11-2017

Synthetic Food Additives in Selected Beverages Sold In the UAE

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SYNTHETIC FOOD ADDITIVES IN SELECTED BEVERAGES SOLD IN THE UAE

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Environmental Sciences

Under the Supervision of Professor Afaf Kamal Eldin

November 2017

Declaration of Original Work

I, Aysha Mohammed AlDhaheri, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled *"Synthetic Food Additives in Selected Beverages Sold in the UAE*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Afaf Kamal Eldin, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Copy 12 of 13

Abstract

The consumption of beverages is increasing around the world with the globalization of the food industry. Synthetic additives can cause a potential risk to human health when they are excessively consumed. Thus, it is important that the level of synthetic food additives do not exceed the CODEX standards and similar to authorized levels in United Arab Emirates (UAE) by Emirates Authority for Standards and Metrology (ESMA). The objectives of this thesis were to determine and analyze selected food additives in non-alcoholic beverages and to ensure that the quantified results comply with CODEX standards. The present research aimed at screening the beverages and related fruit-based juices sold in the UAE market for their contents of food additives with focus on synthetic coloring agents, artificial sweeteners and preservatives. Thirty (30) different beverage samples from two batches including fruit juice, nectar, drink, soft drink, energy drink, flavored water, malts beverages and fresh juice have been analyzed for their contents of synthetic color, artificial sweetener and preservatives. Seventeen (17) synthetic food additives including eleven food colorants, two sweeteners and four preservatives were analyzed using ultra high-performance liquid chromatography diode array detector (UPLC- DAD). The results indicated that the levels of all additives in the studied samples were within the maximum permitted level (MPL) of the CODEX.

Keywords: Beverages, non-alcoholic beverages, drinks, food additives, synthetic color, artificial sweetener, preservatives, ultra performance liquid chromatography (UPLC).

Title and Abstract (in Arabic)

تحديد المضافات الغذائية في المشروبات المباعة في أسواق دولة الإمارات العربية المتحدة

الملخص

أدت تاثيرات العولمة في صناعة الاغذية الى زيادة استهلاك المشروبات والعصائر حول العالم، واستنادا الى التجارب والبحوث العلمية لوحظ وجود مشاكل صحية عند الافراط في استهلاك المضافات الغذائية والتي تهدد صحة المستهلك. ولذلك وجب التاكد من عدم تجاوز المعدل الآمن والمصرح به في دستور الغذاء كودكس ووجب التاكد من مطابقتها مع المواصفات الاماراتية القياسية. الهدف الرئيسي من هذه الاطروحة: دراسة شاملة لتحديد نوعية وكمية المضافات الغذائية في المشروبات المعلبة المباعة في أسواق دولة الامارات ومقارنة نتائج التجربة لتطابقها مع المقاييس المسموح بها لكل من المضافات الغذائية الموجودة في دستور الغذاء كودكس.

شمل هذا البحث عددا من المشروبات المعلبة المباعة في أسواق دولة الامارات لتحديد نوعية المضافات الغذائية الاكثر استخداما في تصنيع المشروبات مثال على ذلك: الملونات الغذائية والمحليات الاصطناعية والمواد الحافظة.

التجربة تمت بتحليل ثلاث مجموعات من المضافات الغذائية الموجودة في (30) ثلاثين عينة مختلفة تشمل: عصائرالفاكهة، النكتار، المشروبات الغازية، مشروبات الطاقة، الماء بالنكهات، شراب الشعير، عصائر الطازجة... الخ، تم تحليلها بعناية فائقة باستخدام تقنية الكروماتوغرافيا سائلة عالي الاداء (كاشف اللوني اسْتِشْرابٌ سَائِلِيٌّ بالضَّغْطِ العالِي ومَنْظُوْمَةٌ كشف ذات صمام ضوئي) (UPLC)، التي تستخدم لفصل المركبات المخلوطة الى مركبات احادية. وتم التحقق من القياس لتطابق 17 من المضافات الغذائية القياسية، تضم 11 عامل لوني غذائي، واثنان من المحليات الاصطناعية و 4 من المواد الحافظة.

بناء على النتائج العملية في عينات العصائر والمشروبات، تبين أن جميع النتائج كانت ضمن المستوى الآمن والمسموح به في دستور الغذاء.

مفاهيم البحث الرئيسية: المشروبات، المشروبات الغير كحولية، العصائر، المضافات الغذائية، الملونات/ صبغات الطعام، المحليات الاصطناعية، المواد الحافظة للاغذية، طرق التحليل تقنية الكروماتوغرافيا سائلة عالي الاداء (كاشف اللوني اسْتِشْرابٌ سَائِلِيٌّ بالضَّغْطِ العالِي ومَنْظُوْمَةٌ كشف ذات صمام ضوئي) (UPLC).

Acknowledgements

Gratefully Allah, the most Gracious and the Merciful in our life.

I wish to acknowledge all those who have contributed to this thesis. Foremost, I would like to express my sincere gratitude to my co-advisor Dr. Sami Ghnimi for initiating this work.

My deepest gratitude extends to my advisor Prof. Afaf Kamal Eldin and co-advisors Dr. Iltaf Shah, and Dr. Muna Bufaroosha for their valuable guidance, encouragement, support which have been of great help in the completion of this M.Sc.thesis.

My sincere thanks to laboratory specialist Dr. Hassan for his assistance throughout my research. My sincere thank extends to all my friends at the UAEU for their encouragement, motivation, wonderful cooperation and friendship. I would like to acknowledge to all the lectures who taught me in all courses for their guidance towards to complete my graduate studies in UAEU. My thanks are extended to the College of Science, College of Food and Agriculture, College of Graduate studies and the library.

Finally, I would like to express my very profound gratitude to my parents, brothers and sisters for providing me with unfailing moral support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Dedication

To my beloved parents and family

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADFCA	Abu Dhabi Food Control Authority
ADI	Acceptable Daily Intake
CDS	Chromatography Data System
CODEX	CODEX Alimentarius
DAD	Diode Array Detector
DMDC	Dimethyl Dicarbonate
EFSA	European Food Safety Authority
EP	European Pharmacopoeia
ESMA	Emirates Authority for Standards and Metrology
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FD&C Act	Federal Food, Drug and Cosmetic Act
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
GRAS	The Generally Recognized as Safe
INS	International Numbering System
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit of Detection
LOQ	Limit of Quantification
MPL	Maximum Permitted Level or the Highest Level of a Food Additive Permitted in Foodstuff
ND	Not Detected
NNS	Non-Nutritive Sweeteners
RSD	Relative Standard Deviation

SCF	Scientific Committee on Food
SD	Standard Deviation
UAE	United Arab Emirates
UPLC	Ultra Performance Liquid Chromatography
USDA	United States Department of Agriculture
USFDA	US Food and Drug Administration
USP	United States Pharmacopeia
WHO	World Health Organization

Chapter 1: Introduction

1.1 Overview

Processed and packaged foods, and beverages have entered the daily routines for many people in today's world, which encourages people towards an easier approach to food consumption with their accelerated lifestyles. In fact, the consumption of beverages is increasing all over the world with the globalization of the food industry. Beverages are more significant in our daily lives as a means to quench our thirst. Instead of water, many people, especially the younger generation, resort to non-alcoholic beverages in many countries including particularly the United Arab Emirates with arid climatic conditions. Nevertheless, the increasing dependence on these processed food and beverages may have negative effects on food additives' levels in the long run with health being the major concern. Obviously, there is a correlation among processed food and beverage intake, lifestyles and health impact.

Despite awareness of the fact that water is an essential part of the body and metabolism, the taste of a beverage seems more appetizing for the average consumer because of enhanced taste which comes from additives. For this reason, it is imperative to review and search into the health implications of particular additives in beverages today. However, soft drink consumption is still a very controversial issue for health. Although, many studies have been conducted about the possible correlation between beverage intake and physical health, results are not conclusive that there in fact is a correlation (Kregiel, 2015). With more emphasis on healthy life styles and exercise, the new generations worldwide are turning to healthy alternatives as substitutes for soft drinks. With this awareness, there is an increase in learning about the ingredients of their food and beverages. However, exact knowledge about food safety especially, the topic of food additives might not be available to the consumer. In other words, the consumer may not be aware of the specific ingredients in the label packaged and\or processed either food or beverage. This is valid especially for international foods as certain additives are allowed in the country of origin of the food or food ingredients might not be accepted or allowed in the country of consumption.

Possible health implications of the consumption of the food additives may have genotoxic or mutagenic disorders leading to increase types of tumor in humans. Studies found that some of the cause mutation cells of bacteria that act as mutagenic and/or carcinogenic agents in humans (Abdelmigid, 2009). However, the excessive addition of preservatives causes heath risk such as chronic intoxication and allergic reaction to pregnant women and children. Therefore, combinations of preservatives are commonly used to prevent the degradation of food should be within the food safety boundary (Xu et al., 2013). According to Kregiel (2015) legislations have been put in place both national and international standards, to ensure that beverage producers are conforming to have consumers trust that the soft drinks they purchase and consume are safe. Food additive safety has received widespread attention recently. While studies conducted attested the overall safety of additives, some studies showed that it should be used within permitted level of additives.

In today’s food industry, food additives are used in most types of processed foods to add color, flavor, taste, texture, increased shelf-life, or certain functionality to prolong the processed food. In addition, proper identification and classification of fruit-based beverages is mandated by law. However, many declared and undeclared food additives including artificial colors, preservatives, and processing-aiding agents are widely used in beverage processing despite the growing demand by consumers for healthy beverage alternatives. In these cases, it is very important that actual fruit content and food additives are all declared label on the package. Regards with Codex defines an additive as any substance added into food that would not be normally consumed as a food by itself and not normally used as a typical ingredient of the food (Codex Alimentarius: CODEX STAN 192-1995). Article “The Safety and Regulatory Status of Food, Drug and Cosmetics colour Additives Exempt from Certification” state that the US Food and Drug Administration (USFDA) established regulations governing and according the uses of colour additives, and the labeling of these products regards the safety of those colour additives has been demonstrated by safety testing programs. Action was well supported with significant toxicological data (Hallagan et al., 1995). In 2008 indicates, a package of regulations that further upgraded the rules in the European Union on food additives, food enzymes and flavorings was adopted. In addition, a regulation establishing a common procedure for their authorization was created (Debeuckelaere, 2015).

Europe has an identification system which was based on “E” numbers for the labeling of the most common additives groups such as antioxidants, colours, emulsifiers, stabilisers, gelling agents, thickeners, preservatives and sweeteners e.g. sorbic acid refers (E200). A common authorization procedure for food additives was established by Regulation (EC) No. 1331/2008 which was evaluated by the European Food Safety Authority (EFSA) by risk assessment, identification hazard, to estimate the exposure to dietary intake and to characterize the risk. EFSA provides scientific opinions and supports advice to adapting European policies and legislation and to the European Commission, European Parliament and European Union (EU) Member States in taking effective and timely risk management decisions commission regulation (EU) No. 257/2010 has set up a programme for the re-evaluation of food additives that was approved before January 2009 in particular, according to the type of food additive, for food colours was set on April 2010, until December 2020 all sweeteners will be approved with different deadlines of each (Regulation (EC) No 1331/2008 of the European parliament and of the council; Colombo et al., 2015).

1.2 Statement of the Problem

Synthetic colorants, preservatives and sweeteners are widely used in beverages to provide the desired appearance, prolonged shelf life, and enhanced flavor and aroma (Ma et al., 2012). Some of these substances may pose a potential risk to human health and can cause allergic reactions to certain populations. As a result, the composition of the ingredients in the food products must be the same as the ingredients listed on the food labels. Therefore, accurate and reliable determining methods for these food additives are necessary for proper use of these additives and assurances for food safety levels (de Andrade et al., 2014). For example, a study conducted in 2003, by the Risk Assessment Section Food and Environmental Hygiene Department in Hong Kong (Risk assessment on artificial sweeteners in beverages, 2003), estimated the exposures to artificial sweeteners from beverages by secondary school students in Hong Kong and assessed the effects on their health studying five artificial sweeteners. It was concluded that exposures to the artificial sweeteners including acesulfame potassium, aspartame, cyclamic acid, saccharin and sucralose from beverages do not pose a health risk to secondary school students in Hong Kong in both average and high consumers. Food control authorities in different countries have the mandate to ensure food safety of foods and beverages. Thereby ensuring that additives are within permitted levels. However, this data is generally not published. Unfortunately, there are relatively few sources of published analytical information concerning the determination of the content levels of these food additives in foodstuff products consumed in the United Arab Emirates (UAE) (Risk assessment on artificial sweeteners in beverages, 2003).

The aim of the present study was to screen thirty (30) different beverages fruit-based juices and related beverages sold in the UAE market for their contents of food additives with focus on synthetic coloring agents, preservatives and sweeteners by using advanced Ultra High Performance Liquid Chromatography (UPLC) with Diode Array Detector analysis (DAD). The safety levels of beverages are detrimental for health and can affect global health and the UAE community. Therefore, the results from this study will be important to the public and food authorities in the UAE and the region.

Research aims to determine whether the percentage of additives in non-alcoholic beverages in the UAE market meet the required safety levels of CODEX-European standards for maximum food additives allowance (Codex Alimentarius, CODEX STAN 192-1995; Commission of the European Communities, 2001).

1.3 Relevant Literature

Beverages are potable liquids, other than water, containing extracts, solutions, or suspensions in water prepared for human consumption. Beverages are generally divided into alcoholic and non-alcoholic beverages with different sub-classifications for non-alcoholic beverages. Beverages are mainly consumed to quench thirst, to feel fresh, and/or to compensate the loss of body fluid due to perspiration. During the last decades, beverages continued to become an important component of our daily diet. Nowadays, many people start their day with a warm nourishing drink (tea, coffee, or chocolate) and refresh themselves throughout the day with different refreshing drinks

(361 Degrees Hospitality, 2014). The beverage industry is growing fast and capturing considerable profit in response to this expanding life-style. Beverages stimulate the palate and act as an aperitif. In the industry of hospitality (restaurants and hotels), non-alcoholic beverages are served to customers either with meals or without. The consumption of non-alcoholic beverages is widespread throughout the globe, processed products are considered to be of great importance not only economically, it has also increased to almost 500% over the past 50 years, especially among the children and teenagers who mostly consume carbonated soft drinks. According to United States Department of Agriculture (USDA) the per capita soft drink consumption in the United States of America (USA) has increased over time (Kostik, 2014).

Beverages and soft drinks take an important part in the total daily intake of beverages with their content of food additives. Therefore, the constant monitoring of their presence in non-alcoholic beverages is needed to ensure compliance with food safety regulations as well as for calculating risk assessment (2012) (361 Degrees Hospitality, 2014).

Over the past years, the consumption of non-alcoholic beverages has grown by about 3.6 percent per year. The total market for commercial beverages was approximately 1.6 trillion liters $\cong$ (565,034,667 tonnes) in 2009, equivalent to (0.0816 tonnes) 231 liters per capita per year. The leading beverage category in market share in 2009 was hot tea at (20.9%), followed by bottled water (15.3%), carbonated soft drinks (12.5%), hot coffee (8.2%), juices/nectars (7.15%), milk (4%), fruit drinks (2.7%), other (2.6%) and flavored milk (0.9%) as shown in Figure 1 (Bailey, 2014).

Kreigel (2015) claimed that the target markets for functional beverages are diverse, and products are often tailored towards particular target markets, for instance, according to age and gender, with a growing focus on children, women and seniors. The non-alcoholic beverage market is expected to grow from roughly $160 billion in 2008 to almost $190 billion by 2020. The relative share of different non-alcoholic beverage categories in the global market is shown in Figure 1 (Bailey, 2014).

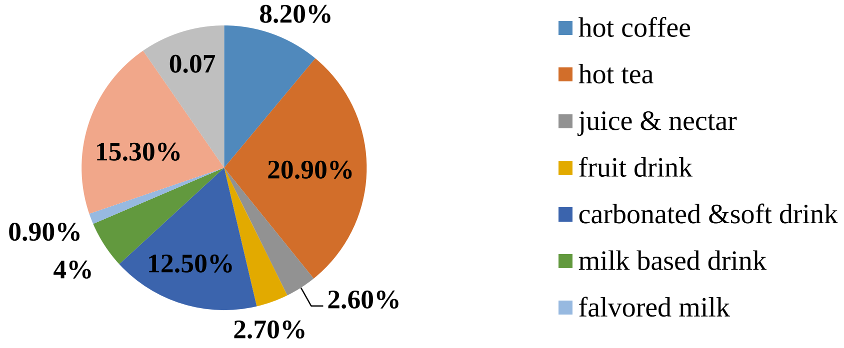


Figure 1: Share of Global Beverage Market, adapted (data from Bailey, 2014)

Similar studies have been conducted in the UAE. The statistical reports from the Department of Finance in Abu Dhabi Emirate have valued data on exported and imported beverages in Abu Dhabi, the capital of the UAE, from 2014 to 2016 showing that the UAE imported 126,143.552 tonnes of beverages, but only exported 35,523.758 tonnes leaving a balance of 90,619.794 tonnes in 2014. In 2015, the UAE imported 142,853.209 tonnes while exporting 50,155.689 tonnes with a balance of 92,697.52 tonnes. Similarly, 139,467.796 tonnes are exported and 50,668.526 tonnes are imported with a balance of 88,799.27 tonnes in 2016. The average between import and export of beverages per kg are around 88,000-92,000 tonnes which is the amount of beverage consumption in Abu Dhabi as represented in Figure 2 (The Department of Finance government Abu-Dhabi, 2016).

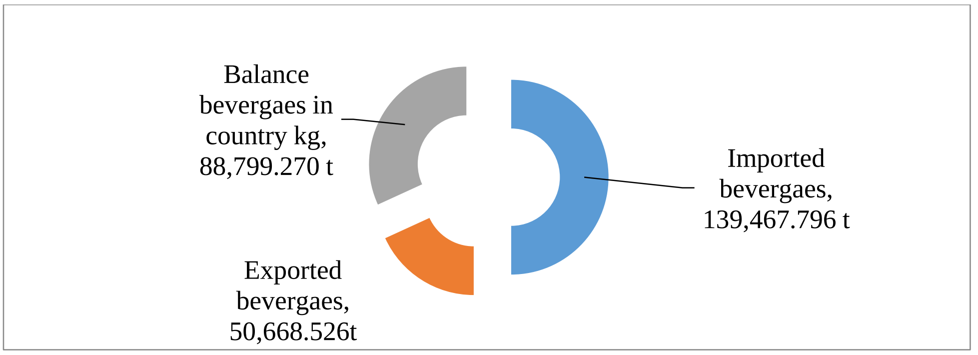


Figure 2: The Balance Beverages Equal Consumption Amount in Abu-Dhabi by tonnes in 2016 (data from The Department of Finance, Abu-Dhabi, 2016)

Regarding data relieved to the types of beverages imported to the UAE, it can be seen that non concentrated mixed juices have the highest percentage with almost eighty five million tonnes followed by natural mineral water of approximately thirty seven million tonnes in 2016. The total tonnes of beverages imported indicate a very high weighing about 140,000 tonnes. Table 1 shows the different categories of beverages imported into the Abu Dhabi, UAE for year 2016. Orange juice, lemon juice, mango juice, mixed juices, and mineral water with flavor or sweetener which were used in this study are listed in Table 1.

