JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

DETERMINATION OF SODIUM BENZOATE AND POTASSIUM SORBATE CONTENTS IN SELECTED PROCESSED PACKED FRUIT JUICES BY UV-VIS SPECTROMETRY

BY: TASEW TEREFE

NOVEMBER, 2021

JIMMA, ETHIOPIA

DETERMINATION OF SODIUM BENZOATE AND POTASSIUM SORBATE CONTENTS IN SELECTED PROCESSED PACKED FRUIT JUICES BY UV-VIS SPECTROMETRY

BY: TASEW TEREFE

ADIVISORS: EPHREM TILAHUN (Ass. Prof)

TSEGAYE GIRMA (PhD)

MSc THESIS SUBMITTED TO THE SCHOOL OF GRADUTE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

NOVEMBER, 2021

JIMMA, ETHIOPIA

DETERMINATION OF SODIUM BENZOATE AND POTASSIUM SORBATE CONTENTS IN SELECTED PROCESSED PACKED FRUIT JUICES BY UV-VIS SPECTROMETRY

MSc THESIS SUBMITTED TO THE SCHOOL OF GRADUTE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY: TASEW TEREFE

ADVISORS:	SIGNATURE	DATE
1) EPHREM TILAHUN (Ass. Prof)		____________
2) TSEGAYE GIRMA (PhD)	____________	____________

EXAMINERS:

	SIGNATURE	DATE
1) ________________________	____________	____________
2) ________________________	____________	____________

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOAC	Association Official Analytical Chemists
FDA	Food Drug Administration
HPLC	High Performance Liquid Chromatography
ISO	International Standard Organization
LOD	Limit of detection
LOQ	Limit of quantification
MPL	Maximum permitted Level
UV-VIS	Ultraviolet -visible

ACKNOWLEDGMENT

First of all I would like to thank my God who never let me down and who helped me to conduct this thesis work. Secondly, I would like to extend my deepest gratitude to my advisors Ephrem Tilahun (Ass. Prof) and Dr. Tsegaye Girma for their valuable comments, suggestions, and assistances during this thesis work. I would also like to give grateful thanks to my friend Belaynesh Gebrie for her kindness and appreciable supporting throughout all activities of this thesis work. I also thank Jimma University, Department of Chemistry for all supports and facilities during conducting this study.

ABSTRACT

Benzoic and sorbic acid, and their salts are most commonly used food preservatives and they are permitted food additives by international laws in processing in restrictive amounts, but their content must be declared and must not exceed the established limits by legislation. The purpose of this study is to determine the amount of sodium benzoate and potassium sorbate in selected packed fruit juices available on markets of Jimma City. The content of these preservatives in six commercially available different brands of packed fruit juices were analysed using UV-Vis spectrophotometer. The concentrations of sodium benzoate and potassium sorbate in all samples were calculated by employing external standard method with a calibration of correlation coefficient of 0.998 and 0.997 from the standards calibration curves. The ranges of concentration of sodium benzoate and potassium sorbate were 19.49 ± 0.87 mg/L to 43.0.3 ± 1.92 mg/L and 23.15 ± 1.36 mg/L to 43.68 ± 0.62 mg/L, respectively. The highest level of sodium benzoate was observed in Alang juice (43.03 mg/L) and the lowest value was in Yoyo mango juice (19.49 mg/L). Similarly, the content of potassium sorbate was the highest in Raubi juice (43.68 mg/L) and the lowest was observed in Yoyo (23.15 mg/L).The level of both preservatives in the analyzed fruit juices were within the Food Drug Administration standard range. The limit of detection and quantification for sodium benzoate analysis was 2.38 mg/L and 7.94 mg/L, respectively and that for potassium sorbate was 1.96 mg/L and 6.54 mg/L, respectively. The UV-Vis Spectrophotometer method was linear in the concentration range of 10 mg/L to 50 mg/L for both sodium benzoate and potassium sorbate and the mean recoveries for the samples ranged from 87-104 %.

