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The effect of artificial colors in food and replacing Them with natural vegetable colors

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Abstract

Artificial colors have a harmful effect on human health, as they cause some diseases, including but not limited to liver and kidney attacks. Therefore, they can be replaced with natural colors from some plants. Some pigments are extracted from these plants, for example chlorophyll pigments in spinach and beta carotene pigments in carrots, using one of the appropriate chemical separation methods using the appropriate solvent. For example, when extracting chlorophyll pigments from the spinach plant, the separation method (simple distillation) is used, using the solvent (isopropyl alcohol), and when separating carotene pigments, the (separation funnel) method is used, using solvents (Ethanol, petroleum ether)
As well as the separation of betalain pigments from beets using the method (separation aqueous) using a polar solvent (water)

Key Words:

Artificial colors, Polar solvent, non-polar solvent, Vegetable colors, Pigments, Separation chemistry.

1. Introduction:

Food, as a basic need for all human and animals, Must become healthy and safe. It is only nutritive when it is pure, fresh and Free from hazardous matters (Jonnaladda et al., 204). Food Coloring by artificial colours is controversial and premier public hazard affecting The quality of life.many industries use banned and synthetic colors to add In varieties of food products (Jeannine, 2003). The Utilization of colors in food confronted with debate when add Up into food, specifically in children nutrition, causing allergic Reactions, respiratory, liver and kidney disorders (Hashem et al., 2010). In addition, synthetic dye contained various heavy Metals such as Pb, Hg, As, Cu, Ni, Mg, Co, etc (protima, 2008). Many food colors are made from petroleum and coal tar Called azo dyes. One of significant azo dye is tartrazine recognized As E102 or FD & C yellow 5, widely used synthetic food color. Tartrazine is a nitrous derivative (azo class) metabolised to Aromatic amines principally by gut microorganisms, azoreductases And liver enzymes playing a minor role in dye reduction(Chung et al., 1992). Tartrazine causes changes in hepatic and renal Parameters in addition to it poses more risk due to the induction of Oxidative pressure through the production of free radical and Allergic reactions in sensitive individuals at high doses. In fact, The mutagenic effect of DNA by tartrazine has been established Through animal studies(Sasaki et al., 2002) . Of great concern, food Colours were used above the acceptable daily intake (ADI) during Various occasions in many developing countries which would Cause serious health complications in human (Rao et al., 2006;Van Beaver et al., 1989).

-The synthetic food colorants showed adverse Effect on human health. It has been reported That consumption of synthetic foods color Additives could sometimes lead toxic effects On liver, kidney and testes.(Naveen, 2006;Abdellah et al., 2015)The Administration of synthetic food colorants Decreased the percentage of high density Lipoprotein cholesterol (HDL-C), Glutathione secretion (GSH), superoxide Dismutase (SoD), and plasma immunesystem and significantly increased plasma Lipid lipoprotein, total cholesterol (LDL-C), Lipid peroxidase, blood glucose, plasma urea And creatinine and increased activities of Alkaline phosphatase, acid phosphatase, and Lactate dehydrogenase.(Reyes et al.,1996)Previous studies Investigated the metabolic and toxicological Disorders induced by the administration of Specific food colorant additives to rats and Other mammals. (Zraly et al.,2006;Al-Shinnawy,2009)The nutritional Hazards of synthetic food colors have been Detected in the liver and kidney.

-Sahar and Manal (Sahar and Manal.,2012)conducted a research to investigate The effect of using colour foods [(Colour fruit juice for 6 – 12 hr) There was also a significant increase in serum creatinine and Albumen. Both low and high colors foods consumed exhibited Significant decrease in liver GSH. The study also revealed that High concentration of colour foods lead to increased number of WBC as the result to the response of the immune system to the Inflammation. Their findings showed that color fruit juice Containing sunset yellow, tartazine and carmosine lead to Significant increase in ALT of serum rats. Therefore, they Concluded that the synthetic colours used in their research have Adverse effects on some of the serum biochemical, liver and Kidney. These results were well supported by the data reported by Mekkawy et al.(Mekkawy et al.,1998), and Amin et al.(Amin et al.,2010), who indicated that rats Which consumed high dose synthetic colours (Tartarzine, Carmoisin, sunset yellow and fast green) showed a significant Increase in serum ALT and AST when compared to control rats. A significant increase in serum ALT and AST may attribute Those changes in liver function to be hepatocellular impairment Level of intracellular enzymes into the blood(Abdel-Rahim etal.,1987). This was more Evident in the histopathological studies. At low dose synthetic Color, the liver revealed a disruption of hepatic cells near the Central vein and hepatocellular damage. These results are in Agreement with the Sharma et al(Sharma et al., 2008).who reported that synthetic Colours have adverse effect on vital organs. The release of a Normally high level of specific tissue enzymes into blood stream Is dependent on both the degree and type of damage exerted By the toxic compound administration(Malki et al.,1980;Westlake et al.,1981).

