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Annals of Biological Research, 2017, 8 (3): 18-23  
(<http://scholarsresearchlibrary.com/archive.html>)



ISSN 0976-1233

CODEN (USA): ABRNBW

# Effects of Umami Substances Monosodium Glutamate (MSG) and Ribonucleotides (GMP/IMP) on Mitotic Index in *Allium cepa* L. Plant

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## ABSTRACT

*In this research the effects of two different food additives like Mono Sodium Glutamate (MSG) and ribonucleotides [inosine 5'-monophosphate (IMP) and Guanosine 5'-monophosphate (GMP)] have been used for determining the genotoxic potential by the meaning of mitotic index analysis. Allium cepa L. root tip test were used for mitotic index measuring. For comparing the effects of three different concentrations of MSG and ribonucleotides (I+G) after 24, 48 and 72 hours exposure time on A. cepa, root tip test were realized. All experiments were carried out under controlled conditions in the laboratory. According to our research results, it seems that different concentrations MSG and ribonucleotides (I+G) applications has been decreased the mitotic index values in A. cepa depending to increased concentrations and 72 hours exposure time respectively in MSG and ribonucleotides (I+G) as %85.43 and %90.78 when compared with the control group. Research results consistently showed that mitotic cell division has been inhibited with different concentrations of MSG and ribonucleotides (I+G) applications depending to the exposure time.*

**Keywords:** Allium cepa, MSG, GMP and IMP, umami, mitotic index

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## INTRODUCTION

Food is one of the vital substance for living creatures as well as for humans. People were able to preserve their foods for a while even in the 3000 B.C., moreover several methods were also used to enhance foods flavors such as

spices and foods were artificially colored more than 3500 years ago [1, 2]. Food additives can add some attributes to the food, such as flavor enhancing, better shelf life and more preserving time span. These substances could contribute to food nutrient values or not. Briefly, in modern time food additives offer better shape, flavor, aroma and attractiveness for customers [3, 4]. Today, increasing population demands more food than ever and advancing food technology needs alternative methods to supply markets. Thus, food sector using more than 4000 kind of food additives [2, 5].

Today, JECFA is examining every chemical compound of food additive to determine their toxicological status. JECFA is an international expert scientific committee, which also has responsibility for the implementation, regulation, and assessment of chemical compound tests. This committee administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). Since 1956 food additives are evaluating for safety of human health by international establishments. This safety assessments brings a new identification: Acceptable Daily Intake (ADI) by this term, food additives are becoming more secure to use for foods [1, 2]. However, all of these efforts are not enough to cease discussions about food additives and their adverse effects [6].

Naturally glutamates are present in human body and have crucial metabolic roles. Nevertheless, glutamates can be found in several meat, vegetable and fish sources. Monosodium glutamate (MSG) is one of the most known and using flavor enhancer of food additives [6, 7]. MSG discovered in 1900s by Japanese scientist Kikunae Ikeda from seaweed [8]. Basically MSG is a glutamic acid salt and contains water, glutamic acid and sodium salt [7, 9, 10]. Today, MSG labelled on food packages as E621 by European Union (EU) [11, 12]. Guanosine 5'-monophosphate or disodium 5'-guanylate (GMP) and inosine 5'-monophosphate or disodium 5'-inosinate (IMP) are purine 5'-nucleotides and food additives like MSG [10, 13, 14]. Guanylic acid was identified by Kuninaka in 1958. This substance was available naturally in broth and black mushroom *shiitake* (*Lentinus edodus*). Inosinic acid was identified by Konosu et.al. like guanylic acid, this substance was also available naturally in beef broth. Both guanylic and inosinic acids were known since 1800s but their taste effects have not known for years. As like in MSG, IMP and GMP are salt form of the inosinic and guanylic acids [15]. These are labelled on food packages as E627 for GMP and E631 for IMP by EU [12].

