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Robust Chemical Testing for Food Safety: Assessing and Identifying Dye Adulterants in Blueberry Juice: a Comprehensive Case Study with Statistical Measures and Correlation Analysis

Bhandare Saurabh Dilip *, Malode Sarika Shivaji

1. Foxabell - Laboratory of Science and Research Studies. Nashik-India- 422101

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ABSTRACT

In the food sector, the problem of food fraud—which encompasses the more focused category of adulteration driven by economic gain—is becoming more widely acknowledged and is raising concerns. Both the government and the industry are accountable for avoiding food adulteration, regardless of where the food danger originated. Food adulteration can result from incidents concerning food safety, food fraud, and food defence, which poses serious risks to the general public's health. In contrast to inadvertent injury in food safety events and intentional harm in food defence incidents, food fraud entails intentional activities for financial gain. Although financially motivated adulteration may have financial motivations, the unusual nature of contaminants frequently makes the related public health hazards more dangerous than traditional food safety threats. Establishing and executing a database that aggregates reports of food component fraud available for publicly accessible sources is an essential task. For governments, agencies, and individual enterprises assessing the possible dangers associated with certain items created in specific regions and those disseminated and sold elsewhere, this database provides essential information and useful data. The paper also describes current analytical tools for identifying food fraud and highlights new developments and trends in the food production industry and the food production bad sector.

1. Introduction

All living organisms need sustenance for survival, and the quality of food plays a crucial role in human health. Unfortunately, in contemporary times, the integrity of food is compromised by numerous deceptive practices. Food adulteration refers to the deliberate degradation of the quality of food available for purchase, achieved through either the inclusion or replacement of a substandard substance or the exclusion of a valuable ingredient [1].

*Corresponding Author:

Bhandare Saurabh Dilip,

Foxabell - Laboratory of Science and Research Studies. Nashik-India- 422101;

saurabh_bhandare@yahoo.com

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The risks associated with food ingredient fraud and economically motivated adulteration are on the rise. However, there is currently no comprehensive collection of information detailing problematic ingredients and the methods used to detect them. The 8th edition of the Food Chemicals Codex by the US Pharmacopeial Convention encompasses 1305 entries, with 1000 of them featuring analytical methods that are sourcable records on food toxicology analysis for food-induced toxicants that damage the health of people [2].

Thus, food adulteration is the deliberate alteration of food's quality, usually for financial or business benefit. The process of manipulating food involves adding ingredients to change its colour, appearance, taste, weight, volume, and shelf life, among other characteristics.

The prevalent targets for adulteration reports were olive oil, milk, honey, and saffron. Additionally, identified potential hazards encompassed problems such as spices being mixed with lead chromate and lead tetraoxide, the substitution of toxic Japanese star anise for Chinese star anise, and the adulteration of high-protein foods with melamine [2].

1.1 Adulteration risks and ingredients at stake

Various ingredients used in the food industry, such as saffron, black pepper, chilli, oregano, turmeric, and fennel seed, face the risk of adulteration. The adulterants include a wide array of substances, from other spices to dried red beet pulp and powdered fruits. The prevalence of adulteration emphasise the importance of quality control measures to maintain the integrity and safety of food products. Vigilance is crucial to addressing the diverse sources of adulterants in the industry [3–5].

1.2 Health impacts of illegal dyes

The health impacts range from genotoxicity to potential carcinogenicity, emphasising the need for stringent regulations and monitoring in the food industry.

It is a serious hazard to public health in addition to being an economic crime [6].

The goal is to reorganise entire food supply chains in order to reduce fraud opportunities, rather than only detecting food fraud. Food Fraud is a newly defined area of food protection that sits between food safety issues (like pesticide residue or Salmonella) and food defence issues (like terrorism or other hostile intent). Food Fraud, in contrast to Food Defence, is deliberate but does not aim to hurt others—rather, its only goal is financial gain. The prevention of food fraud benefits society and the economy in a manner similar to those of initiatives aimed at improving food safety and food defence. Preventive measures like these lessen the number of unauthorised acts that endanger public health, which makes society safer and more secure [7].

The September 11, 2001 terrorist attacks are responsible for the development of food defence as a new discipline within food protection and a separate field of study. Food fraud could develop into a similarly distinct notion, situated between the domains of food safety and food defence, as economically motivated adulteration grows in scope, scale, and recognition [8].

Beyond that, there is social peace and increased customer satisfaction. Increased production of higher-value goods that serve consumers, promote trade, and aid in exports leads to an increase in food security. Food fraud has a significant economic impact since it not only reduces citizen productivity from illness but also erodes consumer confidence, which lowers business activity [7].

1.3 Food risk matrix and fraud impact

The Food Risk Matrix acts as a comprehensive model, integrating key concerns in food protection. It aids in understanding the role of food fraud within the broader context of food protection, considering factors like to factors like: food quality, safety, and defence.0.

The culinary landscape is growing, with consumers worldwide showing a growing interest. However, this industry is not without challenges, as it faces the persistent threat of economically motivated adulteration, illegal dye usage, and potential health risks. The implementation of stringent regulations, oversight by relevant authorities, and comprehensive quality control measures are essential to safeguarding the integrity and safety of herbs and spices in the global food market.

The occurrence of food adulteration is frequently documented and poses a significant potential risk to the safety of food products [9].

Chinese consumers perceive food fraud to be a hazard that represents a food safety risk [10]. On a worldwide scale, the primary consumers of herbs and spices are in Asia and Europe. Nevertheless, there is a growing interest in herbs and spices among consumers in the United States, as indicated by reports from AMCHAM and Trade USA in 2015 [11]. The positive connection between availability and familiarity was observed in relation to attitudes towards traditional food. In the case of European food, mood emerged as a favourable factor influencing attitude [12]. Applying the Theory of Planned Behaviour (TPB) to analyse consumers' intentions to buy food from sustainable sources [13]. For both categories of food, there was a positive correlation between sensory appeal, attitude, and the inclination to make a purchase [12].

