

Analysing The Role of Colours and Preservatives in Food Products: A Qualitative Study

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ABSTRACT

Food additives are substances used in the processing and preparation of various types of food to impart colors, flavors, preservation, and other desirable qualities. This study qualitatively determined different colors and preservatives using various methods. Benzoic acid is a common preservative for beverages and carbonated soft drinks due to its antibacterial properties. Colors are added to make food products more appealing. For instance, the orange color in food comes from carotenoids, a major class of natural colors providing yellow, orange, or red hues. Anthocyanins, another type of natural color, are responsible for the red color in strawberries, specifically from compounds like pelargonidin-3-glucoside and cyanidin-3-glucoside. Curcumin, a phyto-polyphenol with multiple pharmacological properties, gives turmeric its yellow color due to the presence of anthocyanins and carotenoids. These additives were qualitatively analysed using methods such as titrimetric analysis, thin-layer chromatography, and paper chromatography. The results were recorded and compared with existing literature.

KEY WORDS: Food additives, Colors, Preservatives, Benzoic acid, Carotenoids, Anthocyanins, Natural colorants, Synthetic colorants, Titrimetric analysis, Thin-layer chromatography.

INTRODUCTION

Additives

Food additives are used in the preparation and processing of nearly all types of food to enhance flavour, appearance, and other desirable qualities. Simply put, they are substances added to food. According to the Food Protection Committee of the US National Research Council, food additives are defined as “A substance or a mixture of substances other than a basic foodstuff that is present in food as a result of an aspect of production, processing, storage, or packaging.”¹

The US Food and Drug Administration (FDA) defines a food additive as “any substance, the intended use of which results or may reasonably be expected to result-directly or indirectly-in it becoming a component or otherwise affecting the characteristics of any food.”

Table 1: Different type of food additives

Block of numbers	Food additives
E100-E199	Colors
E200-E299	Preservatives
E300-E399	Antioxidants and acidity regulators
E400-E499	Thickeners, stabilizers and emulsifiers
E500-E599	Anticaking agents
E600-E699	Flavor enhancers
E700-E799	Antibiotics
E900-E999	Glazing agents and sweeteners
E1000-E1599	Additional chemicals

Although the term 'food additives' is commonly used today, their use dates to ancient times, likely even before the hunter-gatherer era. While food additives provide many benefits to manufacturers, retailers, and consumers, their use must be approached with caution. Most additives are synthetic chemicals, and modern consumers are increasingly turning to natural ingredients and bio-based additives due to the adverse effects associated with some chemicals. Consequently, plant-derived substances are becoming more popular as preservatives, colorants, flavors, and antibacterial agents.

1) COLORS

According to the US FDA, "A color additive is any dye, pigment, or substance, which when added or applied to a food, drug, or cosmetic, or to the human body, is capable (alone or through reactions with other substances) of imparting color." Food colors are used as additives primarily to enhance sensory effects, particularly the appearance of food².

There are several reasons for adding colors to food:

1. Compensation for Color Loss:

Processing and storage conditions can cause food to lose its natural color, so color additives are used to restore it.

2. Color Consistency:

Natural food colors can vary, so color additives are used to correct these variations.

3. Enhancement of Natural Color:

Color additives can be used to enhance the natural color of food.

4. Addition of Color to Colorless Foods:

Some foods may lack color altogether, and color additives are used to give them an appealing appearance.

There are two types of food colors: certified colors and colors exempt from certification. Certified colors are synthetic compounds that are generally more effective and do not introduce off-flavors to food. Colors derived from natural sources are exempt from certification but are usually more expensive and may impart off-flavors.

The health effects of food colorants are a major concern among consumers and regulatory bodies, making toxicity studies essential. For example, a recent study revealed that Allura Red AC lacks genotoxicity, addressing concerns raised by the European Food Safety Authority. Additionally, research has shown that negative effects caused by synthetic colorants, such as tartrazine, can be mitigated. For instance, a study by Rafati *et al.* demonstrated that the adverse effects of tartrazine in mice could be reduced by the simultaneous administration of vitamin E³.

The list of colors usually used in food manufacturing is stated below.

Curcumin, Riboflavin, Riboflavin-5'-phosphate, Tartrazine, Quinoline yellow, Sunset Yellow FCF, Orange Yellow S, Cochineal, Carminic acid, Carmines, Azorubine, Carmoisine, Amaranth, Ponceau 4R, Cochineal Red A, Erythrosine, Allura Red AC, Patent Blue V, Indigotine, Indigo Carmine, Brilliant Blue FCF, Chlorophylls and chlorophyllins, Copper complexes of chlorophyll and chlorophyllins, Green S, Plain caramel, Caustic sulphite caramel, Ammonia caramel, Sulphite ammonia caramel, Brilliant Black BN, Black PN, Vegetable carbon, Brown FK, Brown HT, Carotenes, Annatto, Bixin, Norbixin, Paprika extract, Capsanthin, Capsorubin, Lycopene, Beta-apo-8'-carotenal (C30), Ethyl ester of beta-apo-8'-carotenoic acid (C30), Lutein, Canthaxanthin, Beetroot Red, Betanin, Anthocyanins, Litholrubine BK⁴.