-Popular concern over adverse neurobehavioral effects of food additives has recently implicated artificial colors as etiologic factors in childhood hyperactivity (hyperkinesis, attention deficit disorder). However, clinical studies using total dietary elimination such as the so-called "Feingold diet" have not shown conclusive or dramatic effects when performed under controlled conditions. Challenge studies, in which identified "re-sponders" to dietary elimination were acutely presented using a cookie or drink containing an estimated 10 common artificial colors, have suggested that some responders may exist, but that any such children are not numerous. In contrast, in vitro and in vivo exposure of rodents to erythro-sin B produces significant neurochemical and behavioral effects. Experimental results identify neural Na, K-ATPase as the possible substrate of erythrosin's action. Further, these findings may suggest a genetic basis for the effects of erythrosin in vivo, with implications for the design of future clinical studies(Slibergeld et al .,1982).

2. The Theoretical Framework

Color,

which contributes so much to the Beauty of Nature, is essential to the attractiveness and acceptability of most products used by modern As long ago as the 25th .(Anliker, R. (1980)) century BC man colored his surroundings and clothes using a limited range of natural colorants of both animal and vegetable origin

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Absorption of light quanta is the precondition for any photochemical "The chromophore. Starting 'with white light, one can consider a 'black body the ideal absorber in a physical sense because all .wavelengths of the light are equally absorbed ,But This situation is not realized in plants however. The actual absorbers preferentially absorb certain wavelengths of the visible light but .are more or less transparent for other wavelengths After such an absorption, the remaining light looks coloured to the human eye. The absorbers are therefore called chromophores

Molecules which have the properties of such absorbers are called pigments. These terms are used here in such a sense that the entire molecule is called pigment whereas the particular part of the molecule which absorbs light is called its (Rüdiger, W. (1986)) .chromophore

Substituents that increase the intensity of the absorption, and possibly thewavelength, are called auxochromes

,auxochromes include (methyl, hydroxyl, alkoxy halogen ,and aminogroups(
(Lashkaripour.A December 2021)
The types of chlorophyll
Chlorophyll is a chlorin pigment, related to the porphyrin containing iron compound known as heme. At the centre of the ring is a magnesium ion,This green pigment is what gives green plants their colour. It is involved in photosynthesis by absorbing energy from visible light. We have chlorophyll (a,b,c1,c2,d,)[26]

But we concerned about chlorophyll (a,b)

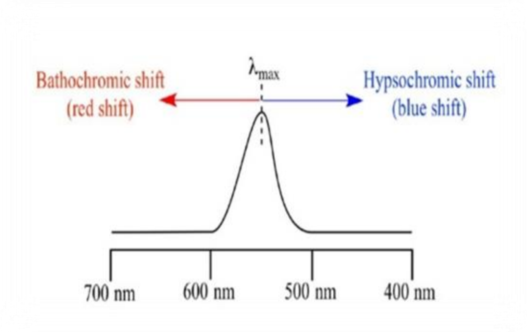
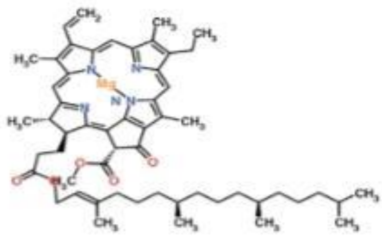


Figure 1:Scheme of oxuchrome

Chlorophyll a,b

Chlorophyll a and b contain methyl group (CH3) and formyl group (CHO) respectively on carbon C-3 of tetrapyrrole ring of the chlorophyll structure.(Radriguez-Amaya, 2018)Chlorophyll a appears blue-green and chlorophyll b yellow-green (Radriguez-Amaya, 2018),Chlorophyll b has been reported to be more heat stable than chlorophyll a(Thorngate,2002)The stability was attributed to electron-withdrawing effect of its C-3 formyl group (Radriguez-Amaya, 2018)Chlorophyll a
This type of chlorophyll is found in almost all photo synthetic organisms, i.e. plants, algae, cyanobacteria, and aquatic species. (Jordan et al.,2001;Nakamura et al.,2003).

