



# Impact of Dietary Components and Food Additives on Lipid Peroxidation (LPO) Product in *Drosophila melanogaster*

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## Abstract

Our goal was to determine the direct relation of acrylamide with the malondialdehyde concentration in whole body of *Drosophila melanogaster* and compare with the result obtaining by supplying other food contaminants. Other food additive used in this study was Monosodium Glutamate and carmoisine. Monosodium Glutamate (MSG) is a flavor enhancer usually used in canned vegetables, Chinese foods and processed meats. However, there is no research conducted yet to prove the direct link between MSG and the above given symptoms. The TBARS assay was performed in triplicate sample by taking 10 flies from all the bottles containing test components (acrylamide formed in 1% charred media, monosodium glutamate and carmoisine) under study and the student t-test was likewise performed to decide the criticalness of the information acquired from the negative geotaxis measure. The outcome demonstrated that all the sustenance contaminants (1% burned nourishment, Monosodium glutamate, and Carmoisine) has an impact on locomotor action of the *Drosophila melanogaster*. In any case, in view of the information got, we can say that both acrylamide containing charred food and carmoisine impacts more to life form contrasted with impact brought about by monosodium glutamate. Based on the data obtained, we can infer that to reduce the oxidative stress, we should avoid the consumption of food containing various food additives to enhance the taste and color to make the food more appealing to eye. We should also avoid eating fried foods to lower the potential risks of getting cancer, neurological diseases and various other pathological conditions.

**Keywords:** *Drosophila melanogaster*; Lipid peroxidation; Acrylamide; Climbing assay

## OPEN ACCESS Introduction

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Every organism requires nutrient in the form of food irrespective of their size. Microorganisms need carbon, nitrogen, and trace elements to acquire healthy life cycle but, in case of human beings and higher vertebrates these nutrients are required in bulk form. Thus, or highly developed organism such as a human being, foods such as vegetables, milk and its derivative products, non-vegetables are usually consumed [1,2]. The organic and inorganic compounds present in these foods are used as energy, regulatory or building substances by these higher organisms. Regrettably, these foods which are consumed by people are a source of toxicants. The consequence of consuming junk food does not stop here but it may lead to unhealthy future generation ACR is a sort 2 alkene a concoction class that incorporates fundamentally related electrophilic ecological contaminations. Naturally inferred class 2 alkenes may act synergistically with endogenously produced unsaturated aldehydes to intensify cell harm and in this manner quicken human sickness/damage forms that include oxidative pressure [3]. ACR is responsible for the death and degradation of distal axons and nerve terminal in the peripheral and central nervous system that leads to cerebella Purkinje cell death (Lopachin 2004). It can also cause tumour formation and leading to cancer in various part of the body in an organism. This neuronal degeneration occurs due to accumulation of lipid peroxidation products like malondialdehyde, 4-Hydroxynonenal and others. In order to determine the pathological condition of an organism, these lipid peroxidation products are used as a biomarker [4,5]. Acrylamide gets converted to glycidamide in the body which can cause mutation and damage to DNA. When acrylamide enters in body fluid then Acrylamide and its breakdown items leave your body generally through urine, little sums may leave through faces, breathed out air, and breast milk. Since, we all know that acrylamide may lead to enhanced oxidative stress thereby affecting overall development of an organism. This can also result in induction of diseases such as diabetes mellitus, cardiovascular diseases. However more data is required to confirm the safety level of acrylamide

and critical involvement in various pathological conditions. Our goal was to determine the direct relation of acrylamide with the malondialdehyde concentration in whole body of *Drosophila melanogaster* and compare with the result obtained by supplying other food contaminants. Other food additive used in this study was Monosodium Glutamate and carmoisine. Monosodium Glutamate (MSG) is a flavor enhancer usually used in canned vegetables, Chinese foods and processed meats. However, there is no research conducted yet to prove the direct link between MSG and the above given symptoms. It is a well-known fact that glutamic acid acts as a neurotransmitter in the brain. To be more specific is an excitatory neurotransmitter. MSG has been labeled as excitotoxin as some people have claimed that MSG causes excessive stimulation of nerve cells. We selected fruit fly to conduct the primary study associated with MSG mediated lipid peroxidation [6]. Carmoisine aggravates the metabolic activities in the human body and modifies the biochemical markers in fundamental organs, for example, kidneys and liver, not just at higher portions it demonstrates poisonous quality even at lower dosages. Many of the countries banned carmoisine because of its toxicity and carcinogenicity but, in India this carmoisine is still permitted and consumed by the peoples frequently [7].