A) PURPOSE OF FOOD

People often associate certain colors with specific flavors, and the color of food can influence the perceived taste, whether it's candy or wine. Sometimes the goal is to mimic a color perceived as natural by consumers, such as adding red coloring to glacé cherries that would otherwise be beige. Other times, it's for visual effect, like the green ketchup Heinz launched in 1999. Color additives are used in foods for various reasons, including^{5, 6}:

- Making food more attractive, appealing, appetizing, and informative
- Offsetting color loss due to exposure to light, air, temperature extremes, moisture, and storage conditions
- Correcting natural variations in color
- Enhancing naturally occurring colors
- Adding color to colorless and "fun" foods
- Allowing consumers to identify products briefly, such as candy flavors or medicine dosages.

B) IMPORTANCE OF FOOD COLOURS

As food should be visually appealing, color plays a crucial role in defining its quality. The color of food is the first characteristic noticed, shaping our expectations of both flavor and quality. Colorants influence flavor identification and can affect the perceived level of sweetness in food. The purposes of adding colorants include^{7, 8}:

1. Overcoming appearance damage caused by processing and preserving product identity
2. Ensuring color uniformity in food products that naturally vary in color
3. Intensifying the colors of certain manufactured foods
4. Protecting flavor and light-sensitive vitamins during storage through a sunscreen effect
5. Serving as a visual indication of quality

C) CLASSIFICATION OF FOOD COLORS

Colors added to food are regulated as food additives. In food, coloring agents are substances that restore or enhance color. Synthetic colorants used commercially are known as certified color additives. The added colorants can be classified into^{9,10}:

Natural Colors: Extracted from animals, vegetables, fruits, minerals, and spices, these colorants are used to color foods. Examples include carotenoids from annatto, paprika, saffron, anthocyanins, caramel, chlorophyll, and turmeric. Carotenoids are the most widely used, followed by red pigments and brown-colored caramels¹¹.

i. Anthocyanins: These water-soluble compounds are responsible for the red to blue color of various fruits and vegetables, such as grapes, redcurrants, blackcurrants, raspberries, strawberries, apples, cherries, red cabbage, and eggplant. They provide orange, red, blue, violet, and magenta colors. The use of anthocyanins dates back to antiquity when Romans used highly colored berries to augment the color of wine¹².

ii. Carotenoids: Widely found in plants and animals, carotenoids are natural pigments that nature produces at an estimated rate of 3.5 tons per second. Over 600 different carotenoids have been identified, many of which are present in our diet. They provide natural yellow, orange, or red colors and are used as non-toxic natural or nature-identical colorants. Chemically, carotenoids are aliphatic or alicyclic members of the terpene group, consisting of eight isoprene units joined tail-to-tail at the center of the molecule. Carotenoids are divided into hydrocarbon carotenes and their oxygenated derivatives, called xanthophylls (e.g., violaxanthin, neoxanthin).

iii. β -Carotene: This yellow pigment was first isolated from carrots, which is why it was named carotene. Beta carotene is found in various fruits and vegetables, including bananas, jackfruit, maize, mango, papaya, pumpkin, watermelon, red pepper, spinach, peaches, apricots, oranges, and broccoli. It imparts a yellow-to-orange color in

foods and is used at concentrations of 0.13% to 2%. The oil-soluble form of β -carotene is commonly used for coloring butter and margarine, while water-soluble nor-bixin products are used in water-based products like ice cream and yogurt^{13, 14}.

Additional natural coloring agents include:

- β -Apo-8'-Carotenal
- Canthaxanthin
- Annatto
- Betalain

Food Colors Permitted by FSSA: Natural coloring agents that may be used include:

- Carotenoids
- Chlorophyll
- Riboflavin (Lactoflavin)
- Caramel
- Annatto
- Saffron
- Curcumin or Turmeric

These natural coloring principles, whether isolated from natural sources or produced synthetically, can be used in or on any article of food as permitted by the rules.

D) ADVANTAGES AND DISADVANTAGES OF COLORS

Advantages of Natural Colours

1. **Minimal Environmental Impact** – Because they come from natural sources, natural dyes are not harmful to the environment, which makes it so appealing for consumers. Natural dyes are biodegradable and disposing them don't cause pollution.
2. **Renewable** – Natural dyes are obtained from renewable sources that can be harnessed without imposing harm to the environment.
3. **Color pay-off** – If you're going for a soft hue or soothing shade, natural dyes can help you achieve that look.
4. **Safe** – Some natural dyes, such as carmine found in lipsticks, will not cause harm or health problems when ingested¹⁵.

