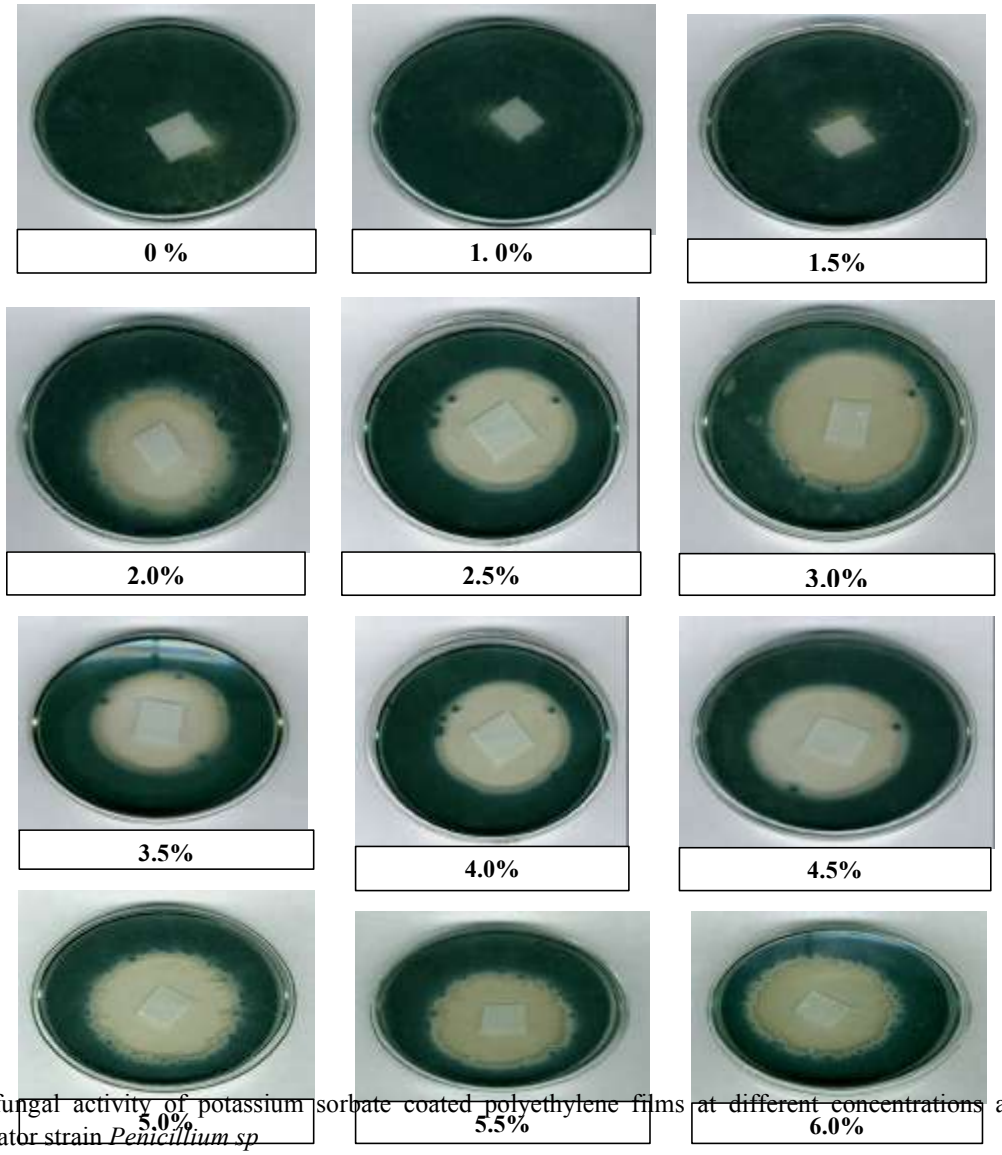

Fig. 1: Antifungal activity of potassium sorbate coated polyethylene films at different concentrations against the indicator strain *Penicillium sp*

Table 1: Effect of potassium sorbate coated films on total bacterial count of Labaneh

	Total bacterial count (CFU/g)			
	Treatment			
Storage(day)	1	2	3	4
Fresh	2×10^4	4.2×10^4	3.1×10^4	$3.9.\times10^4$
10	2.2×10^4	5.3×10^4	2.6×10^4	3.8×10^4
20	2.5×10^4	2.8×10^5	2.1×10^4	2.9×10^4
30	1.1×10^5	3.9×10^5	6.9×10^3	2.1×10^4

1: Control

2: Control+ *Penecillium sp* 1.4×10^6 cfu ml^{-1}/24 cm^2.

