

Original article**A comparative study of the effects of the two preservatives, sodium benzoate and potassium sorbate on *Aspergillus niger* and *Penicillium notatum***

Ahmad Heydaryinia, MSc¹
Masoud Veissi, MSc¹
Ali Sadadi, MD²

¹Department of Nutrition, School of Paramedical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

²School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Address for correspondence:

Masoud Veissi, Department of Nutrition, School of Paramedical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
Tel: +98611 3738254
Fax: +98611 3342414
Email: m_veissi@yahoo.com

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Abstract

Introduction and objective: Sodium benzoate (SB) and potassium sorbate (PS) have long been used as preservatives in foods and other products. The aims of this study were to compare the inhibitory effect of different concentrations of two preservatives SB and PS on *Aspergillus niger* and *Penicillium notatum*, and to study the probability of existing and synergistic effect in combining these two preservative together.

Materials and methods: Preservative effects of separate concentrations (0.02, 0.04, 0.06, 0.08 and 0.1%) and different combinations of SB and PS (0.08 SB + 0.02 PS, 0.06 SB + 0.04 PS, 0.04 SB + 0.06 PS, 0.02 SB + 0.08 PS) on *A. niger* and *P. notatum* in yeast extract sucrose broth at preschedule days i.e. 0, 4, 10, 14, 18, 21, 24, 28 and 40 were investigated.

Results: Sodium benzoate at 0.02% and 0.04% concentrations had no effect on the growth of *A. niger* but the 0.1% concentration had the highest effect. Sodium benzoate at 0.02% had a better effect than the same level of PS on *P. notatum*. Potassium sorbate with all the concentrations had a better effect on *A. niger*: like benzoate, increasing concentrations of sorbate resulted in a higher preservative effect; PS at 0.1% had the highest effect. Combination 0.02 SB+ 0.08 PS and 0.08 SB+ 0.02 PS had the greatest and the least effects respectively.

Conclusion: 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.

Significance and impact of the study: The better use of preservatives includes increase of antimicrobial spectrum, extension of pH range, reduction of toxicity, and prevention of microbial resistance.

Keywords: Sodium benzoate; Potassium sorbate; *Aspergillus niger*; *Penicillium notatum*; Preservative

Introduction

Sodium benzoate (SB) and potassium sorbate (PS) have long been used as preservatives in foods and other products, and their safety has been established. In the US, SB is used at concentrations of 0.03% to 0.1% [1,2], and sorbic acid and its salts are suitable preserving agents to inhibit growth of fungi in fermented products of pH near 4-5 used from 0.01% to 0.02% [1,3-6]. On the other hand for a substance to be approved as preservative, difficult conditions should be met. Because of very high costs involved in safety testing, development of new preservatives seems unlikely [7].

By considering the fact that today only a few compounds are used as preservatives and the numbers of alternative preservatives have not been increased in recent years. In addition, tighter restrictions are being imposed by regulatory authorities, and the use of some additives are banned, and because the demand for food preservative is greater than any other line in history [2,7]. Further evaluation of old preservatives, which have been used for a long time and are known to be safe, would be rational. By doing this, the disadvantages of these substances could be recognized and the best use could be gained from them.

One way to reach this goal, is the combined application of two of more preservatives and probably, emphasis will be put on new uses for old preservatives and new preservatives will not unusual compounds, but rather will be unusual combinations of old compounds as well as Dai *et al.* [8] and Jin *et al.* [9] have reported them. Such combined uses might have some advantages, of them synergism could be denote. Other advantages include increase of antimicrobial spectrum, extension of pH range, reduction of toxicity, and prevention of microbial resistance. Though sodium benzoate and benzoic acid have long been

used as preservative in foods, but with respect to their narrow pH range in which they are effective and the off-flavor. They impart to the foods they are to preserve, and their toxicological properties compared to some other preservatives. There is a trend to replace this substance with other preservatives having better characteristics [10].

The objectives of this study therefore were to compare the inhibitory effects of different concentrations of each preservative on each mold, and to evaluate the effect of equal concentrations of two compounds with each other, and also to study the effect of a specific concentration of one preservative on two organism; and in the end to study the probability of existing any synergistic effect in combining these two preservatives together.

Materials and methods

This work was performed according to the method described by Bullerman [11,12].

