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# Effect of monosodium glutamate (MSG) on alpha-amylase activity

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## ABSTRACT

The effect of monosodium glutamate (MSG) on health has been a matter of controversy since long period of time. A number of reports indicated that it has several adverse and negative health impacts. It has a constituent in commonly available fast foods and restaurant foods all across the world and is known for enhancing the palatability. Several reports have been published over a period of decades indicating its role in various physiological and biological manifestations. The current work is an attempt to asses the effect of this compound on the enzymatic activity of gastrointestinal enzyme in particular the alpha-amylase. The results showed that the MSG inhibits the enzyme in concentration dependent manner. The kinetic studies indicated that the low concentrations of MSG inhibit the alpha-amylase in a noncompetitive manner and in the presence of higher concentrations it follows the mixed type of inhibition. 

## KEYWORDS

Monosodium glutamate;
Alpha-amylase;
Enzyme activity;
Inhibition.

## INTRODUCTION

People all across the world are deeply concerned about the possible health impact of MSG which is generally added in food. It is commonly used as food additive for the taste enhancement in various Chinese and fast foods. It is sodium salt of the non-essential amino acid, i.e. glutamic acid and widely used in the preparation of certain ethnic foods as well as large number of canned food[1]. Excessive use of MSG cause a complex of symptoms, such as, headache, burning sensation, palpitation, chest pain, abdominal distress, asthma like symptoms, thoracic, facial and cervical tightness, etc. They are commonly known as 'Chinese restaurants syndrome' because of its frequent use in Chinese restaurants[2]. They are also known as "monosodium glutamate symptoms complex"[3]. The MSG is generally present in seaweeds, sea tangles and soy sauces as condiments and has a history of its use of at least a thousand years[4].

There are many reports suggesting that the high concentration of MSG induces pain and sensitivity in various skeletal muscles[5-8]. It is also reported that the MSG, glutamic acid, and glutamine are toxic when given parenterally[4]. When the young mice were injected with glutamate, they developed brain lesions, obesity, sterility and stunt growth[9]. It is also known to induce obesity and diabetes in mice, change in the intestinal muscle activity, which may be due to the metabolic alteration as well as MSG action on enteric neurons and/or

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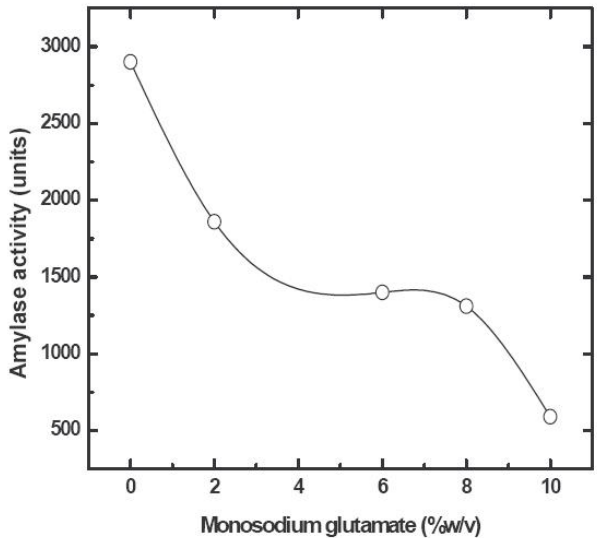


**Figure 1 : Effect of MSG on the activity of microbial alpha-amylase. The activity was determined by estimating the reducing sugar after hydrolysis of starch in the presence of different concentration MSG**

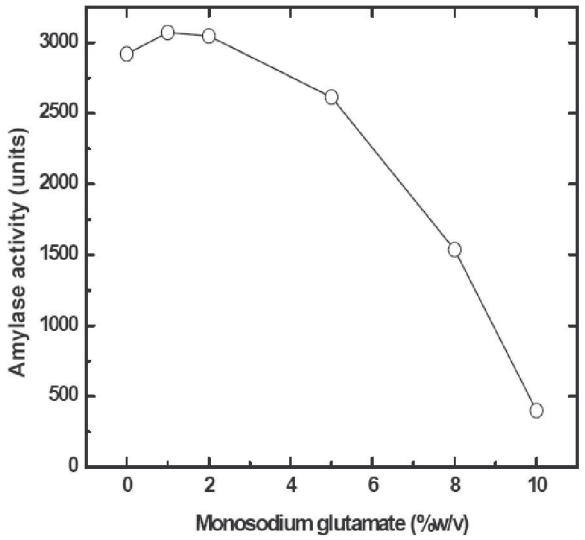


**Figure 2 : Effect of MSG on the activity of human salivary alpha-amylase. The activity was determined by estimating the reducing sugar after hydrolysis of starch in the presence of different concentration MSG**