Previously it had been called chlorophyllα.It is found in all light harvesting complexes and both reaction centers in organisms, photosystem I and photosystem II It absorbs red light from the solar spectrum; the absorption peak is captured at 420nm and 660nm in organic solvents and at 453nm and 670–480nm in photosynthetic cells (in vivo) ,It works as primary donor in the reaction center of Photosystem I and photosystem II(Strain et al .,1963).The molecular formula of chlorophyll a is C55H72MgN4O5; It contains a chlorine ring in which a magnesium ion is surrounded centrally by four nitrogen atoms(Taiz et al .,2006)
The side chains of chlorophyll determine the properties of other types of chlorophyll and cause a change in their ability to be absorbed Solar light.(Raven et al.,2005)
And Chlorophyll A has a hydrophobic tail(Raven et al.,2005).

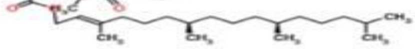


Chlorophyll *a*

Figure (2): structure of chlorophyll a

Chlorophyll b

Previously, was called chlorophyll β. Chlorophyll b has been confirmed to be found in green algae and also in higher plants. This pigment has yellow color



Chlorophyll *b*

in its natural state but absorbs blue light from the solar spectrum. For chlorophyll b the characteristic absorption peak was observed at 453nm and **625nm in vitro and at 480nm and 650nm in vivo(Strain et al .,1963)**

	Chlorophyll a	Chlorophyll b	Chlorophyll c1	Chlorophyll c2	Chlorophyll d
Molecular formula	C55H72O5 N4 Mg	C55H70O6N4Mg	C35H30O5N4Mg	C35H28O5N4Mg	C54H70O6N4Mg
C3 group	-CH=CH3	-CH=CH2	-CH=CH2	-CH=CH2	-CHO
C7 group	-CH3	-CHO	-CH3	-CH3	-CH3
C8 group	-CH2CH3	-CH2CH3	-CH2CH3	-CH=CH2	-CH2CH3
C17 group	CH2CH2COO phyty	CH2CH2COO phyty	-CH=CHCOOH	-CH=CHCOOH	CH2CH2COO phytyl
C17-C18 group bond	Single	Single	Double	Double	Single
	Occurrence universal	Mostly plants	Various algae	Various algae	Cyanobacteria

The chemical formula of chlorophyll b is C55H70MgN4O6 There is only a minor difference between the structures of chlorophyll a and chlorophyll b: in the latter, a group is found in place of CH3 at the C-7 position (Sunil pareek et al.,2017)

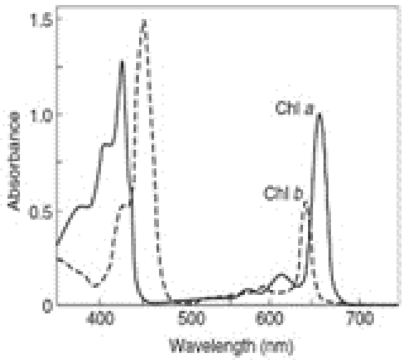
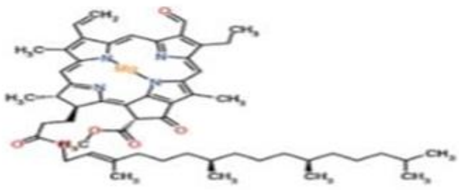


Figure (4):the spectrum of chlorophyll a, b in the uv



Chlorophyll *b*

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Figure (3): structure of chlorophyll b

Carotenoids

Carotenoids are organic pigments found in chloroplast of plants and other organisms(Britton,1995)

Carotenoids can be classified into two groups according to their function: xanthophylls, such as lutein and zeaxanthin and carotenes, such as α-carotene, β-carotene, and lycopene (Saini et al.,2015)Most carotenoids are insoluble in water and soluble in organic solvents such as acetone(Rivera Vélez and Canela i Garayoa,2012)

B-carotene

Beta carotene is one of the most important families of carotenoids,is a strongly red orange-colored pigment , It appears in fruits and vegetables such as carrots and in the human body in the form of vitamin A(Jahnson,2002)The chemical formula of beta carotene is C40 H56 and molecular weight 536.88.(Dutta et al.,2005)

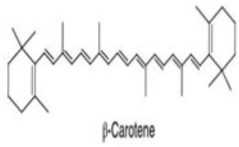


Figure (5): structure of beta carotene

Betanin or phytolaccamin (C24H27N2O13), the water-soluble red pigment happenes in the roots of beetroot, Beta vulgaris L. The color of betanin rely on pH between 4 and 5. It is bright-bluish red, betanin degrades by hydrolysis.(prasad et al ..2023)

Betanin is used to tint food and pharmaceutical products.It is successfully used to color meet substitutes, summer sausage, gelatin, desserts, and dairy products. It can be used to color natural compounds like tomato sauce, puree, and catch-up.(prasad et al .2023)