Table 1: Imported Beverages (diluted and concentrates) into the Border of Abu Dhabi, UAE in 2016

No	Types Beverages	Weight tonnes	No	Types Beverages	Weight tonnes
1	**Mixture juice not concentrated** **Mixture juice other**	84,791.144 8,802.239	**10**	**Ordinary water**	76.068
2	**Natural mineral water**	39,754.465	**11**	**Citrus fruit** **Citrus fruit other**	17.137 17.342
3	**Orange juice prix > 20 Orange juice other**	19.642 1,990.284	**12**	**Mineral water flavored or sweetened**	26.586
4	**Mango juice not concentrated** **Mango juice other**	2,305.760 554.442	**13**	**Mineral water other**	22.145
5	**carrot juice not concentrated** **carrot juice other** **carrot juice other 2**	116.795 0.247 7,332.980	**14**	**Lemon juice**	17.317
6	**Apple juice** **Apple juice other**	0.716 644.002	**15**	**Pineapple juice** **Pineapple juice other**	17.788
7	**Guava juice not concentrated** **Guava juice others**	40.708 190.842	**16**	**Grape fruit**	7.402
8	**Cranberry juice**	102.187	**17**	**Aerated water**	6.157
9	**Artificial mineral water**	83.979	**18**	**Grape juice** **Grape juice other**	3.916
Total beverage weight : approximately 140,000 tonnes					
Source data from the Department of Finance, Abu Dhabi, 2016					

1.3.1 Classification of Non-Alcoholic Beverages

Non-Alcoholic Beverages, potable drinks, are divided into three sub-groups as being thirst quenching: stimulating, refreshing, and nourishing beverages presented in Figure 3. The non-alcoholic beverage category includes fruit and vegetable juices, fruit drinks, carbonated soft drinks, tea, coffee and bottled flavored water etc. (361 Degrees Hospitality, 2014). Regarding alcoholic beverages (CODEX STAN 192-1995), made distilled spirituous beverages including alcohol-free and low-alcoholic counterparts a low-alcoholic beverage (<1% alcohol), a sweet alcoholic beverage (<10% alcohol) and the alcoholic beverages should contain 15% up to 24% of alcohol. In the USA, non-alcoholic beverages should contain less than 0.5% alcohol by volume. While in the UAE, the range for ethanol (alcohol) should not exceed 0.3% in the final product, as an example in energy drink standardized by Emirates Authority for Standards & Metrology (ESMA) (ESMA: Energy Drink. UAE.S 1926:2015).

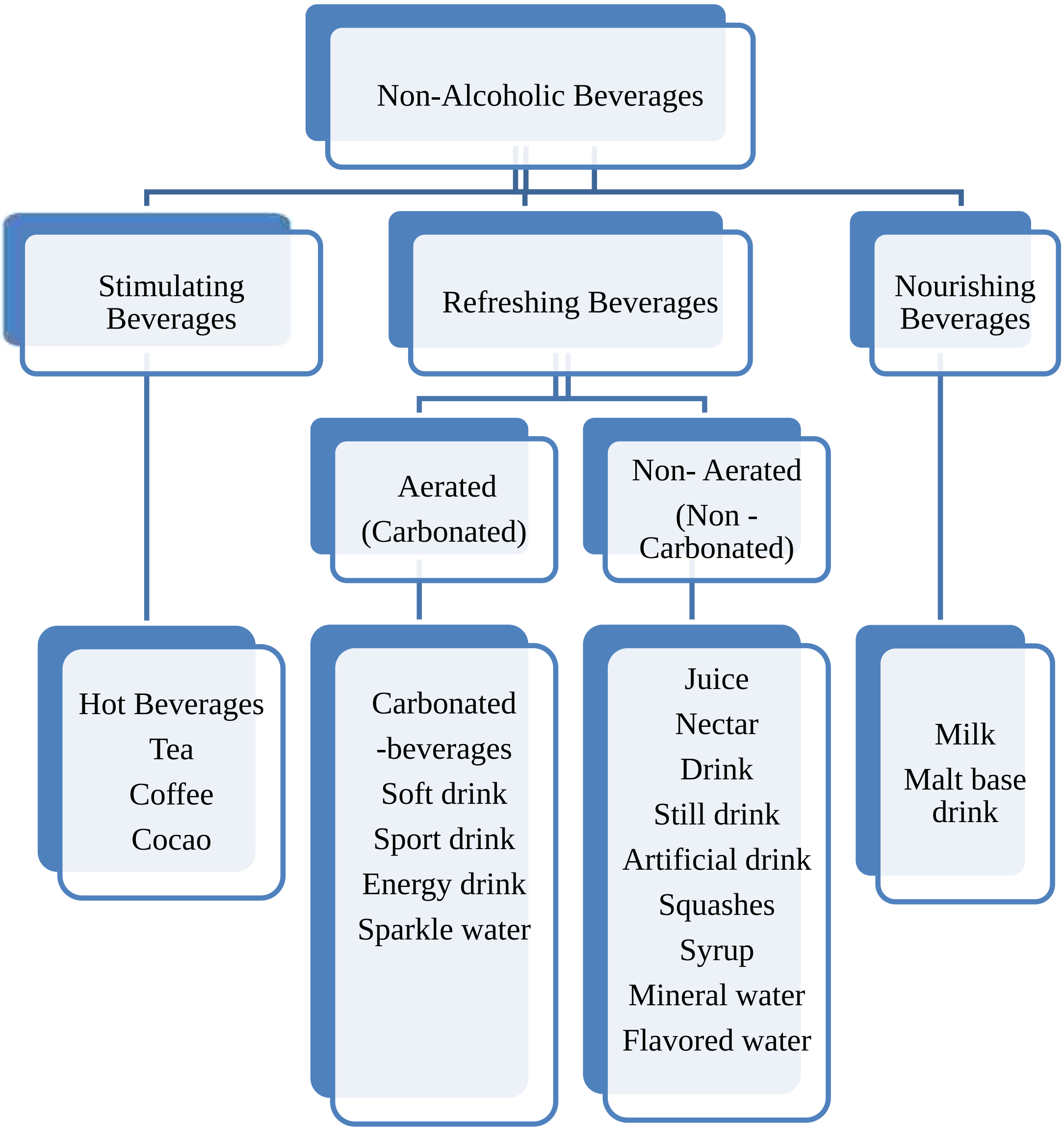


Figure 3: Classification Scheme for Non-Alcoholic Beverages (Note on Non-Alcoholic Beverage)

1.3.1.1 Refreshing Beverages

These are beverages that refresh our body and can be categorized as aerated waters or non-aerated waters as explained below:

- Soft drinks and Aerated beverages: This category includes waters that are charged or aerated with carbonic gas and comprise a combination of water, gas, sugar, and artificial essence. Soda water (carbonated and tonic water), colas

(Pepsi, Coca cola), oranges/lime (Miranda, Fanta, 7 Up, Lime), tonic water, etc. Soft drinks are hygienically canned and can be consumed as chilled, hot, bottled, canned, or open liquids in the form mineral water, juices, squashes, syrups, smoothies, shakes, etc. Mineralized and vitamin-fortified water beverages belong to this category (Note on Non-Alcoholic Beverage).

Kregiel (2015) article state that Sports drinks are products described as “isotonic,” “hypertonic”, or “hypotonic”, still or carbonated, ready to drink, or non-ready to drink powders and concentrates, as well as fruit and non-fruit flavored drinks, whereas, Energy drinks are as the name suggests energy enhancing drinks, mostly carbonated and contain taurine, guarana, glucose, caffeine, exotic herbs and substances and minerals and vitamins (Kregiel, 2015).

Soft and aerated beverages differ with respect to their contents of sugar or sweeteners, colorants, preservatives, and flavoring agents. Soft drink makers also use non-nutritive or artificial sweeteners such as aspartame, acesulfame potassium, saccharin, cyclamate, and sucralose (Kregiel, 2015).

- Juices and Nectars: Juices and nectars are different in the content of fruit juice in the packaged beverage. Juices can be bottled or canned. Juices are 100% pure fruit or vegetable juice without any ingredients other than the permitted minerals and vitamins including less than 2% sweetening agents (Kregiel, 2015).

In other words, juices can legally contain small amounts of added sugar as well as other additives. To protect or stabilize the commercial product, e.g. vitamin C is added to apple juice to stop it from turning brown and protect its natural antioxidants. Juices are prepared, from one or mixed fruits, by suitable processes that maintain the essential physical, chemical, organoleptic, and nutritional characteristics of the fruit from which they came. Juices are pure products with no preservatives, sweeteners, or artificial colors.

Types of juices

(i) Fresh juices: directly obtained from fruit and may or may not contain pulp of the fruit. Examples of fresh juices include orange, mango, grapefruit, pineapple, lime, and tomato juice.

(ii) Reconstituted juices: are diluted from juice concentrates at a bottling plant to prepare juices similar to the original condition regarding the concentration of soluble solids in water. Initially, fruit and vegetable juices are concentrated at low temperatures under vacuum in order to concentrate a product for storage and for shipping to different parts of the world.

(iii) Not-from-concentrate juices (NFC): these are juices that are directly obtained from fruits but are subjected to a slight pasteurization process. NFC Juices do not undergo concentration or dilution during processing and they retain the characteristics of "fresh" juices. If juices are diluted with water (or other liquids), then they cannot be called 'juice', therefore, must be sold as nectars, fruit drinks, or under some other name.

- Nectars: have a certain content of pure juice ranging around 25-99% depending on the laws in the country. Unlike juice, nectars can contain sweeteners, coloring and preservatives, which makes them cheaper than 100% juices. As defined by Kregiel (2015) nectars are diluted fruit\vegetable juices with pulp and have sweetening agents, minerals and vitamins.

- Juice Drinks and Fruit Drinks: both of them must contain a minimum of 5% fruit content and then added water, sugar, food acids, flavours and colours. The current legislation, no difference between Juice Drinks and Fruit Drinks.

- Fruit juice: Low calorie versions of juice drinks using less sugar or replacing the sugars with intensity sweeteners (Beverage descriptors, 2013).

- Still drinks: contain less than 5-25% fruit juice and larger quantities of additives including water, sugar, food acids, flavours, and colours added to mimic fruit juice. Kregiel (2015) defines still drinks as “flavored ready-to-drink, noncarbonated beverages, containing fruit or non-fruit flavors or juice content up to 25%”. Low calorie drinks either contain less sugar or high intensity sweeteners instead of sugar. These beverages are cheap and are highly consumed by lower income populations (Codex Alimentarius, CODEX STAN 247-2005; Neves et al., 2012).

- Syrups and concentrates: are concentrated sweet fruit flavoring that can be diluted to make drinks. They include e.g. Grenadine (pomegranate syrup), Casis (black currant syrup), Citronelle (lemon syrup), Gomme (white sugar syrup), Framboise (raspberry) etc. Syrups are never consumed as such but are diluted or added as flavors to milk before consumption. For example, squash\syrups are described by Kregiel (2015). Non-ready-to-drink products, which are marketed as concentrates to be consumed at home. They include fruit and non-fruit based products and flavors.

- Fruit Syrups: These products are effectively a sub group of cordials except that they need to contain a minimum of 5% fruit juice when diluted as per the instructions.

- Concentrates for carbonated Beverage: are solutions or a suitable solvent or mixtures of salts and include all or some of these components. Flavors taste, smell, color, turbid, materials, preservatives and acids are permitted in soft drinks to give them the taste, smell and color that is required as well as emulsifying, and any other additives and they are all permitted materials.

- Syrups as Standard for Sugars: liquid sucrose, invert sugar solution, invert sugar syrup, fructose syrup, liquid cane sugar, isoglucose and high fructose syrup added to concentrate fruit juice (Codex Alimentarius, CODEX STAN 247-2005).

1.3.1.2 Nourishing Beverages

Usually *nourishing drinks* are associated with fresh juices or milk. Amongst drinks with fruit juices are fresh and tinned orange, mango, grapefruit, pineapple, and lime. In terms of milk based nourishing drinks, cocoa based drinks are like drinking chocolate Ovaltine and Bournvita. These are sweetened powder mixes that dissolve readily in milk to give rich cocoa flavor; while tomato juice is the loner from the vegetable family (Note on Non-Alcoholic Beverage).

1.3.2 Nutritional Value and Safety of Non-Alcoholic Beverages

Consumption of beverages sweetened with sugar and/or high-fructose syrups are linked with obesity and type 2 diabetes (Malik et al., 2010; Vartanian, Schwartz &Brownell, 2007). In addition to sugars and associated calories, a number of synthetic food additives are added to beverages including coloring agents, artificial sweeteners, and preservatives. Synthetic food colors and artificial sweeteners are widely added to beverages to improve their appearance and taste while preservatives are added to maintain consistency and prolong shelf-life. With increased consumption of sugar sweetened beverages, there are increasing concerns about a potential risk to human health such as cardiovascular disease, type 2 diabetes and obesity risks (Malik et al., 2010). Approved food additives are Generally Recognized as Safe (GRAS) status (de Andrade et al., 2014). The maximum usage level is established as the highest level of a food additive permitted in foodstuff to achieve the intended technological effect to avoid such effects on health. So these levels are set by the EFSA through specific directives, e.g. Directive 94/35/EC for sweeteners, Directive 94/36/EC for colors, and Directive 95/2/EC for additives other than colors and sweeteners (Commission of the European Communities, 2001).

It is imperative that consumers know that the beverages purchased are safe and their quality is guaranteed. The expectation is also that the information provided will help to make informed decisions about the purchased product. In fact, beverages are covered by national regulations based on codes and standards. The CODEX Alimentarius "Food Code" was established jointly by the Food Agricultural Organization (FAO) and the World Health Organization (WHO) in 1963 so as to protect consumer health safety and promote food trade. "Codex Alimantarius: international food standards, guidelines and codes of practice contribute to the safety, quality and fairness of this international food trade. Consumers can trust the safety and the quality of food products they buy and importers can trust that the food they ordered will be accordance with their specifications".

GENERAL STANDARD FOR FOOD ADDITIVES CODEX STAN 1992-2016 (CODEX) defines Food Additives as:

"Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities."

According to CODEX, the use of food additives is justified "only when such use has an advantage, does not present an appreciable health risk to consumers, does not mislead the consumer, and serves one or more of the technological functions set out by Codex".

The Codex Alimentarius Commission determines the Maximum permitted Level (MPL) of an additive as the highest concentration of the additive to be functionally effective in a food or food category and agreed to as being safe. More specifically it is defined as *"Maximum usage level = Highest level of a food additive permitted in foodstuff to achieve an intended technological effect. The levels are set in the specific directives: for sweeteners in Directive 94/35/EC, for colours in Directive 94/36/EC and for additives other than colours and sweeteners in Directive 95/2/EC."*

The maximum use level may not always correspond to the optimum, recommended, or typical level of use. For Good Manufacturing Practice (GMP), the optimum or recommended, use level will change for each application of an additive and would depend on the intended technical effect and the specific food in which the additive would be used. This process would consider the type of raw material, food processing and post-manufacture storage, transport and handling by distributors, retailers, and consumers as defined in CODEX 2016 (Codex Alimentarius: CODEX STAN 192-1995).

Similarly, in the EU, beverages are subjected to the EU legislation on microbiological criteria, food additives, and general hygiene requirements for the production, storage and trade of food products. There are four main EU regulations all referred to as “Package on Food Improvement agents” which are the first regulation EC 1331/2008 for authorize procedure for food additives, enzymes, flavoring. Second regulation is EC 1332/2008 on food enzymes. Third regulation EC 1333/2008 list of approved food additives can be used with food categories. The fourth regulation is EC 1334/2008 on flavorings. According to this regulation, the reevaluation of approved additives has to be completed by the end of 2018 except for colors and sweeteners.

These levels are set by the EFSA through specific directives, e.g. Directive 94/35/EC for food sweeteners, Directive 94/36/EC for colors, and Directive 95/2/EC for additives other than colors and sweeteners (Commission of the European Communities, 2001). The maximum usage level is established as the highest level of a food additive permitted in foodstuff to achieve the intended technological effect according to Codex Alimentarius, CODEX STAN 192-1995 shows in Table 2, listed food additives and their maximum permitted level (MPL) of different beverages categories. In addition, the accumulation of these additives in our body could have side effects in the long term if intake is more than the required amounts. Acceptable Daily Intake (ADI) defined by CODEX is *"The amount of a food additive, expressed as mg/kg body weight that can be ingested daily over a lifetime without incurring any appreciable health risk".* The Philosophy of ADI is stop the spread of ideas that excessive intake of minerals, additives or preservatives increases the risk of diseases like cancer. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was established in 1955. JECFA has the responsibility for establishing monographs for the identity and purity of individual food additives. The additives are assigned INS numbers. The work of JECFA with additives feeds into the Codex General Standard for Food Additives (GSFA) food category system. The EFSA sends its opinion to the EC and the member states to identity and characterization of the food additive, the assessment of the biological and toxicological data, a dietary exposure assessment for the European population taking into account other possible sources of dietary exposure, an overall risk assessment establishing a health based guidance value, such as an acceptable daily intake (ADI) value with the contribution of each food category or foodstuff for the use is authorized or has been requested, to the total exposure. This system is a hierarchical system and applies to all foodstuffs. Thus, most of standards are built on the ADI (acceptable daily intake) for additives (Lehto et al., 2017; McAvoy, 2014).

The European Union permitted maximum allowed as Acceptable Daily Intake level of majority food additives have been assigned an Acceptable Daily Intake (ADI) or determined on the basis of other criteria to be safe by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and an International Numbering System (INS) designation by Codex will be considered for inclusion in this Standard (Codex Alimentarius, CODEX STAN 192-1995; Commission of the European

Communities, 2001). In Table 2 listed the ADI values of food additives which were included in this study. Many studies were shown that adverse effects on human health regarding the ADI level, which were mentioned under 1.3.3 synthetic additives in beverages. Approved food additives are given in the GRAS status (de Andrade et al., 2014).

1.3.2.1 Studies of Estimation of Intake of Additives around the World

GCC countries have been developing over the past 20 years. The high incomes have positively affected the standard of living, therefore, contributing to the abundance and diversity of food trade from all over the world. Thus, there has been an increase in the amount of food additives that include preservatives that are consumed.

The study by Alghamdi, Alghamdi & Alwarthan (2005) in Saudi Arabia in Riyadh city has shown that the estimation of daily intake of food additives through beverages consumption depends mainly on both the content of food additives and the amount of consumption of these beverages. It is assumed that two to three bottles of beverages are consumed daily by every adult on average. The size of the beverage bottles varied considerably (125-330 mL), thus, daily consumption rate of 400 mL of the beverage and also an average adult body weight of 70 kg were also assumed. The estimated daily intake of these food additives is solely due to the consumption of beverages and contributions from other foodstuffs such as soft drinks, energy drinks, tea, coffee, etc. This average of the daily intake value of food additives is less than the maximum permissible daily intakes acceptable limit of joint FAO/WHO and within the permitted level comply the food regulations of Saudi Arabian Standards Organization(Alghamdi, Alghamdi & Alwarthan, 2005). While, the current study used similar assumptions related to the CODEX standards level within the safety levels of food additives in UAE beverages content. The results showed that the content levels of additives in beverages and foods are lower than of the authorized additives levels.