Food safety and quality have received more attention recently since they have a significant influence on human health. Food adulteration is a problem that affects food quality and safety globally and is difficult to detect. High-performance liquid chromatography and gas chromatography-mass spectrometry are two examples of sophisticated detection techniques that can accurately identify the kinds and quantities of adulterants in a variety of food products. However, the high expense, ineffectiveness, and difficulty of sample preparation and operation are barriers that prevent these methods from being widely used [14]. The identification of hazardous compounds in food items is necessary since the food business, the government, and consumers all have a shared concern for the safety and quality of food. Traditional methodologies, such as biological techniques and instrumental analysis such as mass spectrometry and chromatography, are renowned for their time-consuming nature and their incapacity to offer spatial distribution data for analytes. Consequently, there is a growing emphasis in the field of food research on the investigation and development of quick, non-destructive, real-time, and aesthetically pleasing detection systems. Furthe, the complementary use of mass spectrometry and spectral imaging optimises each technique's advantages and makes it possible to obtain detailed mass spectrometry and spectrum data for the compounds of interest [15]. Hyperspectral imaging, which combines spectroscopic and imaging technologies, is quickly becoming a real-time, non-destructive way to determine the safety and quality of food [16]. An innovative technology called hyperspectral imaging (HSI) combines spectroscopy, chemometrics, and imaging with other optical sensing techniques. The simultaneous acquisition of spatial and spectral data by the HSI sensor makes it possible to quickly, nondestructively, and contactlessly monitor the physical and chemical characteristics of food products [17]. In conclusion, it is expected that the chances of using imaging technologies in food research will only get better [15]. Because of the wide variety of foods and various food production systems, microbial food safety is a persistent and difficult global concern. The frequency of bacterial pathogen-related foodborne diseases highlights the ongoing danger to human health and safety. Traditional techniques, which include pretreatments like culture, enrichment, and amplification, are not only complex but also costly and time-consuming when it comes to recognising and counting bacteria in complex microbial communities. As a result, quick, on-site detection technologies with high sensitivity and specificity are essential for managing foodborne bacterial infections and ensuring food safety. As a powerful diagnostic tool, hyperspectral imaging (HSI) offers a quick and non-invasive method of pathogen identification [18]. Hyperspectral imaging (HSI) and data analysis techniques were investigated in this study as potential tools for the non-invasive, early identification of *Botrytis cinerea* infection. Over several time periods, hyperspectral images were taken of contaminated and uncontaminated fruits grown in a lab. Moving window smoothing (MWS), standard normal variates (SNV), multiplicative scatter correction (MSC), Savitzky-Golay 1st derivative, and Savitzky-Golay 2nd derivative techniques were used to preprocess the spectral wavelengths between 450 and 900 nm. The method showed that it could detect contaminated samples even before symptoms of illness appeared. The results also showed that kiwifruit characteristics like firmness, soluble solid content (SSC), and titratable acidity (TA) were significantly impacted by gray-mold infection. Moreover, the predictive accuracy of the Savitzky-Golay 1st derivative-CARS-PLSR model reached its peak for kiwifruit firmness, SSC, and TA, attaining determination coefficient (R^2) values of 0.9879, 0.9644, and 0.9797, respectively, in the calibration phase. Correspondingly, the cross-validation R2 values stood at 0.9722, 0.9317, and 0.9500 for firmness, SSC, and TA. This investigation poses the significant potential of hyperspectral imaging (HSI) and chemometric analysis for rapid and non-destructive evaluations of fungal-infected kiwifruits throughout the storage period [19]. In a study with (N) individuals, with (XN) of them being female, participants were recruited from both a farmers market and an organic produce outlet located in a major Australian capital city. To gather data, participants were asked to complete an online questionnaire, which included scales related to the Theory of Planned Behaviour (TPB), such as: attitude, subjective norms, perceived behavioural control, and intention. Additionally, the questionnaire included assessments for positive moral attitude and ethical self-identity, along with inquiries about their motives behind food choices [13]. A questionnaire was distributed in three countries in the spring of 2009, using the Theory of Planned Behaviour (TPB) model to predict the intention to consume ready-to-eat (RTE) meals: Norway (with a sample size of 112), the Netherlands (with a sample size of 99), and Finland (with a sample size of 134). The results of a stepwise hierarchical regression analysis showed that moral attitude had a major impact on RTE meal consumption. The sense of moral obligation, operationalised as a negative feeling of guilt, negatively impacted consumers' intentions to consume ready meals across all three examined countries. The Theory of Planned Behaviour's (TPB) explained variance (R^2) increased when moral considerations were included as an explanatory factor. When moral attitude was included in the TPB model, however, considerable cultural differences emerged in the impact of subjective norms on the consumption of ready meals, indicating non-significant outcomes in both Norway and the Netherlands. This suggests that various cultures have varied social influences on how often they eat prepackaged meals [20].

Three different nations provided survey responses in March 2004: Finland (with a sample size of 270), Italy (with a sample size of 202), and the United Kingdom (with a sample size of 200). Participants' plans to buy organic apples and organic ready-to-cook pizza instead of conventional ones were directly addressed in the questionnaire [21].

International organisations, the general public, and food safety officials are all very concerned about the problem of adulterated olive oil. The European Union (EU) has promoted labelling olive oil with protected designation of origin (PDO) and protected geographical identification (PGI) characteristics to guarantee its authenticity. In order to improve quality control, food safety organisations are concurrently investigating cutting-edge technologies that can function quickly, consistently, and in real-time, either locally or remotely. Of the methods that have been proposed, photonic technologies are the most suitable and promising to tackle this problem [22].