3: Potassium sorbate coated film

4: Potassium sorbate coated film+ *Penecillium sp* 1.4×10^6 cfu ml^{-1}/24 cm^2.

Table 2: Effect of potassium sorbate coated films on yeast and moulds count of Labaneh

	Yeast and mould (CFU/g)			
	Treatment			
Storage(day)	1	2	3	4
Fresh	1.3×10^1	2.5×10^3	1.1×10^1	2.2×10^3
10	2×10^2	3.3×10^3	ND	1.1×10^1
20	4.5×10^2	5.8×10^3	ND	ND
30	1.4×10^3	1.8×10^4	ND	ND

1: Control

2: Control+ *Penecillium sp* 1.4×10^6 cfu ml^{-1}/24 cm^2.

3: Potassium sorbate coated film

4: Potassium sorbate coated film+ *Penecillium sp* 1.4×10^6 cfu ml^{-1}/24 cm^2.

ND: Not Detected

Effect of Potassium Sorbate Coated Films on Microbial Contents of Labaneh: We superficially inoculated *Penecillium sp* to Labaneh 1.4×10^6 cfu ml^{-1}/24 cm^2. Table 1 shows the effect of potassium sorbate coated films on total bacterial count of Labaneh. Potassium sorbate coated films showed important results in reducing total bacterial count from 3.1×10^4 to 6.9×10^3CFU/g (For sample 3) after 30 days of storage at fridge temperature compared to the control sample (1) in which the total bacterial count increased from 2×10^4 to 1.1×10^5 CFU/g over the same storage period. In addition, the coated films inoculated with 1.4×10^6 CFU ml^{-1}/24 cm^2 of *Penicillium* (Sample 4) showed a slight decrease in total bacterial count from 3.9×10^4 to 2.1×10^4 CFU/g after 30 days of storage at fridge temperature compared to the control inoculated with the same number of *Penicillium sp* (Sample 2) in which the total bacterial count increased from 4.2×10^4 to 3.9×10^5 CFU/g over the same storage period. Table 2 shows the effect of potassium sorbate coated films on yeast and moulds count of Labaneh. Once again Potassium sorbate coated films showed great results in affecting total yeast and moulds count. In specific, it totally eliminated the yeast and moulds after 10 days of storage at fridge temperature and thereafter (As shown for sample 3) compared to the control sample (1) in which the total yeast and moulds count increased from 1.3×10^1 to 1.4×10^3 CFU/g after 30 days of storage at fridge temperature. Coated films inoculated with 1.4×10^6 cfu ml^{-1}/24 cm^2 *Penicillium sp*. (Sample 4) showed a great reduction of yeast and moulds count from 2.2×10^3 to 1.1×10^1 CFU/g after 10 days of storage at fridge temperature and became undetected thereafter compared to the control sample (2) in which the total yeast and moulds count increased from 2.5×10^3 to 1.8×10^4 CFU/g after 30 days of storage at fridge temperature. On the other hand, there were no observed spore former, total coliform, total *staphylococci* counts in all samples.

Our results are consistent with [1, 8, 9, 12, 20, 21] and many others who found that potassium sorbate is an

effective preservative acid for many types of food. Other researchers found other materials that can be used to improve the quality of Labaneh and decrease its bacterial count. For example, Keceli *et al.* [22] found that virgin olive oil preserves Labaneh by preventing the growth of yeasts and moulds over its surface. Additionally, they found that the type of vegetable oil covering Labaneh balls was unimportant and sunflower, safflower and cottonseed oil were equally effective in preserving yogurt cheese inoculated with *Kluyveromyces marxianus* subsp *marxianus* and stored for three months at 25°C. Consistently, Ayana and El Deen [23] found that the addition of aromatic oils to Labaneh made from goat milk decrease the viable bacterial count of it and improved its quality.

Overall, testing the antimicrobial packaging system using solutions, agar and food system is a very important topic. It can be argued that the main cause of spoilage of many refrigerated foods is microbial growth on the product surface; consequently 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 improve microbial safety of the product. Our results showed that potassium sorbate is an effective antimicrobial packaging material agent and the package coating solution successfully served as a carrier of the 2.5% of potassium sorbate and reduced the population of the indicator strain *Penicillium sp* on Labaneh. Previous studies showed similar results [24-28].

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