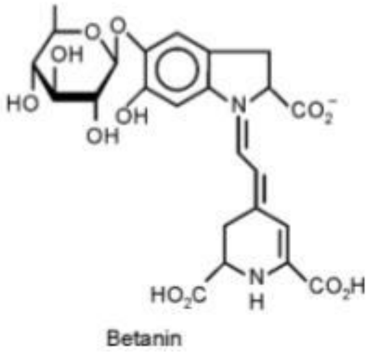


Figure (6): structure of betanine

Betanins or betalains are natural dyes separated from different fruits and vegetables. They are largely used as food colorants in food products like yogurts, ice cream and other products (church.N.1994)

Recent studies have width that betanines have antioxidant, antimicrobial and antiviral activity (Sturzoiu.A.u2011). Beetroot (Beta vulgaris) is the central source of natural red dye, known as "beetroot red". Betanine is the main ingredient of the red colorant extracted from Beta vulgaris Betalain pigments removed from red beet (Beta vulgaris) roots extend a normal alternative to synthetic red dyes. Betalains have been successfully used in commercial food colouring operations for a number of years(church.N.1994) . and continue to be an important source of red colour in the food industry.

Betalains have some features which are beneficial to our health due to which they are used to enrich food products too (Azeredo.H.M.C.2009). They are also used to promote the color and flavor of tomato paste, desserts, jams, jellies, ice cream, sauces, sweets and breakfast cereals (Agrawal.A.2013)

Classification of solvents

Solvent definition :

A solvent can be defined as “a liquid that has the ability to dissolve, suspend or extract other materials, without chemical change to the material or solvent. [Dick . FD , (2006)]

Classification of solvent: [Mondal . S , (2020)]

Based on their chemical structure✓

1- Inorganic solvent:

The most popular Inorganic (not containing carbon) solvents are water (H_2O) and aqueous solutions containing special additives (surfactants, detergents, PH buffers, inhibitors)

2-Other inorganic solvents are liquid anhydride ammonia, concentrated sulfuric acid.

3-Organic solvents:[Mondal . S (2020)]

Oxygenated Solvents•

Oxygenated solvent is an organic solvent, molecules of which contain oxygen.

Examples of oxygenated solvents:

(Alcohols, glycol ethers, methyl acetate, ethyl acetate, ketones, esters, and glycol ether/esters).

•Hydrocarbon solvents:

Molecules of hydrocarbon solvents consist only of Hydrogen and carbon atoms.

Aliphatic solvents

Molecules of aliphatic solvents have straight-chain structure . (Hexane, gasoline, kerosene are aliphatic solvents …)

White spirits (mineral turpentine spirits) White spirit is a mixture of aromatic and paraffinic hydrocarbons.

-Pure aromatic solvents

Molecules of pure aromatic solvents have benzene ring structure.

Examples of pure aromatic solvents are benzene, toluene and xylene.

Halogenated solvents:

Halogenated solvent Is an organic solvent, molecules of which contain halogenic atoms:

Chlorine (Cl), fluorine (F) , bromine (Br) or iodine (I)

Accordingly, to the type of halogen halogenated solvents are classified into the following categories:

Chlorinated solvents:-

The common chlorinated solvents are trichlorethylene ($ClCH-CCl_2$), perchlorethylene)tetrachlorethylene, Cl_2C-CCl_2)

Methylene chloride (CH_2Cl_2), carbon tetrachloride (CCl_4)), chloroform ($CHCl_3$), 1,1,1-trichlorethane (methyl chloroform, CH_3-CCl) .

Fluorocarbon solvents:

Examples of fluorocarbon solvents:

Dichlorofluoromethane (Freon 21, $CHCl_2F$), Trichlorofluoromethane (Freon 11, CCl_3F) ,tetrafluoromethane (Freon 14, CF_4), difluorodichloromethane (freon 12, $CHCl_2F_2$), hydrochlorofluorocarbon (freon 22,HCFC).

Brominated solvents:

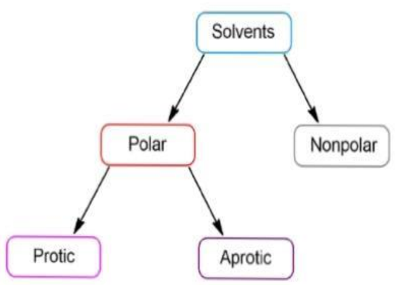
Examples of brominated solvents:

Ethylene dibromide

-2 ,1)dibromoethane, $BrCH_2-CH_2Br$,(methylenechlorobromide (bromochloromethane, CH_2BrCl), methyl bromine (bromomethane, CH_2Br).