Disadvantages of Natural colors

1. **Cost:** A larger amount of natural dyes may be needed in order to dye a specific amount of fabric as opposed to synthetic dyes. For instance, one pound of cotton may be dyed with just five grams of synthetic dye whereas 230 grams of natural dye are needed to dye the same amount of material. Since that is the case, using natural dyes is more expensive than synthetic dyes.
2. **Colour pay-off:** Colour pay-off from natural dyes tend to fade quickly. More so, quality may not be as consistent than what synthetic dyes can deliver.
3. **Availability:** Another issue with natural dyes is their availability. It can be difficult to produce because the availability of raw materials can vary from season to season, place
4. **Harmful Effects:** Natural dyes can also be harmful to some extent. Logwood has ingredients, hematein and haematoxylin, that can have harmful effects when inhaled, ingested, or absorbed through the skin. Bloodroot, another natural dye source, can cause irritation and inflammation when inhaled. More so, natural dyes may need mordants for application. While these substances help the dye stick to fabrics, they can also be toxic. Example of mordants used in natural dyes are aluminium, copper, iron, and chrome.

5. **Sustainability:** While natural dye sources are renewable, sustainability can still be an issue for natural dyes because producing them require vast areas of land¹⁶.

Advantages of synthetic colors

Artificially synthesised colors are less costly to produce, and are attractive in coloring properties, highly concentrated, and they are widely available and have been used in food, paint, coating, textile and plastics industries.

Disadvantages of synthetic food coloring:

- Many countries warn by writing special instructions on the color so that it can be used wisely. For example, In Europe, a few dyes (yellow 5, yellow 6) have a warning text- 'may have an adverse effect on activity and attention in children'. These are related to allergic reactions. Though in India, there are no such warning signals on food dyes to avoid
- They are carcinogenic and can cause cancer as some artificial food colors are also associated with the production of carcinogenic compounds while getting metabolized
- Yellow 3 has been linked with a few allergies (especially those who are sensitive to 'aspirin')
- According to sources, Red 3 dye when tested on mice, created a risk of thyroid tumor /cancer.
- Red 40 dye has been suspected with carcinogen which damages the liver, and stomach in animals. It has also been associated with behavioural issues in children. They may increase hyperactive behavior in some children. It includes chemicals like sodium benzoate linked to hyperactivity.
- Synthetic food color dyes can destroy the nutrients in the food because of their chemical composition
- They can also cause skin irritation and eczema, a type of skin rash, etc
- Artificial food colors can also cause intestinal upset and breathing problems¹⁷.

E) APPLICATION OF FOOD COLORING

- Enhancing naturally occurring colors.
- Protecting flavors and vitamins from damage by light.
- Decorative or artistic purposes such as cake icing
- Masking natural variations in color.
- Offsetting color loss due to light, air, extremes of temperature, moisture, and storage conditions.
- Providing varieties of wholesome and Nutritious food that meets consumer's demand
- Improves taste
- Added to food or drink to change its color
- Improves and maintains nutritional value of food

2)PRESERVATIVES

The term food preservation refers to any one of a number of techniques used to prevent food from spoiling. It includes methods such as canning, pickling, drying and freeze-drying, irradiation, pasteurization, smoking, and the addition of chemical additives. The process prevents or retards spoilage because high temperatures kill or inactivate most kinds of pathogens. The addition of compounds known as BHA and BHT to foods also prevents spoilage in another

different way. These compounds are known to act as antioxidants, preventing chemical reactions that cause the oxidation of food, those results in its spoilage¹⁸.

A) CLASSIFICATION

There are three types of preservatives:

- 1) Natural Food Preservatives.
- 2) Chemical Food Preservative.
- 3) Artificial Preservatives.

B) Preservatives Are Classified According to The Mechanism of Action As.

- A. Antimicrobial agents.
- B. Antioxidants
- C. Chelating agents

C) Role of food preservatives:

Food preservatives play a vital role in preventing deterioration of Food preservatives play a vital role in preventing deterioration of botulism and other organisms that can cause food poisoning. By extension, preservatives reduce food cost, improve convenience, lengthen shelf life and reduce food waste.

D) Side effects of food preservatives:

- Taking large quantity of preservative is also harmful leading to health problem
- People who are sensitive to small amount of particular preservative should not the same
- Research published in the "Journal of the Egyptian Public Health Association" in 2001 discovered that intake of foods preserved with sodium benzoate and sodium nitrite among pregnant rats is associated with a higher death rate in offspring. The results suggest that pregnant women should need caution or avoid the consumption of foods containing these preservatives¹⁶.

ADVANTAGES OF FOOD PRESERVATIVES

- Preservatives decreases the wastage of food.
- It increases the shelf-life of the food products.
- It ensures the availability of out-of-season food materials¹⁷.

DISADVANTAGES OF FOOD PRESERVATIVES

- Preservatives can have allergic consequences: the food additives BHA and BHT have inconclusively been linked to cancer and carcinogenic activity.
- It may cause loss of some nutrients, particularly thiamine and vitamin C.
- Blanching of veges prior to freezing may cause loss of vitamin B and C.
- Increases salt and sugar content of the food¹⁸.

APPLICATIONS OF PRESERVATIVES:

- Prevent deterioration of food.
- Prevent spoilage from mold, yeast and other means of food poisoning.
- Prevention of food borne illness.
- It extends the shelf-life of food products.

ANALYSIS

The analysis of preservatives is done by qualitative and quantitative analysis.