Organisms

The molds used were *A. niger* and *P. notatum*, these genera were chosen because of their high occurrence and importance in comparison to other fungi in the spoilage of food products. These genera are usually compared with others because of their resistance to these preservatives. Also *A. niger* is used in industrial fermentation.

Substrate

In the present study Yeast extract sucrose broth, contained 2% yeast extract and 15% sucrose (YES, Q lab, Canada) was used. The maximum pH for antimicrobial activity of SB and PS is 4.5 and 6-6.5, respectively [1,2,11]. Hence for both preservatives to be effective, the pH of the medium was adjusted to 4.5 with 1m HCl. Prepared medium dispensed in 50ml quantities in to 150, 250ml Erlenmeyer flasks and sterilized

by autoclaving at 121°C for 15mins. Stock solutions of both preservatives, sodium benzoate (Merck, Germany), and potassium sorbate (Klose Cheme, Hamburg) with 10% concentration were prepared and sterilized by filtration through 0.45µm membrane filter.

Inoculum

Inoculum for each mold was prepared by growing the organisms on potato dextrose agar (Merck, Germany). Slants for 7-14 days at ambient temperature (25°C), or until good sporulation had occurred. Spore suspensions of each organism were made by adding 10ml of an aqueous sterile solution of 0.01% Tween 80 (Hopkins and Williams Ltd, UK), to the culture vessel and gently brushing the spores with a flamed wire loop. The spore suspensions were filtered through eight layers of sterile cheese cloth to remove mycelial debris and the volumes adjusted so that each suspension contained approximately 9×10^6 to 10×10^6 spores per ml. Spores enumeration was performed with an improved double Neubauer counting chamber.

Concentration and combinations

The maximum permitted level of SB in foods is 0.1% and this amount for PS is 0.2% [1,3]. Two different preservative equal concentrations are concerned. The 0.02, 0.04, 0.06 and 0.08% concentration were used and higher levels of PS despite their allowance were not included. Thus in designing the combinations, maximum level of SB i.e. 0.1% was gradually reduced and on equal amount of PS was added instead to reach the same 0.1% concentration on the whole. Therefore combinations of two preservatives were as follows: 0.08 SB + 0.02 PS, 0.06 SB + 0.04 PS, 0.04 SB + 0.06 PS, 0.02 SB + 0.08 PS. To observe the synergistic effect, a comparison was made

between combinations and 0.1% concentration of SB and PS.

Addition of preservatives, spore suspensions and comparison of the effect of concentrations and combinations

In this study of each mold while having 50ml of the substrate were divided in to 10 groups and more to make the aforementioned concentrations and combinations. First preservative from the stock solutions and then 0.1ml of spore suspension were added to the flasks. Then all flasks were incubated at ambient temperature for 40 days.

In each group, one flask with spore suspension and without preservatives was considered as control. To evaluate the effect of concentrations and combinations, at preschedule days i.e. 0, 4, 10, 14, 18, 21, 24, 28 and 40, each time 15 flasks were filtered with a Buchner funnel, and the mycelial dried weight were determined. To do this each time 15 Whatman No 4 filter paper (11.5cm diameter), were dried at 80°C for 20h again, cooled in a desiccator and weighted. The difference between the weight of dried filter and this weight was considered to be mycelial dry weight.

Spore suspension enumeration and confidence from their equality

Each suspension was counted 10 times and the mean was considered as the final numbers of spores. These values for *A. niger* and *P. notatum* were 940.3 and 946.9, respectively. By multiplying these amounts to 10,000 the number of spores in 1ml of each suspension would be achieved which are 9403000 and 9469000. In spite of this apparent difference with a student Z test, it was determined that two suspensions were equal ($P > 0.5$).

Results

Sodium benzoate at 0.02% and 0.04% concentrations had no inhibitory growth effect on *A. niger*, but the 0.1% concentration had the highest effect (Fig. 1). PS in all concentrations had a better effect on *A. niger*: like benzoate, increasing concentrations of sorbate resulted in a higher preservative effect, PS at 0.1% had the highest effect (Fig. 2). 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 (Figs. 3, 6). Combination 0.02% SB+0.08% PS and 0.08% SB+0.02% PS had the greatest and the least effects, respectively. In regard to *P. notatum*, growth was observed only in 0.02% concentration of the two preservatives and other concentrations did not show any growth till the end of 40 days experiment. SB at 0.02% had a better effect than the same level of PS on *P. notatum* (Figs. 4, 5).

