USE OF POTASSIUM SORBATE AND NATAMYCIN TO INHIBIT THE GROWTH AND AFLATOXIN PRODUCTION OF *Aspergillus parasiticus* 299 IN IMPROVED BINAGOL

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ABSTRACT

Binagol (pH 6.3) was reformulated using lemon juice to lower the pH to 5.5, 5.0 and 4.5. Sensory evaluation was done to determine the acceptability of the binagol with lower pH, using the original binagol at pH 6.3 as the control. Sensory evaluation showed binagol can be acidified to pH 5.5 without noticeable differences from the original. Potassium sorbate and/or natamycin were used to inhibit growth and aflatoxin production by *Aspergillus parasiticus* in the original and modified binagol. Shelf life was improved by using the inhibitors at the lower pH. At pH 5.5, a combination of potassium sorbate and natamycin extended the shelf life of binagol to 21 days. A synergistic effect of increased aflatoxin inhibition was observed when potassium sorbate and natamycin were combined at half of the effective levels needed for each one alone.

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INTRODUCTION

Binagol, a popular root crop dessert item in Leyte, Philippines, has been found to be an excellent substrate for fungal growth and a good substrate for mycotoxin production (Palomar, 1986). The product spoils quickly but little attempt has been made to improve its shelf life.

Potassium sorbate, a commonly used mold inhibitor in food products, effectively controls *Aspergillus* and other molds when used at low pH (pH 4-6) (Sofos and Busta, 1981). Previous laboratory research had shown that a combination of potassium sorbate and natamycin was effective in

controlling the growth of *Aspergillus parasiticus* and aflatoxin production in yeast extract sucrose broth (Palomar and Bullerman, 1985). Since lemon juice is commonly added as a flavoring agent in many Philippine delicacies (Laquian and Sobreviñas, 1977), it could be used to lower the pH of binagol making sorbate more effective, while at the same time masking the flavor of taro to make it more appealing to a non-taro consumer.

The objectives of the experiments were to modify the binagol recipe by lowering the pH, determine the acceptability of the binagol with lower pH by sensory evaluation and determine the effectiveness of sorbate alone and in combination with natamycin in controlling fungal growth and aflatoxin production in the original and modified binagol.

MATERIALS AND METHODS

Binagol reformulation

Binagol was processed following the procedure described by Palomar (1986). After 20 min of low temperature cooking, juice extracted from a fresh lemon fruit was added to both filling and topping mixtures to bring the pH level to about 5.5. Cooking was continued until the desired consistency was attained. The mixture was molded and then steamed for 30 min. The finished product was refrigerated overnight. However, its temperature was raised to room temperature before it was subjected to sensory evaluation.

Sensory evaluation

The trained sensory panel consisted of 9 Filipinos, 2 Taiwanese and 1 Mexican living in Lincoln, Nebraska, USA who were familiar with either taro or binagol. A hedonic rating scale (Larmond, 1982) was used to determine the degree of acceptability of binagol at pH values of 6.3 (original), 5.5, 5.0, and 4.5.

Application of inhibitors

Product formulation. Binagol was processed according to the procedure previously described. The mixture was divided into three portions: two portions adjusted to either pH 5.5 or 5.0 using fresh lemon juice and one portion kept at pH 6.3 to serve as control.

Preparation and application of inhibitors. Stock solutions of sorbate and natamycin were prepared and added to the mixture to give concentrations of 3000 µg/g for potassium sorbate and 500 µg/g of natamycin. When the two inhibitors were combined, 1500 µg/g potassium sorbate and 250 µg/g natamycin were used. Each sample contained 12.5 g of the filling and 7.5 g of the topping in a 16-oz round glass jar with wide mouth and vinylite lined screw cap seal (VWR Scientific). The jars were loosely covered and steamed for 30 min and allowed to cool at room temperature. The experiment was replicated twice with duplicate samples in each replicate.