While the study in Saudi Arabia focused on adults, the study in Kuwait was conducted on children. In Kuwait, Sawaya, et al. (2008) conducted a study and looked into the distribution and mean levels of artificial colour additives in food items that were found to be most commonly consumed by 5 to 14 year-old children in Kuwait. They then compared the results with GMP and permitted levels in other countries estimating how much children were exposed to artificial color additives. Their target population was public primary and intermediate schools. The population was over 3000 children from 58 schools in Kuwait. A total of 344 food containing artificial colour additives such items as biscuits, cakes, ice cream, candy, chips and puffed snacks, chocolates, drinks and juices, chewing gum, jelly and lollypops that were identified during were analysed for nine permitted and two non-permitted artificial food colour additives by Kuwait’s law. Among the 344 food items analysed, 90%, contained artificial colour additives that are permitted in foods by the Kuwaiti authorities such as tartrazine, sunset yellow, carmoisine, allura red, indigotine, brilliant blue, brilliant black, and brown HT. Around 10% did not contain any artificial food colour additives. Only a few food items contained non-permitted colour additives, e.g., erythrosine and orange G (Sawaya, et al., 2008).

Similary another research was completed in India focusing on children as children have higher consumption vulnerability. Dixit et al. (2011) searched into national-level data covering 16 major states of India on the usage pattern of colours and identified foods where color exceeds ADI limits. From their analysed samples, over 80% contained permitted colours, of which only 48% adhered to the prescribed limit of 100 mg/kg1. Non-permitted colours were found mostly in candyfloss and sugar toy samples. Though sunset yellow FCF (SSYFCF) and tartrazine were the two most popular colours, many samples used a blend of two or more colours. The blend of SSYFCF and tartrazine exceeded the prescribed limit and erythrosine exceeded the respective ADI limits by three- to 12-fold in all five age groups. Dixit and his team concluded that prescribed limit of synthetic colours at 100 mg/kg1 under Indian rules needs to be reviewed and should be governed by consumption profiles of the food commodities to check the unnecessary exposure of excessive colours to those vulnerable in the population that may pose a health risk (Dixit et al., 2011)

Lino, Costa, Pena, Rui, Ferreira, and Cardoso (2008) from Portugal conducted a survey with teenager students looking into the levels of acesulfame-K and aspartame in soft drinks and in light nectars, from which the intake of these intense sweeteners was estimated. The sample population came from a high school in the city of Coimbra, Portugal randomly a mix of male and female students aged between 13-15. A total of 48 samples were chosen in accordance with products available for sale at the canteen of the school and the consumption of the teenagers. When data on content of these sweeteners in soft drinks was analyzed according to flavor, they found that cola drinks had the highest mean levels for both the sweeteners for acesulfame-K and aspartame, respectively. For soft drinks based on mineral water, aspartame was found in 62% of the samples, and 80% of nectars samples contained acesulfame-K and aspartame (Lino et al., 2008).

Based on the idea that in Brazil, there is little data about the exposure to food additives by Lorenzoni, Oliveira & Cladera-Olivera (2012) researched food additives present in products for children. They analysed the information contained on product labels of all foods advertised on the website of a supermarket for products that were directed to children, as well as products generally consumed by children. It was organized in four categories (cereals and cereal products, dairy and meat products, candy and chocolate, beverages). The number of additives present in each product, the percentages of each class of additive present in the different food categories and the presence of artificial dyes in each category were presented in tables. Among the all products (5882) seen on the web site, 8.60% were classified as children products, from which 468 products contained information on their ingredients (and additives) and 438 products contained at least one additive in their formulation. The most used additives were lecithin (45.30%) and citric acid (22.86%) and artificial dyes allura red (9.83%), tartrazine (6.84%), sunset yellow (5.77%), brilliant blue (5.77%). They concluded that although they are widely used, those additives do not represent a risk for children health (Lorenzoni, Oliveira & Cladera-Olivera, 2012).

In a more recent study, Elif Celik et al. (2014) researched the Aspartame levels in Soft drinks consumed in Ankara, Turkey. Although the current thesis did not focus on Aspartame as an additive, their findings are interesting. Celik and her team attempted to determine levels of aspartame in soft drinks and to evaluate whether these amounts were within the Turkish Food Codex values. They used a total number of 90 soft drink samples including 15 from each brand that were collected from supermarkets in Ankara, Turkey. The results showed that average levels of aspartame were found within Turkish Food Codex in all samples.

Interestingly, however, some samples were not found appropriate according to the label information (Celik et al., 2014).

In addition, a number of synthetic food additives are added to beverages including coloring agents, artificial sweeteners, and preservatives. This addition should be within the allowed Authorized levels; but some studies found that sometimes the allowed levels are exceeded (de Andrade et al., 2014; Ma et al., 2012). Synthetic food colors and artificial sweeteners are widely added to beverages to improve their appearance, look, and taste while preservatives are added to maintain consistency and prolong shelf-life of the food product as well as inhibit the growth of microbe and fungi. With increased consumption of beverages, there were studies that show a potential risk to human health due to the ingestion of these additives (Diago et al., 2013).

For example, demonstrated studies illustrate that children may actually consume more colored foods than expected by the regulatory authorities, e.g. in the USA the amount has risen in several colorants from 12 mg/capita/day in 1950 to 62 mg/capita/day in 2010. Moreover, there are new toxicity concerns for some colorants due to their ability to bind to human serum albumin, e.g. sunset yellow (Kus & Eroğlu, 2015), tartrazine (Pan et al., 2011), azorubine (Basu & Kumar, 2014; Datta, Mahapatra & Halder, 2013), allura red (Wang, Zhang & Wang, 2014) and patent blue (Tellier et al., 2013). Hypersensitivity reactions have been reported of blue dys. These issues were considered by the European Council Regulation (EC) No. 1333/2008 on food additives, which did not find a strong evidence for toxicity. Nevertheless, systematic studies need to be performed on the pharmacological, neurodevelopmental and other effects that various colorants or may their mixtures

(Amchova, Kotolova & Ruda-Kucerova, 2015). The use of colorings in EU countries must comply with EC Food Additives Regulation 1333/2008. The EFSA's ANS Panel, has started reassessing of all permitted food colorings. In 2013, the EFSA recommended that new tests be carried out to address the possible genotoxicity of Sunset Yellow FCF, Tartrazine, and Azorubine/Carmoisine (Kregiel, 2015).

These colorant agents can be an allergen, which may cause intolerance in people allergic to salicylates or acetylsalicylic acid (aspirin). It is also a histamine liberator, which may intensify symptoms of asthma. Ponceau 4R is even considered carcinogenic in some countries, including the USA, Norway, and Finland. It is currently on the Food and Drug Administration's FDA list of banned substances in the USA authorized by EFSA (Kregiel, 2015). Consumption of beverages sweetened with sugar and/or high-fructose syrups are linked with obesity and type 2 diabetes, sugars as associated calories (Malik et al., 2010). While, according to United States Regulations, beverages are regulated approved for food color in 2015 and in 2020 it will be approved by FDA, also beverage ingredients must comply with FDA safety requirements for food sweeteners. FDA is regulating additives and listed on the food additive status list which includes additives specified under the Federal Food, Drug and Cosmetic Act (FD&C Act) (Guidance for Industry Food and Drug Administration FDA Records Access Authority Under Sections 414 and 704 of the Federal Food, Drug, and Cosmetic Act: April 2014; Kregiel, 2015).

Table 2: List of Selected Food Additives be used with their Acceptable Daily Intake (ADI) and Maximum permitted Level (MPL)

Food Additives	**ADI (mg/kg bw)**	**MPL mg/kg**	**Food Additives**	**ADI (mg/kg bw)**	**MPL (mg/kg)**
Sunset Yellow FCF	2.5	50-300	**Allura Red AC**	7	300
Tartrazine	7.5	300	**Patent Blue V Sodium Salt**	15	-
Quinoline Yellow 2 S F	10	50	**Acesulfame K**	9	350
Metanil Yellow	-	-	**Sodium Saccharin**	5	80
Amaranth	0.8		**Methyl paraben**	10	200
Ponceau 3R	-	-	**Ethyl paraben**	10	200
Ponceau 4R	4	150	**Butyl paraben**	0-10	200 mg/kg
Erythrosine Extra bluish	0.1	100	**Thiabendazole**	0-0.3	-

ADI for adults: Acceptable daily intake is the maximum allowed daily intake mg per kilogram body weight for Adult (mg/ kg bw) (US Food and Drug Administration; FAO/WHO, 2016; Commission of the European Communities, 2001), MPL: Maximum Permitted Level (Codex Alimentarius, CODEX STAN 192-1995), GMP: Good Manaufaturing Practice.

1.3.2.2 Global Regulation

UAE- GCC Region and the Middle East

Global regulation of food colors is differed among the US, EU, Canada, Mexico, China, Japan, Korea, Australia. GCC countries including United Arab Emirates, Saudi Arabia Bahrain, Kuwait, Oman and Qatar follow the Codex Alimentarius of General Standard for Food Additives GFSA. However other Middle East and Northern Africa countries including Israel, Jordan, Lebanon, Malta and Syria follow EU regulations while, other countries in this region such as Algeria, Djibouti, Egypt, Iran, Iraq, Libya, Morocco, Tunisia, West Bank and Gaza, and Yemen follow their own regulations (McAvoy, 2014). Recently, it was proposed to the World Trade Organization to adopt the Codex Alimentarius GFSA. Yet, the problem with this is that all additives have not fully progressed in the GFSA. So that the countries in MENA follow their own regulation of some type.

The US, Europe and Other Countries

In the US, however, as potential allergen or sensitizer in additives such as cochineal extract, carmine and FD&C Yellow No. 5 so must be declared on all food labels.

Comparatively, the EU states that products must declare the color additives used in food products and ingredients list, giving their full name and/or their E number. In the US, colours are subjected to declare or some certification are required to be by listed names such as FD&C Yellow No. 5. listed by simple name, dropping the FD&C prefix (e.g., Yellow 5 lake). Other alternative names such as E numbers may be added in parenthesis. Colour additives can be excused from certification and can be labeled as ‘artificial color’, ‘artificial color additive’, ‘color added’ or an equally informative term and can be combined with the listed name. For some colours voluntary declaration are recommended. (Lehto et al, 2017) In the US, it is required to label color additives. Certified colors must always be declared by name. It must include the FD&C prefix or the term in the declaration, such as Yellow 5, Blue 1 Lake. Those that are exempt can be referred to as artificial coloring, color added or artificial color added.

The EU demands that color additives be declared by the category name (color) and E number of the specific color. For example: Color (E 171). If it is a flavor with coloring, it needs to be designated by the term “flavoring” or more by the description of the flavor and color. Using the term “coloring food” is not allowed (McAvoy, 2014). There are artificial colors permitted differ by countries. For example, FAO/WHO Codex Alimentarius permits 14 artificial colours, European Union (EU) 15 colours, Japan 12 colours, USA 9 colours and Korea 9 colours (Suh & Choi, 2012). Table 3 below shows some of artificial colours that have been permitted in different countries in Codex Alimentarius European Union, USA, Japan and Korea.

Table 3: Regulations for Permitted Artificial Colours in Different Countries

Color	**E number**	**Codex**	**EU**	**US**	**Japan**	**Korea**
Tartrazine	E102	+	+	+	+	+
Sunset Yellow FCF	E110	+	+	+	+	+
Amaranth	E123	+	+	-	+	+
Erythrosine	E127	+	+	+	+	+
Allura Red AC	E129	+	+	+	+	+
Ponceau 4R	E124	+	+	-	+	+
Quinoline Yellow	E104	+	+	-	-	-
Patent Blue V	E131	-	+	-	-	-

+ permitted, – not permitted (Suh & Choi, 2012).

Comparison of food color regulation in the world

Food Labeling is the written, mark or sign indicating the contents of products. Rules and regulations exist in many countries regarding food labeling. Each country can have varying regulations about what is to be included on the labels. Although consumer awareness of health related risks of artificial colour additives has increased, artificial colours are used more frequently than natural colours in many processed foods (Suh & Choi, 2012). While some require all contents to be mentioned others require less information. The study by McCann et al. (2007) raised awareness about harmful effects of some artificial food colours on children's behavior. Results from studies might be contributed to implementation of warnings, special rules that apply to labeling of products for professional use. For instance, foods containing tartrazine, quinoline yellow, sunset yellow, ponceau 4R, allura red and carmoisine need to be accompanied by warning of potential adverse effects on health. General principle of Codex is to promote the unifying food laws among countries and allowing internationally agreed-upon standards for foods and beverages as this would reduce the barriers for trade and make it easy to transfer food products among countries. This in turn would benefit farmers and help reduce hunger and poverty worldwide (McAvoy, 2014).

1.3.3 Synthetic Additives in Beverages

Food additives are divided into 6 groups including: coloring agents, preservatives, flavoring agents, nutritional additives, texturizing agents and miscellaneous agents. Synthetic color encompasses the azo compounds, the chinophthalon derivatives, the triarylmethane compounds, the xanthenes and the indigos. The preservatives are sub-divided into antimicrobials, antioxidants and antibrowning agents. The flavoring agents include sweeteners, natural and synthetic flavors, and flavor enhancers. As a final the texturizing agents are divided into emulsifiers and stabilizers (Carocho, Morales & Ferreira, 2015).

Food colorings or synthetic colorants, color additive, color agents, dyes, pigments, or other substance include any color added to food and drink. In addition, a synthetic color can be any chemical that reacts with another substance and causes formation of a color (de Boer, 2013; Newsome, Culver & Van Breemen, 2014). Among the reasons for using color additives in food and beverages are compensation of color loss caused by exposure to light, air, temperature and storage conditions; enhancement of natural colors to make the food more attractive; adding color and allowing consumers to identify products on sight (Barrows, Lipman & Bailey, 2003). Although colorants can be classified according to criteria such as origin, solubility and transparency, these categories can overlap.

In the past, materials of natural origin were used to provide color in foods, drugs and cosmetics. Later, it was discovered that materials, mostly coming from plants, could be used to enhance the appearance of products. Therefore, turmeric, paprika and saffron were used for more than just their flavor. Natural and synthetic color additives started being used to color foods, beverages, drugs and cosmetics by the early 1990s. Color is an important characteristic for consumers in terms of choice. Color is important for safety purposes, so color additives are used for a wide variety of purposes in foods, beverages and cosmetics (Clydesdale, 1993; Hallagan, Allen & Borzelleca, 1995).

Artificial sweeteners, non-caloric sweeteners, or sugar substitutes are referred to as non-nutritive sweeteners (NNS). They are 30-13,000 times sweeter in taste compared to sucrose sugar with empty calorie that promotes weight loss (Shankar, Ahuja & Sriram, 2013). According to Shankar et al. (2013) the ADI for acesulfame-K is 15 mg/kg body weight. In the United States, actual consumption is about 20% of the ADI over a lifetime and Kostik (2014) indicates a purity of 98.0%. Daily Intake (ADI) and in accordance with the appropriate regulations is 350 mg/L (Kregiel, 2015). The most commonly used sweeteners (with maximum permitted dosage in the EU) are aspartame (600 mg/L), acesulfame K, sucralose (300 mg/L), and saccharin (80 mg/L) (Kregiel, 2015).

Synthetic preservatives or artificial preservatives are widely used to prevent changes and degradation of food, cosmetics and pharmaceuticals by stopping microbial contamination and preventing fungal attack. Therefore, preservatives are commonly used because of high performance, low cost and wide availability (Li et al., 2008). In addition, the reason that antimicrobial food additives are used as preservatives is to maintain nutritional value with other properties food or beverages. It is important for food safety. However, excessive use could cause human risk (Xu et al., 2013).

1.3.3.1. Colorants (Dyes)

Different classifications of dyes or colorants types are soluble and insoluble which obtained either natural or synthetic (Amchova, 2015) Azo-dyes are synthetic organic color with nitrogen bound. Mostly of synthetic azo-dyes, are widely used in the production of beverages to improve their appearance for consumer acceptability. These synthetic dyes have some advantages over natural food colors because of their stability, high color intensity, and insensitivity to heat, light, and chemical interactions (Scotter, 2015).

Table 4 presents eleven examples of selective synthetic food colorants that are used in beverages including: sunset yellow FCF, tartrazine, quinoline yellow 2 S F, mentanil yellow, amaranth, ponceau 3R, ponceau 4R, erythrosine extra bluish, sulfor hodamine B, allura red AC and patent blue V sodium salt.

Sunset yellow is an orange water soluble anionic monoazo-dye. Toxicity of Sunset Yellow was evaluated by JECFA in 1982 and by the Scientific Committee on Food (SCF) in 1984. It was concluded that the substance is safe at an ADI of (0-2.5) mg/kg of body weight per day. Tartrazine is a yellow water-soluble anionic azo-dye. Examination panels have concluded that the substance is safe at an ADI dose of (0-7.5) mg/kg of body weight per day. Ponceau 4R is a red water-soluble anionic monoazo-dye, also known by more than 100 synonyms including cochineal red A, brilliant scarlet 4R or new coccine. Toxicity of ponceau 4R was evaluated by JECFA in 1983 and SCF in 1984. It was concluded that the substance is safe at an ADI of (0-4) mg/kg of body weight per day. Allura Red is a red water-soluble anionic monoazo-dye. Toxicity of allura red was evaluated extensively by JECFA in 1980 and also by SCF in 1984 and 1989. This colorant was claimed to be safe at ADI of 0-7 mg/kg of body weight per day. In 2009, the EFSA Panel considered all relevant results and recommended further research. Patent blue description is a blue water-soluble anionic triphenylmethan dye. Toxicity of patent blue was evaluated by JECFA in 1970 and 1975 and the SCF in 1983. However, a final ADI dose was determined only by the SCF (0-15) mg/kg of body weight per day. Indigo carmen is a blue water-soluble anionic pyrrole-based dye. Toxicity of indigo carmine was evaluated first by JECFA, which established a temporary ADI of (0-2.5) mg/kg of body weight per day in 1969. This value was increased to a final ADI of (0-5) mg/kg of body weight per day in 1975.

According to Amchova, Kotolova & Ruda-Kucerova (2015), brilliant blue FCF is a blue water-soluble anionic triphenylmethan dye, also known as blue 1. Toxicity of brilliant blue FCF was evaluated by JECFA in 1970 and also the SCF in 1975. Both panels defined the ADI as (0-12.5) mg/kg of body weight per day. In 1984, the available findings from long term studies were revised and the ADI value was adjusted to 10 mg/kg of body weight per day. Green S is a green water-soluble anionic triarylmethane dye. Toxicity of green S was evaluated by JECFA in 1970 and 1975, and by the SCF in 1984. JECFA concluded that the substance is safe at an ADI of (0-25) mg/kg of body weight per day. However, this decision was withdrawn in 1975 and to date has not been re-established (Amchova, Kotolova & Ruda-Kucerova, 2015).

