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Review Article

FOOD COLORANTS AND THEIR TOXICOLOGY: AN OVERVIEW

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Abstract

There are many kinds of colorants, which have been already banned or are used under strict supervision of food safety authorities. Use of artificial colorants over natural ones has increased in modern world. Besides, it has also concerned on importance of toxicological studies. Acceptable daily intake (ADI) of different colorants will define the optimum level of coloring substances on food items. Molecular size of coloring agents and their absorption ratio have significant role in toxico-kinetics. Bigger molecular size has lower absorption capacity and toxicity while vice versa for the smaller one. Higher absorption of smaller molecules can be lowered by binding with carrier molecules to make big enough to block through mucosal layer. Adverse health effects related to many colorants are itching,urticaria, anaphylaxis, hypersensitivity, intolerance reactions, mutagenicity, carcinogenicity, genotoxicity, cytostaticity and cytotoxicity. Color additives seem to be complex subject having direct relationship with consumer safety. Therefore, toxicology of attractive coloring agents might overlook the advantages of colorants. So, toxicologist and food scientist need to collaborate to define the use of different color additives and their safety in future in more scientific way.

Key words: Food, Colorants, toxicology, toxicokinetics, toxicodynamics

INTRODUCTION

Color, as always, is the first impressive aspect in existing things. Not an exception, appearance with respect to color is a driving factor which aestheticizes foods. Prominently, fresh and attractive look of food is a major concern of consumers and Hutchings[1] has also enlightened the issues. Colors of food are attributed to a special compound termed as colorants, which is either natural or artificial. According to statistical data, synthetic colors as safe for additive purposes if used within a limit of ADI.[2] Colors in food have different values according to traditions and localities. For instance, red or different colored eggs prepared during Easter in Europe. Food colorants are used according to specific food habits and cultures of different countries and regions.[1] Use of turmeric powder, as natural food colorant, can be considered as patterned indigenous to South Asian countries. According to USDA[3]- colorants are referred to dye, pigments or other substances which gives color to food, drugs, cosmetic or human body and all color additives need premarket approval by food and drugs administration (FDA) in USA,[3] European food safety authority (EFSA) in Europe,[4] Norwegian committee for food safety (VKM) in Norway,[5] Department of food technology and quality control (DFTQC) in Nepal[6] and other national food safety bodies. Different countries have different food safety regulatory bodies for inspection of color additives whether they are safe or not for human consumption.

FDA classified the color additives as “certifiable” to coal-tar dyes derived from petroleum and “exempt from certification” to color additives from mineral, plants and animal sources.[3]In a way, these can be termed as natural or synthetic color additives. Natural color additive are derived from plant and animal sources; for example saffron, turmeric, grape skin extract, etc. and synthetic color additives can be derived from chemical synthesis, for example tartrazine (FD& C Yellow No. 5),

erythrosine (FD&C Yellow No. 3) etc..[7] Synthetic colorants can be categorized as water soluble and fat soluble colorants.[8]

Popular food and feed colorants like astaxanthine, canthaxanthin, annatto extract, caramel, β-carotene, grape color extracts, paprika and paprika oleoresin, riboflavin, saffron, turmeric and turmeric oleoresin, fruit juices and vegetable juice are some food colorants exempted from certification by FDA.[3] Many synthetic dyes has been a part of food practice in modern periods. Azo dyes with azo group –N=N-, are most versatile dyes, comprises major portion of total dyes used in foods.[9]

There have been many discussions widely on pros and cons of synthetic colorants, though synthetic colorants have benefits in terms of their variation in color profile. Not all the synthetic colorants can be regarded as toxic in terms of health as some might have positive aspects as well. Mostly, the toxicological aspect of synthetic colorants seems to be major concern. Some of the studies mentioned the functional relations of synthetic food colors with behavioral syndromes in children.[10-15] Some synthetic dyes have also been doubted to exhibit genotoxicity.[16] A study conducted in school children from the Nederland also tried to clarify the colorings below ADI might not be toxic.[17] On the other hands, some colorants are under strict control due to their toxicological effects. The colorants like Sudan Red 1 was prohibited for food use in Europe.[18] Not only specific colorants but also the impurities needs to be taken into consideration for food colorants. EU directives have defined the specified level which should not be exceeded (Table 1).[18] The issues of using prohibited food colorings and malpractice on food trades[19-21] are extremely serious in many regions and countries, especially in developing and politically unstable countries due to political, cultural and poor law enforcement issues.[20-23] For these reasons, toxicological aspects of colorants need to be addressed and treated in a strict legal manner by national or international food safety authorities. Besides these scary sides, it might not be a wise to exclude the positive and important aspects of coloring agents as well. Considering all these points, this review aims to give toxicological overview of food colorants.