Umami is a distinctive taste which was discovered by Ikeda in 1908 and established with MSG, IMP and GMP [15, 16]. This characteristic taste also known as fifth taste; was not recognized by western world for a long time. Then several experiments has been done and some studies such as psychophysical and electrophysiological studies

showed that umami is independent of the known sweetness, sourness, bitterness, and saltiness of four classical basic tastes [17]. Along with that, MSG and nucleotides (GMP and/or IMP) showed synergetic effects on taste [18, 19]. The ADI was not specified for MSG, IMP and GMP by JECFA [12]. The ADI decide of JECFA and effects of umami substances still controversial [20–22]. Today, umami taste or its compounds such as MSG, IMP and GMP are using in food sector like soups, chips, bakery and snacks [23]. These substances can be found commercially in public market.

*A. cepa* is one of the reliable, short term and known assay that also approved by WHO [24, 25]. One of the utmost feature of *A. cepa* is its large chromosomes and also less chromosome numbers ( $2n=16$ ). Moreover, prokaryotes may show DNA damages, but eukaryote plants like *A. cepa* may show extended genetic damages like chromosomal mutations [26]. This assay was first offered by Levan in 1938 with using *A. cepa* root tips [27]. However, first *A. cepa* adaptation was conducted by Fiskesjö in 1985 [28]. Since then many of tests were done with this assay.

In this research, we aimed to show commercial and ready to use umami substances MSG and IMP-GMP combination (I+G) effects on *Allium cepa* root tips. MSG and I+G experiments was conducted separately with *A. cepa* root tip test to evaluate these substances genetically damage levels.

## MATERIALS AND METHODS

### Plant material and chemicals

The certified onion bulbs (*A. cepa*,  $2n=16$ ) were purchased from Ceylan Agricultural Company (Turkey). Chemicals of MSG and ribonucleotides (I+G) were purchased from Kimbiyotek Chemical Substances Company (Turkey).

### In vitro assay

Onions were selected equally-sized, and immersed in glass test tubes. Onions were leaved in distilled water to germinate for 3 days. The germination process was conducted in controlled chamber at  $25\pm 2$  °C with 16/8 photoperiod using three replicates each of which included five onions bulbs. MSG was dissolved in distilled water and different concentrations were prepared as 0.125, 0.25 and 0.50% g/L. Ribonucleotides (I+G) was dissolved in distilled water and and different concentrations were prepared as 0.20, 0.40 and 0.80% g/L [29].

At the end of the germination, onion roots were growth approximately 2-3 cm. Right after of germination, control group was immersed into distilled water and any treatment was not applied. MSG and I+G experimental series were conducted for 24, 48 and 72 hours. Each time period was conducted with 0.125, 0.25 and 0.50% g/L MSG

solution. Bulbs were immersed into the glass test tubes and each concentration which included control groups was performed with five onions bulbs. I+G experiment was consisted with 0.20, 0.40 and 0,80% g/L I+G solutions for each time period. Bulbs have been immersed into the glass test tubes and each concentration, included control groups was performed with five onion bulbs. Due to evaporation, all concentrations were added to adjust initial amount [30].

The root tips were taken after 24, 48 and 72 hours into the mixture of ethanol/glacial acid (3:1) solution for fixation, and the duration was overnight at +4°C. Then, root tips were hydrolyzed in 1N HCl, at 60°C for 8 minutes and rinsed with distilled water. After the hydrolysis process, root tips were immersed into 2% (w/v) aceto-carmin to stain for overnight at +4°C. To assess the effect of MSG, I+G on mitotic index (MI), more than 1000 cells were scored in each slide five times, for control, MSG and I+G groups. The slide preparations were performed as one root tip per slide. Totally five slides (more than 5000 cells) were prepared per MSG, I+G and control. The slides were evaluated by observing the cells in prophase, metaphase, anaphase, telophase and aberrations by light microscopy at 1000X magnification with Motic brand BA210 model microscope.

Assessment of cytotoxicity was based on the MI. Mitotic index formula estimated with:

$$MI = \left( \frac{\text{Number of Dividing Cells}}{\text{Total Number of Observed Cells}} \right) \times 100$$

5000 cells were scored in MSG, I+G and control groups. Statistical significance for each group were compared with *Student's -t test* for mitotic index using MS Excel 2016. Statistically significance value was considered as  $P < 0.05$  and standard error was displayed as mean  $\pm$ SE.