Table 4: Selected Colorants used in Beverages

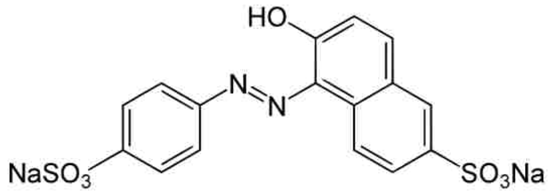
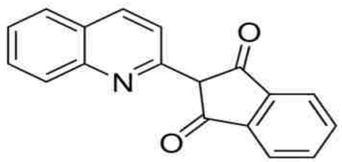
No	Trivial Name	E-Number	Food Color	Chemical Structure IUPAC Name	CAS Registry Number
1	Sunset Yellow FCF	E 110	Yellow - 3	 Disodium-6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate	2783-94-0
2	Tartrazine	E 102	Yellow - 4	 Trisodium(4*E*)-5-oxo-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)-hydrazono] -3-pyrazolecarboxylate	1934-21-0
3	Quinoline Yellow 2 S F	-	Yellow	 2-quinolin-2-ylindene-1,3-dione	83-08-9

Table 4: Selected Colorants used in Beverages (Continued)

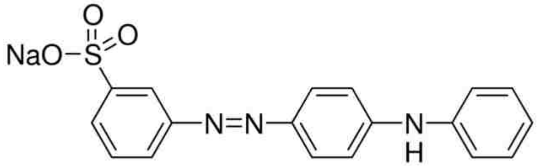
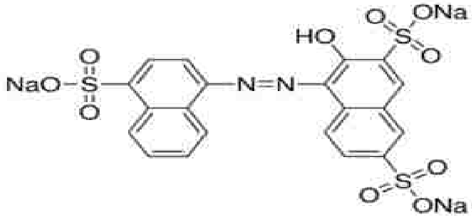
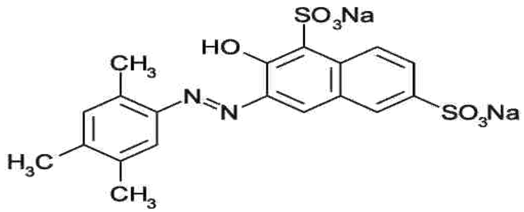
No	Trivial Name	E-Number	Food Color	Chemical Structure IUPAC Name	CAS Registry Number
4	Metanil Yellow		Acid Yellow-36	 Sodium 3-(4-Anilinophenylazo) benzenesulfonate	587-98-4
5	Amaranth Red	E 123	Red - 9	 Trisodium (4*E*)-3-oxo-4-[(4-sulfonato-1-naphthyl)-hydrazono]-naphthalene-2,7-disulfonate	915-67-3
6	Ponceau 3R Dark red		Red -7	 Disodium 3-Hydroxy-4-[(2,4,5-trimethylphenyl)azo]-naphthalene-2,7-disulfonate	3564-09-8

Table 4: Selected Colorants used in Beverages (Continued)

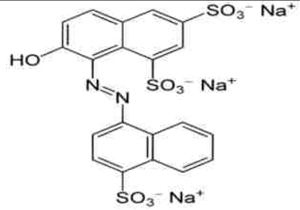
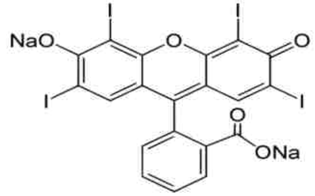
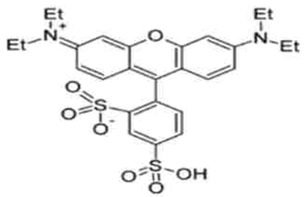
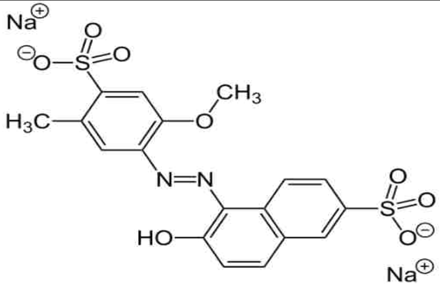
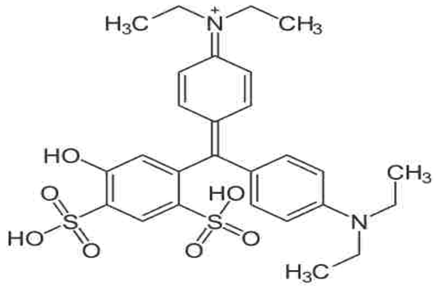
No	Trivial Name	E-Number	Food Color	Chemical Structure IUPAC Name	CAS Registry Number
7	Ponceau 4R Cochineal Red A	E 124		 Trisodium (8Z)-7-oxo-8-[(4-sulfonatonaphthalen-1-yl) hydrazinylidene] naphthalene-1,3-disulfonate	2611-82-7
8	Erythrosine Extra bluish	E 127	Red No.3	 Disodium 2-(6-Hydroxy-2,4,5,7-tetraiodo-3-oxo-xanthen-9-yl) benzoate	16423-68-0
9	Sulfor-hodamine B	-	Kiton Red 620	 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl) -5-sulfo-benzenesulfonate	3520-42-1

Table 4: Selected Colorants used in Beverages (Continued)

No	Trivial Name	E-Number	Food Color	Chemical Structure IUPAC Name	CAS Registry Number
10	Allura Red AC Food Red 17	E 129	Red-17	 Disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonate	25956-17-6
11	Patent Blue V Sodium Salt	E 131	Blue-5	 Sodium or calcium salt of [4-(α-(4-diethylaminophenyl)-5-hydroxy-2,4-disulfophenylmethylidene)-2,5-cyclohexadien-1-ylidene] diethylammonium hydroxide	20262-76-4

1.3.3.2 Sweeteners

Sweeteners are sugar substitutes that provide a sweet taste resembling that of sugar while containing significantly less energy. Therefore, they are called sugar substitutes. According to Food and Environmental Hygiene Department, Risk assessment on artificial sweeteners in beverages (2003), Hong Kong market surveys also reported that the soft drink industry has been identified as the biggest user of artificial sweeteners worldwide. Today sugar free products are popular, as they have less calorie content. It is for this reason that the food industry uses various artificial sweeteners which are low in calorie content instead of high calorie sugar (Chattopadhyay, Raychaudhuri & Chakraborty, 2014). U.S. Food and Drug Administration have approved aspartame, acesulfame-k, neotame, cyclamate and alitame for use as per acceptable daily intake ADI value mg/kg body weight.

As mentioned previously, artificial sweeteners are generally used to control calorie intake and in certain medical conditions such as diabetes and hyperglycemia. Aspartame, sodium cyclamate, acesulfame K, and sodium saccharin are the most common ones, and are marketed in many countries around the world (Kostik, 2014; Serdar and Knežević, 2011). However, the usage of the artificial sweeteners in the food industry has provoked strong controversy because of their possible carcinogenic effects (Shankar et al., 2013; Kostik, 2014).

According to Chattopadhyay, Raychaudhuri and Chakraborty (2014), the breakdown products of these sweeteners in the body have controversial health and metabolic effects have not been proven. In contrast, rare sugars which are monosaccharides have not known health effects, because it does not metabolize in the body, yet show same sweet taste and bulk property as sugar.

First type, acesulfame-k**,** which was developed as a sweetener by the pharmaceutical company, Hoechst in 1967 by (Clauss & Jensen, 1973), is not metabolized in the human body, thus, it provides no calories and does not influence potassium intake despite its potassium content (ADA, 2004). This high-intensity sweetener is about 200 times sweeter than the table sugar sucrose. Acesulfame-K is known to be toxic if consumed in very large doses, because human exposure to this breakdown product would be negligible. Regardless, the USFDA concluded that no further testing of it was necessary. ADI for acesulfame-K is 15 mg/kg of body weight (Shankar, Ahuja & Sriram, 2013).

The second type of sweetener most commonly used for more than 100 years is sodium saccharin, a non-nutritive sweetener, which was discovered by discovered by Remsen and Fahlberg in 1879 at John Hopkins University, (Shankar, Ahuja & Sriram, 2013). Saccharin (E954) is 300 times sweeter than sucrose but leaves a bitter/metallic aftertaste. Usage of saccharin in foods dates back to 1907. This sweetener is permitted in more than 100 countries around the world. The Food and Drug Administration tried to ban saccharin in 1977, because the results on animal studies rats specifically showed that it caused bladder cancer. However, there have been many studies conducted on saccharin since then and there is no evidence to show a clear relationship between saccharin consumption and health risks in humans when taken in normal doses. Some studies have shown a correlation between consumption and cancer incidence (Chattopadhyay, Raychaudhuri & Chakraborty, 2014; Weihrauch & Diehl, 2004). Saccharin is currently permitted for use under regulation that specifies the amounts of saccharin allowed in beverages, processed food, and sugar substitute. It also requires that the product level must be stated in the declaration and specify the amount used (Chattopadhyay, Raychaudhuri & Chakraborty, 2014; Kroger, Meister & Kava, 2006). ADI for saccharin is set at 5 mg/kg body weight per day for adults and children (Shankar, Ahuja & Sriram, 2013).

Different types of sweeteners are available including;

(i) Natural sweeteners, e.g. fructose syrups, date sugar, agave nectar, fruit juice concentrates, honey, maple syrup, molasses, etc.

(ii) Sugar alcohols, e.g. erythritol, lactitol, maltitol, isomaltitol, mannitol, sorbitol, xylitol, etc.

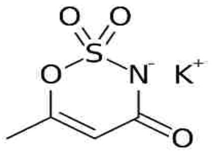
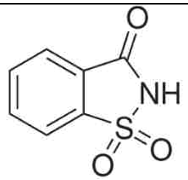
(iii) Novel sweeteners, e.g. Tagatose (Naturlose), Trehalose, Stevia, etc.

(iv) Artificial sweeteners, e.g. acesulfame potassium, aspartame, neotame, saccharin, sucralose, advantame, etc. (Chattopadhyay, Raychaudhuri & Chakraborty, 2014)

Sweeteners can be divided into two categories, natural and synthetic sweeteners also known as intense sweeteners. Synthetic sweeteners cannot be metabolized in the human body and provide no or little calories; therefore, they are also named nonnutritive sweeteners or artificial low-calorie sweeteners are the most thoroughly tested and important function sweetness in food (Chang & Yeh, 2014). Products including aspartame and saccharin have undergone several rounds of risk assessment by the FDA and EFSA, in relation to a number of potential safety concerns for consumers, including carcinogenicity as well as its effects on body weight gain, glycemic control and effects on the gut microbiome (Roberts, 2016). Yet, another study showed the effect on the metabolic system in youth (Brown, De Banate & Rother, 2010). In the EU, artificial sweeteners must be indicated in the food label by the name (e.g. 'Sweetener aspartame') or E-number (e.g. 'Sweetener E951') to make it clear to the consumer that the food product contains sweetener.

Products containing aspartame should have the additional notice ‘contains a source of phenylalanine (The food labelling regulations 1996, 1997). Table 5 gives two examples of sweeteners that are used in this study of UAE beverages.

Table 5: Selected Sweeteners (Sugar Substitutes) used in Beverages

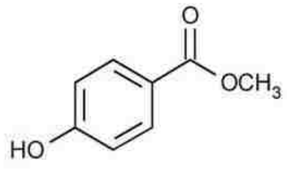
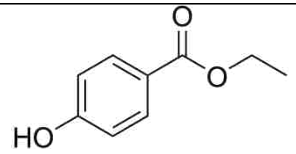
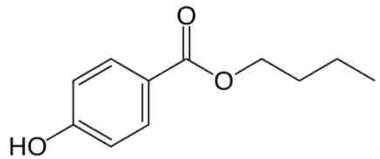
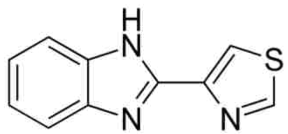
No	**Trivial Name**	**E-Number**	**Chemical Structure** **IUPAC Name**	**CAS Registry Number**
1	Acesulfame K (Acesulfame Potassium)	E 950	 Potassium 6-methyl-2, 2-dioxo-2*H*-1, 2λ^{6}, 3-oxathiazin-4-olate	55589-62-3
2	Sodium Saccharin	E 954	 2*H*-1λ^{6}, 2-Benzothiazol-1, 1, 3-trione	81-07-2

1.3.3.3 Preservatives

Beverages and drinks have high levels of water activity acidity, sugars contents, which are a suitable environment that allows microbial growth. The use of preservatives allows the food products to have a longer shelf life by inhibiting the growth of microorganisms (yeasts, mold and bacteria). A preservative can be defined as a substance or a chemical that is added to foods and beverages to prevent their spoilage by microbial growth or by undesirable chemical changes. Preservative food additives reduce the risk of foodborne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality (Kregiel, 2015) in her article "Health Safety of Soft Drinks: Contents, Containers, and Microorganisms" indicates that chemical preservatives are used to improve the microbiological stability of soft drinks. The types of chemical preservatives that can be used depend on the chemical and physical properties of both the preservative and the beverage. The pH of the product, the presence of vitamins, the packaging, and the conditions of storage will determine what types of preservative should be used to prevent microbial growth. According to Kregiel, Sorbates, benzoates, and dimethyl dicarbonate (DMDC) are permitted in ready-to-drink beverages in Europe. Sorbates are effective preservatives against bacteria, yeasts, and molds. Benzoates, on the other hand, react with ascorbic acid (vitamin C) and form benzene, especially if they are stored for extended periods at high temperatures. DMDC is commonly used as a preservative in cold sterilized soft drinks. DMDC is very reactive and rapidly breaks down when added to a substrate, such as a water based beverage. Food preservative can work as ‘‘hygiene" when added to food, protected against micro-organisms, infectious agents and pathogens, therefore allergic risks could have to humans (Maier et al., 2010).

Food additives have long been suspected to be associated with increased hyperactivity in children (Eigenmann & Haenggeli, 2004). Studies show that sulfite (sulfur dioxide) worked to prolong the shelf life of the baked food, but has had an adverse effect on the human ingestion (Lien et al., 2016). The harmlessness of colorants and preservatives has been tested. Therefore, food additives are generally seen as safe (Maier et al., 2010; Parke & Lewis 1992). Yet, their effect on health, the immune system especially, is controversial. Recent studies have shown that, food preservatives sodium sulfite and sorbic acid (Winkler et al., 2006) and popular colorant beet root extract (Winkler et al., 2005) were found to possess suppressive activities on Th1-type immunity in vitro. Although, these observations were found in vitro only, they indicate that the employment of assays with greater sensitivity is able to demonstrate a potential immunomodulatory capacity of such compounds (Maier et al., 2010). Table 6 gives four examples of selected preservatives that are used in UAE beverages.

Table 6: Selected Preservatives used in Beverages

No	Trivial Name	E-Number	Chemical Structure IUPAC Name ()	CAS Registry Number
1	Methyl paraben	E218	 Methyl 4-hydroxybenzoate	99-76-3
2	Ethyl paraben	E214	 Ethyl 4-hydroxybenzoate	120-47-8
3	Butyl paraben	-	 Butyl 4-hydroxybenzoate	94-26-8
4	Thiabendazole	E233	 4-(1*H*-1, 3-Benzodiazol-2-yl)-1, 3-thiazole	148-79-8

Chapter 2: Materials and Methods

2.1 Samples Collection

Thirty beverage samples including fruit juice, nectar, drinks, soft drinks, energy drink, aerated water, flavored water, carbonated malts, carbonated drinks etc. from different batch numbers were selected randomly of different brands from those commercially available at the supermarkets in the city of Al-Ain in the Emirate of Abu-Dhabi, UAE. The samples, segregated into groups depending on the type of their class, were purchased from two batches and have been coded and listed below in Table 7.

Table 7: List of Non-Alcoholic Beverages Sub-Groups Studies

Non-Alcoholic Beverages Class Sub Groups	**Categories**	**Total**
Refreshing (Non-Areated/ Carbonated)	Drink	**10**
	Flavored water	**2**
	Nectar	**2**
	Juice	**3**
Refreshing (Areated /Carbonated)	Carbonated soft drinks	**6**
	Energy Drink	**3**
	Nutritive drink Ion supply	**2**
	Carbonated drink with vitamins &mineral	**1**
Nourishing	Malt beverages	**1**
Total		**30**

Each sample was collected from two different (date of production /batches) (Total 60 beverage samples).

2.2 Chemicals and Reagents

All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). Eighteen synthetic food additives including eleven coloring agents, two sweeteners and five preservatives were used. The eleven color agents included: tartrazine, amaranth, allura red AC, ponceau 4R, ponceau 3R, sunset yellow FCF, erythrosine extra bluish, quinoline yellow 2 S F, sulforhodamin B, mentanil yellow and patent blue V sodium salts. The two types of sweeteners were acesulfame K and sodium sccharin. The five Preservatives comprised of: thiabendozole, ethyl paraben, methyl paraben, and butyl paraben. These reagents (purity > 85%) were used as standards without further purification. Ethanol, methanol, acetone, and acetonitrile were of ultra high performance liquid chromatography (UPLC) grade. Water was purified using Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3 Preparation of Standard Solutions and Analysis

Seventeen stock solutions with a concentration of 10 mg/mL were prepared by dissolving solid analyte in deionized water in volumetric flask except for thiabendozole; ethyl paraben; quinoline yellow; butyl paraben and methyl paraben, which were dissolved in 10 mL methanol. Some of the reagents such as quinoline yellow 2 S F, butyl paraben, and methyl paraben were sonicated in ultrasonic device for 2 minutes to be dissolved. The seventeen stock standard solutions were stored individually in a refrigerator until used for the experiment. The stock solutions were diluted with water to prepare calibration solutions having seven concentrations, while (0) was blank of pure distilled water, (approximately 0, 0.15, 0.30, 0.625, 1.25, 2.5, 5.0, and 10.0 µg/mL) (Gao et al., 2013; Yoshioka & Ichihashi, 2008).

2.4 Sample Preparation and Analysis

All of the beverage samples were injected as it is into the Ultra Performance Liquid Chromatograph (UPLC) with Diode Array Detector (DAD) instrument, except mango juice, nectar juice, melon milk juice, and fresh juices samples were diluted with deionized water (1:1, v/v). Some of thick juicy beverage samples such as mango juice, melon milk juice, and nectar juice was filtered by using syringe with membrane filter (0.45 µl) and the residual solvent liquid of beverages. Other samples including soft drinks, aerated beverages, energy drinks and sparkling beverages were degassed for ten minutes in vials before being injected into the UPLC with DAD. Approximately, 0.1 g of the sample was weighed and organized in vials in a tray that were analyzed by using UPLC. Samples were analyzed on the same day, however the duplicated analyses were repeated at different days. All thirty samples were duplicated in two batches.

2.5 UPLC Analysis

The UPLC system was Dionex UltiMate® 3000 (Thermo Fisher Scientific, MA) with an automated sample injector and a variable wave length detector. Separations were done using column a ZORBAX Eclipse XDB-C18 Rapid Resolution HT (150 mm × 4.6 mm i.d., 1.8 µm, Agilent Technologies, CA, USA). The column was kept at 50 ºC in the column oven. Solvent A was 0.1 mol/L of ammonium acetate aqueous solution (pH 6.7) and solvent B was methanol-acetonitrile (50:50, v/v). In gradient-elution analysis, the initial mobile phase was 3% of solvent B, increased linearly to 60% in 18 min and held at 60% for 2 min. A return to the initial conditions was carried out in 10 min. The flow rate was set at

1.5 mL/min and the injection volume was 5 μl. The column elution was monitored at 450, 490, 520, and 620 nm for the yellow, orange, red, and blue colors respectively.