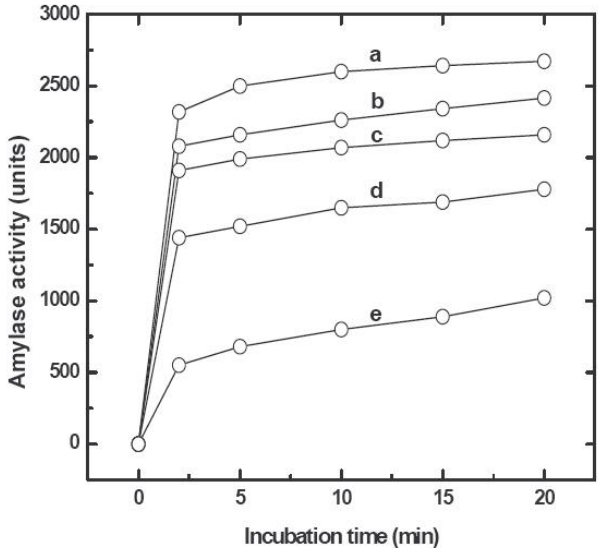


**Figure 3 : Effect of incubation period on the activity of human salivary alpha-amylase in the presence of different concentrations of MSG at 37ºC. The curves are represented as; (a) Control (b) 2% (c) 5% (d) 8% and (e) 10% (w/v) concentrations of MSG**

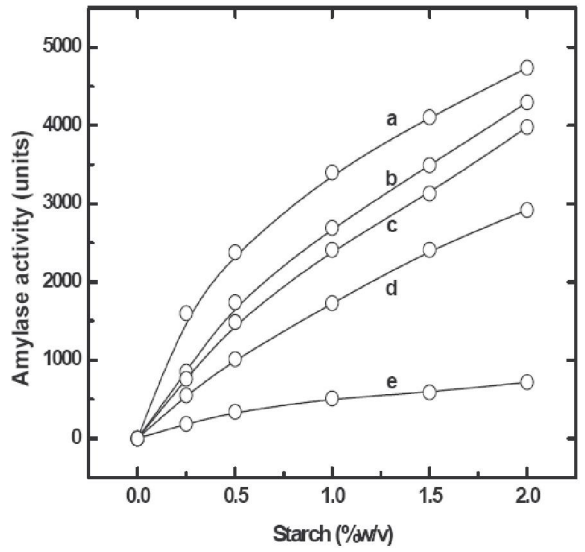


**Figure 4 : Effect of substrate concentration on the activity of human salivary alpha-amylase in the presence of different concentrations of MSG. The curves a, b, c, d, and e represent the enzyme activity in presence of 0.0% (control), 2%, 5%, 8% and 10% (w/v) concentrations of MSG**

smooth muscle receptors[10,11]. The physiological and biological responses of MSG vary among different individuals. Some people react vigorously even to 1 to 2 grams dose but many people develop no symptoms even they are given 12 gram. It is reported that the L isomer of glutamate induce symptoms but D form does not[4]. The inclusion of MSG in food is known to increase in the food intake, which subsequently leads to metabolic disorder associated with oxidative stress and change in glucose, insulin, leptin and triglycerol level and it has adverse effect on mature neuronal cell culture[12,13]. This is mainly because the brain tissues have remarkable ability to accumulate glutamate due to presence of glutamate transporter in the plasma membrane of both glial cells and neurons[14].

Although several reports contradict the safety aspects of MSG in food, a more knowledge is needed for a rational and qualitative understanding both for the

advantage and as a risk. Many food and MSG producing industries keep advertising its positive attributes in the consumer market and many a time they have been biased while addressing the quantitative perspectives of safety issues. Despite the wide public acceptance of MSG as a taste enhancer, no reports exist today indicating its effect on the activity of gastro intestinal enzymes. The present work is an attempt to understand the effect of MSG on the activity of alpha- amylase, a crucial enzyme in carbohydrate metabolism in the living system.