Organism. Aspergillus parasiticus (NRRL 2999) was used as the aflatoxin-producing test strain. The organism was grown on potato dextrose agar (PDA) slants for 10 days until well-sporulated. The spores were loosened by gently brushing the surface of the slants, and were collected in a test tube with sterile phosphate buffer. The spore suspensions were filtered twice through a sterile cheesecloth and adjusted to give a final concentration of approximately 10^3 conidia/ml. The samples were surface inoculated with 0.1 ml of the inoculum using an automatic syringe.

Incubation and sampling. The inoculated binagol was incubated at 25°C. Samples were taken at 0, 7, 14 and 21 days of incubation. Initiation of fungal growth (measure of shelf life of the product) and sporulation were observed visually and noted. The degree of growth and sporulation were recorded at every sampling period. Samples were heated in an autoclave at 121°C for 60 s, cooled and stored in the refrigerator prior to analyses.

Aflatoxin extraction and analysis. The method of Waltking (1970) was modified and adapted for extraction and analysis of aflatoxin. Samples were transferred from the glass jars to pint jars and 50 ml methanol: water (55:45 vol/vol) solution was added. The samples were blended for 60 s at high speed using a blender (Oster), then filtered into a 125-ml Erlenmeyer flask using Whatman #4 filter paper. Twenty ml hexane was added to the filtrate, and the mixture was shaken for 20 min using a Burrell wrist action shaker. Twenty-five ml of the aqueous methanol (bottom) layer was transferred to a separatory funnel. The methanol filtrates were extracted 3 times with 25 ml chloroform, and the chloroform extracts were pooled and allowed to evaporate to about 2 ml. The extracts were transferred to 4-g vials, allowed to evaporate to dryness at room temperature and stored in the freezer until ready for aflatoxin analysis.

Aflatoxin analysis was done using thin-layer chromatography (TLC) plates (20 x 20 cm, 0.5 mm thick silica gel G/HR, J.T. Baker Chemical Co., Pittsburg, NJ) with toluene-ethyl acetate-formic acid (T:E:F) (60:30:10) as the developing solvent. Quantification of the toxin was done using a spectrodensitometer Model 3000 (Schoeffel Instrument Corp., Westwood, NJ) equipped with SDC 300 density computer and HP 3380 integrator (Hewlett Packard, Avondale, PA) by comparing a quantitative standard with the unknown samples.

RESULTS AND DISCUSSION

Sensory evaluation of modified binagol

Sensory evaluation showed that the binagol could be acidified to pH 5.5 using lemon juice without affecting its acceptability. However, binagol acidified to pH 5.0 and 4.5 were significantly less acceptable than the original. The taro flavor seemed less noticeable when lemon juice was added but some taste panelists noticed an increasing softness in texture of the binagol as the amount of lemon juice was increased. The sourness became intense at the lower pH level, especially at pH 4.5.

Shelf-life

Quality of food products is related to shelf life. Table 1 shows the number of days before the appearance of mold is detected in binagol when potassium sorbate and/or natamycin are used. Standard binagol (pH 6.3) in the control had an average room temperature shelf life of only 2 days. This is in agreement with the shelf life of binagol in the Philippines. Further, lowering pH of binagol without mold inhibitors did not increase the shelf life. Also, potassium sorbate when used alone without lowering the pH did not increase the shelf life of the control. However, sorbate increased the shelf life of binagol from 2.5 days to 15 and 21 days at pH 5.5 and 5.0, respectively. Natamycin alone increased the shelf life from 2 days in the control to 7-8 days regardless of the pH of the binagol. A synergistic effect was observed when potassium sorbate and natamycin were used together, since one-half the effective amount of each inhibitor used together gave longer shelf life than when these were used alone.

Aflatoxin production

Table 2 shows the amount of aflatoxin produced by *A. parasiticus* in the original binagol (pH 6.3). There was an increasing amount of toxin produced

Table 1. Mean shelf life of binagol containing potassium sorbate (KS) and/or natamycin (NAT).

Inhibitors	Conc. µg/g	Shelf Life (Days)[1] pH 6.3	pH 5.5	pH 5.0
Control		2.0	2.0	2.0
KS	3000	2.5	15.0	21.0
NAT	500	7.0	8.0	8.0
KS + NAT	1500 + 250	7.0	>21.0	>21.0

[1]Number of days to the appearance of mold growth.