2.6 Spectra Photometer Absorptions

The absorption spectra of the food colors, sweeteners and preservatives were recorded at wavelength between 200 and 700 nm by using UV-visible spectrophotometer (VARIAN Cary, 50 Conc UV-Visible spectrophotometer, Samsung). Therefore, peak identification was done by comparing the retention times and absorption spectra of the samples with peak's food color standards. Verified the absorption for the food color standards were detected between 200 to 700nm within visible and invisible light range. In contrary, colorless sweeteners standard and preservatives standard were detected within 200 to 400 nm in UV. Spectra photometer for each analytes of standard solution was used to finalize the optimum absorption of wave length (λ) between 235 to 319 nm light range that used in UPLC. Appendix 1 listed the sixteen absorption spectra of the food colors, sweeteners and preservatives by using UV visible spectrophotometer.

As a result, Quantification was made with reference to the calibration curves of standard solutions. Thermo Scientific Chromeleon 7.2 Chromatography Data System (CDS) software was employed to plot the calibration curve for each standard as well as to calculate the concentration of analyses in each solution (Gao et al., 2013; Yoshioka & Ichihashi, 2008).

2.7 Method

The developed chromatographic method was through determination of the precision, limit of detection (LOD), limit of quantification (LOQ), linearity, sensitivity/specificity, precision and repeatability for determined the quantification of additives in beverages samples. The Noise (N): Unwanted baseline fluctuations in the absence of analyte signal was obtained from injection of blank observed in chromatogram. The obtained noise was used to determine the limits of LOD and LOQ as follows. LOD or the minimum concentration of analyte that can be detected with a specific method at a known confidence level was determined as (3 *S/N), where, S/N = Signal-to-noise ratio = (magnitude of the signal)/(magnitude of the noise). Analytical values below LOD are expressed as not detected (ND). LOQ was determined as (10*S/N) using peak heights, where, S/N = Signal-to-noise ratio. Trace was used to express uncertainty values ranging between the LOD and the LOQ. The sets of standards having different concentrations (approximately 0, 0.15, 0.30, 0.625, 1.25, 2.5, 5.0, and 10.0 µg/mL) was injected to establish the calibration curves. Appendix 2 showed the calibration curves for each food additive including the three groups' colorant, sweetener and preservatives. The calibration curve model was $y = ax + b$, linear response, weighting scheme $1/y$, where y-peak area and x-concentration. (Vlase et al., 2014).

2.8 Statistical Analysis

The statistical analysis was done by using Microsoft Excel® (Microsoft Office 2013). All the results from the experiment were expressed as the mean followed by corresponding standard errors and compared by analysis of variance. The differences between means were considered by statistically test.

Chapter 3: Results and Discussion

The aim of this study was to identify and quantify seventeen different food additives (including eleven colorants, two sweeteners, and five preservatives) in thirty selected beverages commercially obtained from Al-Ain city of Abu-Dhabi, UAE. Before analysis, methods were performed focusing on limit of detection (LOD), limit of quantification (LOQ), linearity range, precision, selectivity and specifity and repeatability.

3.1 Separation of Mixed Food Additives by Ultra Performance Liquid Chromatography (UPLC)

Simultaneous analysis of the seventeen food additives (including eleven colorants, two sweeteners, and four preservatives was performed by UPLC with DAD. The seventeen food additives were well separated within 25 minutes as shown in Figure 4, and standards were mentioned in Table 8. The elution order of the synthetic food additives followed their polarities or functional groups: hydroxyl groups, sulfonate group etc. Generally, compounds containing azo groups tended to elute earlier than triphenylmethane groups and xanthene groups (Scotter, 2015).

Standards were detected and quantified at different wavelengths to maximize sensitivity. The absorption spectra for sixteen standards of food additives were obtained using a spectra photometer (Appendix 1).

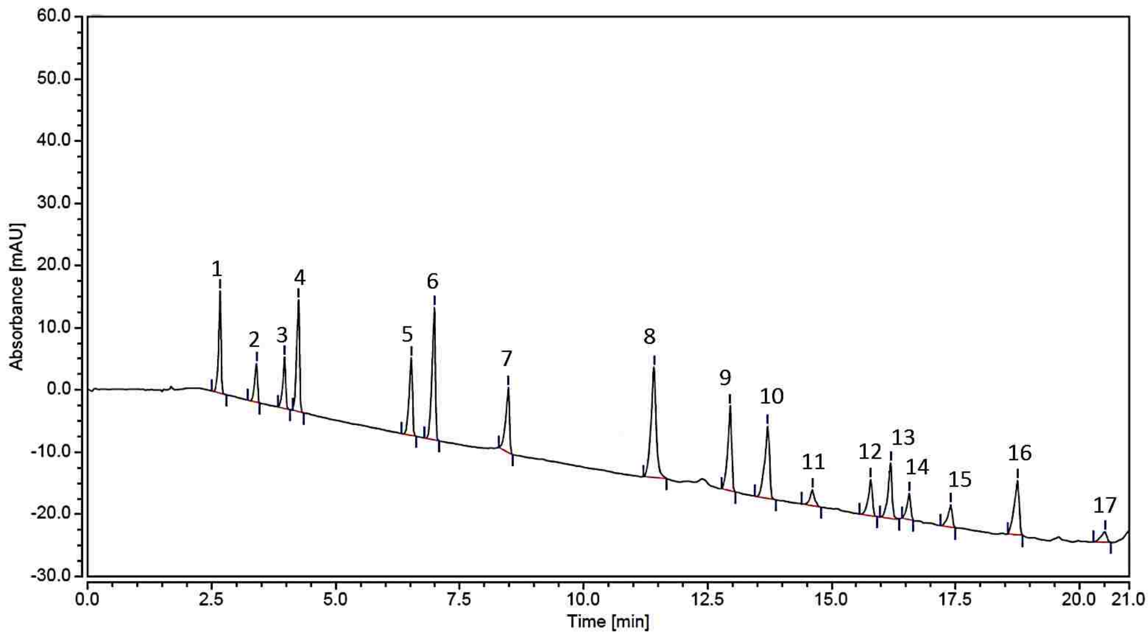


Figure 4: UPLC Chromatogram at Wavelength: 235-319 λ (nm) of Mixed Standard Solution for 18 Compounds

1: Acesulfame K, 2: Tartrazine, 3: Sodium Saccharine, 4: Amaranth, 5: Ponceau 4R, 6: Sunset yellow FCF, 7: Allura red AC, 8: Thiabendozole, 9: Ponceau 3 R, 10: Ethyl paraben, 11: Methyl Paraben, 12: Erythrosine extra bluish, 13: Sulforhodamine B, 14: Patent blue V Sodium salts, 15: Mentanil yellow, 16: Butyl paraben, 17: Quinoline Yellow 2 S F)

3.2 Result and Statistical Analysis for Food Additive

The absorption spectra of the food colors, sweeteners and preservatives were recorded wavelength between 200 and 800 nm. Therefore, Peak identification was done by comparing the retention times and absorption spectra of the samples with peak's food color standards. Spectra photometer for each analytes of standard solution was used to verifiy the limit of wave length (λ) between 235 and 319 nm in UPLC. Appendix 2 listed seventeen absorption spectra of the food colors, sweeteners and preservatives by using UV visible spectrophotometer.

The calibration curve and response factors for each additive (analyte) including threes groups' synthetic colorants, sweeteners and preservatives are given in Appendix 2 and summarized in Table 8. The calibration ranges for all compounds covered the concentrations with excellent correlation coefficients for the relationships between concentration and peak area ($R^2 > 0.98$) as shown in Table 8, where the X-axis represent the concentration of each additives (ppm) or (µg/mL) and the Y-axis represent the peak area absorption unit (AU).

In the case of chromatographic separation, resolution factors should be obtained for critical separation peak resolution and resolution formula regard of both the United States Pharmacopoeia (USP) and European Pharmacopoeia (EP) result in Table 8 (Ermer & Miller, 2006), (Physical Tests / (621) Chromatography1).

The asymmetries of the mean of three peaks large, medium and small were noticed only in the upper part of peak area and peak height. The calculation formulas were dealt for asymmetry (Ermer & Miller, 2006). While the program was given out the data results in Table 8.

Table 8: Calibration Curves, Resolution and Asymmetry for the Seventeen Food Additives

No	Food additives	wave length λ (nm)	Mean ± (SD)			Regressions Equation	Correlation Coefficient R^2
			Retention Time (min)	Resolution	Asymmetry		
1	**Acesulfame K.**	235	2.67±0.002	8.14±0.159	0.77±0.019	y = 0.2703x - 0.0003	0.9998
2	**Tartrazine**	254	3.39±0.007	5.87±0.027	0.77±0.032	y = 0.0398x + 0.0014	0.9997
3	**Sodium Saccharine**	254	3.97±0.004	2.8±0.032	0.78±0.037	y = 0.0556x + 0.0012	0.9997
4	**Amaranth**	235	4.25±0.006	20.44±0.138	0.77±0.018	y = 0.1303x + 0.0023	0.9998
5	**Ponceau 4R**	235	6.52±0.003	3.88±0.064	0.76±0.017	y = 0.0956x + 0.0028	0.9997
6	**Sunset yellow FCF**	235	6.98±0.004	11.92±0.162	0.75±0.019	y = 0.1589x + 0.0052	0.9997
7	**Allura red AC**	235	8.48±0.004	16.43±0.276	0.72±0.034	y = 0.0911x - 0.0006	0.9991
8	**Thiabendozole**	319	11.42±0.008	10.23±0.190	0.93±0.078	y = 0.8543x - 0.2192	0.9974
9	**Ponceau 3 R**	235	12.95±0.006	5.01±0.140	0.78±0.057	y = 0.1376x + 0.0041	0.9985
10	**Ethyl paraben**	254	13.70±0.009	5.80±0.125	0.75±0.019	y = 0.2895x - 0.011	0.9994
11	**Methyl Paraben**	235	14.60±0.007	8.44±0.061	1.02±0.061	y = 0.023x + 0.0068	0.9921
12	**Erythrosine extra bluish**	235	15.78±0.007	3.01±0.114	0.81±0.096	y = 0.0512x - 0.0096	0.9971
13	**Sulforhodamine B**	235	16.18±0.005	3.05±0.115	0.83±0.082	y = 0.1038x - 0.0804	0.9839

Table 8: Calibration Curves, Resolution and Asymmetry for the Seventeen Food Additives (Continued)

No	Food additives	wave length λ (nm)	Mean ± (SD)			Regressions Equation	Correlation Coefficient R^2
			Retention Time (min)	Resolution	Asymmetry		
14	**Patent blue V Sodium salts**	235	16.55±0.014	5.97±0.509	1.07±0.331	y = 0.0241x - 0.0009	0.9984
15	**Mentanil yellow**	235	17.39±0.012	7.28±1.333	0.76±0.064	y = 0.0555x + 0.0057	0.9996
16	**Butyl paraben**	254	18.74±0.010	8.08±1.302	0.85±0.113	y = 0.4193x + 0.2598	0.9942
17	**Quinoline Yellow 2 S F**	319	20.49±0.014	n.a	0.69±0.088	y = 0.0609x - 0.0077	0.996

Y: peak area of response (mAU), x: concentration of additives ppm (10 µg/mL), n.a: not applicable, N: 18 number of additives.
A general form of a linear regression function is given Eq: (Y = Xb + e) or (y = b0 +þ b1x1 +þ b2x2 +þ ...+ þ bkxk +þ e).
Terms for Eq: y = response, xi = factor or monomial-term like xi 2 or xixj, b0 = regression constant, bj = regression coefficiente = inexplicable error, (Ermer & Miller, 2006).

3.3 Determination of the Limit of Detection LOD and the Limit of Quantification

The quantification results of the food additives in thirty beverage samples were shown in Table 9. The results show that the LOD is significantly lower than LOQ for all additive samples. For sodium saccharine and amaranth are at identical values of LOD 0.22 and LOQ of 0.74 (µg/mL or mg/L). Thiabendozole was found to be lowest both for LOD and LOQ µg/mL for butyl paraben was found to be 9.03 mg/L. The LOD and LOQ for each of the analytes (compounds) was calculated from six replicate injections using Excel® (Microsoft Office 2013).

Table 9: Determination of LOD and LOQ (µg/mL or mg/L) for Food Additives (Anlaytes) by UPLC Analysis

No	Food Additives	LOD	LOQ
1	**Acesulfame K.**	0.11	0.36
2	**Tartrazine**	0.42	1.39
3	**Sodium Saccharine**	0.22	0.74
4	**Amaranth**	0.22	0.74
5	**Ponceau 4R**	0.56	1.88
6	**Sunset yellow FCF**	0.20	0.68
7	**Allura red AC**	0.23	0.76
8	**Thiabendozole**	0.05	0.15
9	**Ponceau 3 R**	0.20	0.66
10	**Ethyl paraben**	0.12	0.38
11	**Methyl Paraben**	0.20	0.67
12	**Erythrosine extra bluish**	0.65	2.15
13	**Sulforhodamine B**	1.81	6.03
14	**Patent blue V Sodium salts**	1.08	3.60
15	**Mentanil yellow**	0.63	2.10
16	**Butyl paraben**	2.71	9.03
17	**Quinoline Yellow 2 S F**	0.74	2.47

3.4 Test Examined in Real Samples

Two samples from each of thirty beverages were purchased and each of the samples was analyzed in duplicate. Repeatability of the samples consists of two batches followed with duplicated injection into the UPLC machine. The result shows precisions of approximately 98%. While there are different batches for the same product with difference result can be due to the procedures doing place in the factory e.g. when diluted the food additives into beverages tank to be well homogenized in the same batched and same production dates, but many show light variations in different batches within the approved limits of food additives.

The chromatogram for the real samples and the chromatogram of additive standards were compared. The selectivity and sensitivity represented in peak shapes, resolution, separation in terms of retention times limits. The UPLC method for separation of peak in single run in beverages was found which represent in peaks and retention times shown in Figure 5, Figure 6 and Figure 7.

3.4.1 Chromatograms of Real Samples of UAE Beverages

Figure 5 shows clear peak shapes of compounds (food additive) in real beverage sample from UAE market which are similar to the peak in the chromatogram in Figure 4 that represented standard solutions. In addition, retention time (in minutes) were 2.66 (Acesulfame K), 3.03 (Tartrazine), 3.75 (Sodium Saccharine), 16.18 (Sulforhodamine B), and 18.67 (Butyl paraben), therefore these five compounds provide stable retention time and along with the standard solutions in Table 8.

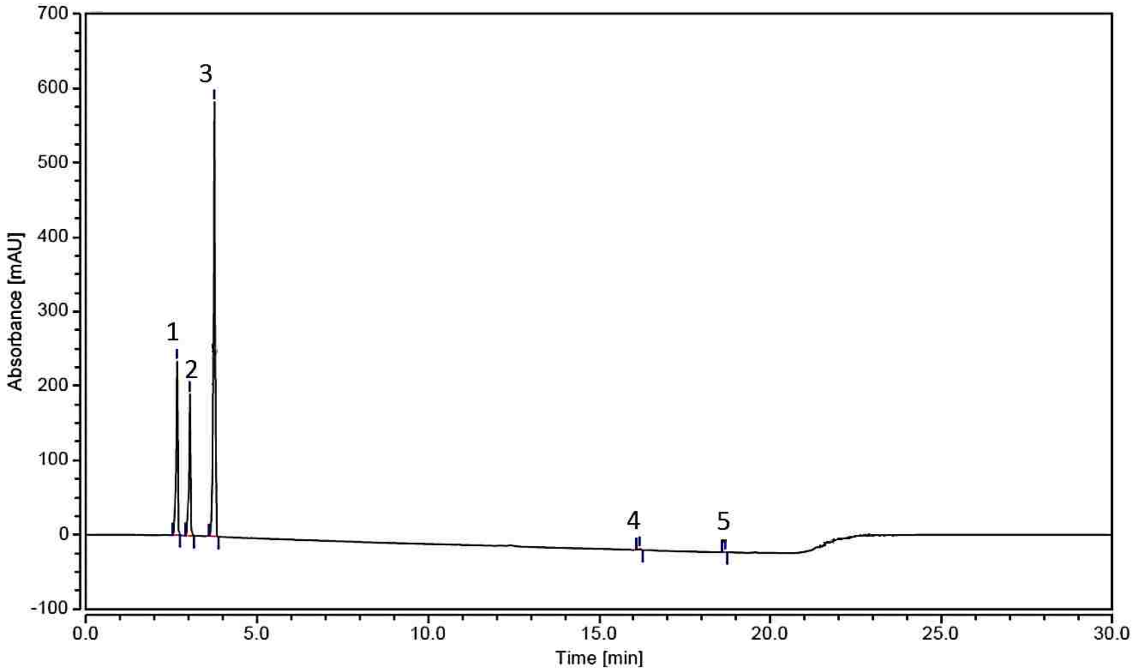


Figure 5: UPLC Chromatogram at Wavelength λ 235.0 (nm) in Real Sample no X1 Beverages from UAE Market

List of peak of additives: 1: Acesulfame K, 2: Tartrazine, 3: Sodium Saccharine, 4: Sulforhodamine B, 5: Butyl paraben

Figure 6 shows clear peak shapes of compounds (food additive) in real beverage sample from UAE market which are similar to the peak in the chromatogram in Figure 6 that represented standard solutions. In addition, retention time (in minutes) were Acesufame K (2.66), Sulforhodamine B (16.24), Butyl paraben. (18.51), was found and represent precision, therefore, these three compounds provide stable retention time along with the standard solutions in Table 8.

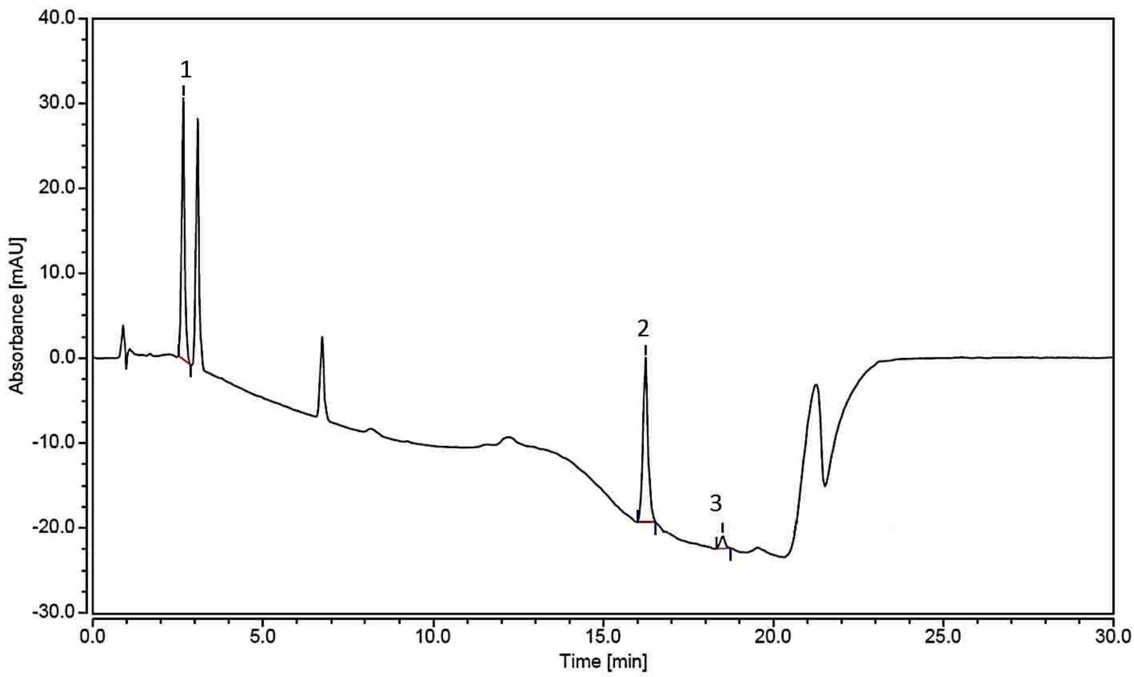


Figure 6: UPLC Chromatogram at Wavelength λ 235.0 (nm) in Real Sample no X2 Beverages from UAE Market

List of peak of additives: 1: Acesufame K, 2: Sulforhodamine B, 3: Butyl paraben

Figure 7 shows clear peak shapes of compounds (food additive) in real beverage sample from UAE market which are similar to the peak in the chromatogram in Figure 7 that represented standard solutions. In addition, retention time (in minutes) were Tartrazine (3.38), Sodium Saccharine (3.86), Sunset yellow FCF (6.99), Sulforhodamine B (16.24), Butyl paraben (18.51), therefore, these five compounds provide stable retention time along with represent in standard solutions in Table 8.