Iodinated solvents:
Examples of Iodinated solvents:
n-butyl iodide (1-iodobutane, $CH_3CH_2CH_2CH_2I$),
methyl iodide (iodomethane, CH_3I), ethyliodide (iodoethane, C_2H_5I),
n-propyl iodide (1-iodopropane, $CH_3CH_2CH_2I$).
Solvents can be classified Into two categories according to polarity : [Rao . M , (2024)]

Polar and non-polar



Polar solvents contain bonds between atoms with very different electronegativities, such as oxygen and hydrogen, And have large dipole moments. [Rao . M , (2024)]

•Non-polar solvents contain bonds between atoms with similar electronegativities, Such as carbon and hydrogen. [Rao . M , (2024)]

These polar solvents can form hydrogen bonds with water to dissolve in water Whereas non-polar solvents are not capable of strong hydrogen bonds.

Polar solvents can be classified into Protic solvents and aprotic solvent:
Polar protic solvent: - [Rao . M , (2024)]

Polar protic solvents are capable of hydrogen bonding because they contain at least one hydrogen atom connected Directly to an electronegative atom (such as O-H or N-H bonds).
They solvate cations and anions very well.
Polar Protic solvents are water, ethanol, methanol, ammonia, acetic acid, and other solvent.

•Polar protic solvents:- [Rao . M , (2024)]
Tend to have high dielectric constants and high dipole moments.
•Furthermore, since they possess O-H bond or N-H bond, they can also participate In hydrogen bonding.
These solvents Can also serve as acids (sources of protons) and weak nucleophiles (forming bonds with strong electrophiles).

Polar Aprotic solvent:- [Rao . M , (2024)]

Polar aprotic solvents contain no hydrogen atoms connected directly to an electronegative atom and they are not Capable of hydrogen bonding. These are acetone, dimethyl sulfoxide,
(N,N-dimethylformamide), acetonitrile, HMF (hydroxymethylfurfural), crown ethers etc.

•An aprotic solvent is a solvent that has no O-H bond or N-H bond.
•The "a" means "without", and "protic" refers to protons or hydrogen atoms.

•The specific meaning of aprotic Is that the molecule has no H atom on O atom or N atom.

This means that the molecules cannot form H-bonds with themselves, but they may accept H-bonds from other Molecules.

•For example, acetone does not have an O-H group, but it has a C=O group that can participate in H-bonding.

Thus, Acetone Is an aprotic solvent.

#These solvents are used to separate dyes from natural plants, as in this reference:

•Alot of Bioactive components are extracted using aqueous Methanol, ethanol, or water. [[Hegdon.S , Haghayegh.G.H , (2014)].

•Aqueous extraction method [Kasiri.M.B and Safapour.S ,(2013)]
Which uses water for extraction with or without The addition of salt/acid/alkali/alcohol to the Extraction bath.

•Solvent extraction method[
Such as acetone, Petroleum ether, chloroform, ethanol, methanol, Or a mixture of solvents such as a mixture of Ethanol and methanol, a mixture of water and Alcohol, and so on, depending on their nature [Kasiri.M.B , Safapour.S (2013)
Muthu.S.S ,(2017)] ,

Both water-soluble and water-insoluble Compounds can be extracted from plant resources Using the water/alcohol extraction technique.
Solvent Extraction requires appropriate extraction Equipment, such as the Soxhlet extractor, and Solvents such as alcohol, hexane, or benzene.[Kasiri.M.B , Safapour.S (2013)]

An alcohol solvent, acid, or alkali is used to Improve the collection of glycosides and colour Bodies. Purification of extracted colours, solvent Removal by distillation, and reuse are simplified.[Muthu.S.S (2017)]
In general, ethanol is favoured because, the Dye production is good, the amount of water Required is minor and the extraction is carried out At a lower temperature.[Werede.E,(2019) ,Samantha.A.K , at al ,(2020)]

•Acid and alkali extraction[Samantha.A.K at al,(2020)]

The majority of natural colours are glycosides, Which may be extracted in either acidic or alkaline Conditions. The extraction of tesu natural dye from Tesu flowers uses an acidic hydrolysis process. Alkaline solutions are appropriate for dyes with Phenolic groups in their structure.

In the practical experiments that were carried out in this research, some suitable solvents were used to separate the dye from carrots and spinach:

We have used isopropyl alcohol to separate the green dye from spinach

But have used ethyl alcohol and petroleum ether to separate the orange dye from carrot
..