## MATERIALS AND METHODS

### Materials

Salivary alpha-amylase was prepared from the human saliva (crude). The microbial alpha-amylase was procured from Biocon India, Pvt. Ltd, Bangalore, India. NaOH, $CuSO_4.5H_2O$, 3,5-dinitrosalysilic acid, citric acid and starch were procured from Himedia laboratories, India. Potassium sodium tartarate, Folincialcalteu reagent, $CaCl_2$ and Sodium citrate were obtained from SD Fine Chemical Company, India. MSG was procured from local market which is commonly available. Quartz double distilled was used throughout the experiment.

### Methods

### Alpha-amylase assay

The human salivary and microbial alpha-amylase activity was determined using Bernfeld method[15] for estimation of reducing sugar produced after alpha-amylase catalysis. The enzyme solution was prepared in 50mM citrate buffer pH 6.5 containing 2mM of $CaCl_2$. 1 ml of enzyme solution was mixed with 1ml of 1% (w/v) starch solution incubated for 5 min at 37ºC. The enzymatic reaction was terminated by addition of 2ml of 1% alkaline dinitrosalysilic acid solution. Thereafter the solutions were subjected to boiling water bath for 10 min, after cooling the final volume was made to 20ml with addition of double distilled water and mixed thoroughly by vertexing. The absorbance was recorded at 540nm. The units of enzyme activity were determined by using maltose standard plot. One unit of enzyme was defined as the number of micromole of reducing sugar equivalent released in catalytic reaction under assay condition.

### Protein estimation

The protein concentrations in enzyme preparations were determined using Lowry's method[16]. The 1 ml of enzyme solution mixed with 0.1N NaOH solution, followed by addition of 5ml of $CuSO_4$ solution and incubated at 30ºC for 10 min. Then 0.5ml of diluted Folin-Ciacalteu reagent was added and kept the test tubes in dark for 30 min and absorbance was recorded at 660nm. The standard was prepared using bovine serum albumin to determine the concentrations of proteins.

### Determination of alpha-amylase activity in presence of MSG

The enzyme samples (salivary and microbial alpha-amylases) were incubated in presence of different concentrations (2%, 5%, 8% and 10% (w/v)) of MSG for a minimum period 12 hrs at 10ºC. The activity of the above enzymes was determined using above method using starch as substrate. Appropriate controls were introduced to eliminate the effect of MSG on the spectral properties of reducing sugars. The pH of all the solutions was maintained uniform throughout.

### Kinetic study of alpha-amylase in presence of MSG

The kinetic constants such as apparent $K_M$ and $V_{max}$ of human salivary alpha-amylase were determined using rate of enzyme catalysis in presence of different concentrations of substrate and MSG. The rate constant of enzymatic reaction was determined in presence of 0.2%, 0.5%, 1.0%, 1.5% and 2.0% (w/v) starch solution and reciprocal plots were used to determine the apparent $K_M$ and $V_{max}$. All the above experiments were carried out in triplet and repeated twice to check the reliability on the data.

## RESULTS AND DISCUSSION

### Effect of MSG on microbial alpha-amylase activity

Microbial alpha-amylase constitutes a major share in carbohydrate and starch based industries and formulation of specialty foods. Since various food formulations are consist of MSG, it is imperative to study its

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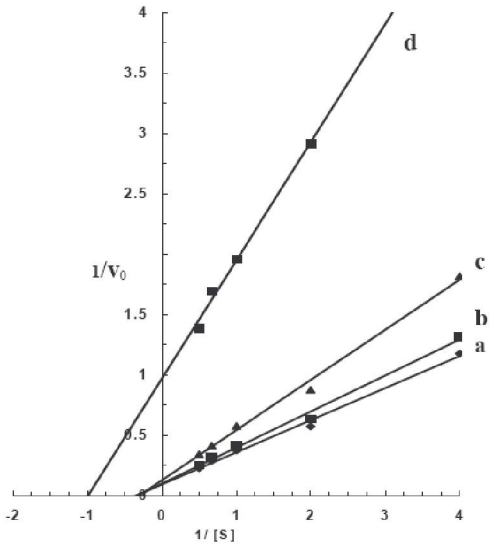


