REVIEW ARTICLE

Caramel Color Paradox: Is it a Safe Natural Food or a Chemical?

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Abstract

Caramel is one of the most extensively used food colors which is added to multiple food items including colas, soy sauces, seasonings, breads, grilled meat, coffee, roasted foods, pet foods, cereals and beverages etc. Although caramel colors are generally considered as safe, a potentially toxic chemical 4-Methylimidazole (4-MEI) is generated during the production of caramel color which is enlisted as a human carcinogen by the World Health Organization. The exposure to 4-MEI from consumption of foods containing added caramel color has been found to be much higher than that from foods containing 4-MEI from thermal treatment. It is paradoxical that inspite of the concerns regarding the presence of 4-MEI in caramel colors, there are not many studies available about health hazards of its chronic consumption which is veiled in a variety of food items being consumed by humans unapprised of its long-term dangers on health and tumor susceptibility.

Keywords: Caramel; 4-Metlyimidazole; Toxic; Carcinogen.

Introduction

Worldwide coloring of food is done on an exceptionally great scale during processing of food. These additives when added to food or drink impart color either independently or during reactions with other ingredients present in food. These are used in the form of a dye, pigment or any added substance and are found in varied forms including liquid, powder, gel or paste.[1] The coloring of food is used in commercial food production as well as in domestic cooking. These are added to foods with the aim of compensating for the loss of color due to exposure to light, air, moisture, extremes of temperature and storage conditions, to provide color to colorless food, or/and to enhance colors that occur naturally.[2]

Commercially, a large number of food dyes are being used including caramel colors (E150), turmeric (E100), paprika (E160c), cochineal (E120), chlorophyllin (E140), saffron (E160a), annatto (E160b), betanin (E162) and lycopene (E160d).[3] Caramel is one of the most extensively used food colors which is added to impart color to multiple food items including colas, soy sauces, seasonings, breads, grilled meat, coffee, roasted foods, pet foods, cereals and beverages etc.[2-4] Although caramel color is generally recognized as safe food additive, it is a paradox since a harmful chemical 4-methylimidazole (4-MEI) is generated during production of caramel colors which is considered as toxic and carcinogenic for various body organs.[5] 4-MEI is not acknowledged to occur as a natural product and is in fact enlisted as a human carcinogen by the International Agency for Research on Cancer (IARC), World Health Organization (WHO). It is a paradox that although alarm bells had been ringing regarding presence of 4-MEI in caramel colors for more than a decade, still there are not many studies available about the health hazards associated with its chronic consumption. Consequently, unapprised of its long-term dangers, humans continue to consume 4-MEI which is veiled in a variety of food items.

Classification of Caramel Color

As per the Code of Federal Regulations (21 CFR 73.85) caramel color is a dark-brown liquid or solid resulting from carefully controlled heat treatment of food grade carbohydrates.[2,5] Caramel colors are divided into four classes depending on the nature of food grade reactants used in its manufacturing as shown in **Table I**.[5] The plain caramel *i.e*., class I is prepared by heating carbohydrates with or without acids or alkalies; no

Table I Classification of Caramel Colors[5]

Class	*Type of caramel*
I	Plain or spirit caramel
II	Caustic sulfite process caramel
III	Ammonia processs caramel or beer caramel, baker's and confectioner's caramel
IV	Sulfite ammonia process caramel or soft drink caramel, or acid-proof caramel

ammonium or sulfite compounds are used. Class II caramel colors are prepared by heating carbohydrates with or without acids or alkalies in the presence of sulfite compounds; no ammonium compounds are used. Among these, the class III and IV of caramel colors are formed by the controlled heat treatment of carbohydrates in presence of ammonium compounds. Class III caramel colors are also known as *ammonia caramel* and are used in bakery products, beer, soy sauce, gravy and other products. Class IV caramel colors are *sulfite ammonia caramel* and are used in pet foods, soups, colas and some of the soft drinks. The class III and IV caramel colors contain a potentially toxic chemical 4-methylimidazole (4-MEI) which has been considered as toxic on multiple body organs.[5]

What is 4-Methylimidazole?