Analysis of Preservatives:

Single preservative, but more often combinations of preservatives, are commonly used in pharmaceuticals, cosmetics, biological samples, food, wood, and plastics products to prevent alteration and degradation of the product formulations. However, these preservatives may be harmful to consumer due to their tendency to induce allergic contact. Hence the simultaneous determination of these preservatives in commercial pharmaceutical products is particularly important both for quality assurance and consumer safety. Therefore, analytical methodologies developed for the quantification of preservatives in these matrices are usually designed to overcome the problems associated with interferences which are originated from other constituents. Because of the circumstances for which they are used in pharmaceutical products, preservatives are usually found as minor components in complex matrices¹⁴.

Quantitative Method of analysis:

- A. Titrimetric Method
- B. Spectrophotometric method
- C. HPLC Method

Qualitative method

1. Ferric Chloride Test
2. Modified Mohler's Test
3. Ester formation test for carboxylic acid

COLORS ANALYSIS

Synthetic color usage in foods and beverages is more frequent today. The consumption of edibles mixed with nonpermitted colors, may lead to potential health hazards. Even, permitted food colors can prove to be toxic if used or consumed indiscriminately. The regulations related to synthetic colors in food consider the health of human and the adulterations of foods and economic needs. The used methods of detection can be used in the area of food quality control to detect whether non-permitted synthetic colors are present or not in food. Efficient analytical methods are required for evaluating toxicity and authenticity in order to determine whether synthetic colors present and the level of permitted synthetic colors in foods, to confirm the absence of added colors in food and to check the stability of colors during processing and storage. This study revealed that frequency of occurrence of synthetic colors and non-permitted colors is still high. Therefore, a systematic approach is needed to evaluate the level of permitted colors in foods and to determine the frequency of occurrence of non-permitted colors in other foods¹⁵.

Quantitative Method of analysis:

1. Paper chromatographic Method
2. Thin layer chromatographic method
3. HPLC Method
4. UV spectroscopic method

Literature collected from different sources for this work:

Ikechukwu P. Ejidike *et al.*, reported the Determination of Benzoic Acid, Saccharin, and Caffeine in Carbonated Soft Drinks by HPLC 2019. B. Xiaoping Li reported the Two-Stage Course-Embedded Determination of Caffeine and Related Compounds by HPLC in Caffeine Containing Food, Beverages and (or) Related Products 2017. C. M. S. Jankulovska *et al.* reported the high-performance liquid chromatography method for determination of preservatives in beverages 2017. D. Yahya S *et al.*, reported the Determination of three dyes in commercial soft drinks using HLA/GO and liquid chromatography.2009.

Our aim is to determine the color, preservatives residues in food products. Objectives of the study, collection of Literature, Extraction of food additives, to evaluate the efficacy of food additives by standard methods (Qualitative Analysis) available in literatures¹⁶.

MATERIALS AND METHODS

Preparation of Sample:

A) Beverages and liquid products: The purchased soft drink was first opened and degassed by undergoing water bath ultra-sonication for 24 hours using an ultra sonicator. Filter the sample and use the filtrate for determination.

(B) Jams, Jellies, Preservatives and Marmalades: Mix 150 gm of sample with 300 ml saturated sodium chloride solution. Add 15 gm pulverized sodium chloride. Add 10 ml of 10% sodium hydroxide solution. Transfer to 500 ml volumetric flask and dilute to volume with saturated sodium chloride solution. Let it stand for 2 hrs. with frequent shaking, filter and use the filtrate for determination.

Benzoic Acid:

QUALITATIVE METHODS

A) Ferric Chloride Test:

Acidify the food product with hydrochloric acid (1+3) and extract with diethyl ether. Evaporate the solvent on a hot water bath removing last traces of solvent under a current of air. Dissolve the residue in few ml of hot water and add few drops of 0.5% ferric chloride solution.

B) Ester formation test:

Take 0.2gms of sample in a test tube and add 3ml of ethyl alcohol and two drops of concentrated sulphuric acid. Heat the test tube on the water bath for 5mins.

C) Benzoic acid test:

Take 0.5gms sample in a test tube add 1ml of water. Add 1drop of phenolphthalein solution and 0.2ml of ammonium solution to it. Boil the solution to remove excess of ammonia. Cool and add ferric chloride solution.

QUANTITATIVE METHODS:

(A) Titrimetric Method:

Benzoic acid is determined by acid base titration. An acid-base titration is an experimental procedure used to determine the unknown concentration of an acid or base by precisely neutralizing it with an acid or base of known concentration

Principle:

Benzoic acid is separated from a known quantity of the sample by saturating with sodium chloride and then acidifying with dilute hydrochloric acid and extracting with chloroform. The chloroform layer is made mineral acid free, and the solvent is removed by evaporation. The residue is dissolved in neutral alcohol and the amount of benzoic acid is determined by titration against standard alkali.