The theory of planned behaviour (TPB) is more predictive of intentions to abstain from driving after drinking when it is expanded to include moral norms and descriptive norms [23]. The application of the Theory of Planned Behaviour (TPB), incorporating moral norms, anticipated regret, and past behaviour, was employed to forecast the intention of surpassing the posted speed limit on various roads and objectively evaluate speeding havior. All assessments, excluding behaviour, were obtained through self-report questionnaires that addressed diverse driving scenarios [24]. In Norway, 4000 people between the ages of 18 and 70 received survey questionnaires; 1025 of them successfully completed them. The studies were limited to persons with a driver's licence who reported drinking alcohol at least once or twice a year; this produced a sample of 879 people, with a mean age of 43.9 years and 46.6% of men and 30.3% of those under 35. Expanding the theory of planned behavior (TPB) by incorporating moral norm and descriptive norm enhances its predictive capability for intentions to refrain from drinking and driving. The results showed that 10% of the variation in intentions for the whole sample could be explained by the Theory of Planned Behaviour (TPB) factors. Further, even after accounting for the influence of TPB components, the extension factors added an extra 2% to the explained variation. The most significant predictor of intents was found to be perceived behavioural control (β=0.24, p<0.001), with descriptive norm coming in second (β=-0.12, p<0.001) [23].

In the period under investigation, the most common reports included mycotoxin residues, pesticide residues, and harmful microorganisms. Herbs and spices, fruits and vegetables, and nuts and seeds were the product categories that were most often noted. The products that were sent in for examination originated in South America, Africa, and Asia [25].

Food safety is the application of controls to mitigate hazards related to food handling and processing, with the main objective being the assurance that food is fit for human consumption and poses no health risks. A growing desire for less processed foods, a decrease in the use of preservatives, and increased consumer consciousness are the main factors driving the growing emphasis on food safety [26].

From the procurement of raw materials to the ultimate delivery of their products to customers, food processing companies face a multitude of obstacles in ensuring the safety of their offerings. Food hazards are unquestionably common in the food industry and can enter the food chain in a number of ways. Unlike open food processing equipment (OFPE), which exposes the food and its contact surfaces to the environment (EHEDG, 2020), closed food processing equipment (CFPE) processes food in a sealed and regulated environment, reducing the risk of food contamination. The safety of food products can therefore be jeopardised by chemical, physical, or biological dangers through a variety of channels, including as waste management, building design, pests, ventilation systems, and human activity. As such, there is an urgent requirement for an extensive analysis of the consumed food products. Thus, in order to guarantee a safe and clean processing environment, a thorough analysis of food contamination pathways and related mitigation techniques are urgently required [27–28].

The herb and spice industry, with an estimated value of around US$4 billion, is experiencing ongoing expansion. However, it faces persistent risks from individuals engaged in economically driven adulteration. The vulnerability to adulteration spans the extensive and intricate supply chains associated with herbs and spices, providing criminals with opportunities to compromise the integrity of the products at various stages. That is, the intricate and vast supply chains that are linked to herbs and spices are susceptible to adulteration, which presents thieves with multiple possibilities to undermine the product's quality [11]. Paprika oleoresin is generally a colouring agent in food [4]. Paprika oleoresin, sometimes called paprika extract or oleoresin paprika, is a semi-viscous liquid that is made from dried red peppers or paprika and has a rich, deep red colour. Its primary uses include adding colour and flavour to food items and acting as an antioxidant. Solvent extraction is the method used to remove the flavour and colour components from paprika (*Capsicum annuum L.*). The main colouring agents in the oleoresin are capsanthin and capsorubin, with other coloured compounds present [5].

Paprika finds application as a colouring agent in a diverse range of food products, including meat items, confectionery, vegetable oils, snacks, surimi, seasonings, soups, sauces, salad dressings, marinades, processed cheese, bakery goods, fruit preparations, convenient foods, and canned products as a colouring agent or colouring ingredient [5].

The Food Risk Matrix integrates key aspects of concern in food protection. It serves or functions as a model that facilitates comprehension of the impact of food fraud within the broader framework of food protection, encompassing factors like food quality, safety, and defence [11].

According to the Grocery Manufacturers Association (GMA), fraudulent activities within the global food industry could result in losses ranging from $10 billion to $15 billion annually, impacting around 10% of all commercially available food products [29].

The use of common illegal dyes in food presents potential health risks, as highlighted by specific examples and their associated impacts. Sudan 1, for instance, found in spices such as: cayenne pepper, turmeric, chilli, paprika, and curry, has been identified as genotoxic and carcinogenic in rats. Sudan 4, another illegal dye present in curry, turmeric, chilli, paprika, and sumac, poses potential genotoxic and possibly carcinogenic effects. Para Red, identified in chilli, cayenne pepper, and paprika, is also considered potentially genotoxic and carcinogenic. Orange II, found in chilli, safflower, sumac, and paprika, is potentially genotoxic, with insufficient data on its carcinogenicity. Methyl yellow, associated with curry, is possibly carcinogenic to humans. Additionally, Rhodamine B, present in sumac, chilli, paprika, turmeric, and curry, is considered potentially genotoxic and potentially carcinogenic. These revelations highlight the importance of stringent regulations and monitoring in the food industry to ensure the safety and well-being of consumers [11].

1.4 EU and US regulations to control fraud in the herb and spice industry

In the General Food Law Regulation (EC) 178/2002 (EU, 2002), the general principles and requirements of food law and procedures of food safety are outlined.

1.5 The European Food Safety Authority (EFSA)

EFSA was established legally in 2002 under the General Food Law, following a number of food crises in the late 1990s. EFSA provides scientific advice and communicates risks within the food chain [11]. In the United States, the FDA and the US Department of Agriculture (USDA) are the principle federal agencies working on food safety. Border protection and import authorities, as well as food safety, food defence and food quality authorities broadly look after food fraud across a number of federal agencies [29].