Extraction methods of natural dyes

Extraction of colour from natural resources is a complicated process. They need specific technique to extract color from their original sources. The different techniques for extraction of natural colourants are as follows:

Acidic or alkaline extraction:

The dye is extracted under an acidic or alkaline medium. This can also be used as diluted acid or alkali which helps in hydrolysis of glycosides resulting in better extraction. The acidic method is used to extract the natural tesu dye from the tesu flower. Alkaline medium is used to extract colors that contain phenolic groups. (Chungkrang et al., 2021)

By fermentation:

In this extraction method, micro-organisms present raw materials are used to carry out the fermentation and thus help the extracting method

The disadvantages of this method Time consuming, bad smell due to microbial action (Chungkrang et al., 2021)

Enzymatic Extraction:

Commercially available enzymes including cellulase,amylase have been used to loosen the binding material present in plants (Chungkrang et al., 2021)

Supercritical CO2 Extraction

Supercritical fluid is the most complex process but has the advantages of both liquids and gasses, high density and viscosity, lower surface tension and higher solubility, which enhance rapidly with the increase in pressure. It can penetrate the matrix of extraction materials and be a very effective extraction mechanism (Prabhu and Bhute,2012). Extraction separation and solvent removal are combined into one unit that identifies the process flow and improves the production efficiency. In addition, it also has a few advantages, such as fast extraction speed, good selectivity, extraction and segregation can be carried out at room temperature or a low temperature (Borges et al .,2012). There is no residual solvent pollution (Luinstra GA , 2008)

Ultrasonic Extraction

Ultrasound is supposed to be one of the most efficient and energy conservative sources of power and assists the extraction as well as much faster (Sivakumar et al.,2009)

Ultrasonic extraction is one of the modern extraction. There are significant differences in the ability to absorb microwave energy in different parts of biological cells (Rahman et al.,2013) .Therefore, the cells undergo deformation under microwave irradiation. Microwave-assisted extraction (MAE) has the advantages of having good quality, high yield, high selectivity, low time and low energy consumption. (Gomes et al., 2020; Xiangyuan et al.,2011)

Aqueous extraction method

is a traditional method for extracting plants and other materials (Muthu,S.S,2017). Which uses water for extraction with or without the addition of (salt, acid alkali, alcohol). (Kasiri and Safapour.,2013)Typically, aqueous extraction was used to extract colours from plants and other materials(MIAH et al.,2016). the dye-containing material is first broken into small pieces or powdered and filtered ,To increase extraction efficiency,the dyecontaining material is first broken into small pieces or powdered and filtered. (Muthu,S.S,2017;Mohan et al.,2020;Samanta et al.,2020; Xiaofeng,N,2012) It is then immersed in water in metal containers for a long amount of time, usually overnight, to break down the cell structure before being boiled to extract the dye solution, which is then filtered to remove non-dye plant remnants. The boiling and filtering step is repeated to remove as much colour as feasible. (Muthu,S.S,2017); Samanta et al.,2020; Xiaofeng,N,2012; Kumbasar, E.P.A ,2011; Zubairu and Mshelia,2015)

The disadvantage of this extraction approach during the boiling process some of the dye decomposes. As a result, only dyes that do not break down at boiling temperatures are acceptable for this procedure. The molecules should be soluble in water.(Samanta et al.,2020)

It includes a long extraction time, a huge amount of water required, the usage of high temperatures, and limited dye yield since only water-soluble colour components are extracted, but many dyes have low water solubility. (Muthu,S.S,2017; Werede, E 2019)

Solvent extraction method

Natural colouring materials can also be extracted using organic solvents such as (petroleum ether, ethanol) or a mixture of solvents such as a mixture of (ethanol and methanol) and so on, depending on their nature(Muthu,S.S,2017; Werede, E 2019). Both water-soluble and water-insoluble compounds can be extracted from plant resources using the water/alcohol extraction technique. Solvent Extraction requires appropriate extraction equipment, such as the Soxhlet extractor. (Kasiri, M.B. and Safapour, S ,2013).An alcohol solvent, acid, or alkali is used to improve the colour bodies.

(Muthu,S.S,2017) In general, ethanol is favoured because, the dye production is good, the amount of water required is minor and the extraction is carried out at a lower temperature.(Werede, E 2019; Samanta et al.,2020)As a result, the extraction yield is higher as compared to the aqueous approach because a greater number of chemicals and colouring ingredients may be extracted. (Muthu,S.S,2017; MIAH et al.,2016)

The limitations of this technique are toxic residues and the greenhouse gas effect (Muthu,S.S,2017; Werede, E 2019)

Chromatography

is based on the principle where molecules in mixture, so we divided it into three categories: (Cuatrecasas et al., 1968; Porath J ,1997)

- **Stationary phase**:

This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".