Procedure:

Pipette 100 ml to 200 ml of the filtrate into a 250 ml separating funnel. Neutralize to litmus paper using hydrochloric acid (1+3) and add 5 ml excess. Extract carefully with 40,30,30 and 20 ml portions of chloroform. Avoid formation of emulsion by shaking gently with rotatory motion. If emulsion forms, break it by stirring chloroform solution with a glass rod after each extraction, but do not drain any of the emulsion with chloroform layer. Transfer the combined chloroform extract into a separating funnel and wash it free from mineral acid by shaking gently and rinsing with water. Drain off the water phase. Dry the chloroform layer over anhydrous sodium sulphate and distil off the solvent. Remove the last traces of the solvent under a current of air at room temperature. Dry the residue overnight or until no residue of acetic acid is detected if the product is a ketchup. Dissolve residue in 30-50 ml of alcohol neutralized to phenolphthalein and titrate with 0.05N sodium hydroxide.

Calculate the benzoic acid contents as follows:

Benzoic acid (ppm) = $122 \times \text{Titre} \times \text{Dilution} \times 1000 \times \text{ml of 0.05N sodium hydroxide} / \text{Weight of sample} \times \text{aliquot taken (100 or 200ml of filtrate)}$ ¹⁸

ANTI OXIDANTS:

Preparation Of Sample:

A) Beverages and liquid products: The purchased soft drink was first opened and degassed by undergoing water bath, ultra-sonication for 24 hours using an ultra sonicator. Filter the sample and use the filtrate for determination.

Thin Layer Chromatographic Detection of Antioxidants:

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.

Principle:

The sample oil is dissolved in petroleum ether and extracted with acetonitrile. Acetonitrile extract is evaporated in vacuum in a rotary evaporator at a temperature not exceeding 40°C. The residue is dissolved in alcohol, applied to TLC plates and after development, spots are visualized by spraying with Gibb's reagent.

Procedure:

Dissolve 10 gm of oil or melted fat in 100 ml of petroleum ether and transfer into a 250 ml separatory funnel. Add 25 ml acetonitrile saturated with petroleum ether to the separator and shake gently. Run off the acetonitrile into a second separator and repeat the extraction three times. Transfer acetonitrile extracts to rotary evaporation flask and evaporate at less than 40°C temperature just to dryness. Dissolve the residue in 2 ml methanol, filter if not entirely soluble. Prepare 20 × 20 cm silica gel G plates with a 0.25 mm layer using 30 gm in a slurry with 60 ml 1% citric acid solution. Dry the plates in air, activate at 30°C for 1 hour and store in a desiccator. Saturate the developing chamber with a freshly prepared solvent mixture. Line the tank with filter paper, allow to stabilize for 1-2 hrs. in the dark. Apply 10 to 20µg extract solution along with standards (4µl) 2 cm apart on a start line 2cm above the bottom edge. Develop the plate to a distance of 15 cm and allow it to air dry. Spray with Gibb's reagent and dry at 103 ± 2°C for 15 min Compare the color and Rf values with standards. Cool the plate and place in a tank containing ammonia and note the characteristic color change¹⁶.

Rf value = distance travelled by compound ÷ distance travelled by solvent front

COLORS

Sample Preparation (Oranges)

Take few ml of juice and check the acidity. As most of the soft drinks are acidic, otherwise slightly acidify with acetic acid.

Test for Anthocyanins

The presence of anthocyanins has been demonstrated by adding 2 mL of sample with 2 mL of 2 N HCl. The appearance of a pink-red color that turns purplish blue after addition of ammonia indicates the presence anthocyanins.

Extraction

Introduce about 20 cm length of woollen thread into a beaker containing about 35 ml of the prepared acidified solution of the sample and boil for a few min till the woollen thread is dyed. Take out the woollen thread and wash it with tap water.

Transfer the washed woolen thread to a small beaker containing dilute ammonia and heat again. If the color is stripped by the alkali, the presence of an acid synthetic dye is indicated. Remove the woollen thread. Make the liquid slightly acidic and boil with a fresh piece of woolen thread. Continue boiling until the color is taken by the woolen thread. Extract the dye from the woolen thread again with a small volume of dilute ammonia, filter through a small plug of cotton and concentrate the filtrate over a hot water bath. This double stripping technique usually gives a pure color extract.

Natural colors may also dye the wool during the first treatment, but the color is not usually removed by ammonia. Basic dyes can be extracted by making the food alkaline, with ammonia, boiling with wool and then stripping with dilute acetic acid. At present, all the permitted water-soluble synthetic dyes are acidic, hence an indication of the presence of a basic dye suggests that an unpermitted color is present¹⁶.

QUANTITATIVE TEST BY PAPER CHROMATOGRAPHY

Draw a pencil-line parallel to the bottom edge of the paper (Whatman No.1) at about 2 cm distance. Spot the concentrated solution of the unknown dye on the line together with a series of spots (about 2 cm apart) of aqueous solutions of standard permitted dyes of similar color and dry. Run the chromatogram, by ascending technique, using a solvent Iso-butanol-ethanol water (1: 2: 1, v/v) is often helpful for general purposes. Identify the color in the sample by matching its spot with the spot of the standard color and confirm by co spotting¹⁷.