Various ingredients commonly used in the food industry face the risk of adulteration, posing challenges to product authenticity and quality. Saffron, a highly sought-after spice, is susceptible to adulterants such as: *Carthamus tinctorius, Chrysanthemum x morifolium, Zea mays, Nelumba nucifera, Safflower, Arnica montana L., Bixa orellana L., Calendula officinalis L., Crocus vernus L., Curcuma longa L., and Hemerocallis sp.* Black pepper, another widely utilised spice, is at risk of being adulterated with Chilli. Chilli itself faces potential adulteration with dried red beet pulp and powdered *Ziziphus nummularia* fruits. Oregano may be compromised by adulterants like *Satureja montana L.* and *Origanum majorana L.*, while olive leaves might be adulterated with *Cistus incanus L., Rubus caesius L., and Rhus coriaria L.* Turmeric, a staple spice, may be adulterated with *Curcuma zedoaria* or *Curcuma malabarica*. Fennel seed, another commonly used ingredient, faces the risk of adulteration with *Anethum gravelones* fruit (AGF) and *Cuminum cyminum* fruit (CCF). Similarly, Chinese star anise may be adulterated with Japanese anise. The prevalence of such adulteration highlights the importance of vigilance and quality control measures in the food industry to ensure the integrity and safety of products. Sources of adulterants [11].

Coffee stands as a widely consumed food item with significant economic significance for the countries involved in its production and export. Coffee adulteration involves the common practice of blending expensive coffee beans with more affordable ones. The adulteration of roasted coffee serves as a tactic aimed at cost reduction. [30] Visual inspection can distinguish the beans, but once ground and roasted, differentiation becomes challenging. Additionally, high-quality and rare coffee varieties, like Kona coffee, Blue Mountain, Tanzanian Peaberry, and Kopi luwak, may be substituted with cheaper alternatives. Adulteration extends to foreign fillers and byproducts mixed with pure coffee products. Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffea canephora*) are the two commercially significant species, differing in quality [31–33].

1.6 Permitted colours or dye adulterants in food sources

Traditional applications of synthetic dyes have presented a significant challenge to the global environment, particularly evident in the pollution of ecosystems through their presence in textile effluents over the past few decades. The harmful and non-biodegradable characteristics of these dyes pose substantial risks to soil fertility, crop yield, and human well-being. Consequently, the focus has shifted towards bio-dyes or natural dyes, which are economically viable, environmentally friendly, and non-toxic colourants derived from plants, animals, and microbes, suitable for textile dyeing [34].

Increasingly, synthetic dyes, crafted from organic compounds, are assuming crucial roles in contemporary society, finding applications not only in industrial sectors such as the paint industry but also in scientific laboratories for purposes like fluorescent tracers and photoredox catalysts. Chemists have devised a multitude of strategies to streamline the synthesis of intricate synthetic organic dyes. A variety of dyes, including anthraquinones, aryl amines, azo dyes, BODIPY, carbazoles, cyanines, fluoresceins, oxazines, phenothiazines, rhodamines, squaraines, thiophene dyes, etc [35]. Synthetic dyes are occasionally termed 'coal tar dyes' because they are produced from materials traditionally derived exclusively from coal tar. These substances are all derived from the hydrocarbon benzene (C_6H_6), a hexagonal arrangement of 6 carbon atoms, each bonded to a hydrogen atom, forming the foundational structure. Coal tar dyes are synthetic colourants derived from coal tar, which is a byproduct of the coal-to-coke process. These dyes are part of a large group of chemicals known as coal tar colours or coal tar dyes, and they have been historically used as colour additives in various products, including textiles, cosmetics, and foods. Several significant biological stains, like carmine and hematoxylin, are part of the natural group dyes. Dyes can be categorised into acid and basic groups, and a blend of these groups has the potential to create a stain that is neutral. Trusted producers and suppliers of biological stains employ quality assurance procedures to assess the effectiveness of their products, aligning with the protocols recommended by the Biological Stain Commission in the United States [36].

Azo dyes, essential synthetic colourants employed across diverse industries, contribute to environmental inefficiency during the fabric dyeing process. This inefficiency arises from the release of around 10%–15% of the dyes into the environment, resulting in the generation of intensely coloured wastewater [37].

1.7 Examples of coal tar dyes include

Tartrazine (Yellow #5)

A yellow dye commonly used in food products, beverages, and medications.

Allura Red AC (Red #40)

A red dye widely used in foods, beverages, and pharmaceuticals.

Brilliant Blue FCF (Blue #1)

A blue dye used in various products, including food and cosmetics.

Quinoline Yellow, a synthetic dye employed as a colouring agent in confectionery, soft drinks, and various food and beverage items, is sanctioned for food use in Australia and Europe but remains prohibited in the United States and Canada. The inclusion of Quinoline Yellow in food products has become a subject of recent controversy, sparked by the McCann study, which suggested a potential connection between hyperactivity in children and their dietary exposure to a combination of colours, including Quinoline Yellow [3].

2. Research investigation

Case study

Adulterated Blueberry juice

Concerns regarding food products' quality and authenticity have grown recently, and food adulteration is now a common problem. This case study explores the adulteration of blueberry juice and reveals the employment of dishonest techniques that may be harmful to one's health with consumption of such juices from the market place.

Adulteration components

The adulteration involves substituting blueberry extract with an artificial colourant, leading to potential stomach discomfort upon excessive consumption. The colourant is ingeniously blended with a matrix of banana peel scrape-out powder to form the pulp. Basil seeds, saccharin (a sugar substitute), and artificial flavour and essence further contribute to the deceptive composition.

Medical concerns

The consequences of consuming this adulterated blueberry juice are not trivial. The artificial colourant used in the adulteration has been linked to stomach discomfort, especially when consumed in large quantities. Saccharin, being a sugar substitute, may have implications for individuals with specific health conditions, and the use of artificial flavours raises concerns about the overall nutritional value of the beverage.

Case analysis

A notable incident involved an individual falling sick after consuming a supposedly reputable blueberry juice from a well-known market. This case raises questions about the integrity of the supply chain and the adequacy of regulatory measures for ensuring food safety.