It is artificially synthesized from disodium salt and it is reddish brown crystal or a dark maroon powder. It is added in certain food products like marzipan, Swiss rolls, sweets, jellies, soda, brown sauce, packet soups etc. Carmoisine can likewise cause prejudice responses in individuals with aspirin intolerance. Different responses to carmoisine can incorporate a rash like annoy rash, and water maintenance.

### Climbing assay

The devastating neurodegenerative issue, for example, Parkinson's infection, amyotrophic lateral sclerosis, and inherited spastic paraplegia are progressively perceived in aged people. Till now there is no treatment has been developed for these neurodegenerative diseases. The boundless clinical utilization of genome-wide, impartial hereditary tests, for example, entire exome sequencing has prompted an expanding number of qualities being embroiled in human locomotor disorder [8]. The climbing assay is the financially affordable and dependable test that would permit high throughput examination that would, in any case, be sufficiently sensitive to identify little changes in motor performance [9]. The climbing assay is also termed as negative geotaxis since it focuses on the natural tendency of the fly to climb. Since, various agents mediate the oxidative stress in the body and thereby causing greater damage to every organ. This investigation centre around impact of nourishment added substances on malondialdehyde fixation in *Drosophila melanogaster*, expanded dimensions of malondialdehyde causes neurodegenerative infections. In this examination, three unique media (Carmoisine, Monosodium glutamate and Scorched sustenance) were used for the growth of *Drosophila melanogaster* [10,11].

## Materials and Methods

### Procurement and maintenance of culture

*Drosophila* culture was obtained from genetic department of IADC on 25<sup>th</sup> January and maintained till the start of this study (Table 1).

### Media preparation

All the components of media were weighed for 60 ml and mixed in warm distilled water. The media was heated at boiling temperature

**Table 1:** Media composition for *Drosophila melanogaster* culture.

Rava	80 g
Jaggery	70 g
Yeast	15 g
Agar	09 g
Propionic acid	4.4 ml
Water	1000 ml

for 5 min in conical flasks and then it was subjected to sterilization at 121°C for 15 min. After sterilization propionic acid was incorporated in the media. The media was then poured directly into drosophila culture bottles under sterile condition. The poured bottles were allowed to cool down to room temperature for solidifying of the media. Before transferring the flies in those culturing bottles, inner surface of all the bottles are allowed to dry (the moisture droplets) [12]. The better way to do so is to keep the bottles overnight, after pouring. Finally, approximately 10 *Drosophila melanogaster* were transferred to freshly prepared media from the stock culture.

### Experiment conditions

The flies obtained were grown on four different media at room temperature as follows:

1. Media with no test agent - Control
2. Media with 1% burned of it - Test 1
3. Media with Carmoisine 14720 (25 mg) - Test 3
4. Media with MSG (25 mg) - Test 4

### Temperature

The *Drosophila melanogaster* were grown at room temperature and routinely they were trans.

### TBARS assay

- 1) Ten males from each of the replicate of each treatment group were homogenized in 1000 µL of Phosphate Buffered Saline (PBS) containing 5 mM Butylated Hydroxytoluene (BHT).
- 2) All homogenates were centrifuged at 6.000 rpm for 10 min to 4°C.
- 3) Add 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the 2 mM MDA standard solution into separate tubes and make it up to 1 ml wit distilled water.
- 4) 1 ml sample containing tissue homogenate, MDA standards and blank were added.
- 5) 1 ml of 0.5% TBA reagent containing 20% TCA and 2.5N HCL were added.
- 6) Each tube was closed and incubated at 95°C for 1 h. They were removed and cooled in an ice bath for 20 min.
- 7) They were mixed and centrifuged at 3000 rpm × 10 min.
- 8) The absorbance at 532 nm was measured in the supernatants.
- 9) The results are expressed in nanomoles of MDA/mg of protein.

### Formula

$$(Sa/Sv) \times 4 \times D = C$$

Whereas:

Sa - Amount of MDA in unknown sample (n mole) from

standard curve

Sv - Sample volume ( $\mu\text{l}$ ) or amount ( $\mu\text{g}$ ) added into the wells

4 - Correction factor for using 200 ( $\mu\text{l}$ ) of 800 ( $\mu\text{l}$ ) reaction

D - Dilution factor

C - Concentration of MDA in sample

### Assessment of locomotor activity

1) Prior to start of the experiment, the triplicate cultures of *Drosophila melanogaster* were collected.