Rf value = distance travelled by compound ÷ distance travelled by solvent front

Sample Preparation (Strawberry)

The preliminary treatment involves removing interfering substances and obtaining the dye in acid solution.

Test For Anthocyanins

The presence of anthocyanins has been demonstrated by adding 2 mL of sample with 2 mL of 2 N HCl. Observe the color change.

Extraction

Introduce about 20 cm length of woollen thread into a beaker containing about 35 ml of the prepared acidified solution of the sample and boil for a few min till the woollen thread is dyed. Take out the woollen thread and wash it with tap water. Transfer the washed woollen thread to a small beaker containing dilute ammonia and heat again. If the color is stripped by the alkali, the presence of an acid synthetic dye is indicated. Remove the woollen thread. Make the liquid slightly acidic and boil with a fresh piece of woollen thread. Continue boiling until the color is taken by the woollen thread. Extract the dye from the woollen thread again with a small volume of dilute ammonia, filter through a small plug of cotton and concentrate the filtrate over a hot water bath. This double stripping technique usually gives a pure color extract.

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QUANTITATIVE TEST BY PAPER CHROMATOGRAPHY

Principle

The principle involved can be partition chromatography or adsorption chromatography. Partition chromatography because the substances are partitioned or distributed between liquid phases. The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of liquids takes place.

Procedure

Draw a pencil-line parallel to the bottom edge of the paper (Whatman No.1) at about 2 cm distance. Spot the concentrated solution of the unknown dye on the line together with a series of spots (about 2 cm apart) of aqueous solutions of standard permitted dyes of similar color and dry. Run the chromatogram, by ascending technique, using a solvent n-butanol-distil water-glacial acetic acid (20: 12: 10, v/v) is often helpful for general purposes. Identify the color in the sample by matching its spot with the spot of the standard color and confirm by co spotting.

Rf value = distance travelled by compound ÷ distance travelled by solvent front

Sample Preparation :(curcumin)

Take a pinch of turmeric powder and dissolve it in 10ml of ethanol. Stir well and then filter the solution. Use the filtrate for the test procedure.

Qualitative Test

Evaporate an alcoholic extract of the material almost to dryness on the water bath with a piece of filter paper. Moisten the dried paper with a few drops of weak solution of boric acid to which some drops of hydrochloric acid have been added. Dry the paper again. If turmeric is present, the dry paper will be cherry red in color which changes to bluish green by a drop of sodium hydroxide or ammonium hydroxide.

Test for Flavonoids

Flavonoids were characterized by said reaction to cyanidin. A volume of 2 mL of the plant extract was evaporated to dryness. After cooling, the residue was taken up in 5 mL twice diluted hydrochloric alcohol in a test tube. Then, two to three magnesium turnings were added. The addition of three drops of isoamyl alcohol intensifies a pink-orange or violet.

Test For phenols

Ferric Chloride test:

Dissolve the given organic compounds in water. Add neutral solution of ferric chloride slowly dropwise. Observe the change in color.

Test for tannins:

Ferric chloride test:

A quantity (1 ml) of the filtrate was diluted with distilled water and added 2 drops of ferric chloride. Observe the color change

Extraction

15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent. The extraction experiment was carried out at 60 °C within 8 h. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator (Stuart RE300) under vacuum at 35 °C. The residue (oleoresin) was dried and weighed; then dissolved in 10 ml methanol. In all extraction experiments acetone was used as the extraction solvent due to its high solubilization capacity.

QUANTITATIVE ANALYSIS BY THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.

Principle:

Thin layer chromatography is based on the principle of separation through adsorption type. The separation relies on the relative affinity of compounds towards both the phases. The compound in the mobile phase moves over the surface of the stationary phase. the movement occurs in such a way that the compounds which have higher affinity towards stationary phase moves slowly while the other travels fast. Therefore, the separation of mixture attend¹¹.

Procedure:

Thin layer chromatographic studies of curcumin, precoated Silica gel aluminium plates (20 X 20cm) were used. Curcumin was separated using n-hexane: ethyl acetate [7:3]. The color and Rf values were recorded using spraying the plates with 1% alcoholic KOH solution¹⁸.

$$\text{Rf value} = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent front}}$$

RESULTS AND DISCUSSION

1)Preservatives

A) Benzoic Acid

Sample 1 (Beverages):

Benzoic acid is a commonly used chemical preservative in food and beverages, especially in carbonated beverages, as it presents its strongest antibacterial activity Benzoic acid is a commonly used antimicrobial preservative in food and beverages, especially in carbonated beverages, as it presents its strongest antibacterial activity.IT can be determined by qualitative and quantitative methods.

