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SHORT COMMUNICATION

ANTI-FUNGAL ACTIVITIES OF *m*-IODOBENZOIC ACID AND SOME OF ITS METAL DERIVATIVES ON BREAD MUCOR

U.B. Eke*, F.O. Nwosu and F.C. Adiatu

Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

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ABSTRACT. The anti-fungal activities of alkali and alkaline earth metal iodobenzoates were studied. Calcium iodobenzoate exhibited the highest anti-fungal activities of 74.60% inhibition for 15 ppm while sodium iodobenzoate exhibited the least inhibition of 61.64%. An optimum concentration of all the metal complexes for inhibition was found to be 75 ppm. Although the use of these metals complexes as food preservatives may only be fungi-static and not fungi-toxic, their use in bread preparation might extend the shelf life of bread from 24 hours to 96 hours.

KEY WORDS: Anti-fungal activities, Alkali metal iodobenzoates, Alkaline earth metal iodobenzoates, *m*-Iodobenzoic acid, Bread mucor

INTRODUCTION

Bread intake as a daily diet for man has increased in many parts of the world [1]. The habit of eating bread has spread from the Mediterranean basin to the whole world. Bread is now available in at least all the small cities of most developing countries. Its advantage of needing no further preparation and its superior nutrient value with protein and vitamin contents higher than those of staple foods like rice, yam, potatoes and cassava cannot be over emphasized. Bread is also an ideal food material for further fortification with vitamins, minerals and protein. Despite the high utility of bread, its shelf life has been reported as 24 hours [1].

The by-products of alkali and alkaline earth metal benzoates are not injurious to the body of humans as they are easily excreted. In the body, benzoic acid combines with the amino acid, glycine and is excreted as hippuric acid. The presence of iodine in the metal complexes will help supplement any insufficiency of iodine and thereby prevent goiter. Sodium and potassium are involved in transmission of nerve impulses and muscle contraction including heart beat. Whenever these alkali metals are in excess, they are excreted out easily via urine and sweat. The importance of calcium cannot be over emphasized in strong bone and teeth formation. Magnesium is found in the body as $Mg_3(PO_4)_2$ and also exist in ionic forms in tissues where it plays a part in many biochemical reactions involved in energy utilization [2] .

Fungi constitute a large group of heterotrophic organisms that ingest on their food by producing highly active digestive substances that cause rapid deterioration of organic materials such as wood fabrics, carbohydrates and leather in damp environment. It has been observed that many fungi tolerate high osmotic pressure and survived in concentrated salt or sugar solution as well as extreme pH [3]. Mucor species are the major species of fungi that grow on processed carbohydrate foods like bread [4]. Bread mucor breaks and absorbs organic molecules or materials for their nourishment.

Certain complexes of phenol compounds, quaternary ammonium halides, benzoic acid and derivatives like *m*-iodobenzoate have been reported to serve as food preservatives [5]. These

*Corresponding author. E-mail: ekeub@yahoo.co.uk

complexes inhibit fungal growth on finished products like bread. Some metal complexes of benzoic acid and their derivatives have been found to exhibit anti-fungal activity [6-8]. They also show inhibition towards bacteria such as *E. coli* and *B. subtilis* and yeast *S. cerevisiae* and *H. anomalia* [9].

The solubility of metal complexes depend on the presence of the hydrophilic groups such as –COOH, $–SO_3H$, $–NH_2$, –OH and –Cl [10]. The relationship between a selected single IR band and antimicrobial activity has also been studied [11]. Benzoic acid is stable under ordinary conditions as it is widely used as food preservatives. This study is concerned with the preparation of metal complex that has ability of extending the shelf life of bread. It will also determine whether the complexes act as fungi-static or fungi-toxic. The correlation between the individual molecule of the metal complexes and their fungal activity are also studied.