Key words: Preservatives, juice, sorbate, benzoate, UV-Vis Spectroscopy

1. INTRODUCTION

Fruit juices are consumed globally, not merely owing to their flavor, taste, and freshness, but also because of their valuable wellbeing properties while consumed on regular basis [1]. Fruit and vegetables are good sources of oligosaccharides, fiber and beet juice [2]. Preservatives are natural or synthetic substances that are added to the fruit juices, vegetables, prepared food items, cosmotics and pharmaceuticals in order to increase their shelf life and maintain their quality and safety by inhibiting their fermentation, acidification, microbial contamination and decomposing by the action of microorganisms, enzymes or physical agents [3]. Its high utilization by the food industry is due to the growing demand for chemically stable, safe and durable foods [4]. Preservatives may be natural (salt and sugar) or chemical, and this is the most effective type in preservation for longer periods [5].

The most commonly used preservatives are: benzoic acid, sorbic acid, propionic acid, sodium benzoate, and potassium sorbate. However, sodium benzoate and potassium sorbate are used more often because of their greater solubility in water than benzoic acid and ascorbic acid, respectively. These compounds are most active in foods, juices and beverages at low pH value (≤4.5) and essentially ineffective in foods at neutral pH values [6]. Similar to other classes of food additives, preservatives must meet criteria for their use, since they are chemicals and may cause adverse health effects [7]. Due to the pleasant taste and ease of availability, the demand of commercial juices is increasing all over the world. However, many juices products on the markets contain a variety of preservatives which on their own are not necessarily dangerous, but in combination with other ingredients can form extremely unhealthy, dangerous chemicals of benzene which causes cancer. Over dosage of preservatives in juices or over consumption of packed juices are also associated with diabetic, obesity and dental caries due to the presence of high sugar contents in juices [8].

Like other countries in the world, consumption of juices is now a very common habit in Ethiopia. Juices are especially very popular to the children. So, the composition of juices should be regulated by legislations and different foods must contain a certain amount of permitted preservatives to have a long shelf-life and the maximum permitted levels for the sodium benzoate present in juices is 130 mg/L. The maximum permitted levels for potassium sorbate in fruit juices is 200 mg/L [9].

In order to maintain or improve the reliability of these determinations is a crucial aspect on which a great deal of effort is spent. The use of food additives is limited by specific regulations. Determination of juices preservatives in juices is mandatory all over the world for ensuring juices safety and quality. Therefore, the objectives of this study was to determine the contents of sodium benzoate and potassium sorbate in selected processed packed fruit juices samples by using UV-Vis spectrophotometer.

1.2 Statement of problems

Processed packed juices are consumed by people regardless of age, religion, gender and culture. Most processed packed juices are imported from abroad, even though there are some companies that produce packed juices in Ethiopia. Different types of processed packed juices available in the market contain different types of additives, among which preservatives have the greater important role. These additives apply to increase the shelf life of packed juices by maintaining its original quality and safety. The processed packed juices are commonly consumed by children, sick and injured persons in Ethiopia without any recommended dosage. Over dose of preservatives in packed juices and over consumption of packed juices can be associated with obesity, diabetes and dental caries and cancer [8]. On the other hand, use of preservatives in the processed packed fruit juices less than the recommended limits may affect the storage time as well as the quality and safety of the products by the action of microorganisms. The consumers may not have awareness about the quality and quantity of preservatives present in processed packed fruit juices and also the maximum permitted levels of preservatives available in commercially available processed packed fruit juices [9]. The contents of these preservatives must be declared and must not be exceed the permissible levels. Therefore, this study was conducted to explore the contents of commonly used preservatives (sodium benzoate and potassium sorbate) in selected processed packed fruit juices available in Jimma city market and compare their levels with respect to FDA guideline.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was to deternime the contents of sodium benzoate and potassium sorbate in selected processed packed fruit juices available in Jimma market.