- **Mobile phase**:

This phase is always composed of "liquid" or a "gaseous component."

- **Separated molecules**

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids. However, ion-exchange chromatography is more effective in the separation of macromolecules as nucleic acids, and proteins.

Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis

gas liquid chromatography is utilized in the separation of (alcohol, ester, lipid) while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins.

Agarose-gel chromatography is used for the purification of DNA, RNA particles. (Gerberding SJ and Byers CH ,1998)

If mobile phase is liquid, it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GS)(Donald et al., 2006). The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achive a satisfactory separation within a suitable time interval (Laurence et al.,1989)

3.Methods of Research and the tools used

The deductive method was used in collecting data for this research, where the problem of artificial colors and the diseases they cause to humans was identified. Then the dye was defined, then its components of chromophores and exochromes were discussed, and then some pigments were identified, such as chlorophyll A and B pigments, which It gives us the green color in spinach, and there are also many carotene pigments, including beta-carotene, which has an orange color, which was extracted from carrots, and the red pigment betanin, from beets. Then, solvents were defined and divided in terms of polarity into polar and non-polar solvents, and they were divided. In terms of chemical composition, it is divided into organic and inorganic solvents. Some types of separation methods that can be used to extract these dyes using appropriate solvents were also discussed, including acid or alkali extraction, fermentation, enzymatic extraction, supercritical carbon dioxide, and ultrasound. , aqueous, chromatographic and solvent extraction .

Among the tools that were used were experiments, where chlorophyll a and b were separated from spinach, using isopropyl alcohol, obtaining a green color. Experiments were also used to separate beta-carotene from carrots, using ethyl alcohol, petroleum ether, and obtaining an orange color.

As well as in separating the betanin pigment from beets using water and obtaining the red color.

This was done by following the laboratory analysis method.

4. Results of Research

Through practical experiments, we achieved the separation of chlorophyll a and b from spinach using the following tools and chemicals (beaker -hot plate) by Using the solvent (isopropyl alcohol) This was done by placing two cups of water in a pot on the fire until it boils, then placing two spinach leaves in the pot for a period of time ,Two minutes, then we put the two spinach leaves in a cup containing 70% isopropyl alcohol for an hour, then we drain the water in another cup and thus we obtain the chlorophyll pigment from the spinach. The results of the research reached the use of the green color resulting from the separation of the chlorophyll a and b pigments from the spinach in dyeing. The following food product

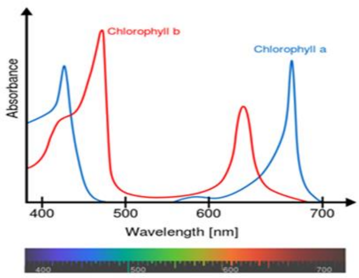
Also, beta-carotene was separated from the carrots using the following chemical tools (beaker – simple distillation device -aluminum foil) by using the appropriate solvent(Ethyl alcohol and petroleum ether)This was done by peeling the carrots, cutting them, then mixing them in the blender, then putting the mixture in the beaker and putting 240 ml of ethanol on it, then We put 10 ml of water on them, then we cover the beaker with a piece of aluminum foil. We put the beaker in a water bath for an hour, then stir the mixture for 10 minutes, then we take the beaker out of the water bath, then we take 10 ml of the extract at a concentration of 1 N and put it in a separating funnel. Then we add 10 ml of petroleum ether to the same funnel and shake them gently, and finally we get beta-carotene with petroleum ether.

We will notice the presence of petroleum ether in the lower layers at the bottom of the funnel; We will filter it and then take the beta-carotene from the upper layers. The results were obtained in using the orange color resulting from the separation of the beta-carotene pigment from carrots in dyeing the following food product.

To separate the betalain from the beets using the following chemical tools: (the baker), using the appropriate solvent, which is (water). This was done by heating a cup of water and placing pieces of the beets and then leaving them to cool. The results were obtained in the use of the red color resulting from the separation of the betalain pigment from the beets. In dyeing the following food product:

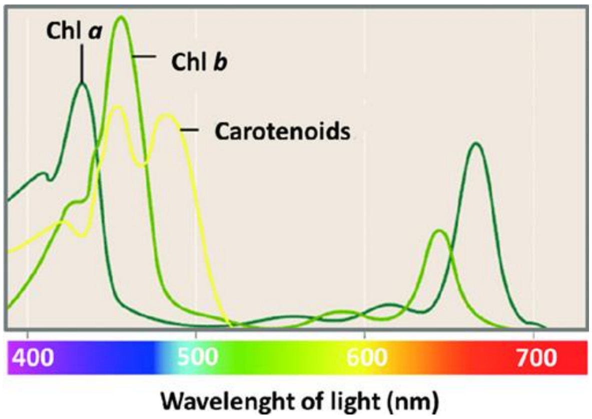
5. Interpretation of Results

-The chlorophyll in the spinach plant absorbs the red and blue colors found in light,



but it does not have the ability to absorb the green color, so

it reflects it, which gives the spinach plants the green color that we extracted from them and used as a natural dye for food coloring.