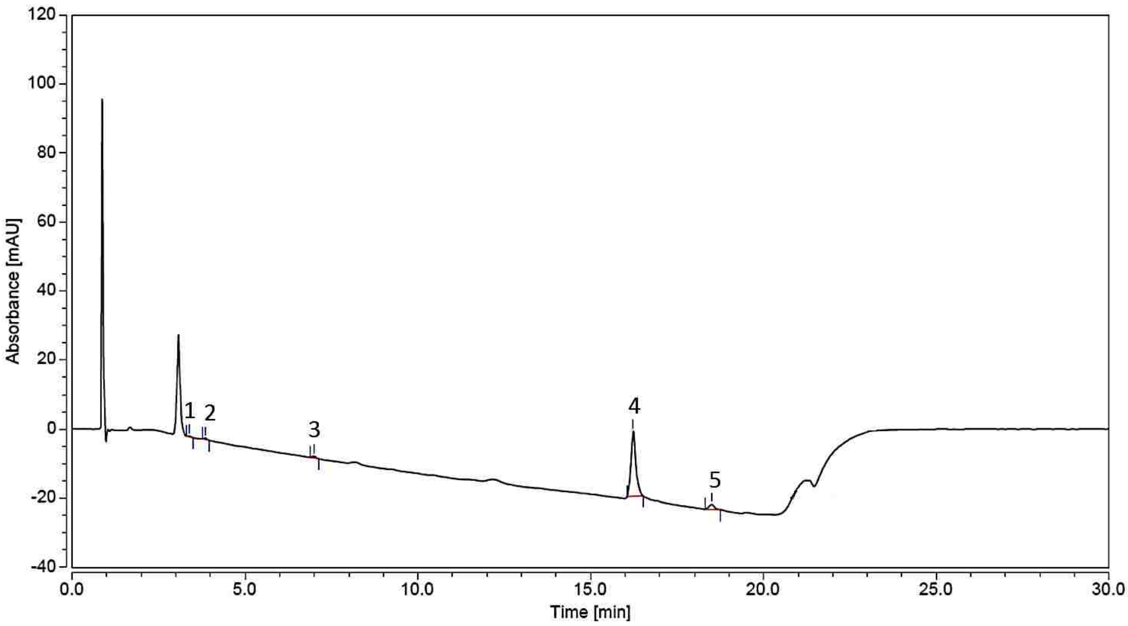


Figure 7: UPLC Chromatogram at Wavelength λ 235.0 (nm) in Real Sample no X 3 Beverages from UAE Market

List of Peak of Additives: 1: Tartrazine, 2: Sodium Saccharine, 3: Sunset yellow FCF, 4: Sulforhodamine B, 5: Butyl paraben.

3.4.2 Level of Additives in Beverages Samples in UAE

Thirty samples including nine drinks, seven carbonated drink (soft drinks), three fresh juices, three energy drinks, two nectars, two types of flavored water, two nutritive drinks, and one carbonated drink fortified with vitamins were purchased from two different batches. The selection of the examined compounds was focused on those additives that cause side effects and which are the most frequently applied for enrichment in the beverages in the food industry. However, a potential risk to human health was raised due to the ingestion of these compounds, especially when they are excessively consumed at different ages. Thus, the determination of synthetic food additives is required to ensure the food safety. Therefore, the following compounds were examined, observed and the measurements were recorded while conducting the procedures described in the methods. Results and the objective of scientific research summarize the collected data with the statistical treatment. The average mean ± SD mg/L contents of synthetics food additives are shown below the explanation of each in Table 10 and Table 11 respectively, with the correlated data in Table 9. The findings of the thirty samples with duplication indicated that the mean of quantification result of the food additives' synthetic color was 14, while the quantification result of the food additives' sweeteners was 7.

3.5 Mathematic Calculations

The results in Table 10 and Table 11 respectively, show the units of the quantified beverages were converted to mg/L and compared with MPL which were carried out in the mean beverages of the duplicated batches, however, the method for the food additives compounds was not sensitive enough and therefore, the presented results are not conclusive. The LOD, LOQ in Table 9 of food additives values obtained from UPLC analysis are in µg/mL. Also the food additives values of the UAE beverages which were detected in µg/mL unit

In Table 10**,** the quantified mean µg/mL (or mg/L) was found only in three synthetic colors tartrazine, sunset yellow FCF and ponceau 3 R as Standard Deviation SD < 1.7. Standard Deviation for the most acceptable result is SD < 2%, if N = 5. The noticeable error of the variation result of SD ±5.98 for tartrazine in the drink categories can be observed. This is most probably because the tested number and trials are very high. Similar reasons are for sunset yellow FCF SD ±11.39 of 14.32 µg/mL mean quantified color in drink categories. For the carbonated soft drinks the SD was ±5.3 which is above 2% mean of the sunset yellow. This variation in SD ± is most likely due to the processing procedure in the factory.

For example, in nectars, juices and soft drinks, sunset yellow can be added to alter the color appearance. As for carbonated soft drinks, the reason for SD of 5.336 could be due to using different concentration recipes and ingredients from different companies which may reflect different color quality for different products. In addition, the source of raw material (concentrated juice) include the additives which can come from various suppliers or by using a variant concentration level of additives from their original country, while when diluted in the local factory in Abu Dhabi, only water and sugar are added. Yet, the percentage of the additive depends on the origin country suppliers.

Furthermore, the quality controller in the factory checks the qualitative and quantitative standards of the product during the processing steps and the mixing of the concentration which may vary from one batch to the other batch caused by human error in the product procedure.

Table 10: The Mean µg/mL ± SD of Synthetic Colorant in Quantified Real Samples

Categories Class	**N**	**The Mean of Synthetic Colorant ± SD**		
		Tartrazine	**Sunset yellow FCF**	**Ponceau 3 R**
Drink	10	5.2±5.9	14.3 ±11.3	2.3±0.4
Fresh Juice	2	ND-Trace	1.7	ND
Fresh Nectar	1	2.1	12.8	ND
Nectar	1	ND	Trace*	ND
Carbonated soft drinks	6	4.7±0.8	29.9±5.3	ND
Carbonated drink with vitamins &mineral	1	14.9	ND	ND

N = number sample, with SD < 2%, for n = 5. ND synthetic colorants result for amaranth, ponceau 4 R, allura red AC, erthyrosin extra bluish, sulfor hodamine B, patent blue V Sodium Salt, metanil yellow and quinoline yellow. 2 S F *Trace sunset yellow result for Categories: Nectar, and ND
ND synthetic colorants result for Categories: Flavored water, Juice, Energy Drink, Nutritive drink Ion supply, Malt beverages

Table 11 the results showed that SD for saccharin is within the limits < 2. However, acesulfame-K SD ±6.772 for carbonated soft drinks and SD ±12.263 for energy drinks referred to 1 out of 3 was brand of a light energy drink which could explain the difference. The explanation for the uncontrolled homogenized additives in different batches with different production dates of carbonated soft drinks. If the factory had proper standard procedures other than manual error in weighted additives while preparing serial batches the difference would not become noticeable.

Table 11: The Mean µg/mL ± SD of Sweetener in Quantified Real Samples

Categories (Class)	N	The Mean of Sweetener ± SD	
		Acesulfame K	Sodium Saccharin
Drink	10	ND	2.0±1.8
Flavored water	2	62.9	ND
Fresh Nectar	1	2.1	3.9
Nectar	1	ND	ND-Trace*
Juice	1		1.1
Carbonated soft drinks	6	14.1±6.8	ND-Trace
Energy Drink	3	16.9±12.3	ND
Carbonated drink with vitamins &mineral	1	4.3	ND

N: number sample, * Trace result Sodium saccharin for Categories: Nectar
ND synthetic sweetener result for Categories: Fresh Juice, Nutritive drink Ion supply, Malt beverages

Thirty samples of random commercial brands of beverages were purchased for the analysis from a shop in Al-Ain city in Abu Dhabi Emirate. The samples from two batches with different date of production were segregated into groups depending on the type of their class. The samples included (drinks, soft drinks, juice, carbonated beverages, energy drinks, and flavored water, etc) respectively, describe zero quantitative result out of four preservatives additives, nothing. Still not confirm were not found in real beverages samples studies.

Table 12 represents the overall results of additives found in 30 beverage samples used in this study. For his research a sample of 30 different beverages sold in AlAin supermarkets were used to detect 3 groups of additives; synthetic colorants, sweeteners and preservatives. Overall, the results showed that poncceau 3R from the synthetic colorant was not detected in 28 out of 30 samples, while other synthetic colorants such as tartazine and sunset yellow FCF was detected in 21 samples out of 30. The trace sample for tartazine was 4, whereas, sunset yellow FCF was 2. For sweeteners, both acesulfame K and sodium saccharin were not detected value (ND) founded in 24 samples out of 30, but 4 trace samples of sodium saccharin were found in compared to 1 trace values of accesulfame K in the 30 analyzed samples.

As for the comparison of the number of positively quantified samples with those of labeled quantified samples, the results showed that sunset yellow FCF was found in 7 samples, but was labeled on 3. Tartrazine was found in 5 beverages samples, yet only labeled in 2. Similarly, 5 positively quantified samples of acesulfame K were identified, however, 3 were labeled as such. Sodium saccharin was not labeled at all of quantification values, but was detected in 2 samples out of 30. likewise, result poncceau3R was not labeled at 2 quantified samples out of 30. As for preservative, there are no quantified results of mean (µg/mL) for the preservative in the real samples. Whereas, thiabenzadazole and methyl paraben were traced as unreliable value in all examined beverages samples. Nevertheless, butyl paraben and ethyl paraben was not detected values (ND) for all tested beverages. In Table 12 indicates the results of non-labeled quantified real samples. Misleading labeling in terms of naming of additives In other words, indicating on the label of product that the additive is within the limit.

Table 12: Results of Additives Found in 30 Beverage Samples used in this Study

Food additives Group	Additives compounds	ND Samples	N Trace Samples	N positives Quantified Samples	N Label of Quantified Sample	N Not labeled of Quantified Sample
Synthetic Colorant	**Tartrazine**	21	4	5	2	3
	Sunset yellow FCF	21	2	7	3	4
	Poncceau 3 R	28	-	2	-	2
Sweeteners	**Acesulfame K**	24	1	5	3	2
	Sodium Saccharin	24	4	2	-	2
Preservative	**-**	-	-	-	-	-

ND : not Detected, N: number

Table 13 represents the comparison of relevant study between UAE and Macedonia for the common synthetic food additive such as synthetic colorants which are permitted within the safety levels in beverages. Regarding the UAE thirty (30) samples of beverages were tested in comparison to a range of (78-665) samples in Macedonia. It is essential to note that all beverage products in the UAE are imported and demotic, and the concentration range of synthetic colorants is within the CODEX level. In fact, they are below the MPL (Maximum Permitted Level). While the Macedonia study has a mix of imported and domestic beverages in their market. For Macedonia, 8 out of 560 samples for imported soft drinks are above the MPL. Also 3 samples out of 105 for domestic soft drinks are above the MPL (Kostik, 2014). In the category of synthetics color for tartrazine E102, the concentration range detected was 1.7-8.1 (µg/mL or mg/L) for the UAE and 1.0-68.3 mg/L for imported beverages and 1.0-43.3 mg/L for domestic beverages in Macedonia all result within the CODEX and MPL. Kostik indicated that sunset yellow FCF was found in 46.43% of tested imported samples (260 samples), and in 100% of tested domestic brands (105 samples). Eight of the imported and three of domestic tested samples exceeded the establish MPL of 50 mg/L. For, sunset yellow FCF E104 the concentration range detected was 0.8-30 (µg/mL or mg/L) for the UAE beverages within the CODEX level while 1.0-70 for imported beverages and 1.0-80 mg/L for domestic beverages a total of 11 samples above the MPL in Macedonia.

Table 13 represents the comparison in terms of declaration of presence of additives on labels, the ratio of non-labeled quantified samples is 6 out of 602 in Macedonia and 3 out of 30 for the UAE for tartrazine E102. The ratio is similar for sunset yellow FCF E104 with 5 out of 665 for Macedonia and 4 out of 30 for the UAE.

Comparison analytical results in the UAE with Macedonia study within one of the comprehensive schemes regulating the use of food colors, the European Union (Directive 94/ 36/EC, 1994) issued legal provisions for foods. The above regulations set the scene for the analytical chemist who has to test for the levels of dyes added to food. The reasons of adding color in many countries have established strict regulations for the allowable kinds and concentrations of synthetic pigments.

In addition, safety evaluation of all additives used in foods must be declared in the list of ingredients in accordance with Council Directive 2000/13/EC (Kostik, 2014).

Studies (Sawaya et al.) show that Kuwait has the highest level of these colorants for all groups not only in beverages, but also in all food products around of 334 food items. In fact, it is higher than the Kuwaiti authorized levels in 2008 (Sawaya et al., 2008).

Table 13: Comparative Results for Presence of Colorants between UAE and Macedonia Beverages

Food additives Groups	Name of additives	N Beverages samples		N positives Samples Quantified		Concentration Range Detected		Maximum permitted level		Not labeled of quantified sample	
Comparison		**UAE**	**Macedonia** I+D	**UAE**	**Macedonia** I+D	**UAE mg/L**	**Macedonia mg/L**	**UAE / CODEX mg/kg**	**Macedonia mg/L**	**UAE**	**Macedonia** I+D
Synthetics Color	**Tartazine E102**	30	602	5	331	1.7-8.1	I: 1.0-68.3 D: 1.0-43.3	50	100	3	6
	Sunset yellow FCF E104	30	665	7	365	0.8-30	I: 1.0-70.1 D: 1.0-80.3	100-300	50	4	5

I: Imported, D: Domestic, N: number, where; µg/mL (tested sample) converted ~ mg/L.

Table 14 shows the comparative results for presence of food additives specifically sweeteners in beverages for UAE, Macedonia and Portugal. However, the study in Portugal (2008) excluded sodium saccharin and only included Acesulfame K. The UAE conducted the experiment with 30 samples, Macedonia with 143 and Portugal with 48. Results showed that in the UAE, 5 out of 30 samples were found to be positively quantified, whereas, in Macedonia 116 out of 143 samples and 38 out of 48 samples in Portugal were found to be quantified. In other words, in Macedonia and Portugal, acesulfame-K was found in nearly 80% of the beverage samples with comparison of approximately 17% in beverages in the UAE. Permitted levels by CODEX for the UAE is 600 and 350 for both Macedonia and Portugal respectively. 2 out of 30 beverage samples were found to be not labeled in the UAE, whereas all beverages products were found to be labeled in the other countries mentioned above. Sodium saccharin was identified and quantified in only 2 samples in the UAE and in 62 samples in Macedonia. The permitted levels for Sodium saccharin is 300 in the UAE and 80 in Macedonia.

In the category of sweeteners, the concentration range, of acesulfame-K (E950) was detected 0.6-31.4 µg/mL (mg/L) for the UAE, 180.5-330.1 mg/L for imported beverages and 202.4-34.6 mg/L for domestic beverages in Macedonia and levels between 35-356 mg/L in Portugal all within the MPL. For saccharin sodium (E954), the concentration range detected was 0.8-3 (µg/mL or mg/L) for the UAE and 55.8-77.9 mg/L for imported beverages in Macedonia all within the CODEX MPL. However, one sample exceed the range 67.8-115.3 mg/L was found for domestic beverages in Macedonia which is over 80 the MPL level and all the UAE beverages within the CODEX level. It is essential to note that all beverage products in the UAE are imported and demotic, and the concentration range of synthetic sweeteners is within the CODEX level. Table 14 shows a summary of the results for presence of food additives in studies beverages of UAE. In terms of declaration of presence of additives on food labels. Result for non-labeled quantified samples is 2 out 5 of each acesulfame K and2 out 2 sodium saccharin in UAE. Similar for sodium saccharin the quantified samples non labeled was 3 out of 62 in Macedonia (Kostik, 2014; Lino et al., 2008). Table 15 shows the comparative results for presence of additives specifically sweeteners acesulfame-K and sodium saccharin in soft drinks for UAE, Romania. Macedonia include (Imported +Domestic), and Portugal. As mentioned in Table 14, Portugal excluded sodium saccharin. Results show that in soft drinks, acesulfame-K was quantified in 5 out of 6 samples in the UAE, 116 out of 143 in Macedonia, 24 out of 30 samples in Romania and 28 out of 38 in Portugal. The percentages for Romania and Portugal are found to be high. As for Sodium Saccharin, 2 samples out of 6 for the UAE, 65 out of 78 for Macedonia and 16 out of the 30 samples in Romania were quantified. Six samples of carbonated soft drinks were tested in the UAE and 30 in Romania study. For Acesulfame-K E950, the concentration range detected was ND-14.90 (µg/mL or mg/L) in the UAE, while in Romania was 0-268.51 mg/L. Both cases acesulfame-K and sodium saccharin (or saccharin-Na) the results were below the maximum level that allowed in soft drinks UAE, Romania, Macedonia and Portugal markets. (Oroian et al., 2013; Kostik, 2014; Lino et al., 2008). The comparison of the data analyzed in this study with that of CODEX, show that the maximum limit of food additives present in different non-alcoholic beverages is below the maximum limit indicated by CODEX. For example, one (person who is 60 kg) needs to consume 4 bottles of a specific soft drink (e.g. XX) so as to reach the maximum levels as acceptable in daily allowance for human consumption. For other drinks, consumption must reach 7 to 8 drinks per day to exceed the maximum levels. According FAO/WHO in 1999 to reach ADI, the Average Daily intake of specific additives, we must consider the body weight and the compilation of additives that come from other consumption of food products not just soft drinks (Sawaya et al., 2008). Most studies conducted are related to maximum levels of safety. For example, fresh juices, sunset yellow FCF is quantified at 0.8 µg\mL; ratio to CODEX maximum limit is between 50 and 300 mg/kg which means one must consume approximately 62 bottle of fresh juices daily to reach the limit MPL and for ADI limit is 2.5 mg/kg body weight. Similarly, diet soft drinks which have sweeteners, have 14.9 µg/mL per bottle. So as to reach the maximum level of acesulfame-K 600 mg/kg of CODEX level, the MPL approximately equal 42 bottles, the maximum permitted level for acesulfame-K in soft drinks is 350 mg/L. This level was not exceeded and with a concentration mg/L (Lino et al., 2008). Similar to the study conducted by Sawaya et al., (2008), to assess exposure to artificial colour additives, the average daily intakes of permitted artificial colour additives and other components for varying age groups were calculated by multiplying the average amounts of coloured foods and beverages consumed by the average levels of the colour or any additives other components in those foods and beverages, and dividing the result by the average body weights for each age group, males and females. Exposure estimates were compared with the corresponding acceptable daily intakes (ADIs) as set by the FAO/WHO (1999) (Sawaya et al., 2008).