1.3.2 Specific objectives

The specific objectives of this study was

- To determine concentrations of sodium benzoate and potassium sorbate in selected processed packed fruit juices
- To compare the concentrations of sodium benzoate and potassium sorbate in selected processed packed fruit juices with other similar works
- To compare the concentrations of sodium benzoate and potassium sorbate obtained in selected processed packed fruit juices with the maximum permissible levels set by international community.

1.4 Significance of the study

There are different types of processed packed fruit juices in the markets, those are important as the sources of energy and as well they are consumed all over the world without any restriction. Processed packed fruit juices contain different types of additives those are used to maintain the safety of the fruit juices. It is very important to know the amount of preservatives present in the processed fruit juices and compare their quantity against the permitted limits. If the contents of preservatives are above or below the permitted levels in processed packed fruit juices, they can be associated with some health risks. Over dosage of preservatives in fruit juices and excessive consumption of juices may cause different side effects. Hence, this study was intended to evaluate the concentrations of the two commonly used preservatives in packed juices. The obtained results may help the community to create awareness about possible health risks of frequent use intake of processed packed fruit juices, to emphasize to customers to use fruit juices properly by considering their side effecs of the fruit juices; it is also used as a precursor and reference for the future researchrers.

2. LITERATURE REVIEW

2.1 Fruit juice

Fruit juice is the unfermented but fermentable liquid from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means including post-harvest surface treatments. It should not have added ingredients, except minerals and vitamins for the purpose of fortification and permitted additives [10].

2.2 Classification Fruit juices

Fruit juices can be classified based on the flavour, appearance, obtaining method, as well as storage conditions [11].

Table 1.Classification of fruit juices

Criteria	Name
Flavour	Single
	Mixed
Appearance	Clear
	Cloudy
Obtaining method	From concentrated
	Not from concentrate
Storage conditions	Chilled
	Ambient

2.3 Types of chemicals used in food processing

2.3.1 Additives

Food additives are substances added intentionally to foodstuff, to increase the durability of the product and enhance or modify its properties, including its appearance, flavor or structure, provided it does not detract from its nutritional value. Food additives can be of natural or synthetic origin, usually without appreciable nutritional value, that are added to food in small amounts during the manufacture (industrial change or during packaging) and they are introduced by food manufacturers into food products to serve a certain technological or sensory function and make them more marketable [12].

Food additive is any substance not normally consumed as a food itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results [12]. Because food additives are essential in the food industry, such as food colorings, flavors, taste enhancers and preservatives, to promote a free and fair market of safe quality food products within the permitted level [13]. Food additives are also necessary for food manufacturers to make sure that the type and amount of a particular additive is suitable to use and does not exceed the acceptable safety limits specially for those people, who by nature of their diet, may consume high amounts of a particular food product such as children. Food additives are used in snack foods, fruit juices and beverages which are consumed heavily by children [14].

The majority of food additives do not provide any nutritional benefits to the consumer; therefore there are two factors that strongly influence the use of a particular food additive, which are safety and technological needs. Any food manufacturer has a priority to provide a wide range of safe and attractive products at affordable prices all year round in order to meet consumer requirements for quality, convenience, variety, preservation, coloring, and sweetening. It would be impossible to do this without the use of food additives. Different types of additives are used for different purposes and they are grouped according to their primary function [12, 13].

Several studies have investigated the prevalence of adverse reactions to food additives [12]. Many doubts have been raised about approximately 200 food additives, which for certain consumers have been claimed to cause intolerance or allergic reactions, or to significantly increase risks of serious long term harms. It has been estimated that the true prevalence of intolerance to food additives is about 2% in adults and up to 20% in children, and for food additives from 0.01 to 0.23% [15]. According to the Food and Drug Administration, a food additive is any substance added to food, including preservatives, coloring, flavor enhancers, thickeners, stabilizers, nutrients and sweeteners. Examples of some chemicals of food additives are sucrose (sugar), glucose, fructose, sorbitol, mannitol, corn syrup, high fructose corn syrup, saccharin and aspartame, used for sweetening; or annato or beta carotene, used for food coloring [16].