2) A batch of 10 flies was transferred in cylindrical tube with a mark at the height of 8 cm.

3) The flies were kept for 4 min for acclimatization of flies in cylindrical boiling tube.

4) Till that time, stopwatch was arranged and kept ready to begin with the experiment.

5) Once flies became favourable to cylindrical tube environment, the tubes were tapped to settle all the flies at the bottom of the tube.

6) The stopwatch was started at the same time and the number of flies was counted that crossed the 8 cm mark within 12 second.

7) Finally, the experiments were repeated twice with the same batch of flies.

8) If a fly climbed back down or falls, then that fly was recorded as -1 and the next fly to cross the target line recorded as the same number as the fly that climbed back down or fell. For example, if the 7<sup>th</sup> fly falls below the target line, the next fly to cross the line (the 8<sup>th</sup> fly) is considered the 7<sup>th</sup> fly and not the 8<sup>th</sup>.

9) The performance was analyzed at 12 sec. data point and student t- test was performed for the two groups and ANOVA can be performed for multiple groups.

## Results and Discussion

The *Drosophila* culture used for the study on food additives was obtained from department of genetics, Indian Academy Degree College (Autonomous), Bengaluru. We successfully maintained the *Drosophila* stock for a month on the modified media of Gajju's media. Three different test compounds such as Acrylamide, Monosodium Glutamate and Carmoisine were fed to *Drosophila melanogaster* throughout their life cycle [13-17] (Table 2). The climbing assay was performed by taking 10 flies from each triplicate culture. The outcomes demonstrated that the fruit flies which were grown on media with no test compound (control) were profoundly dynamic and 8.19 numbers of flies on an average crossed the 8 cm line within 12 seconds amid experimentation. While, *Drosophila melanogaster* grew on the media supplemented with 1% burned food demonstrated that 5.56 number of flies that crossed the comparative target, which is not as much as control [17] (Table 3). Another group of flies that consumed media supplemented with monosodium glutamate and Carmoisine, an average of 6.78 and 6.67 numbers of flies crossed the 8 cm line in glass cylinder contrasted with control. Graph 1 represents locomotion activity of *Drosophila melanogaster* in negative geotaxis assay. The student t-test was likewise performed to decide the criticalness of the information acquired from the negative geotaxis measure. The understudy t-test among control and 1% scorched sustenance indicated t-detail estimation of 4.589242, which was more

**Table 2:** Negative geotaxis assay.

	Total no. of flies	No. of flies climbed				Percentage
		1	2	3	4	
Control	10	1	9	6	8	76.67%
		2	7	10	8	83.33%
		3	8	9	9	86.67%
1% burned food	10	1	7	6	4	56.67%
		2	7	7	5	63.33%
		3	6	5	3	46.67%
MSG	10	1	7	7	8	73.33%
		2	6	7	6	63.33%
		3	8	7	5	66.67%
Carmoisine	10	1	8	5	7	66.67%
		2	7	6	8	70.00%
		3	5	8	6	63.33%

**Table 3:** Student t- test of climbing assay.

Sample	Control	1% charred food	Monosodium Glutamate (MSG)	Carmoisine
1	7.6	5.67	7.33	6.67
2	8.33	6.33	6.33	7
3	8.66	4.67	6.67	6.33
Average	8.197	5.557	6.777	6.667
t Stat		4.589242	3.308096	4.156613
t Critical one-tail		2.353363	2.131847	2.353363

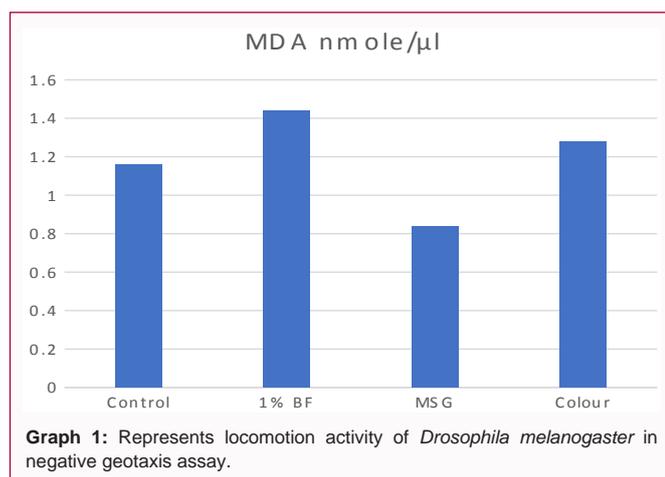
**Table 4:** MDA standard values.