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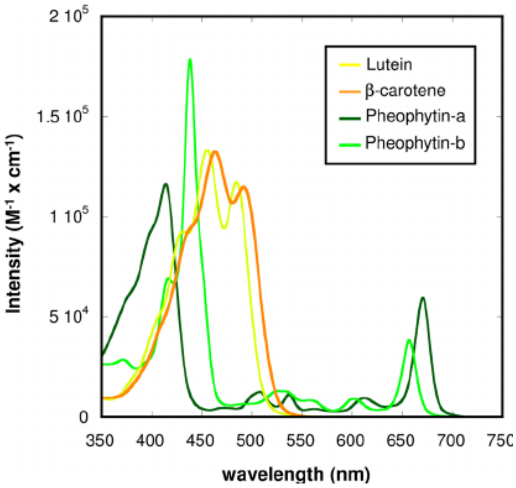
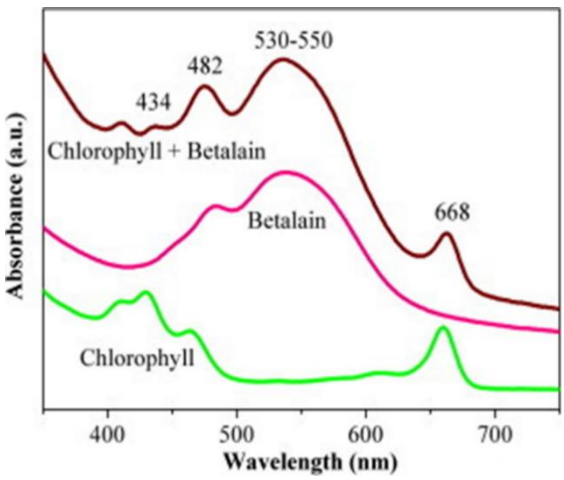
- The beta-carotene pigment found in the carrot plant has the ability to absorb the blue color, but it does not have the ability to absorb the orange color. Therefore, it absorbs the blue color and appears in its complementary color, which is orange. That color is extracted and used as a natural dye to color food in a healthy way.



Beta-carotene pigments are absorbed between the two regions 462-490 nm



Also, the betanin pigment found in beet plants has the ability to absorb the green color and does not have the ability to absorb the red color, so it absorbs the green color and reverses the red color, so the beets appear red. This color is extracted and used as a natural dye for healthy food coloring., which absorb in the region between 535-540nm

6. Conclusion

Food is what affects human health the most, as it is used on a daily basis more than once. Therefore, it was necessary for us to take care that the food is healthy, beneficial, and free of artificial colors, as the results of scientific research indicate that there are severe damages that may be caused by these artificial colors, such as liver damage. And the kidneys.Therefore, in this research we discussed how to replace artificial colors with natural colors that do not cause harm to human health, contribute it's colors to the beauty of nature and the reason for its appearance is due to the presence of chromophores (pulling groups) The presence of oxochrome leads to an increase in the depth of color (donating groups) , which are divided into : bathochromic shift (Red shift) Hypsochromic shift (Blue shift),There are many types of chlorophyll, including chlorophyll A and B, which are pigments that we extracted from spinach and give us the green color used in dyeing foods. Likewise, there are many carotene pigments, including beta-carotene that we extracted from carrots, which gives us the natural orange color used in food dyes as an alternative to artificial colors. Betanin, a pigment found in beets, gives us its natural red colour and Solvents are divided in terms of polarity into polar and non-polar solvents, and they are divided in terms of chemical composition into organic and inorganic. We used a polar inorganic solvent such as water to separate the betanin pigment from beets, and we used the solvent isopropyl alcohol, which is organic and polar, to extract the green pigment from spinach. The solvent petroleum ether, which is non-polar and organic, is used to extract the orange dye from the carrots.

We can extract some dyes from natural vegetables using extraction methods such as,

Acid or alkali extraction, fermentation, enzymatic extraction, supercritical CO2, ultrasound, aqueous, chromatographic and solvent extraction.

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