2.3.2 Preservatives

Preservatives are types of additives that are added to food items in order to preserve them for spoiling. Foods preservatives are substances that can prevent or delay changes caused by the action of microorganisms, enzymes and/or physical agentsonce they are added to a given food [3]. Their high utilization by the food industry is due to the growing demand for chemically stable, safe and durable foods [3]. Preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods and to prolong the shelf life and quality of foods [4]. Preservatives are known as antimicrobial agents, used to extend the shelf-life of foods by protecting them against deterioration caused by microorganisms and most of the preservatives focus on reducing or preventing the growth of fungi, moulds and yeasts, thus one can refer preservatives as a type of additives for extending shelf life of different types of foods [3].

Preservatives are commonly added to many food products, such as soda, fruit juice, soy s propionic acid auce, jams and jellies, and other condiments, to inhibit decay. Preservatives can be obtained from natural sources like salt, sugar, vinegar and citrus juice or can be human-made or synthetic. The most commonly used preservatives are: benzoic acid, sorbic acid, sodium benzoate, potassium sorbate, sulfur dioxide, propionic acid, nitrites propionic acid and nitrites are normally preferred and used as food preservatives [6]. Since the early 1900s, sodium benzoate and potassium sorbate have been widely used worldwide as a preservative due to their antimicrobial properties combined with their low toxicity and taste [17]. Similarly, to other classes of food additives, preservatives must meet criteria for their use, since they are chemicals and may cause adverse health effects [7]. Recent toxicological studies indicate that certain concentrations of synthetic preservatives and their continuous use may be potentially mutagenic and/or genotoxic [18].

The Food and Drug Administration (FDA) regulates the uses of benzoate and sorbate as preservatives in the USA. The FDA lists benzoate and sorbate as substances that are generally recognized as safe with a maximum permitted concentration of 0.13% in accordance with good manufacturing or feeding practices [19]. Similarly, benzoate and sorbate are regulated in Europe by the European Union Legislation with a limit of 0.005% to 0.13% and 0.005% to 0.2% in juice products respectively. If higher concentrations of sodium benzoate and potassium sorbate are used 0.13% and 0.2%, then alterations in taste may occur in juices. On the other hand, concentrations sodium benzoate and potassium sorbate less than 0.005% will have little inhibitory effect. Therefore, a reliable testing method is required to assure that the concentration of benzoate/ sorbate are within product and regulatory specifications [19].

2.3.2.1 Sodium benzoate

Sodium benzoate is a synthetic chemical produced when benzoic acid, which is found naturally in some fruits and spices, is combined with sodium hydroxide. Sodium benzoate is found in carbonated sodas, fruit juice products, salad dressings, and fermented foods such as vinegar, wine, and pickles [20].

Table 2 Physico -Chemical Properties of Sodium benzoate

Data Parameter	Value
Chemical Formula	$C_7H_5O_2Na$
Molecular Weight	144.11 g.mol-1
Physical State at 25 °C	Crystalline solid
Color	white compound
Odor	sweet Synonyms
Solubility	in water 87 g/L (25 ºC)
Vapor Pressure	0.3513 mm Hg at 25 °C
Melting Point	43 °C
Boiling Point	181.8 °C

It is typically used as a preservative in some products from the cosmetic, pharmaceutical and food industries [6]. In the pharmaceutical industry, it is used in the treatment of various diseases such as disorders of the urea cycle, liver diseases and multiple sclerosis [21]. In the food industry, sodium benzoate is used in foods and beverages, as it is effective to inhibit the growth of fungi and bacteria during storage, besides providing easy application [22]. Sodium benzoate has been used for many years as a preservative because of its good stability and excellent solubility in water [6].