## RESULTS AND DISCUSSION

In this research, we investigated the effects of umami substances (MSG and I+G) on *Allium cepa* root tips. MSG was applied to root tip cells with concentration of 0.125%, 0.25% and 0.50% (g/L) for each 24, 48 and 72 hours. The mitotic index was declined in *A. cepa* root tip cells after expose to MSG ( $P < 0,05$ ) in terms of increasing time duration and concentration, compared to negative control (Figure 1).

MSG Concentration (g/L)	Time of Exposure		
	24 h	48 h	72 h
0-Control	9.74±0.41	9.14±0.40	8.92±0.39
0.125	5.90±0.33	4.74±0.29	3.65±0.25
0.25	5.46±0.32	3.56±0.26	2.09±0.20
0.50	3.26±0.25	2.62±0.22	1.30±0.16

Figure 1: Mitotic index results in *A. cepa* root tip cells after MSG application.

I+G was applied to root tip cells with concentration of 0.20%, 0.40% and 0.80% (g/L) for each 24, 48 and 72 hours. The mitotic index was declined in *A. cepa* root tip cells after expose to I+G ( $P < 0.05$ ) in terms of increasing time duration and concentration, compared to negative control (Figure 2).

Ribonucleotides (I+G) Concentration (g/L)	Time of Exposure		
	24 h	48 h	72 h
0-Control	10.39±0.42	9.28±0.40	10.30±0.41
0.20	6.36±0.33	3.21±0.24	2.40±0.21
0.40	5.77±0.32	3.47±0.26	1.86±0.19
0.80	4.29±0.28	2.27±0.20	0.95±0.13

Figure 2: Mitotic index results in *A. cepa* root tip cells after ribonucleotides (I+G) application.

The highest decrease on mitotic index has been found as 85.43% at 72 hours after 0.50% g/L of MSG application. Along with that, the highest decreasing effect on mitotic index has been found as 90.78% at 72 hours after 0.80% g/L I+G application. These effects clearly show that mitotic index was declined dramatically compared to control groups. Alongside of this, mitotic index was declined by means of increasing time duration for a concentration. For instance, figure 1 shows that the mitotic index for 0.125% g/L concentration of MSG was declined when compared with control group as 5.90±0.33, 4.74±0.29 and 3.65±0.25 for 24, 48 and 72 hours, respectively. These values are supporting evidences the reliability of *A. cepa* root tip tests [31]. Mitotic index is a key value for the assessment of cytotoxicity [26]. Since MI is a considerable parameter for evaluation of cytotoxicity, many of studies were conducted for this intend [32, 33]. As mentioned in some studies, excessive application of MSG can damage vital organs [7]. Moreover, MSG can cause depressive-like behaviors therefore the effects are not limited by physically [34]. Decreasing of MI on *A. cepa* may support these findings by means of the harmful for plant cells. Some publications showed that the effects of umami are safe. Furthermore JECFA did not specify an ADI for umami substances [17, 35]. Even if these substances accepted as safe, several studies mentioned that MSG is inducing to eat more and triggering obesity by excess food intake [7, 36].

## CONCLUSION

In conclusion, declining of MI by MSG and I+G shows that these substances can be assumed as cytotoxic agent. These research results showed that consistently changing levels of MI and differences depending to the flavoring agents with exposure time. It is important for the consumers should be aware of the food additives in the food they use. The present study suggesting the importance of recognition and controlled use of food additives. Consumers should be informed about food additives and their effects. Awareness of the issue can contribute public health by positive way.

**ACKNOWLEDGEMENT**

This research is a part of Miss. Nursel DURAN's MSc Thesis which is continues in Biological Science in Graduate School of Natural and Applied Sciences in Canakkale Onsekiz Mart University under the supervisory of Prof. Dr. Cüneyt AKI. The authors would like to thank Evren Erten, for comments and language proofreading.

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