EXPERIMENTAL

Regents and instruments

All chemicals used were of analytical grade unless otherwise specified. The reagent used included: sodium hydroxide (minimum assay 99%, BDH), potassium hydroxide (minimum assay 99%, BDH), calcium hydroxide (minimum assay 99%, BDH), barium hydroxide-octahydrate (minimum assay 97%, BDH), magnesium sulfate (minimum assay 99%, BDH), *m*-iodobenzoic acid (Aldrich). The instruments used comprised of: mounted gallenkamp autoclave (England), Atomic Absorption Spectrometer ALPHA 4 Chem. Tech. Analytical with graphite atomizer (U.K.), Gallenkamp oven (England). Atomic absorption spectroscopy studies were performed at the Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of metal complexes [9]

The alkali (Na, K) or Ca metal complexes of *m*-iodobenzoic acid were prepared by dissolving separately 0.175 g (0.004 mole) NaOH, 0.246 g (0.004 mole) KOH or 0.046 g (0.006 mole) $Ca(OH)_2$ in 200 mL distilled water contained in conical flask. To each of the salt solutions, 1.0 g of *m*-iodobenzoic acid was then added separately to the salt solution and gently heated to 90 ^{0}C with stirring for 30 - 60 minutes.

The solution was then reduced to half its original volume and (in the case of the sodium complex to about 12 mL). The solution was left overnight to crystallize (sodium complex crystallized immediately). Sodium iodobenzoate was brown coloured while the calcium complex was a very light yellow complex.

The compounds crystallized as fine needles, which were recrystalized by dissolving each complex in warm distilled water. Each solution was allowed to cool and fine crystals were obtained and stored in reagent bottles.

Magnesium iodobenzoate was prepared [9] by dissolving 0.049 g (0.0002 mole) $Ba(OH)_2.8H_2O$ in 200 mL distilled water in a conical flask. A *m*-iodobenzoic acid (1 g, 0.004 moles) was added to the hydroxide solution and heated to 90 ^{0}C with stirring. It was concentrated to half its original volume and left overnight to form barium diiodobenzoate. About 0.053 g of $MgSO_4$ was dissolved in 200 mL distilled water, 1 g (0.003 moles) barium diiodobenzoate crystals were added to the $MgSO_4$ solution above, and heated to 90 ^{o}C with stirring [9].

The mixture was filtered to remove the insoluble barium sulphate and the filtrate concentrated. The solution was left overnight to obtain magnesium diiodobenzoate crystals which were filtered and re-dissolved in a small quantity of warm distilled water. It was left

overnight to obtain crystals, which were weighed and kept in reagent bottles and stored in a desiccator.

The solubility test of each complex was also carried out using distilled water, methanol, chloroform and acetone.

Preparation of culture medium and metal complexes stock solution

The culture medium used is PDA (potato dextrose agar) and was prepared locally [3]. About 50 g of peeled sliced fresh potatoes in 100 mL distilled water in a conical flask was boiled for 25 minutes and filtered using muslin cloth and made up to the 200 mL mark of a volumetric flask with distilled water. Then 95 g of prepared PDA powder was dissolved in 250 mL of sterilized water by heating it. It was allowed to cool to room temperature. The mixture was then covered with a cotton wool and aluminium foil; and autoclaved at 121 ^{0}C and 15 Psi for 20 minutes and kept in a refrigerator for preservation.

An amount of 0.1 g of each metal complex was dissolved in 100 mL of distilled water to give 1000 ppm stock solution. Other concentrations were obtained by dilution. The different concentrations of the complex (preservative) were mixed with the culture medium that was already mixed with streptomycin which is an anti-bacterial agent. A culture medium containing streptomycin without the preservative was used as the control.

Studies on inhibition of microbial growth

A range of concentrations of the metal complexes (0.40 - 15 ppm) were prepared from the 1000 ppm stock solution. A 5 mL portion of each concentration was mixed with 10 mL of a culture medium (PDA) already poisoned with streptomycin. The mixture was poured into a plate and allowed to set at room temperature. Bread mucor obtained from stale bread kept in a damp environment for 36 - 48 hours was then inoculated directly in the center of the plate by the use of a sterilized needle. The plate was then covered immediately and kept in an oven at 37 ^{0}C and readings that measure the diameter in centimeter of microfungal growth and number of colonies were taken. The same measurements were also carried out at higher concentrations of 50, 75 and 100 ppm of the metal iodobenzoates complexes.

RESULTS AND DISCUSSION

From Table 1, it is observed that the metal complexes were soluble and those that were slightly soluble became soluble on warming. This may imply that the metal complexes contain some hydrophilic groups like carboxylate and iodo groups that enhance their solubility in polar solvents in line with views reported in literature [10]. The complexes with alkali metals gave added advantage to their solubility as they were soluble at lower temperature. Most of the metal complexes prepared (preservatives) were found to be soluble in polar solvents.

Table 1. Solubility test for prepared metal complexes.