2.3.2.2 Potassium sorbate

Potassium sorbate is the potassium salt of the sorbic acid, which disassociates in solution to ionic potassium and sorbic acid, which inhibits oxidative and fermentative in foods[23]. It is a preservative and antimicrobial agent that is widely utilized in cosmetics, pharmaceuticals juices and food products [24].**Table 3. Physio Chemical Properties of Potassium Sorbate**

PROPERTY	Characteristic/Value
Molecular Formula:	$C_6H_7KO_2$
Molecular Weight:	150.22
Percent Composition:	C 47.97%, K 26.03%, O 21.30%, H 4.70%
Physical state at 25°C/1 Atm.	Crystals
Color	White
Odor	Characteristic
Density/Specific Gravity	1.363 g/ml at 25°C
Melting point	Decomposes above 270°C
Boiling point	446°
Solubility	At 20°C: water 58.2%; alcohol 6.5%
Vapor pressure	At 20°C<0.01 mm; At 143°C: 50 mm
Flammability	Not found
Storage stability	Stable incompatible with strong oxidizing agents
Corrosion characteristics	Not corrosive to glass
Air half life	2.6 hrs
Soil half life	416 hrs

Table 4. Permitted preservatives used in fruit juices [19]

Preservatives	Applications	Commonly used levels
Sulphites and sulphur dioxide	Sulphur dioxide gas and the sodium or potassium salts of sulphite, bisulphite or metabisulphite are the most commonly used forms. Sulphurous acid inhibits yeasts	0.005-0.2%
Ascorbic acid	Sorbic acid and sodium and potassium sorbate are widely used to inhibit the growth of moulds and yeasts. The activity of sorbic acid increases as the pH decreases. Sorbic acid and its salts are practically tasteless and odourless in foods when used at levels less than 0.3%.	0.05-0.2%
Benzoic acid	Benzoic acid, in the form of sodium benzoate is a widely used preservative. It occurs naturally in cranberries, cinnamon and cloves and is well suited for used in acid foods. It is often used in combination with sorbic acid at levels from 0.05-0.1% b y weight.	0.03-0.2%
Citric acid	Citric acid is the main acid found naturally in citrus fruits. It is widely used in carbonated beverages and as an acidifier of foods. It is a less effective anti-microbial agent than other acids	No limit

2.3.2.3 Classification of preservatives

Preservatives are classified as class I (natural preservatives) such as salt, sugar, vinegar, honey and edible oil and class II (chemical or synthetic preservatives) such as benzoate, sorbate, nitrites, and nitrates of sodium or potassium, sulfites, glutamates and glycerides. The food standards regulation require that not more than one class II preservatives should be used in one particular food item. People consuming or using items containing more than one preservative are at risk of exposure to multiple chemical. Both natural and synthetic preservatives are categorized into two (2) types [25].

2.3.2.3.1. Antimicrobials

These destroy or delay the growth of bacteria, yeast and mold. For example nitrites and nitrates prevent botulism in meat products. Sulfur dioxide prevents further degradation in fruits, wine and Beer. Benzoates and sorbates are anti- fungal agents used in jams, salads, cheese and pickles [26].

2.3.2.3.2. Anti- oxidants

Antioxidant which extend the shelf-life of foods by protecting them against oxidation; Carriers, used to disperse, dilute, dissolve or otherwise physically modify a food additive, without altering its technological function and without the very substances exerting any technological effect, in order to facilitate its handling, application or use; Acidifying substances, used to increase the acidity of a foodstuff or confer them a sour taste and is widely used in beverages to give them an acid or sour taste similar to the taste of the fruit; Acidity regulators, which alter or control the pH of foodstuffs; prevent foods from oxidising, or going rancid. [27]. Anti-oxidants are including: Ethyl, propyl, octyl and dodecyl gallates, butylated hydroxyanisole, tertiary butyl hydroquinone and resin guaic, ascorbic acid, are permitted under FSS, Rules and Regulation [28].