According Portuguese legislation (Decreto-Lei n 394/98 de 10 de Dezembro de 199), law contributes to Health concern and consciousness in terms of declaration of presence of additives on labels. However, all ingredients included in the food or beverages are required to be listed on the food labels for consumer right and ethical value in order to prevent health consequences on the consumer.

Table 14: Comparative Results for Presence of Sweeteners in Beverages among UAE, Macedonia and Portugal

Food additives Groups	**Name of additives**	**N Beverages samples**			**N positive Samples Quantified**			**Concentration Range Detected**			**Maximum permitted level**			**Not labeled of quantified sample**		
Comparison		**UAE**	**Macedonia I+D**	**Portugal**	**UAE**	**Macedonia I+D**	**Portugal**	**UAE mg/L**	**Macedonia mg/L**	**Portugal mg/L**	**UAE / CODEX mg/kg**	**Macedonia mg/L**	**Portugal**	**UAE**	**Macedonia I+D**	**Portugal**
Sweeteners	**Acesulfame-K E950**	30	143	48	5	116	38	0.6-31.4	I: 180.5-330.1 D: 202.4-34.6	35-356	600	350	350	2	-	
	Saccharin Sodium E954	30	78		2	62		0.8-3	I: 55.8-77.9 D: 67.8-115.3		300	80		2	3	

Macedonia study included beverages and soft drinks, N: number.

Table 15: Comparative Results for Presence of Sweeteners in Carbonated Soft Drinks among UAE, Macedonia, Romania and Portugal

Food additives Groups	**Name of additives**	**N Beverages samples**				**N positive Samples Quantified**				**Concentration Range Detected**				**Maximum permitted level**				**Not labeled of quantified sample**			
Comparison		**UAE**	**Macedonia** I+D	**Romania**	**Portugal**	**UAE**	**Macedonia** I+D	**Romania**	**Portugal**	**UAE mg/L**	**Macedonia mg/L**	**Romania mg/L**	**Portugal mg/L**	**UAE / CODEX mg/kg**	**Macedonia mg/L**	**Romania**	**Portugal mg/L**	**UAE**	**Macedonia** I+D	**Romania**	**Portugal**
Sweeteners	**Acesulfame-K E950**	6	143	30	38	5	116	24	28	0.6-31.4	I: 180.5-330.1 D: 202.4-34.6	0-268.51	3.7-182	600	350	200	350	2	-		
	Saccharin Sodium E954	6	78	30		2	65	16		0.8-3	I: 55.8-77.9 D: 67.8-115.3	0-83.75		300	80	200		2	3		

Macedonia study were include soft drinks (Imported +Domestic) , N :Number

Chapter 4: Conclusion and Recommendation

4.1 Conclusion

The purpose of the current study was to determine the quantification of the seventeen artificial food additives in the thirty beverages which were selected randomly from Abu-Dhabi, Al-Ain city supermarkets. The analytical method used for the determination of the synthetic colorants, sweetener and preservatives in beverages gave reliable and reproducible results with efficient detection limits and short analysis time for the analysis of the food additives extraction in UHPL chromatographic run. This method showed partial validity, linearity, repeatability, and specificity parameters with missing the sensitivity. Based on public databases relevant studies matching our purposes, the results of synthetic colorant additives tartrazine and sunset yellow FCF were quantified in UAE beverages and compared with relevant results in Macedonia. Other comparative studies among UAE, Macedonia, Romania and Portugal serves in similar topic aspect of human health risks of exposure to acesulfame-K and saccharine are widely used in food industry. Results for two types of sweeteners were below the regulation level UAE carbonated soft drinks in Abudhabi, which were similar to those in Macedonia, Romania and Portugal beverages and carbonated drinks. In addition, there were no quantified results for preservative in the real samples which are within safety level in UAE.

Today these findings of food additives synthetic colors, sweeteners and preservatives in beverages covered in this study and sold in the UAE market are controlled and comply with the approved level of CODEX. Yet, results are still not confirmed, due to lack of sensitivity of the method.

Although the number of drink samples analyzed is very small scale research “pilot study” or “pilot experiment “, the data presented in this study gave a basic outline about the content levels in beverages most consumed in the UAE.

Among the 30 beverages analysed, only a few contained non-labeled colour additives and sweeteners, but preservatives were not detected. This could have been due to mislabeling of the beverages by food manufactures. All of these findings indicate that mislabeling of food items may constitute an issue that warrants further investigation by the food control authorities in the UAE.

In the future, the findings of this study might be enhanced the domestic food law and could be formulized, which will assist in creating their own trade food law. Therefore, it might advance the legislations and regulation rules as (Abu Dhabi Food Control Authority Food Law No. 2 of 2008) Abu Dhabi Food Control Authority (ADFCA) for Abu-Dhabi, UAE and associations with ESMA. In addition, this study may cooperate with the Authority controller and provide valuable insight into the function of the additive levels in beverages as a specialized topic. Retrospectively, there is a need to ensure the safety of food products due to concern for consumer health. To avoid misleading labeling in terms of naming or codes of additives, there also must be a declaration of the MPL and ADI. For that reason, it is essential the public need awareness about side effects of accumulation of additives in their body.

In conclusion, UAE imposed taxes on September 2017 on soft drink by increasing the price 50%, as a precaution step to reduce health risks by discouraging the highly consumption.

4.2 Research Implications and Future Research

Follow-up studies benefiting from available resources and upcoming results of this research show a level of additives being used in beverages in the UAE. Following future studies, with additional survey needs to be undertaken in order to determine the range of real consumption of beverages include different types of food and drinks especially in UAE. This will lead us to determine if the side effects are due to over-consumption of food additives in beverages or from other processed foods. Finally, this study focused on only thirty samples which does not cover the whole spectrum of food additives e.g. enhancer, flavor, caffeine, thickeners, emulsifiers, stabilizers, etc. Which can be used in processed foods or beverage . Therefore, the results are representative for small representative beverages group and further research can give insight regarding the wider spectrum of other products such as candies and canned foods.

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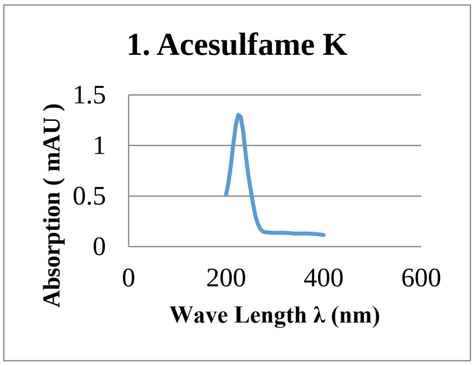
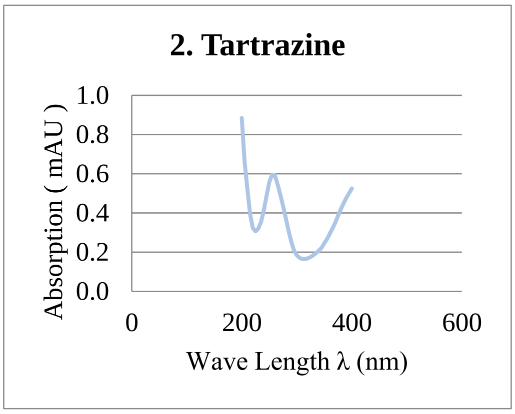
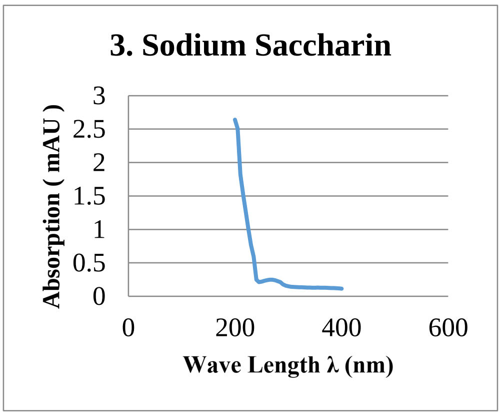
Appendix 1

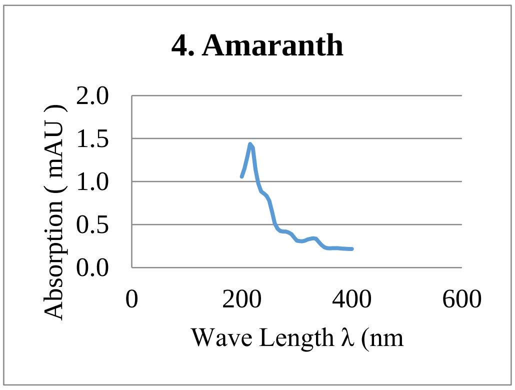
The absorption spectra of the food colors, sweeteners and preservatives by using UV visible Spectra photometer wavelength between 200 and 700 nm.

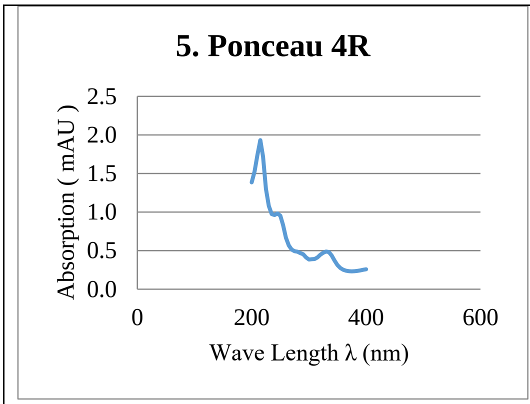
*Additives were prepared by dissolving solid analyte in deionized water except for thiabendozole; ethyl paraben; sulfor hodamine B; butyl paraben and methyl paraben, which were dissolved in 10 mL methanol.