Qualitative Test

Table 2: Qualitative Test for Beverages

S.No	Test	Observation	Inference
1	Ferric Chloride Test: Sample +HCl (1+3) And Extract with Diethyl Other Evaporate the Solvent Dissolve Residue in Few ml Of Hot Water Add Few Drops Of 0.5% Ferric Chloride Solution.	Salmon Color Precipitate of Ferric Benzoate is Observed	Presence of Benzoic Acid
2	Ester Formation Test: 0.2g Of Sample+3n Of Ethyl Alcohol +2drops Of Conc. Sulphuric Acid Heat on Water Bath.	Fruity Smell is Produced	Presence of Benzoic Acid
3	Benzoic Acid Test: 0.5g Of Sample +1ml Water +1 drop Of Phenolphthalein +0.2ml Of Ammonia Solution Boil the Solution Cool and Add Ferric Chloride Solution.	Puff Color Precipitate is Produced	Presence of Benzoic Acid



Fig 1: Test For Benzoic Acid;



Fig2: Ester Formation Test;



Fig 3 :Ferric Chloride Test

QUANTITATIVE ANALYSIS:

Titrimetric Method

Benzoic acid is separated from a known quantity of the sample by saturating with sodium chloride and then acidifying with dilute hydrochloric acid and extracting with chloroform. The chloroform layer is made mineral acid free, and the solvent is removed by evaporation. The residue is dissolved in neutral alcohol and the amount of benzoic acid is determined by titration against standard alkali.

Benzoic acid (ppm) = $122 \times \text{Titre} \times \text{Dilution} \times 1000 \times \text{ml of 0.05N sodium hydroxide} / \text{Weight of sample} \times \text{aliquot taken (100 or 200ml of filtrate)}$

$$= 122 \times 0.7 \times 40 \times 1000 \times 0.4 / 15 \times 100 = 910.93 \text{ ppm or } = 0.0910\% \text{ (X \% = X ppm/10000)}$$

The amount of benzoic acid in soft drinks was found to be 0.0910%.



Fig 4: Titrimetric analysis

Sample- 2 (jellies):

Benzoic acid:

Benzoic acid is a commonly used chemical preservative in food and beverages, especially in carbonated beverages, as it presents its strongest antibacterial activity. Benzoic acid is a commonly used antimicrobial preservative in food and beverages, especially in carbonated beverages, as it presents its strongest antibacterial activity. It can be determined by qualitative and quantitative methods.

Qualitative analysis

Table 3: Qualitative Analysis- Jellies

S. No	Test	Observation	Inference
1.	FERRIC CHLORIDE TEST: Sample +HCL (1+3) and extract with diethyl ether. Evaporate the solvent. Dissolve residue in few ml of hot water add few drops of 0.5% ferric chloride solution.	Salmon color precipitate of ferric benzoate is observed	Presence of benzoic acid
2	ESTER FORMATION TEST: 0.2g of sample+3ml of ethyl alcohol +2drops of conc. sulphuric acid. Heat on a water bath.	Fruity smell is produced	Presence of benzoic acid
3	BENZOIC ACID TEST: 0.5g of sample+1ml water + 1drop of phenolphthalein + 0.2ml of ammonia	Puff color precipitate is produced	Presence of benzoic acid

	solution. Boil the solution. Cool and add ferric chloride solution.		
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Fig 5: Test for benzoic acid; Fig 6: Ester formation test; Fig 7: Ferric Chloride Test

QUANTITATIVE ANALYSIS:

Titrimetric Method:

Benzoic acid (ppm)=

$122 \times \text{Titre} \times \text{Dilution} \times 1000 \times \text{ml of 0.05N sodium hydroxide} / \text{Weight of sample} \times \text{aliquot taken (100 or 200ml of filtrate)}$

Weight of sample \times aliquot taken (100 or 200ml of filtrate)

$$= 122 \times 0.6 \times 40 \times 1000 \times 0.4 / 15 \times 100$$

$$= 780.8 \text{ ppm or } 0.07808\% \text{ (X \% = X ppm / 10000)}$$

The amount of benzoic acid in jellies was found to be 0.07808%.



Fig 8: Titrimetric analysis

ANTI OXIDANTS

Sample (synthetic orange juice)

Antioxidants are used to preserve food by inhibiting atmospheric oxidation and prevent it from breakdown and become rancid.

Thin layer chromatography

Thin layer chromatography is based on the principle of separation through adsorption type. The separation relies on the relative affinity of compounds towards both the phases. The compound in the mobile phase moves over the surface of the stationary phase. The movement occurs in such a way that the compounds which have higher affinity towards stationary phase moves slowly while the other travels fast. Therefore, the separation of mixture attend.

Rf value = distance travelled by compound/distance travelled by solvent front

$$=1.9/4.2 = 0.45$$

COLORS

Sample-1(orange)

Test for Anthocyanins

Sample + 2 mL of 2 N HCl

Observation:

Appearance of a pink-red color that turns purplish blue after addition of ammonia

Inference:

Indicate the presence anthocyanins.