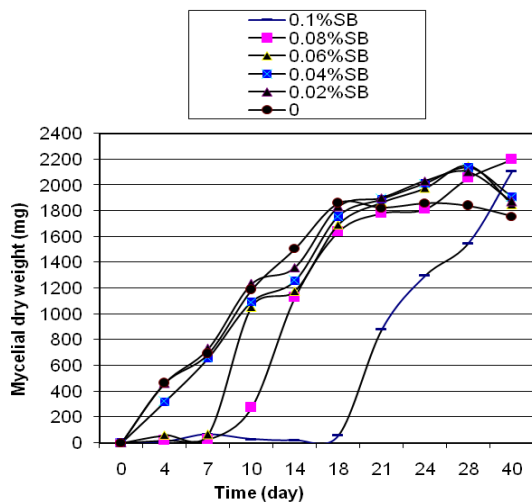


Fig. 1: Effect of different concentration of SB on *A. niger* growth in YES broth

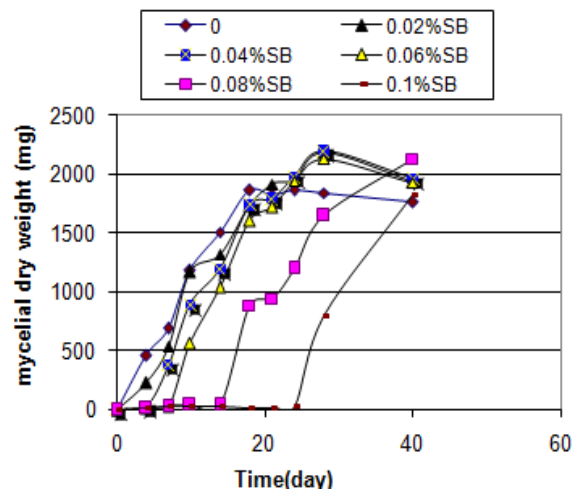


Fig. 2: Effect of different concentration of PS on *A. niger* growth in YES broth

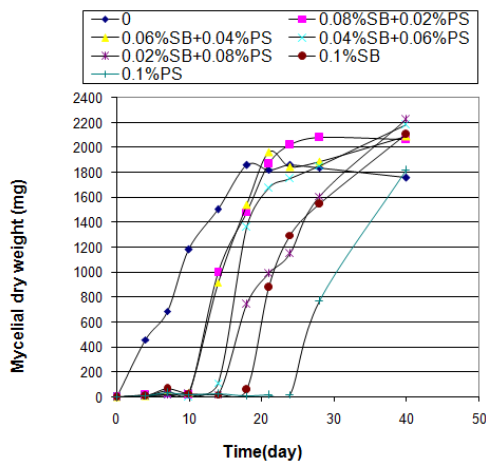


Fig. 3: Effects of different combination of SB and PS on *A. niger* in YES broth

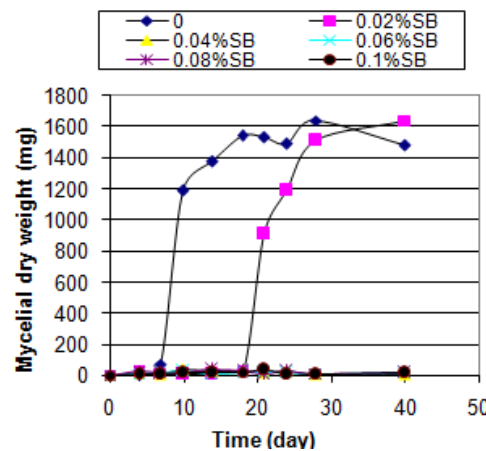


Fig. 4: Effects of different concentration of SB on *P. notatum* growth in YES broth