Metal complex / Solvents	Acetone	Methanol	Chloroform	Water
Na – complex	Insoluble	Slightly soluble	Insoluble	Soluble at 40 ^{0}C
K – complex	Insoluble	Slightly soluble	Insoluble	Soluble at 37 ^{0}C
Ca – complex	Insoluble	Insoluble	Insoluble	Soluble at 50 ^{0}C
Mg – complex	Insoluble	Insoluble	Insoluble	Soluble at 43 ^{0}C
m – Iodobenzoic acid	Insoluble	Insoluble	Insoluble	Soluble at 87 ^{0}C

Since microbes grow in damp environment, it is necessary that the food preservatives should be soluble. If it is insoluble in polar solvent, then moulds will grow in the aqueous phase rendering the preservative inactive. The colour of medium are generally not changed when mixed with these metal complexes which is added advantage for the complexes as food preservatives. From the atomic absorption spectroscopic test shown in Table 2, the experimental amount of alkali metals were observed to be less than that of theoretical amount while those of alkaline earth metals, Mg and Ca are more than those of their theoretical amount. This may probably be due to the absorption of water by the complexes. Most probably, the alkaline-earth metal complexes appear unstable on storage. However, it is not clear that the anti-fungal activities of the alkaline earth metal complexes are due to the decomposed products since the antifungal studies were done soon after preparation of the complexes whereas the AAS analysis were done after some period of storage.

Table 2. Percentage yield of complexes, experimental and theoretical amount of metal in metal iodobenzoate complexes.

Complexes without absorbed water	Complexes with varying amount of absorbed water	Yield (%)	Experi-mental amount (%)	Theoretical amount without absorbed water	Theoretical amount with varying amount of absorbed water
Na – complex IC_6H_4COONa	Na – complex $IC_6H_4COONa.5H_2O$	82.3	6.24	8.52	6.38
K – complex IC_6H_4COOK	K – Complex $IC_6H_4COOK.5H_2O$	80.4	9.82	13.64	10.37
Mg – complex $(IC_6H_4COO)_2Mg$	Mg – complex $(IC_6H_4COO)_2Mg$	62.1	6.75	4.66	-
Ca – complex $(IC_6H_4COO)_2Ca$	Ca – complex $(IC_6H_4COO)_2Ca$	60.5	12.83	7.49	-

From Table 3, the number of colonies of fungus developed varied inversely with increase in solution concentration of all metal complexes for 0.40 - 15 ppm range. This implies that as the concentrations of the different complexes increase, their percentage inhibition against fungal growth increase. Thus the concentrations of 0.40, 0.50, 0.60, 0.75, 10.00 and 15.00 ppm of sodium iodobenzoate exhibited 4.11, 5.48, 5.48, 12.31, 52.05 and 61.64% inhibition, respectively, against fungal growth of bread mucor. The percentage inhibition of 6.85, 8.22, 6.85, 15.07, 49.32 and 65.75% were exhibited by 0.40, 0.50, 0.60, 0.75, 10.00 and 15.00 ppm concentrations of potassium iodobenzoate, respectively. The same concentrations in ppm of calcium iodobenzoate showed percentage inhibition of 4.11 - 74.60% range against fungal activities. Similarly, the concentrations of 0.40, 0.50, 0.60, 0.75, 10.00 and 15.00 ppm of magnesium iodobenzoate showed 6.85, 10.96, 10.96, 17.81, 56.16 and 67.12% inhibition, respectively, against fugal growth of bread mucor. The percentage inhibition of the *m*-iodobenzoic acid was however found to be as high as 68% for 10 ppm and 15 ppm. From Table 3, it was also found that there were increases in percentage inhibition for all the preservatives (metal complexes) except for the *m*-iodobenzoate that remained as 68.49% inhibition against fungal growth of bread mucor for 10 ppm and 15 ppm. For the 15 ppm concentrations of prepared metal complexes; 61.64, 65.75, 74.60, 67.12 and 68.49% inhibition against fungal growth of bread mucor were found, respectively, for sodium, potassium, calcium, magnesium complexes and *m*-iodobenzoic acid, respectively. Thus the increasing order of percentage inhibition of these preservatives might be suggested to be: calcium iodobenzoate > *m*-iodobenzoic acid > magnesium iodobenzoate > potassium iodobenzoate > sodium iodobenzoate.