Paper Chromatography:

Mobile Phase: iso-butanol: ethanol: water (1:2:1)

R_f value = distance travelled by compound/distance travelled by solvent front

$$= 2/3.9 = 0.512$$



Fig 9: Paper Chromatogram of orange

Sample 2 (strawberry)

Test for Anthocyanins

sample + 2 mL of 2 N HCl

Observation:

appearance of a pink-red color that turns purplish blue after addition of ammonia

Inference:

indicate the presence anthocyanins

Paper chromatography:

Mobile phase =n-butanol: distiller water: glacial acetic acid (20:12:10)

Rf value=distance travelled by compound/distance travelled by solvent front

$$=2.3/3.9 = 0.589$$



Fig 10: paper chromatogram of strawberry

Sample-3 (curcumin)

Qualitative test:

Sample is evaporated on the filter paper almost to dryness. Add few drops of weak solution of boric acid and HCL. Dry the paper

Observation:

Cherry red color is produced which changes to bluish green by adding a drop of sodium hydroxide.

Inference:

Indicates the presence of turmeric.

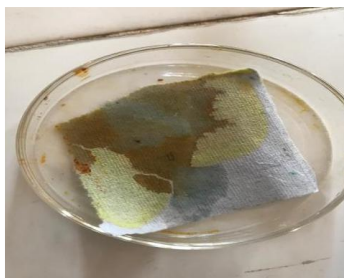


Fig 11: Test of curcumin

Table 4: Qualitative analysis - curcumin

S.No	Test	Observation	Inference
1.	Test for flavonoids: Evaporate 2ml of sample+ 5ml HCl in a test tube +2-3 magnesium turnings + 3ml of isoamyl alcohol	Pink, orange color changes to violet color	Presence of flavonoids
2.	Test for Phenols: Ferric chloride test	Appearance of red	Presence of phenols
3.	Test For Tannins Ferric chloride test	Transient greenish to black color is produced	Presence of tannins

Quantitative test

Thin layer chromatography

Stationary phase: silica gel G

mobile phase= n hexane: ethyl acetate (7:3)

Spraying reagenr: 1% alchloic KOH Solution

Rf value=distance travelled by compound/distance travelled by solvent front

$$=0.8/3 = 0.26$$



Fig 12: TLC of curcumin

Table 5: Products used to determine preservatives and color in food products.**PRODUCTS USED TO DETERMINE PRESERVATIVES IN FOOD PRODUCTS**

S. No	Product Name	Preservative	Calculated Percentage	Percentage Acceptance
01	Just Jelly	Benzoic acid	0.0780%	0.05-0.1%
02	Thumbs up	Benzoic acid	0.0910%	0.05-0.1%

PRODUCTS USED TO DETERMINE COLORS IN FOOD PRODUCTS

S. No	Product Name	Colours	Calculated Rf Value	Standard Rf Value
01	Pulpy orange	anthocyanin	0.512	0.32-0.62
02	Just jelly	anthocyanin	0.589	0.32-0.62
03	Turmeric powder	curcumin	0.260	0.280

SUMMARY AND CONCLUSION

The present study focuses on the qualitative determination of colors and preservatives in food products. The analysis was conducted according to standard procedures available in the literature. The chapters include an introduction to colors and preservatives, a literature review on previous investigations, the aim and scope of the work, experimental procedures for determining colors and preservatives, and the results of the experiments.

Benzoic acid, a commonly used preservative in food and beverages, especially in carbonated drinks, is known for its strong antibacterial activity. Both qualitative and quantitative analyses of benzoic acid were conducted, and the results were compared with existing literature.

The orange color in foods is mainly due to carotenoids, a major class of natural pigments. Carotenoids are widely distributed in the plant kingdom and can also be found in animals, bacteria, algae, molds, and yeast. Over 700 carotenoids have been identified, providing natural yellow, orange, or red colors to foods like tomatoes, carrots, red peppers, oranges, and pumpkins. However, the color of some varieties, such as blood orange juice, is primarily due to anthocyanins, red-blue water-soluble pigments. The red color of strawberries is attributed to anthocyanin compounds, mainly pelargonidin-3-glucoside and cyanidin-3-glucoside. Strawberries also contain a group of phenolic compounds, including flavonoids represented as anthocyanins, phenolic acids, lignans, stilbenes, tannins, and coumarins, with anthocyanins being responsible for the red to blue color and possessing antioxidant properties.

Curcumin, a phyto-polyphenol pigment isolated from the plant *Curcuma longa* (commonly known as turmeric), has various pharmacological properties. Curcumin provides turmeric with its yellow color and contains anthocyanins, carotenoids, and a wide variety of phytochemicals, including dimethoxy curcumin, zingiberene, curcumenol, eugenol, and turmerones. Curcumin is hydrophobic and freely soluble in ethanol and acetone. It possesses anti-inflammatory properties by inhibiting cyclooxygenase enzymes (COX) and other enzymes involved in inflammation, contributing to its observed antineoplastic properties, such as inhibiting tumor cell proliferation and suppressing chemically induced carcinogenesis. Curcumin is a beta-diketone with two hydrogens substituted by feruloyl groups, and it acts as a metabolite, anti-inflammatory agent, antineoplastic agent, hepatoprotective agent, flavoring agent, biological pigment, nutraceutical, antifungal agent, dye, lipoxygenase inhibitor, radical scavenger, immunomodulator, and neuroprotective agent. It derives from ferulic acid.

The colors and preservatives were determined qualitatively according to the procedures mentioned in the literature, and the results were reported. Further research is required to advance the analysis.



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