Adulteration methods

The blueberry pulp, a key component of the juice, was found to be adulterated with *Ziziphus mauritiana* pulp instead of authentic blueberry. The inclusion of banana peel scrape-out powder as a forming agent in the adulteration of blueberry juice can potentially lead to severe stomach discomfort. This is attributed to the cellulose and fibre content of the banana peel, along with certain starchy and inedible components. The ingestion of such a concoction introduces a combination of indigestible elements, contributing to gastrointestinal distress and the health risks associated with deceptive food practices.

This substitution not only undermines the nutritional value but also indicates a deliberate effort to deceive consumers.

The introduction of an artificial colour with the potential to visibly stain vomit and remaining food material imparts a pronounced blue hue, leaving a lasting impression. This conspicuous colouration extends to the inner portions of the mouth, notably the tongue, intensifying the visual impact. Beyond the aesthetic concern, such robust and persistent colouring raises alarming health considerations. The ingestion of a substance with such potent colouring agents poses a risk of adverse health effects, possibly leading to significant and detrimental health conditions for the individual.

The afflicted individual not only endured stomach discomfort but also reported urinary issues. (The affected individual also complained of discomfort during urination, highlighting an additional dimension to the health implications of consuming the adulterated blueberry juice. including a noticeable light bluish colouration in the discharged urine.) This additional manifestation of discolouration in bodily excretions suggests a systemic impact, indicating that the adulterated blueberry juice may have repercussions beyond gastrointestinal distress, affecting various physiological processes and reinforcing concerns about the potential health hazards associated with the deceptive consumption of such market consumable products.

In summary, the present case study highlights the significance of strict quality control and regulatory measures aimed at protecting customers against deceptive food practices. In addition to compromising the product's quality, adulterating blueberry juice with artificial colourants, sugar alternatives, and fruit pulp substitutes may be harmful to your health. It is a harsh reminder of the need for increased attention to detail and oversight in the food business in order to guarantee customer safety and well-being.

Generally from a study report; that measured the amount of flavonoids excreted in the urine and the serum levels after eating guava, pineapple, and pomelo using liquid chromatography-mass spectrometry (LC-MS/MS). After an overnight fast and a 24-hour diet free of flavonoids, 200 g of fresh fruit were given to healthy volunteers in separate groups. The results show that only the glucuronic-conjugated metabolites of myricetin, kaempferol, luteolin, and quercetin were detected after fruit consumption [38].

3. Research methodology

3.1 Case study on adulterated blueberry juice: 1. Goal

Examine and assess the adulteration of blueberry juice, paying particular attention to the ingredients and potential health hazards.

3.2 Statistical measure

A statistical measure of people who consumed blueberry juice at a juice center could involve various metrics depending on the specific information. [39]

3.3 Percentage of total customers

This measure would represent the proportion of customers who chose blueberry juice out of the total number of customers at the juice center. It is calculated as:

Percentage

$$\text{Percentage} = \frac{\textit{Number of Blueberry Juice Consumers}}{\textit{Total Number of Customers}} \times \mathbf{100} \quad (1)$$

3.4 Frequency distribution

This involves categorising the number of people who consumed blueberry juice into different ranges (e.g., daily, weekly, monthly) and creating a frequency distribution table.

3.5 Average consumption per customer

Calculate the average number of blueberry juice servings per customer. This would give an idea of the typical consumption pattern.

3.6 Time series analysis

Analysing the data over time to identify trends or patterns in the consumption of blueberry juice. This could involve daily, weekly, or monthly breakdowns.

3.7 Demographic analysis

The data based on demographics (age, gender, etc.) to understand which groups are more likely to consume blueberry juice.

3.8 Correlation with other factors

Correlation between the consumption of blueberry juice and other factors like the time of day, weather, or promotional activities. Table 6.

3.9 Vital statistics

Vital statistics refer to numerical data collected and analysed regarding vital events in a population, primarily encompassing births, deaths, marriages, and divorces. These statistics provide crucial information for understanding demographic trends, health patterns, and population dynamics. Vital statistics play a significant role in public health planning, policy formulation, and demographic research, offering insights into the overall well-being and structure of a population.

3.10 Death rate and ratio

The total number of regular population visiting and have the blueberry juice was regularly studied and statistically tally marked for the presence.

The study produced results that could be further documented and predicted for the analysis of the severity of the adulterant.

Annual crude death

Total number or deaths during the year/ total population as of mid of the year. k

Annual crude death

Annual crude death

$$rate: = \frac{\text{Total number or deaths during the year}}{\text{total population as of mid of the year.}} \times k \quad (2)$$

3.11 Correlation coefficient r

The correlation coefficient "r" is a statistical measure that quantifies the strength and direction of a linear relationship between two variables.

It ranges from -1 to 1, where:

i. -1 indicates a perfect negative linear relationship,

ii. 0 indicates no linear relationship, and

iii. 1 indicates a perfect positive linear relationship.

The correlation coefficient is used to assess how closely the data points in a scatter plot align with a straight line, indicating the degree of association between the variables.

3.12 Chemical and scientific laboratory investigations

In the chemical laboratory, scientists and researchers carefully plan experiments to support their technical discoveries and inquiries. Therefore, chemical laboratory investigations are organised carefully in order to understand the complexities of molecular interactions, compositions, and transformations. These studies apply chemical principles.

In this laboratory experimental investigation, a comprehensive analysis was undertaken to scrutinise the dyes commonly employed for artificially colouring food materials. The study involved a meticulous examination of these dyes through various chemical analysis techniques and methodologies within the laboratory setting. The investigation aimed to assess the safety, authenticity, and adherence to regulatory standards of these colourants in the food industry. By employing rigorous laboratory findings and methodologies, the study provided critical insights into the chemical nature of food dyes, contributing valuable information for both scientific understanding and regulatory considerations in the food sector.

4. Result

4.1 Examination and assessment data for the people who consumed blue berry juice.

A statistical measure of people who consumed blueberry juice at a juice center could involve various metrics depending on the specific information(**Table 1**).