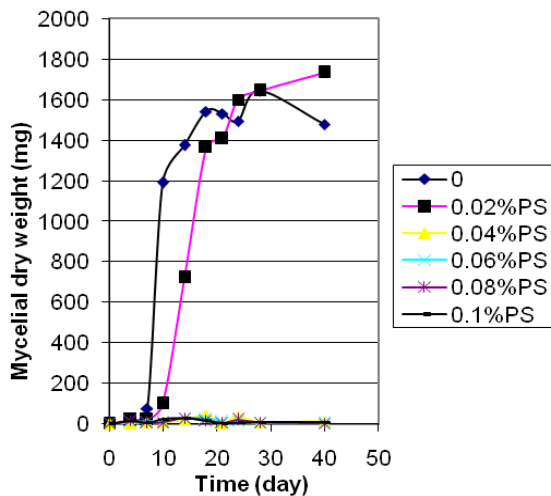


Fig. 5: Effects of different concentration of PS on *P. notatum* growth in YES broth

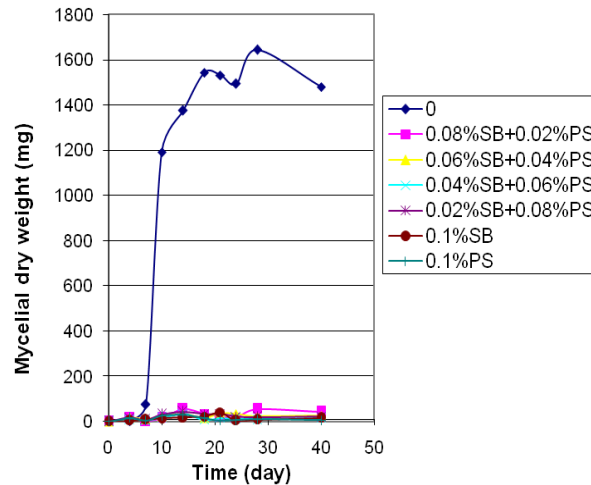


Fig. 6: Effects of different combination of SB and PS on *P. notatum* growth in YES broth

Discussion

With regard to *P. notatum*, it was observed that the 0.02% concentration of SB was more effective than its PS counterpart and it was also noticed that only sodium benzoate at this concentration had a significant difference from control. According to the report of Liewen and Marth [13], some species in genus *Penicillium* including *P. notatum* maintained their growth ability in the presence of 7100ppm or even 9000ppm sorbate. On the other hand *Aspergillus* species, isolated from the same source could not grow in the presence of 2000ppm, of this substance [13].

Aspergillus niger has previously been shown to require the activity of a phenylacrylic acid decarboxylase for the decarboxylation of the weak acid preservative sorbic acid [14]. Based on the report of Mann and Beuchat [15], about the ability of *A. niger* and some *Penicillium* species isolated from Parmesan cheese, they grow in the presence of 3500ppm sorbate or 3000ppm calcium propionate and degrade this substance to 1,3- pentadiene, a volatile compound with an extreme hydrocarbon odor. Results of their study showed that preservative systems containing a reduced concentration of PS, in

combination with other antimycotics, have potential for controlling the growth of molds though to be capable of producing 1,3-pentadiene.

Palou *et al.* [16] have reported the best compounds for the control of major postharvest diseases of stone fruit were 200 mM PS, 200mM SB and 200mM sodium sorbate. Study of Palou *et al.* [16] also showed mixtures of fludioxonil with PS, SB were not synergistic in their effect on brown rot, gray mold and sour rot. According to the report of Valencia- Chamorro *et al.* [17] among all organic acid salts tested, PS and SB were the most effective salts in controlling Both *P. digitatum* and *P. italicum* on citrus fruit, but the use of mixtures of parabens or organic acid didn't provide an additive or synergistic effect for mold inhibition when compared to the use of single chemicals.

Palou *et al.* [18] have also reported that PS, SB 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 PS or SB alone. By considering the fact that *P. notatum* used in this experiment had not been exposed to

any amount of sorbate or benzoate earlier, to become resistant towards them. This difference could be due to better action of benzoate or higher intrinsic resistance of *Penicillium* to sorbate or both.

Conclusion

Regarding the combinations, no synergistic effect has been observed by combining these two substances together, and no combination in the prevention of growth was better than the 0.1% concentrations of the two preservatives, with regard to *P. notatum*, growth was observed only in 0.02% concentration of the two preservatives and other concentrations did not show any growth till the end of 40 days experiment. Sodium benzoate at 0.02% had a better effect than the same level of PS on *P. notatum*.

Conflict of interest statement: All authors declare that they have no conflict of interest.

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