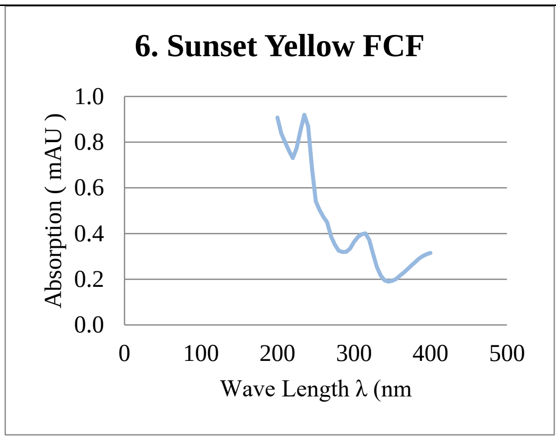


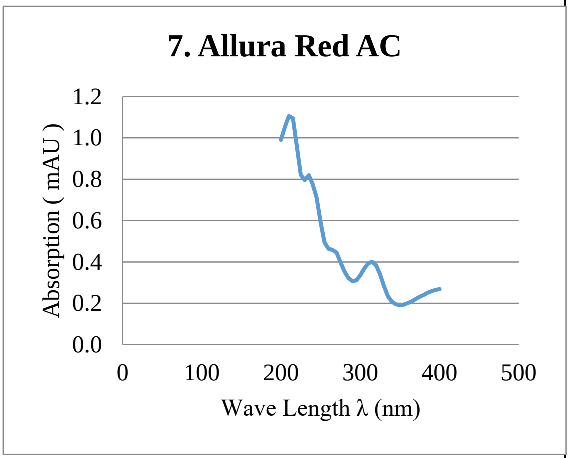


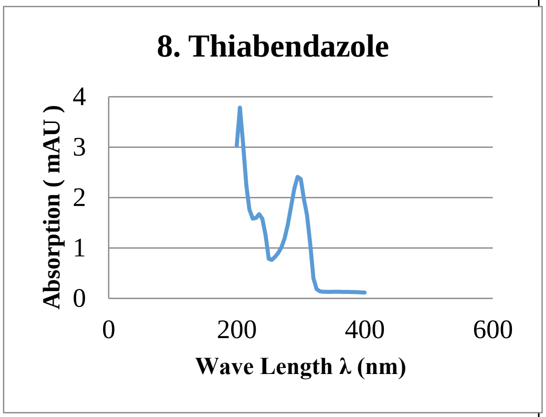


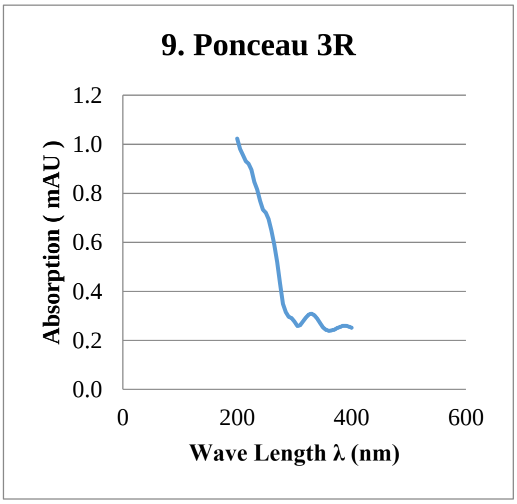


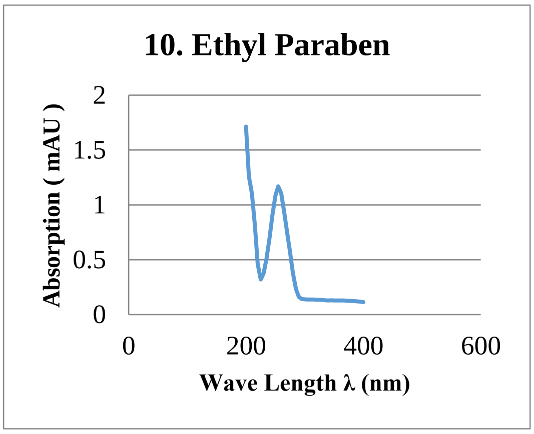

5. Ponceau 4R
Absorption (mAU)
2.5
2.0
1.5
1.0
0.5
0.0
0
200
400
600
Wave Length λ (nm)

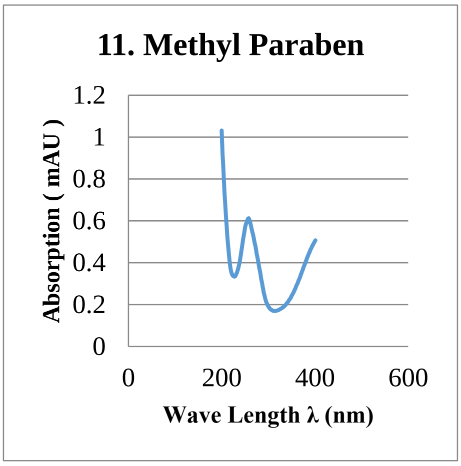

6. Sunset Yellow FCF
Absorption (mAU)
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
Wave Length λ (nm

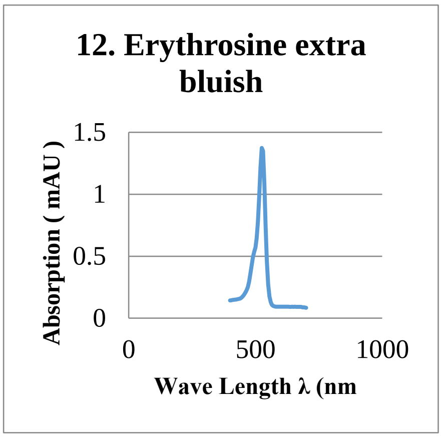

7. Allura Red AC
Absorption (mAU)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
Wave Length λ (nm)

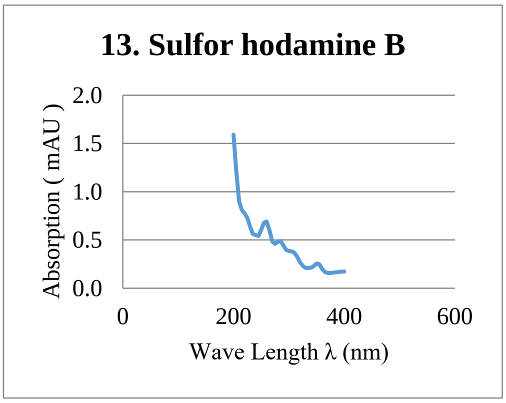

8. Thiabendazole
Absorption (mAU)
4
3
2
1
0
0
200
400
600
Wave Length λ (nm)

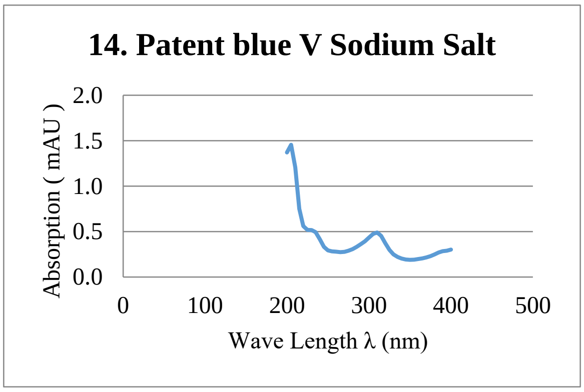

9. Ponceau 3R
Absorption (mAU)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
Wave Length λ (nm)

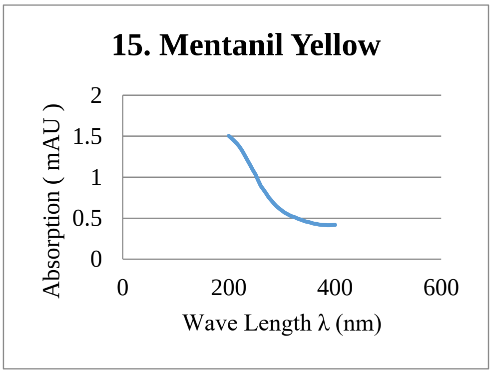

10. Ethyl Paraben
Absorption (mAU)
2
1.5
1
0.5
0
0
200
400
600
Wave Length λ (nm)

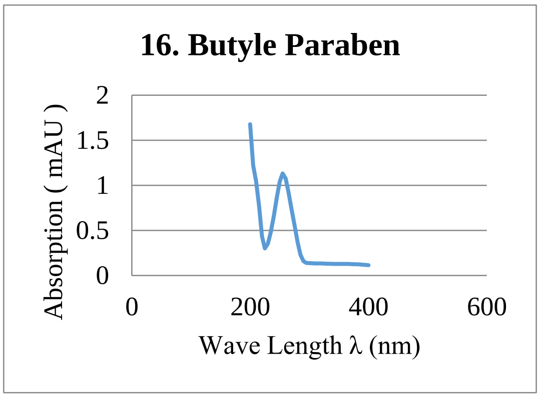

11. Methyl Paraben
Absorption (mAU)
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
Wave Length λ (nm)


12. Erythrosine extra bluish
Absorption (mAU)
1.5
1
0.5
0
0
500
1000
Wave Length λ (nm


13. Sulfor hodamine B
Absorption (mAU)
2.0
1.5
1.0
0.5
0.0
0
200
400
600
Wave Length λ (nm)


14. Patent blue V Sodium Salt
Absorption (mAU)
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Wave Length λ (nm)

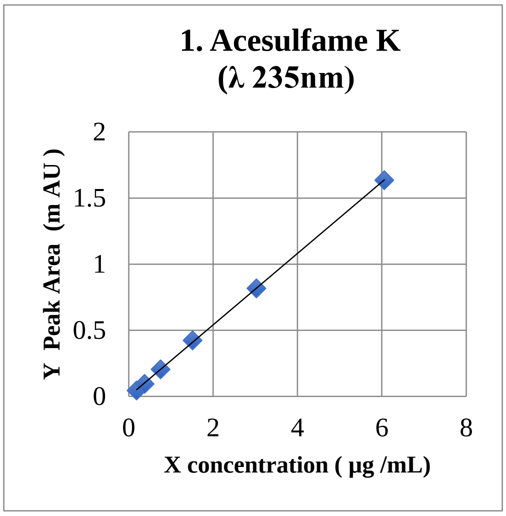

15. Mentanil Yellow
Absorption (mAU)
2
1.5
1
0.5
0
0
200
400
600
Wave Length λ (nm)

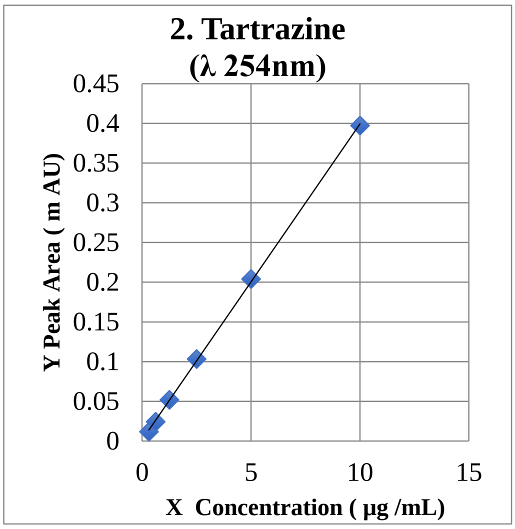

16. Butyle Paraben
Absorption (mAU)
2
1.5
1
0.5
0
0
200
400
600
Wave Length λ (nm)

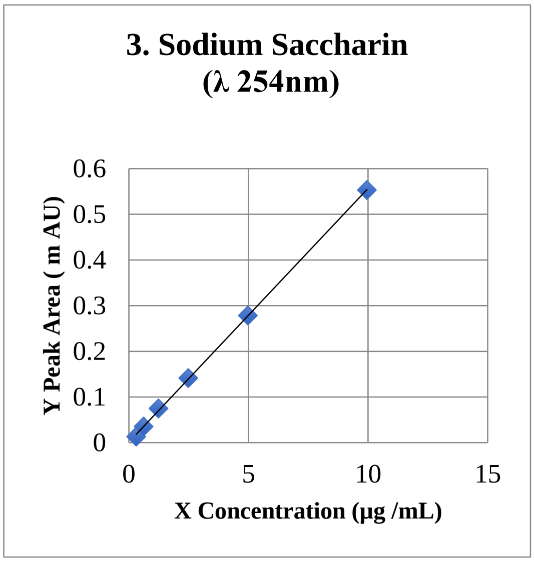
Appendix 2

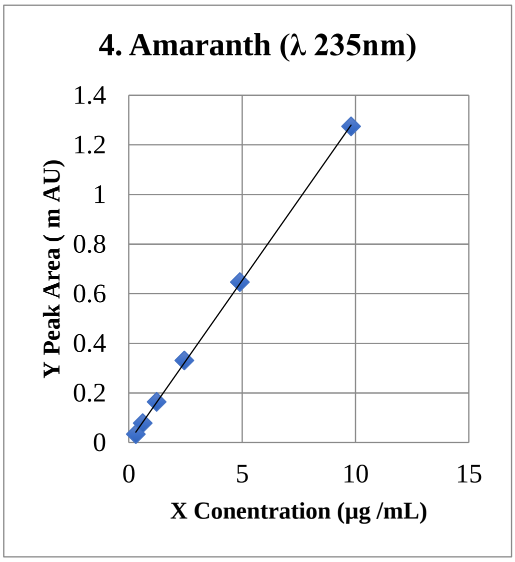
Calibration curves for each food additive including the three groups' colorant, sweetener and preservatives.

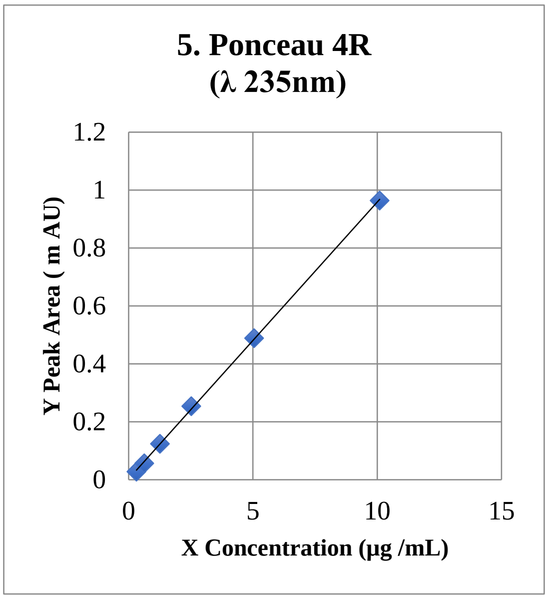
*Where the X-axis represent the concentration of the each additives (ppm)or (µg/mL) and the Y-axis represent the peak area absorption Unite (AU).

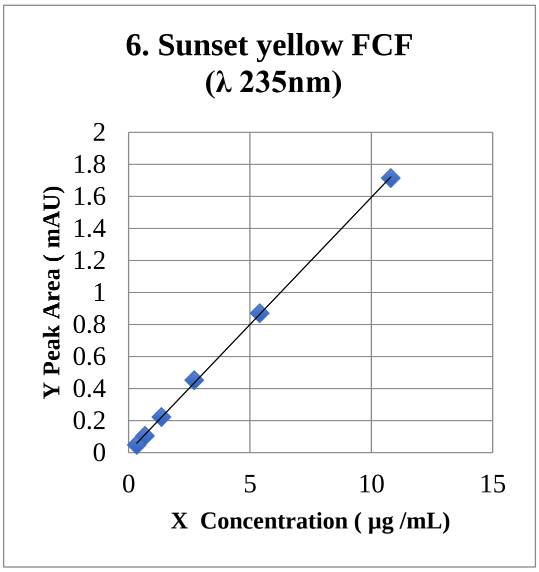


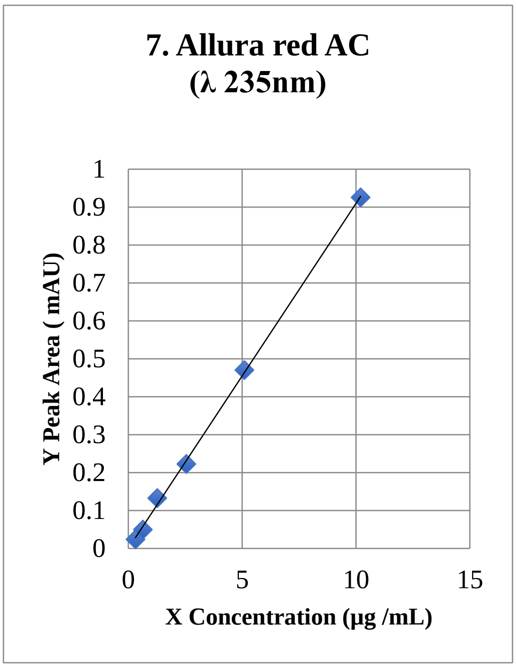


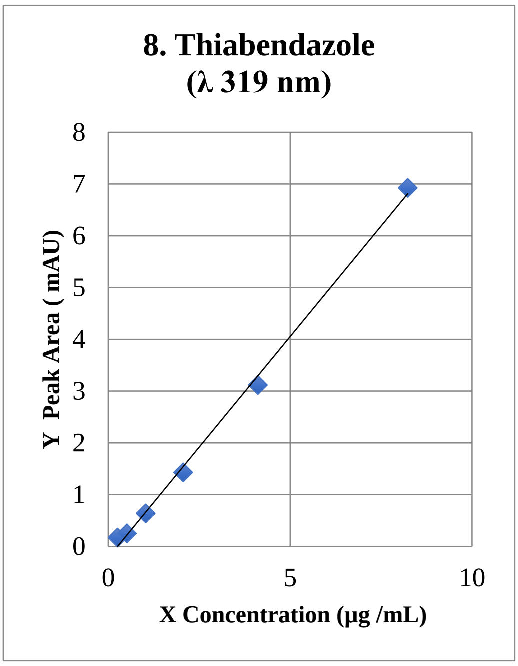


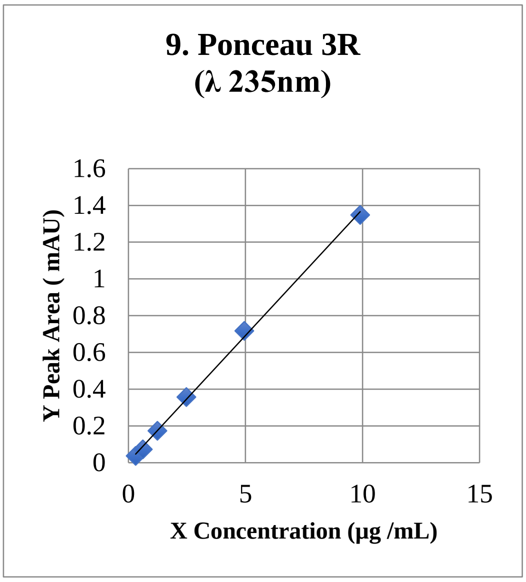


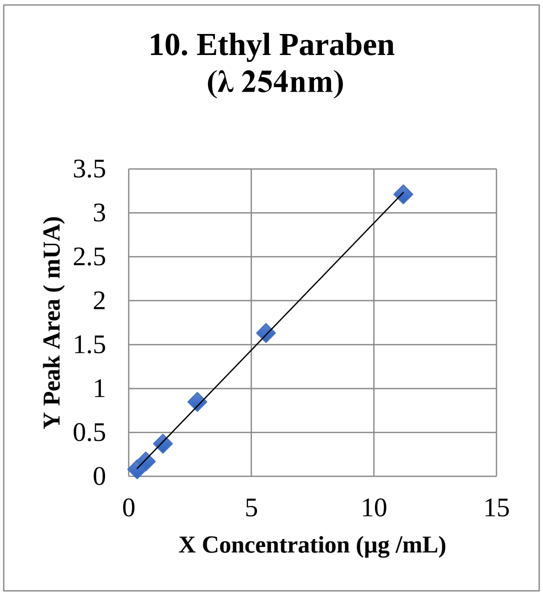

5. Ponceau 4R
(λ 235nm)
Y Peak Area (m AU)
X Concentration (μg /mL)
0
0.2
0.4
0.6
0.8
1
1.2
0
5
10
15

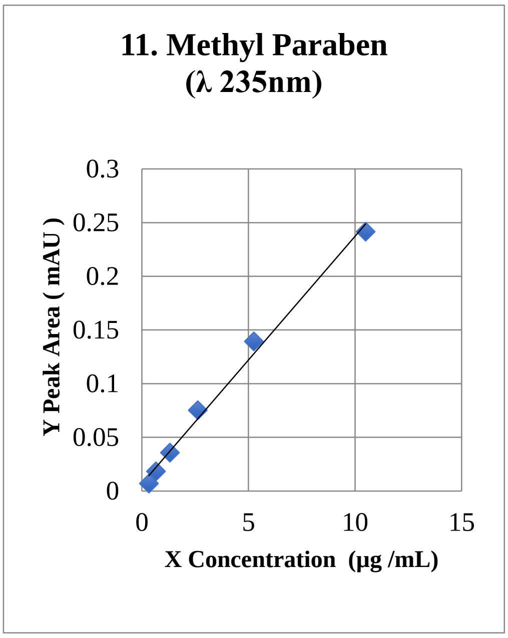

6. Sunset yellow FCF
(λ 235nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
5
10
15

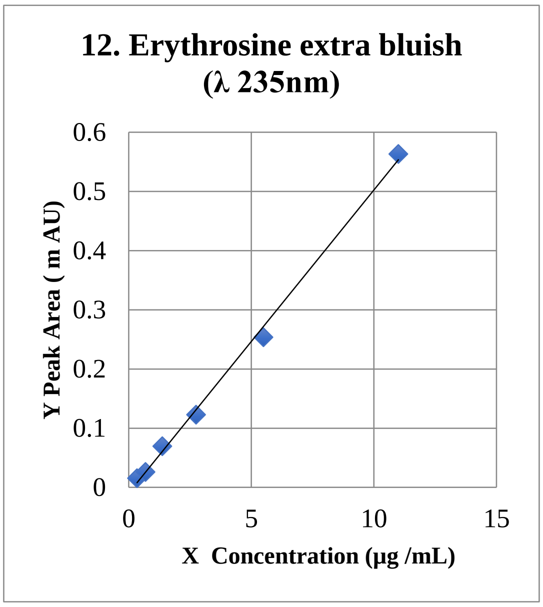

7. Allura red AC
(λ 235nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15

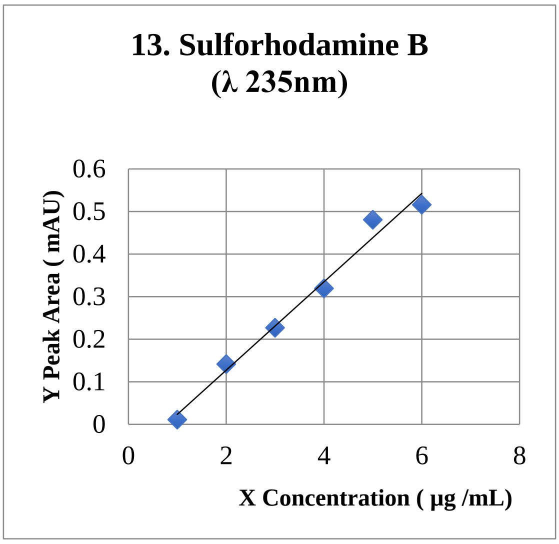
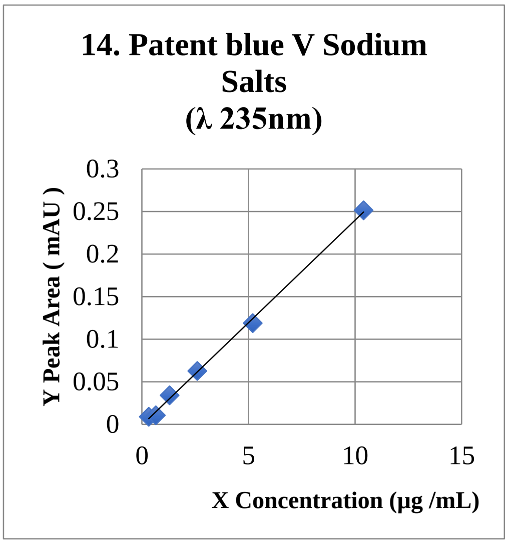
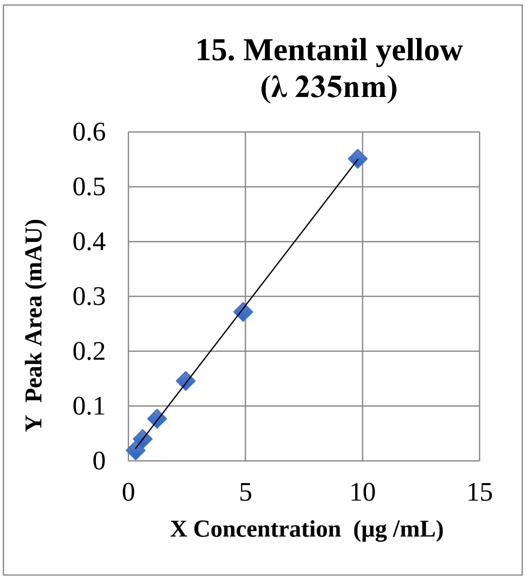
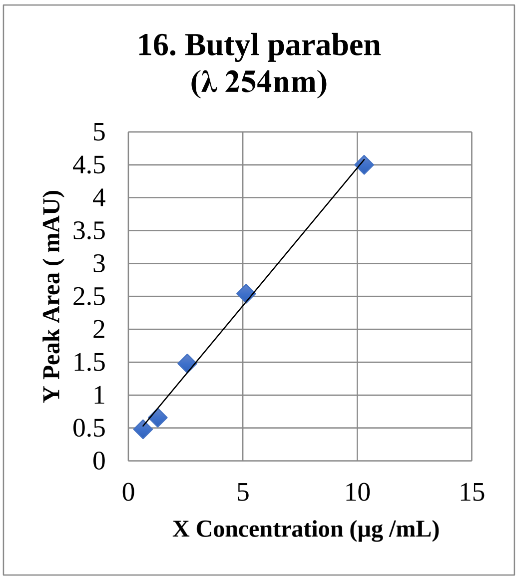

8. Thiabendazole
(λ 319 nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
1
2
3
4
5
6
7
8
0
5
10

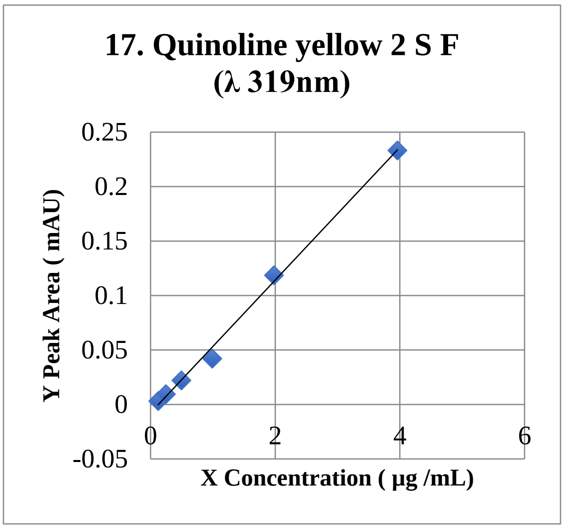

9. Ponceau 3R
(λ 235nm)
Y Peak Area (mAU)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
X Concentration (µg /mL)


10. Ethyl Paraben
(λ 254nm)
Y Peak Area (mUA)
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
X Concentration (µg /mL)


11. Methyl Paraben
(λ 235nm)
Y Peak Area (mAU)
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
X Concentration (µg /mL)


12. Erythrosine extra bluish
(λ 235nm)
Y Peak Area (m AU)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
X Concentration (µg /mL)

13. Sulforhodamine B
(λ 235nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
8	14. Patent blue V Sodium Salts
(λ 235nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
0.05
0.1
0.15
0.2
0.25
0.3
0
5
10
15
15. Mentanil yellow
(λ 235nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15	16. Butyl paraben
(λ 254nm)
Y Peak Area (mAU)
X Concentration (μg /mL)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
5
10
15


17. Quinoline yellow 2 S F
(λ 319nm)
0.25
0.2
0.15
0.1
0.05
0
-0.05
Y Peak Area (mAU)
0
2
4
6
X Concentration (μg /mL)