Table 1. Statistical measure.

Parameter	Total count
Juice consumers	Count of the consumers
Blueberry consumers	46
Total consumers	102

4.2 Percentage

Percentage = (46/102) ×100 **(data from Table 1)**

Calculating the percentage:

Percentage = 0.45098×100 Percentage = 0.45098×100

Percentage ≈ 45.1%

Therefore, the percentage of total customers who chose blueberry juice out of the total number of customers is approximately 45.1%.

4.3 Frequency distribution

Table categorises the number of people who consumed blueberry juice into different ranges, specifically daily, weekly, and monthly, along with the corresponding frequencies (**Table 2**). (**Figure 1**)

Table 2. Frequency distribution table for the given data on blueberry juice consumption.

Frequency Range	Number of Consumers
Daily	50
Weekly	347

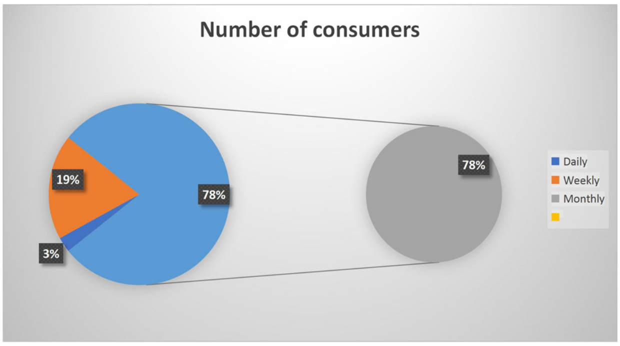

Figure 1. Representation of number of consumers on daily, weekly, monthly biases.

4.4. Average consumption per customer

To calculate the average consumption of blueberry juice per customer, with an applicable formula: (3)

Average consumption per custome r=*Total consumption/Number of customers* (3)

Given that the average consumption is 250 ml of juice and the number of consumers in each category (daily, weekly, monthly), the total consumption can be calculated as follows:

4.5 Total consumption

Total consumption = (Daily consumers × Daily consumption per customer) + (Weekly consumers × weekly consumption per customer) + (Monthly consumers × Monthly consumption per customer) (4)

4.6 Substitute the given values

a. **Total Consumption**=(50×250ml)+(347×250ml)+(1457×250ml)

b. **Total Consumption**=12500ml+86750ml+364250ml

c. Total Consumption=463500ml.

4.7 To find the average consumption per customer use the formula

Average consumption per customer = *Total consumption/Number of customers* (5)

Given that there are 50 + 347 + 1457 = 1854 customers in total:

Average consumption per customer = 463500 ml/1854

Average consumption per customer ≈ 250 ml

Therefore, the average consumption per customer is approximately 250 ml.

4.8 Time series analysis

i. Daily consumption (per day):
   a. 50 consumers daily consumption per day.

ii. Weekly consumption (per day):
   a. To obtain a daily equivalent from the weekly data, you can divide the weekly consumption by the number of days in a week.
   Assuming a 7-day week: 347 consumers7 days/week≈49.57 consumers/day7 days/week347 consumers≈49.57 consumers/day

iii. Monthly consumption (per day):
   a. Similarly, to obtain a daily equivalent from the monthly data, you can divide the monthly consumption by the average number of days in a month.
   Assuming an average of 30 days in a month: 1457 consumers30 days/month≈48.57 consumers/day30 days/month1457 consumers≈48.57 consumers/day

4.9 Demographic analysis

Age Below 15

Consumers in this age group who consume up to 15 units of blueberry juice.

Age Above 15

Consumers in this age group who consume up to 30 units of blueberry juice.

4.10 Correlation with other factors

Base consumption (without Rain or Winter)

Based on the previous information, let's assume a base consumption of 250 ml per customer.

Reduction during rain

There is a 15% reduction in consumption during rain. Therefore, the adjusted consumption during rain would be: Adjusted Consumption during Rain=Base Consumption×(1−Reduction Percentage)Adjusted Consumption during Rain=Base Consumption×(1−Reduction Percentage) Adjusted Consumption during Rain=250 ml×(1−0.15) Adjusted Consumption during Rain=250ml×(1−0.15) Adjusted Consumption during Rain≈212.5 ml Adjusted Consumption during Rain≈212.5 ml.

Reduction during winter

Similarly, there is a 15% reduction in consumption during winter. Therefore, the adjusted consumption during winter would be: Adjusted Consumption during Winter=Base Consumption×(1−Reduction Percentage) Adjusted Consumption during Winter=Base Consumption×(1−Reduction Percentage) Adjusted Consumption during Winter=250 ml×(1−0.15)Adjusted Consumption during Winter=250ml×(1−0.15) Adjusted Consumption during Winter≈212.5 mlAdjusted Consumption during Winter≈212.5 ml.

This analysis accounts for a 15% reduction in blueberry juice consumption during both rain and winter.

4.11 Vital statistics

Death rate and ratio

The study of statistical measures revealed that 30 people regularly visited and had blueberry juice very often, with a frequency of 10 in a month's time. The number of health issues reported by them was very common, such as: vomiting, at a frequency of 1; the expected reasons known were excess drinking and eating. However, weight gain could be observed in such people, who were seen with submental fullness and also had swollen faces as a result of visual study. Some of them with shy havior thus reported having dysuria as a generalised micturition habit that was discussed, as their complaints usually had no idea of the cause or were unreported.