Standard nm/mg	O.D. at 532 nm
0	0
4	0.45
8	0.8
12	1.3
16	1.7
20	2.1

prominent than the basic esteem 2.353363. Thus, it can be said that the information acquired from the climbing assay has its significance [19-22]. Similar significance value 4.156613 obtained for the Carmoisine consumed fruit flies because, the t- stat value is greater than the critical value showed that it leads accumulation of Malondialdehyde. However, the t- stat value for Monosodium glutamate was found to be 3.308096, which is higher than the critical value 2.353363 - the data has significant value. The outcome demonstrated that all the sustenance contaminants (1% burned nourishment, Monosodium glutamate, and Carmoisine) has an impact on locomotor action of the *Drosophila melanogaster* [23]. In any case, in view of the information got, we can say that both acrylamide containing charred food and Carmoisine impacts more to life form contrasted with impact brought about by Monosodium glutamate [24]. The TBARS assay was performed in triplicate sample by taking 10 flies from all the bottles containing test components (acrylamide formed in 1% charred media, monosodium glutamate and carmoisine) under study [25-29] (Table 4, 5). The result obtained from TBARS assay demonstrated that the malondialdehyde formation in control, 1% charred food, monosodium glutamate and carmoisine was found to be 1.16

**Table 5:** Malondialdehyde concentration of control and test samples.

Day and Date	Control	1% BF	MSG	Carmoisine
14 <sup>th</sup> 09-04-2019	0.028	0.03	0.017	0.028
	0.037	0.034	0.03	0.047
	0.037	0.059	0.028	0.036
Average	0.034	0.041	0.025	0.037
Unknown conc.	0.29	0.36	0.21	0.32
MDA n mole/ $\mu$ l	1.16	1.44	0.84	1.28



nm/ $\mu$ l, 1.44 nm/ $\mu$ l, 0.84 nm/ $\mu$ l and 1.28 nm/ $\mu$ l respectively. The concentration of malondialdehyde estimated from the flies that were consumed acrylamide formed in 1% charred food while frying in oil at high temperature was higher than control showing that oxidative stress is enhanced. We found malondialdehyde concentration in fruit flies fed with monosodium glutamate showed less malondialdehyde accumulation thereby having less oxidative stress on drosophila compared to lipid peroxidation product generated due to consumption of charred food. Further study is required to gain more insight and to answer why the Monosodium glutamate has less effect on the lipid peroxidation [30-35]. However, the effect of carmoisine on malondialdehyde formation in *Drosophila melanogaster* found to be slightly higher than the control group. Hence, we can say that the reactive oxygen species may lead to various pathological conditions [36-43]. Based on the data obtained, we can infer that to reduce the oxidative stress, we should avoid the consumption of food containing various food additives to enhance the taste and color to make the food more appealing to eye. We should also avoid eating fried foods to lower the potential risks of getting cancer, neurological diseases and various other pathological conditions [44-48]. More the consumption of unhealthy diet, the more pathological conditions may occur in future generations.

## Conclusion

The findings of this study validated accumulation of malondialdehyde due to naturally formed toxic agents and added food contaminants in diet in a TBARS assay. The naturally formed acrylamide in 1% charred food and Carmoisine added in media showed higher accumulation of malondialdehyde. However, the malondialdehyde concentration found in *Drosophila Melanogaster* consumed media supplemented with Monosodium glutamate was higher than control but less than *Drosophila* fed with 1% burned food and Carmoisine. The similar results obtained from climbing assay

demonstrated that higher accumulation of malondialdehyde resulted in reduced locomotion of *Drosophila melanogaster*. Flies grown on media with no food additives (control) showed higher performance in negative geotaxis assay. Whereas, the flies grew on media with test compounds showed affected locomotor activity.

The study concludes by stating that further studies have to be carried out to identify the impact of long-term exposure of these food contaminants and underlying mechanism at molecular level which may lead to various pathological conditions.

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