Table 1.EU directives for colorants to satisfy the following criteria. Coloring matter should not contain following inorganic and organic impurities:

Impurities	Levels (should not exceeded)	Impurities	Levels (should not exceeded)
Inorganic	**mg.kg-1**	Ti	ND
As	5	U	ND
Pb	20	Chromates	ND
Sb	100	Soluble combinations of Ba	ND
Cu	100	**Organic**	
Zn	100	2-napthyl amine	ND
Cr	100	benzidine	ND
BaSO4	100	amino-4-diphenyl or their derivative	ND
Taken together(Sb,Cu,Zn,Cr,BaSO4)	200	polycyclic aromatic hydrocarbon	ND
Cd	ND	Synthetic organic coloring matters[A]	0.70%
Hg	ND	Synthetic organic coloring matters[B]	0.75%
Se	ND	Synthetic organic coloring matters[C]	4%
Te	ND	sulphonated organic coloring matters[D]	0.72%

ND: Non-Detectable. A: Synthetic organic colorants not more than 0.70% of free aromatic amine, B: not more than 0.75% of synthetic intermediate products other than aromatic amine, C: not more than 4% of accessory coloring matters, and D: not more than 0.72% of substances extractable by diethyl ether.[18]

ESSENCE AND TRENDS OF COLORANTS

Colorants can make food elegant and fancy in appearance. Use of colorants makes them popular day by day. In 1996, one of the study projected annual growth of colorant industries up to 5-10%.[24] Synthetic dyes are commonly used nowadays but growing trend towards natural colorants to make healthier life style seems to be elaborated in coming years.[25-26] Productions of bacterial[27-28] and marine fungal[25] pigments are trending in recent years due to biodegradability and compatibility with milieu than synthetic colorants. The pigments produced from non-mycotoxigenic fungi are termed as GRAS (Generally Recognized as Safe).[25]This will widen the concept of pigment and color production from alternative resources for example from marine resource, which might have to tremendous possibilities for mass production. This might consequently results to disparage market of synthetic colorants in future.

Many natural foods, vegetables, fruits and their products lose the natural color during processing and storage. In these cases, colorants can be a part of food processing routines to improve product quality along with other food preservation and processing measures. Some studies suggested on antioxidant potentiality of food colorants[1,26,29] and extension of shelf life of food. Additionally, some natural pigments are important part of body metabolism and essential to run normal and healthy physiological function in animals[1]. For example, carotenoid is well known as pro-vitamin A and plays important role in immune systems. Likewise, colorants astaxanthine and canthaxanthin are the important ingredients in fish feed to impart red color in fillet giving better impressions.[30] Red color of Atlantic salmon fillet which is due to astaxanthine and is a very important quality attribute.[31] Beside this, natural colorants flavonoids[32-33] and carotenoids[33-34] are regarded as a strong compounds that quenches singlet oxygen and acts as strong antioxidant. It is well understood that reactive singlet oxygen can cause harm to the molecules and then cells. Antioxidant plays against this reactive oxygen to stop series of chain reaction and consequently prevent the cell from damage.[33-35]