Annual crude death

$$\boldsymbol{rate}: = \frac{\text{Total number or deaths during the year}}{\text{total population as of mid of the year.}} \times k = \frac{0}{30} \times 1000$$

Annual crude death $\boldsymbol{rate}: = 0$ (6)

4.12. Correlation coefficient r

The sample data collected for dysuria as micturition habit, and increased systolic blood pressure (mmHg), in a pool of 14 patients who visited doctor upon their complaint about unusual micturition habits. About 10 patients were, prescribed medicines with measure of weight and check up of their, systolic blood pressure. And four patients were simply prescribed with medications and were as to have simple lemon home made lemonade or orange juice as a cure. The cause of dysuria may be various depending on the cause such as: bacterial infection or due to cationic release in the urinary track, that is sensitive to the cationic groups. ***(Chemical irritants:*** *Certain substances, such as harsh soaps or bubble baths, can irritate the urethra and cause dysuria.)*

Tabulated summary of the generalised causes of dysuria a significant medical condition that requires prompt medical attention, before the case worsens（**Table 3**）.

A prescription analysis of patients with dysuria condition and were prescribed with medicine, have a level of pain been examine at the level of mild to severe. The threshold at which physiological or psychological effect begins to be produced were categorised at 3 levels: mild, moderate and severe.

Mild is denoted with English numeral one, moderate is denoted with English numeral two, and severe is denoted with English numeral 3. The scores were determined by examining the prescription or diagnosis of the patient medicinal case report or through report history. The patient diagnosed with mild pain was given score of 1 and the patient diagnosed with severe pain was scored with English numeral 3, rest of them were moderate and were scored with English numeral 2.

The data of sysuria and systolic blood pressure in mmHg obtained from the prescriptions have been quoted(**Table 4**).

Table 3. Tabulated summary of the generalised causes of dysuria a significant medical condition that requires prompt medical attention, before the case worsens.

Cause	Description
Urinary Tract Infections (UTIs)	Infections affecting the urinary tract, including the bladder (cystitis) or urethra, often caused by bacterial infections.
Sexually Transmitted Infections (STIs)	STIs such as gonorrhea or chlamydia can cause inflammation and pain during urination.
Urinary stones	Kidney stones or bladder stones may cause irritation and pain as they pass through the urinary tract.
Prostatitis	Inflammation of the prostate gland, often due to infection, leading to dysuria in men.
Interstitial cystitis	A chronic condition involving inflammation of the bladder lining, causing pain during urination.
Urethritis	Inflammation of the urethra, resulting from infections or irritants, contributing to dysuria.
Vaginal infections	In women, infections like yeast infections or bacterial vaginosis can cause painful urination.
Bladder dysfunction	Conditions like overactive bladder or interstitial cystitis can lead to dysuria.
Trauma or injury	Injuries to the urinary tract, such as those from catheterisation or surgery, may result in painful urination.

Table 4. The data of sysuria and systolic blood pressure in mmHg obtained from the prescriptions have been quoted.

Sr. no of patients	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Dysuria.	1	3	3	3	2	2	1	1	1	1
Systolic blood pressure (mmHg)	130 mmHg	150 mmHg	148 mmHg	166 mmHg	140 mmHg	142 mmHg	131 mmHg	132 mmHg	134 mmHg	128 mmHg

Measuring the central tendency of the data

The mean (average) of the systolic blood pressure values can be calculated. The given data represents the systolic blood pressure for 10 patients experiencing dysuria (**Table 4).**

$$\text{Mean} = \frac{Sum\ of\ all\ values}{Number\ of\ values} \tag{7}$$

$$\text{Mean} = \frac{130+150+148+166+140+142+131+132+134+128}{10}$$

$$\text{Mean} = \frac{130+150+148+166+140+142+131+132+134+128}{10} = {}^{1401}/_{10}$$

Mean=140.1mmHg

Therefore, the mean systolic blood pressure for the given data is 140.1 mmHg.

The mode and median for the given systolic blood pressure data, was calculated for the provided values in such order as below:

Values: 128,130,131,132,134,140,142,148,150,166.

Median:

Since there are 10 values, the median will be the average of the 5th and 6th values.

Median=140+142/2

Median= 282/2

Median=141 mmHg

Mode:

The mode is the value(s) that occur with the highest frequency. In this case, all values appear only once, so there is no mode.

Therefore, for the given systolic blood pressure data:

Median: 141 mmHg

Mode: There is no mode.

To calculate the mean, median, and mode for the given values of patient suffering from dysuria: (Table 4).

The data was arranged as such: 1,3,3,3,2,2,1,1,1,1.

Mean (Average):

$$\text{Mean} = \frac{Sum\ of\ all\ values}{Number\ of\ values} \tag{8}$$

$$\text{Mean} = \frac{1+3+3+3+2+2+1+1+1+1}{10}$$

$$\text{Mean} = \frac{18}{10}$$

Mean=1.8

Median:

Since there are 10 values, the median will be the average of the 5th and 6th values when the data is sorted statistically.

Data: 1,1,1,1,2,2,3,3,3,3.

Median=$\frac{2+2}{2}$

Median = 4/2

Median = 2

Mode:

The mode is the value(s) that occur with the highest frequency. In this case, the mode is 1, as it appears most frequently.

Therefore, for the given values of Dysuria:

Mean: 1.8

Median: 2

Mode: 1

Correlation coefficient r

Patients records of dysuria and systolic blood pressure (mmHg)(**Table 5**).

$$\textbf{Mean } \bar{X} = \bar{X} = \Sigma X_i/n \tag{9}$$

$$\textbf{Mean } \bar{Y} = \bar{Y} = \Sigma Y_i/n \tag{10}$$

Mean $\bar{X}$ = 1.8 (as it is not an integer, the value of A is 2) (data from Table 5.)

Mean $\bar{Y}$ = 140.1 mmHg (as it is not an integer, the value of B is 140 mmHg) (data from Table 5.)

So, the correlation coefficient r can be calculated using following formula mathematically:

$$r = \frac{n.\sum dxdy - \sum dx.\sum dy}{\sqrt{n.\sum dx^2 - (\sum dx)^2}.\sqrt{n.\sum dy^2 - (\sum dy)^2}}$$

$$= \frac{10\times 89 - 2\times 9}{\sqrt{10\times 8\times 4}\sqrt{10\times 1265\times 81}} = \frac{872}{319.678} \approx 2.724 \tag{11}$$

The correlation coefficient is non negative.