4-MEI [IUPAC name: 4-methyl-1H-imidazole (4-methylimidazole)] is a nitrogen containing heterocyclic compound with molecular formula $H_3C–C_3H_3N_2$ or $C_4H_6N_2$. It is derived from imidazole through replacement of the hydrogen in position 4 by a methyl group (**Fig. 1**). It is slightly yellowish colored solid at room temperature with a density of 1.02 g/cm^3, melting point 46-48°C, boiling point 263°C, flash point 157°C and is soluble in water and alcohol.[6,7]

GENERATION OF 4-METHYLIMIDAZOLE

4-MEI is known to be formed by browning of foods including grilled meat, soy sauces and coffee by way of the Maillard reaction between a reducing sugar and ammonia containing compounds which is the typical caramel color preparation method for beverages.[8] 4-MEI is generated during commercial production of caramel colors and also as a byproduct of fermentation in food products.[9] It has been established that ammonolysis of methylglyoxal, which is one of the glucose degradation products, leads to formation of formamide, which subsequently reacts with 2-aminopropanal formed from methylglyoxal to produce 4-MEI.[10] Furthermore, thermolysis or fermentation of natural products such as caramel colors and soy sauces may also lead to production of this compound.[2] It is even detected in ammoniated hay forage used for livestock.[11,12]

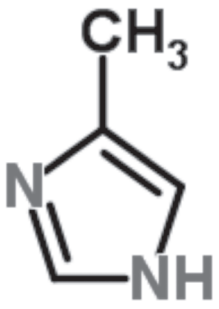

Fig. 1 Structure of 4-Methylimidazole.

COMMERCIAL PRODUCTION OF 4-MEI AND ITS USES

Commercial production of 4-MEI occurs by cyclo-condensation of aldehyde and ammonia with methylglyoxal.[4,5] Exposure to the chemical can occur in workers by inhalation or dermal contact during its production. 4-MEI is used in the industry for manufacturing of a variety of products which include[13]

- Pharmaceuticals, agricultural chemicals, rubber, photothermographic chemicals, dyes and pigments
- Oven cleaners containing imidazole-phenoxy-alkanal
- Epoxy resin hardeners as cross-linking agent which are used in plastination,
- Corrosion inhibitor for cooling water in heat exchangers and
- Raw material for preparation of inks and paper dyes

PHARMACOKINETICS

Animal studies show that following oral administration, 4-MEI is absorbed in rodents. It has a huge volume of distribution and is distributed in the blood and also in the extravascular compartments to liver, intestines and kidneys. A study was undertaken to understand the metabolism and toxicokinetics of 4-MEI in F344 male rats. The rats were administered C-14 radiolabeled 4-MEI orally in the dose of 50 mg/kg body weight. It was found that about 85% of the radioactivity was recovered in urine within 48 hours of administration but the elimination of radioactivity via respiration, fecal or biliary route was negligible. In the same study, rats were administered with oral doses of 5, 50 and 150 mg/kg of 4-MEI and found that the compound was readily absorbed with bioavailability of about 60-70%. They also described a two-compartment process after an intravenous dose of 5mg/kg body weight in which the estimated half-life was 1.8 hours and apparent

volume of distribution was 2.3 litre/kg body weight.[13]

In another study the metabolism and distribution of 4-MEI in goats and heifers has been described. They administered 4-MEI in a dose of 20mg/kg body weight in both goats and heifers. They found that the volume of distribution was same in both the animals. Although goats metabolized 4-MEI to a much higher extent than heifers in which the major part of the compound was excreted unchanged in urine. This report recommended important species-specific variations in metabolism of 4-MEI.[14]

Exposure to 4-MEI in Humans and Animals

Humans possibly might be exposed to 4-MEI, by the consumption of foods containing caramel colors, tobacco smoke, and milk of animals fed with ammoniated forage.[4,16] It has even been detected in the condensate of smoke of cigarettes.[5] Ammonization of hay is undertaken so as to increase the non-protein nitrogen content in feeds of animals and is a regularly used practice in animal farms.[14] Detectable levels of the chemical were also found in the milk of pregnant and post-partum cows exposed to 4-MEI.[17]

Apparently, exposure to 4-MEI is a matter of great concern in human and veterinary toxicology. Various studies done to report the toxicity of 4-MEI revealed differences in the tumor producing activity of the compound. A study done under the aegis of the National Toxicology Program (NTP, 2007), gives clear evidence of carcinogenic activity of 4-MEI in male and female B6C3F1 mice. It reported an increased incidence of alveolar or bronchiolar neoplasm after exposure to 4-MEI.[18] Paradoxically, an anti-carcinogenic effects of 4-MEI in tumors of adrenal medulla and pituitary gland in male rats as well as reproductive organs in female rats has been reported.[9] It was suggested that the decrease in tumor rates was because of reduction in body weight of animals due to 4-MEI exposure. However, a review of the literature on carcinogenic potential of 4-MEI concluded that reduced body weight was only partially responsible for reduction in tumor rates, thereby indicating tumor preventing action of 4-MEI itself.[19]