Table 2. Effect of potassium sorbate and/or natamycin on total aflatoxin production (µg/g binagol) by *A. parasiticus* at 25°C and different pH.

pH	Time (day)	Treatment: Control	KS (3000 µg/g)	NAT (500 µg/g)	KS + NAT (1500 + 250 µg/g)
6.3	4	5.4	2.3	ND[1]	ND
	7	3.9	1.9	ND	ND
	14	10.6	11.3	2.0	4.7
	21	19.4	49.1	29.8	16.2
5.5	4	5.2	ND	ND	ND
	7	9.8	ND	ND	ND
	14	10.5	ND	16.8	ND
	21	10.4	23.4	18.1	ND
5.0	4	3.9	ND	ND	ND
	7	9.9	ND	ND	ND
	14	7.6	ND	8.8	ND
	21	6.6	ND	21.8	ND

[1]ND = Not detected.

by the control until the 21st day of incubation except for a slight decrease seen from the 4th to the 7th day. Tsai (1985) observed peak aflatoxin production in rice at about 4 days which decreased on the 7th day. The time of higher aflatoxin production was delayed in the presence of inhibitors. When potassium sorbate was present alone, there was minimal aflatoxin production observed during the first two samplings but higher values than the control were observed especially on the 21st day. Natamycin alone prevented aflatoxin production at 4 and 7 days, allowed reduced production at 14 days, but higher production than the control at 21 days. Natamycin + potassium sorbate-treated binagol had undetectable toxin levels at days 4 and 7, and reduced amounts of aflatoxin at days 14 and 21. Cultures to which low levels of inhibitors had been added sometimes produced higher amounts of aflatoxin than cultures without inhibitor, but at later times than when peak production occurs in untreated cultures (Tsai, 1985). Even high levels of sorbate (3000 μg/g) used at this high pH (6.3) may produce such an effect because the sorbate dissociates at the higher pH and is less effective, essentially because of low concentration of undissociated molecules.

At pH 5.5 binagol treated with potassium sorbate had undetectable toxin levels until the 21st day. Binagol made with natamycin had 16.8μg aflatoxin/g sample on the 14th day which increased to 18.2μg/g on the 21st day. Aflatoxin was undetectable through the 21st day of sampling when potassium sorbate and natamycin were combined at 50% of their effective levels. A synergistic or additive effect on aflatoxin production was observed using a combination of the two mold inhibitors. A similar synergistic effect on aflatoxin production has been observed in a broth system using lower concentrations of potassium sorbate and natamycin (Palomar and Bullerman, 1985).

Sorbate, alone or in combination with natamycin, completely inhibited aflatoxin production at pH 5.0. Sorbate is most effective at pH values approaching its dissociation constant (pKa) of 4.75 (Sofos and Busta, 1981). Consequently, sorbate was also most effective in the binagol with the lower pH. It can be seen that potassium sorbate used at a level of 3000μg/g (0.3%) prevented growth and aflatoxin production by *A. parasiticus* in binagol, acidified to pH 5.0. However, at this pH the binagol might be too sour, therefore sucrose or corn syrup might be added to mask the sour flavor. Addition of sugar or syrup might also have a beneficial effect by lowering the water activity (A_w). Growth and aflatoxin production by *A. parasiticus* were also prevented at pH 5.0 using a combination of potassium sorbate

and natamycin at 1500 and 250 µg/ml, respectively. However, the use of natamycin alone did not prevent aflatoxin production at 14 and 21 days. The activity of natamycin alone was not appreciably affected by pH.

This study has shown that the pH of binagol can be lowered to pH 5.5 and still produce a consumer acceptable product. At this pH, treatment of the binagol with a combination of 1500 µg/g of potassium sorbate and 250 µg/g natamycin can completely prevent mold growth and aflatoxin production for at least 21 days. Mold growth and aflatoxin production could be prevented for up to 21 days if the pH could be lowered to 5.0 using 3000 µg/g of potassium sorbate alone, or the combination of sorbate and natamycin.

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