The determined rank coefficient of correlation is r ≈2.724 (calculated from data given in Table 6.)

The Pearson r is usually rounded off to two decimal places. Because r = 2.72, that is 3; it means that there is strong positive correlation between X and Y, that is *Dysuria* and Systolic blood pressure (mmHg).

Table 5. Data table results of performed outcomes.

Sr. no of patients	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Dysuria.	1	3	3	3	2	2	1	1	1	1
Systolic blood pressure (mmHg).	130 mmHg	150 mmHg	148 mmHg	166 mmHg	140 mmHg	142 mmHg	131 mmHg	132 mmHg	134 mmHg	128 mmHg

Table 6. Correlation calculation performed and denoted as below in the tabular format.

X	Y	dx=x-A=x-2	dy=y-A=y-140	dx2	dy2	dx.dy
1	130	-1	-10	1	100	10
3	150	1	10	1	100	10
3	148	1	8	1	64	8
3	166	1	26	1	676	26
2	140	0	0	0	0	0
2	142	0	2	0	0	0
1	131	-1	-9	1	81	9
1	132	-1	-8	1	64	8
1	134	-1	-6	1	36	6
1	128	-1	-12	1	144	12
-	-	-	-	-	-	-
18	1401	Σdx=-2	Σdy=-9	$\Sigma dx^2 = 8$	$\Sigma dy^2 = 1265$	Σ dx.dy = 89

Therefore, the study implies a robust positive correlation between X and Y, specifically Dysuria and Systolic blood pressure (mmHg). That means that it is on the reason that the increased in Systolic blood pressure due to Dysuria is a non-bacterial or viral response or as considered point i to ix in **(table 3)** but is a response to the increased in the chemical contaminant, that is the cations type of contaminants that can delay and give dysuria to the person is clear from the above correlation data.

So through the prediction it can be stated that it can be cationic colouring agents:

Example crystal violet: A cationic dye is commonly used. Or,

Methylene Blue: Another cationic dye often employed for highlighting may have been used accidently instead of permitted colours.

Other natural colourants can be sweetened Indigo in the food. (As an adulterant or contaminant).

Therefore, a laboratory chemical investigation was focused to identity such dye material that can harm the body and its detection.

4.13 Chemical and scientific laboratory investigations

A test to identity such adulterated juices with dye instead of fruit juices was studied by analysing it by protonation to form conjugate acid of the particular base.

An example of such type includes NH_3+protonation→NH_4^++Cl^-

The outcome of the conducted test on the standard substance reveals a distinctive colouration indicative of protonation, both in the standard sample and in the adulterated fruit juices. This observation serves as confirmation for the presence of artificial dyes, particularly in significant quantities or those of a potentially toxic nature. To establish a standard, all accessible grades and brands of dyes underwent the test, while a similar examination was conducted on the adulterated juices. The findings distinguish between adulterated fruit juices, which lack specific branding and may be potentially unhealthy, and those that are deemed safe for consumption.

The test results indicate a noticeable change in colouration, transitioning from blue to curdy red, suggesting the presence of artificial dye. This stark colour difference was observed in contrast to the blueberry extract, which was also subjected to testing but did not exhibit a similar colouration. The distinct colour change further confirms the presence of artificial dye in the sample, highlighting a divergence from the natural colour properties observed in the blueberry extract (**Figure 2).**

Figure 2. Test results showing positive results for the verification of tampered fluids/juices.

5. Discussion

A multidisciplinary approach including statistical measurements, time series analysis, demographic profiling, correlation analyses, and critical statistics was used in the extensive case study on contaminated blueberry juice.

Understanding consumer preferences was aided by the statistical metrics' insights into consumption trends. A more sophisticated understanding of the elements driving consumption was provided by correlation assessments, whereas demographic analysis identified possible marketing target groups. Vital statistics highlight the importance of quality control because they have an impact on health.

The correlation between dysuria and systolic blood pressure, supported by chemical testing, hinted at potential health risks associated with cationic contaminants. The laboratory method presented, involving protonation tests, emerged as a practical and cost-effective means of identifying dye adulterants.

The findings discuss the importance of stringent quality control measures in the food industry to ensure consumer safety and well-being.

6. Conclusion

The laboratory investigation or the study was successful in identifying fruit juices that had been tampered with and had artificial colouring agents rather than the original fruit pulp. Such juices are visually appealing and can't be identified visually or with taste and therefore have to undergo chemical tests. The distinct colour shift—especially from blue to curdy red—is a good way to tell if artificial dye is present. This information is essential for differentiating between fruit juices that are safe to consume and those that could be dangerous. It is advised to conduct more oversight to guarantee the security and legitimacy of food items. Laboratory instruments such as: U.V. visible spectrophotometer, colourimeter can be utilised to draw the conclusion with reference to the standard fruit pulp. Without the need for costly, large-scale devices or outside interventions, the present test is practical and appropriate in every setting. When working with large sample sizes, its robustness, affordability, and high accuracy make it an essential chemical test for the accurate identification of colour adulterants. Its importance also goes to being a significant and unique technique for identifying artificial pigments in fruit juices. Further, comparable analyses can be performed on other types of liquids besides blueberries, including strawberry, mango, orange, rose syrup, peach, and watermelon juices. Reciprocal outcomes will follow, indicating modifications in protonation to hydroxyfication. Thus, the protonation test proves to be a valuable tool for the identification of artificial dye adulterants in fruit juices. Its portability, cost-effectiveness, robustness, and accuracy make it a significant chemical test with practical applications. Further exploration of its utility across a range of fruit juices and subsequent standardisation will contribute to its broader adoption in quality control measures for the food and beverage industry.

Author contributions

Both authors, SDB and SSM, contributed to conceptualisation, methodology, formal analysis, perform the manual calculations, investigation, writing (original draft), and visualisation.

Conflict of interest

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