These natural pigments flavonoids and carotenoids have been mentioned as anti-carcinogenic compounds.[33,36] Flavonoids have demonstrated for the reversal role against multi-drug resistance.[36] Carotenoids (lyco-red) extracted from tomato peels supplemented in ice cream have been reported as an antioxidant and radical scavenging activity.[37] Other colorant betanin, a natural red colorant, has antioxidant defense systems and gene-regulatory activity along with the prevention of DNA damages.[38] Focusing health concerns and pros of natural colorants, peoples have been preferring natural food colorants over synthetic in past decades[38] and these trends seems to increase further more in coming years. All these positive outcomes of natural food colorants are not full reasons behind increasing demand for natural colorants. Moreover, headlines over big media like New York Times[39], abc News[40] and Fox News[41] on food colorants have given high attentions in past years. New York Times also has also tried to make people aware to the safety sides of natural food colorants against artificial ones.[39] At the same time, consumer are also showing their awareness to the health effect and legislation also comes into play to avoid falsified impression of poor quality food products. Sound knowledge for the use of correct and proper level of colorants in food is necessary for the manufacturers and consumers. For example, it is not normal to have black or green colored straw berry ice cream. It is normal to have pinkish strawberry color in strawberry flavored ice cream. Similarly, vanilla ice cream is preferred when it is white not when it is coffee colored. Therefore, food colors should be used according to trends, cultures, habits, routines, rituals and daily life. Food colorants are now parts of everyday life and cannot be excluded. Illegal food trade practices resulting in high amount of colorants' residues[20] in junk foods are good examples. Soda drinks are also examples of having potential carcinogenic compounds 4-methylimidazole (4-MEI).[40] Therefore; it is very easy to imagine the awful situation on future if illegal use of synthetic colorants continues in food trade.

TOXICOKINETICS OF COLORANTS

Eating natural colorants through food matrix might not be harmful but the situation is not same for synthetic colorants. Thus, it is extremely important to know the level of synthetic colorants in foods. The total daily dietary intake level which causes no harm to our body is termed as acceptable daily intake (ADI).[42]It is not always easy to predict the recommended level of ADI or half life of toxic food colorants for human. The study conducted in different test animals cannot be always extrapolate for humanbeings.[43] Natural colorants like chlorophyll have no ADI but when magnesium in chlorophyll is replaced by copper then ADI is 0-15 $mg.kg^{-1}$ of body weight.[44] That is why there are limitations on the toxicokinetic studies of colorants due to limited number of colorants approved for human ingestions.[45]

Toxicity in the human body is driven by absorption (Figure 1) of colorants through gastrointestinal tract. Absorption of colorants is affected by their molecular size, for instance, some colorants are too big to pass through mucosal wall and hence they have very less chances of toxicity.[46] Lower molecular size molecules easily pass through mucosal wall and rate of absorption is high as compared to high molecular size ones. Higher absorptions is directly associated with high toxicity. Therefore, toxicity of colorants can be minimized by binding smaller color bodies (chromophores) with carrier molecules. This makes molecules big enough to get absorbed in to the body without affecting coloring properties of chromophores.[46] Most of artificial colorants FD & C Blue 1, Blue 2, Green 3 and Orange B are excreted in feces with very less absorption when fed orally in rats.[47] On the other hand, some food dyes like Citrus Red 2, FD & C Red 40, FD&C Yello 5 (Tartrazine), Yellow 6 (Sunset Yellow) are broken down by gastrointestinal bacteria and produces byproducts. These byproducts might get absorbed and hence to cause negative health effects.[47]The toxicological process of colorants after exposure and digestive byproducts has been presented in figure 1.[48] Exposure here is defined by the level of colorant in food or amount we ingested. Generally, synthetic food dyes like azo are more stable in wide pH range of foods, exposure to light and oxygen and adverse situation than natural food dyes.[49-52] Stability in adverse conditions make them

available in food at a considerable higher level than natural colorants in case of equal inclusion before food processing. Therefore, serious consideration is taken on the level of synthetic colorants added in foods over natural colorants.