The highest percentage inhibition of 74.60% was exhibited by 15 ppm concentration of calcium iodobenzoate. This might probably be attributed to the presence of two iodine atoms present in the two *m*-iodobenzoates of the calcium complex. The same explanation may be responsible for the better antifungal effect of the megnessium iodobenzoate when compared with their alkali metal congeners. It is however observed that the percentage inhibition value of *m*-iodobenzoic acid is almost the same as that of magnesium iodobenzoate. It could be suggested that the effect of two iodine atoms of Mg complex may not be more than the combined effect of one iodine atom in *m*-iodobenzoic acid and that contributed from its carboxylic acid group. It may be explained further that the –COOH presence, lowers the pH of the medium to about 2.4, thereby inhibiting the growth of the moulds which thrive best between pH 4.0 - 6.0 and at temperature of 30 ^{0}C [7].

Table 3. Data for number of colony and percentage inhibition of microbial growth after 72 hours.

Concentration of metal complex (ppm)	Na-complex		K-complex		Ca-complex		Mg-complex		*m*-iodo-benzoic acid		Culture medium only	
	NC	% I	NC	% I	NC	% I	NC	% I	NC	% I	NC	% I
0.04	70	4.11	68	6.85	70	4.11	68	6.85	69	5.48	73	100
0.50	69	5.48	67	8.22	67	8.22	65	10.96	65	10.96	-	-
0.60	69	5.48	68	6.85	65	10.96	65	10.96	58	20.55	-	-
0.75	64	12.33	62	15.07	55	24.66	60	17.81	55	24.66	-	-
10.00	35	52.05	32	49.32	28	61.64	32	56.16	23	68.49	-	-
15.00	28	61.64	25	65.75	20	74.60	24	67.12	23	68.49	-	-

NC: Number of colonies formed after 72 hours of inoculation.
% I: percentage inhibition of microbial growth after 72 hours of inoculation.

$$\% \text{ I} = \frac{\text{Number of colonies in control} - \text{Number of colonies in sample}}{\text{Number of colonies in control}} \times 100$$

Table 4. Data for extent of fungus coverage on different culture media for higher concentration of metal complexes.

Metal complex	Concent-ration (ppm)	Extent of coverage in diameter (cm)						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Na - complex	50	-	-	-	0.1	2.0	3.0	10.0
	75	-	-	-	-	1.0	2.0	4.0
	100	-	-	-	1.5	3.0	3.5	5.0
K - complex	50	-	-	-	-	2.0	3.0	10.0
	75	-	-	-	-	-	0.1	3.0
	100	-	-	-	-	0.1	3.5	10.0
Ca - complex	50	-	-	-	0.1	3.0	4.5	5.0
	75	-	-	-	-	1.0	1.5	1.5
	100	-	-	-	-	2.0	4.0	10,0
Mg - complex	50	-	-	-	0.1	3.5	4.0	6.0
	75	-	-	-	-	1.5	1.5	2.5
	100	-	-	-	-	3.0	4.5	10.0

Table 4 shows the extent of fungal growth up to 7 days for metal iodobenzoates for higher concentrations of 50, 75, and 100 ppm. After more than 7 days, there was full coverage of the culture media poisoned with sodium and potassium preservatives at 50 ppm concentration. Similar observations were made for the potassium and calcium complexes at 100 ppm

concentrations. For each of the complexes 75 ppm showed to be the optimum concentration as at the end of 7 days because little or no fungal growth was observed.

The data in Table 4 also indicate that from 1- 4 days, the fungi did not grow at all but showed up again from 5^{th} days after inoculation of fungi into the culture media. Thus, these preservatives (metal iodobenzoates) could be regarded as fungi-static and not fungi-toxic. However, the shelf life of bread that was 24 hours could be extended to 96 hours with the use of these metal iodobenzoates as preservatives.

CONCLUSION

Metal complexes of potassium, sodium, calcium and magnesium of *m*-iodobenzoic acids were prepared. Calcium iodobenzoate exhibited the highest anti fungal activities on bread mucor. It showed 74.60% inhibition for 15 ppm concentration while sodium iodobenzoate showed the least inhibition of 61.64%. The optimum concentrations of the metal complexes for inhibition against fungal growth were found to be 75 ppm for all the preservatives. The metal complexes were found to be fungi-static but not fungi-toxic. However the use of these metal complexes could extend the shelf life of bread from 24 hours to 96 hours.

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