**Figure 5 : Reciprocal plot (Lineweaver-Burk plot) of human salivary alpha-amylase activity in the presence of different concentration of MSG. The curves a, b, c, and d represent the enzyme activity in presence of 0.0% (control), 5%, 8% and 10% (w/v) concentrations of MSG**

effect the activity of gastro intestinal enzymes. In the present investigation our study was confined to study the effect of MSG on activity of microbial and human salivary alpha-amylases. Now days this enzyme, particularly from the microbial sources, is commonly used in the preparation of various digestive ads. MSG was found to be inhibitory for the alpha-amylase in concentration dependent manner. As shown in Figure 1 the enzyme activity is severally affected due to presence of MSG. With increasing concentration of MSG the enzyme activity was found to be linearly decreased. In presence of 2%, 6%, 8% and 10% (w/v) concentrations of MSG the enzyme activity was found to be 1750, 1500, 1400 and 600 units compared to the control value of 2800 units. This is indicative of inhibitory effect of MSG on the alpha-amylase.

## Effect of MSG on salivary alpha-amylase

After studying the effect of MSG on microbial alpha-amylase the study was extended to investigate its effect on human salivary alpha-amylase. Since it is the first carbohydrate hydrolyzing enzyme which encounter with the carbohydrate in the digestive process. It plays a major role in the initial digestion of carbohydrate after ingestion of food. Therefore it was felt essential to study the effect of MGS on human salivary alpha-amylase activity. In both the cases (microbial as well as salivary alpha-amylases) the MSG was found to be inhibitory but the over all response was found to be significantly different. As shown in figure 2 the enzyme activity was found to be inhibited in presence of higher concentration of MSG. In presence of low concentration of MSG the salivary alpha-amylase showed a marginal increase in the activity which is a matter of further investigation. In presence of 5%, 8% and 10 % (w/v) concentrations of MSG the enzyme activity was found to be 2750, 1500, 450 units compared to control value of 2800 units. It is clear from the above data that human salivary alpha-amylase behaves differently from the microbial alpha-amylase and it is comparatively less sensitive to MSG.

## Kinetic study of alpha-amylase inhibition in presence of MSG

Kinetic approach was adopted in order to understand the mechanism of MSG induced inhibition of alpha-amylases. As shown in Figure 3 with increasing concentration of substrate the rate of enzyme catalysis is marginally diminished. This was correct only at relatively lower concentration of MSG. In the presence of higher concentration of MSG (10%) there no significant increase in the enzyme activity was observed. Enzyme activity was measured in presence of different concentrations of substrate and MSG. It was found that with increasing concentration of substrate the inhibitory effect of MSG was found to be marginally nullified (Figure 4). This shows that the nature of enzyme is competitive, means the MSG complete for the active site therefore with increasing concentration of substrate the inhibitory effect is getting minimized. The Figure 5 showed the Lineweaver-Burk plot and it indicates that the $K_M$ is found to be unchanged in presence of 5% and 8% (w/v) concentrations of MSG and it is similar to control. On the other hand the $V_{max}$ is continued to decrease with increasing concentration of MSG. Further increase in the MSG concentration (10%) results increase in the $K_M$ value and decrease in $V_{max}$, which indicates the decrease in enzyme-substance affinity[17]. This could be mainly due to change in solution properties at higher concentrations of solutes (MSG) which leads to change in surface properties of the enzyme such as hydration and preferential interaction parameters[18-20] which subsequently results change in the enzyme activity.

## CONCLUSION

From the above results is clear that both the alpha-amylase were inhibited by MSG in concentration dependent manner. The concentration of MSG at which the enzymes showed the inhibition is relatively higher compared to its concentration used in food formulations and at that concentration these enzyme did not shown any inhibition. In the first instance it looks that concentration of MSG used in food is too less to affect the activity of alpha-amylase, however it is imperative to address the safety aspects of MSG and it needed more understanding about the nature of interaction with various enzymes under different conditions of pH, temperature and ionic strength.

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