Natural colorant like curcumin is less absorbed with possible complex absorption kinetics[53-54] but rapidly metabolized to water soluble compounds and excreted.[54] Acute toxicity are not a major problem with natural and food colorants which are approved by food safety bodies. Generally, life-long adverse effects of colorants are expressed based on ADI in mg/Kg body weight. ADI is more common term than LD50 during expressing the toxicological information of food colorants because, LD50 indicates the acute oral toxicity.[55-56] In case of commonly used artificial colorants, it is not common to have serious acute toxicity and poisoning unless the case of intolerance. For lethal effects of food dyes like azo, many grams need to be consumed to get acute toxicity.[56] The reason behind this might be that azo dyes are water soluble and metabolized in the liver.[57] This makes them easily excreted from the body.[16,58-60] Additionally, azoreductase enzyme found in different microorganisms and mammals, breaks the azo linkage.[16,58-60] Lastly these azo dyes are metabolized in liver and kidney, then excreted through urine.[57]

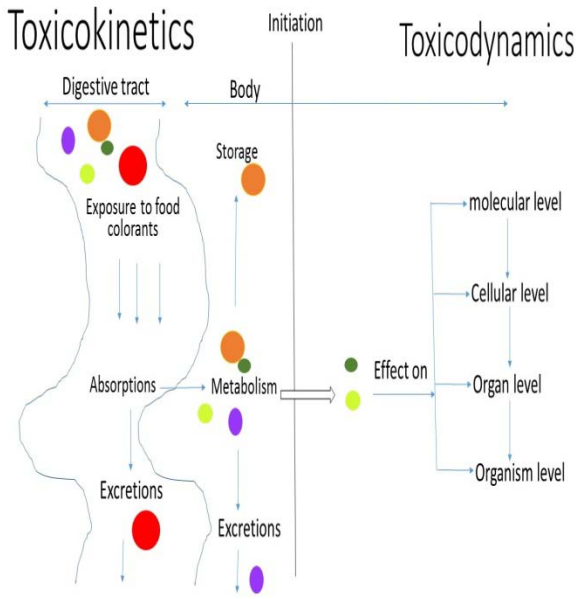

Figure 1. Picture showing general toxicological process of food colorants (Modified figure, original figure from JHSPH).[48]

Colorants which are less water soluble are subjected to store in our body for longer period. This results in a long-run negative health effects. One of the previous pharmacokinetic study suggested on bioavailability and level of one colorants in serum was affected by level of other colorants in diets.[45] Study suggested specifically on natural colorants beta-carotene and canthxanthine (natural pigments), as canthaxanthin level in serum is discouraged by beta-carotene.[45] Based on this, it can be proposed a hypothesis as combining different natural colorants with various synthetic colorants might lower the toxic effects of synthetic colorants. There might be possibilities for combining various natural food colorants with different synthetic colorants and study their toxicokinetics role on human subjects. At this stage, this hypothesis needs thorough and extensive research to draw conclusions.

TOXICODYNAMICS OF COLORANTS

Toxicological response of colorants at cellular or organism level might be different for different colorants. Responses like allergic reaction are not common for synthetic colorants but natural colorants may contain proteins, as an example annatto and carmine, which can cause allergy.[7] Other side effects resulted from colorants are itching,[5]urticaria, anaphylaxis,[61-62] hypersensitivity[13] and intolerance reactions[7]. Furthermore, some colorants have been strongly associated with mutagenicity, carcinogenicity, genotoxicity, cytostaticity and cytotoxicity.[63] Safety assessment for colorants on short and long term toxicity studies needs to first addressed for humans.[5]

CONCLUSION

Color additives have been used as traditions and are important in many cultures, societies and countries since ancient time. Food safety authorities from different countries have listed different types of colorants which can be used but they can be over walked by different legislations and traditions. There might be a chance to approve new types of color in future by authorities. However, study on toxicological effect and production cost must be considered. Synthetic pigments might be cost effective over natural colors but health effects and consumer demands of natural colorants are important factors. Optimization of the production cost and minimizations of health risk of consumers are challenging issues. Natural food colorants can also be produced in cost effective way.

On the other hand, using colorants to make old and damaged food products fresh are forbidden.[1]Despite making attractive looks, many synthetic colorants have been associated with allergy, hypersensitivity, mutagenicity, carcinogenicity and other toxicological effects. In contrast, natural food colorants are rarely associated with toxicological effects. Therefore, uses of color additive are still question mark in many food products and are directly related to consumers' health and safety. Some colors have short term and some have long term toxicological effects. Toxicological science of food colorants cannot overlook the positive function and advantages of colorants. So, toxicologist and food scientist needs to collaborate to define on the use of different color additives and their safety.

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