The health effects of this compound are a matter of immense concern in the recent years, in view of the fact that 4-MEI has been identified as a carcinogen.[14] World Health Organization (WHO) International Agency for Research on Cancer (IARC) has added 4-MEI among the list of human carcinogens occurring in alcoholic beverages based on the fact that it may cause cancer of the lung in mice when administered orally.[20] For this reason, many researchers and regulatory agencies have become focused on its presence in foods and beverages. Reports of the toxicity of 4-MEI in animals put forward that it may cause adverse effects on human consumers as well.[15] Toxic and deleterious effects of 4-MEI due to occupational exposure or consumption of foods containing caramel colors continues to remain a cause of worry. The chemical 4-MEI is formed in Class III and Class IV caramel colors that are made using ammonium compounds. 4-MEI can also form in food through Maillard reaction between reducing sugars and amino acids during cooking, roasting or dry-heating. In a study spanning from 2013-2015, the USFDA analyzed an excess of 700 food and beverage samples for the presence of 4-MEI. The samples incorporated foods containing additional caramel color as well as foods that are not labeled as containing added caramel color, but which may contain 4-MEI consequential to thermal treatment. It was reported that the exposure to 4-MEI from consumption of foods containing added caramel color was higher than that from foods that contain 4-MEI from thermal treatment for all population groups. Amongst the foods which contain added caramel, Cola-type carbonated beverages were the highest contributors whereas coffee was the highest contributor in foods where 4-MEI could form through thermal treatment.[21]

Should 4-MEI be banned?

The United States Center for Science had submitted a petition in the public interest more than a decade ago to bar the use of caramel colorings produced with ammonia or ammonia sulfite process which contain 4-MEI and related chemicals.[5] It was suggested in the petition that the Food and Drug Administration (FDA) should change the name "*caramel coloring" to "chemically modified caramel coloring" or "ammonia-sulfite process caramel coloring".* Moreover, it was even stated that the food products which contain any type of caramel color should not be labeled as natural. Presently not enough documentation is available about the effects of chronic exposure to 4-MEI than exposure to many other chemicals. The U.S. Food & Drug Administration – which regulates food and beverages – is presently in the process of assessing the cancer risk from 4-MEI. Some caramel colorings used in manufacture of some of soft drinks and other beverages may contain significant amounts of 4-MEI.[22] Single serving of carbonated malt cola has in excess of 300micrograms. Pepsi and Diet Pepsi have around 24-31 micrograms, whereas Coca Cola and Diet Coke contain 3.6-4.1 microgram per serving. 4-MEI levels in these carbonated soft drinks are hovering much above Prop65 levels. It has been suggested that there is no safe limit of 4-MEI but since a threshold of Prop65 (29micograms/day) has been set consumption must be not more than 3micrograms/day. In India, there is no such report available on the level of 4-MEI in caramel colors being

used in food and beverage industry. There are no studies / reports available regarding feeding animals on massive doses even for durations ranging to more than 2 years. [23]

An update on caramel color suggested that caramel colors are not genotoxic or carcinogenic, and exposure estimates indicate that intake of caramel colors and constituents do not pose undue safety risks .[24] On the contrary, an in-vitro study demonstrated that 4-MEI produced sister chromatid exchange, chromosome aberrations and micronuclei induction in human peripheral lymphocytes; however, result of an in vivo study showed that there was no increase in the frequencies of micronucleated erythrocytes in the bone marrow of male rats or mice administered with, 4-MEI by intraperitoneal injection, or in peripheral blood samples from male and female mice which were dosed with 4-MEI in feed for upto 14 weeks [25] In NTP bioassays, 4-MEI fed to groups of male and female mice in diet, containing increased incidence of pulmonary alveolar/bronchiolar adenoma in all dosed groups of females, and alveolar/bronchiolar adenoma or carcinoma (combined) in males fed 1250 ppm and in females fed 625 and 1250 ppm. Also, there was equivocal corroboration in females based on modest increases in the incidence of mononuclear cell leukemia. Although there are some animal studies available, there were no epidemiologic studies assessing human cancer risk of 4-MEI.[26] Although alarm bells are ringing vis-à-vis health hazard susceptibility of cumulative effects of 4-MEI on human body, further studies on a larger scale are required to assess the same. There is an urgent need for 4-MEI sparing during manufacture of caramel colors, lest we may have to deal with the health hazards of chronic 4-MEI consumption.

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