2.3.2.4 Uses of preservatives

Preservatives are substances that able to inhibit or stop the growth of microorganisms and to increase the shell life of juices [3]. Preservatives are commonly added to many food products, such as soda, fruit juice, soy sauce, jams and jellies, and other condiments, to inhibit decay, since the early 1900s, sodium benzoate and potassium sorbate have been widely used worldwide as a preservative due to their antimicrobial properties combined with their low toxicity and taste and sodium benzoate and potassium sorbate are the most effective in an acidic environment ($pH \leq 4.5$) and are not recommended for use at higher pH [6]. Benzoic acid and ascorbic acid is effective antimicrobial agent for the purpose of preservation [29]. However, sodium benzoate and potassium sorbate are more effective and preferred because they are more soluble than benzoic acid and ascorbic acid [29].

Preservatives are natural or synthetic substances that are added to fruits, vegetables, prepared from food items, cosmetics and pharmaceuticals in order to increase their shelf life and maintain their freshness, color, flavor, quality and safety by inhibiting, retarding or arresting their fermentation, acidification, microbial contamination and decomposition, there are many preservatives used in different juices. The most commonly preservatives used in juices are include: benzoic acid, ascorbic acid, citric acid, sodium benzoate and potassium sorbate [3]. It is typically used as a preservative in some products from the cosmetic, pharmaceutical and food industries [6]. In the pharmaceutical industry, it is used in the treatment of various diseases such as disorders of the urea cycle, liver diseases and multiple sclerosis [21]. In the food industry, sodium benzoate is used in foods and beverages, as it is effective to inhibit the growth of fungi and bacteria during storage, besides providing easy application [22]. Sodium benzoate has been used for many years as a preservative because of its good stability and excellent solubility in water [6].

2.3.2.5 Effects of preservatives

In combination with ascorbic acid (vitamin C), sodium benzoate and potassium sorbate may form benzene, a known carcinogen. FDA, heat, light and shelf life can affect the rate at which benzene is formed [30]. The American Academy of Pediatrics as of 2017 says that fruit juice should not be given to children under age one due to the lack of nutritional benefit [31]. For children ages one to six, intake of fruit juice should be limited to less than 4–6 oz (110–170 g) per day (about a half to three-quarters of a cup) due to its high sugar and low fiber content compared to fruit. Overconsumption of fruit juices may reduce nutrient intake compared to eating whole fruits, and may produce diarrhea, gas, abdominal pain, bloating, or tooth decay [32**]**. Overconsumption of fruits and fruit juice may contribute to dental decay and cavities via the effect of fruit acids on tooth enamel [33]. Longitudinal prospective cohort studies showed a significantly increased risk of type 2 diabetes when juices with added sugars were consumed compared to eating whole fruits [34]. Overconsumption of fruit juice with added sugars has also been linked to childhood obesity. The American Journal of Public Health proposed that the Healthy Hunger-Free Kids Act of 2010 in the United States eliminate 100% fruit juices and substitute instead with whole fruits [35].

While IDA is almost unlikely to be exceeded for average consumers, large daily consumers of soft drinks and juices may exceed the ADI [3] As soon as it is consumed; sodium benzoate/Potassium sorbate is rapidly absorbed by the gastrointestinal tract, and then conjugated with glycine to form pyruvate in the liver [36]. The ingestion of these preservative cause an increase in the serum of benzoate/sorbate and also of pyruvate [7]. The resulting hippuric acid is rapidly excreted in the urine within the first 6 h, and the remaining dose is completely eliminated within 2 to 3 days. In addition to that, it has been proved that certain preservatives, especially antimicrobial agents, may cause allergies, urticaria [37].Behavioral disorder such as hyperactivity and attentiondeficit/hyperactivity disorder as well as being toxic and genotoxic when consumed above the ADI [21].

2.3.2.6 Mechanism of preservation

The inhibitory action of preservatives is due to their interfering with the mechanism of cell division, permeability of cell membrane and activity of enzymes [38]. The mechanism starts with the absorption of benzoic acid into the cell. If the intercellular pH changes to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase decrease sharply which inhibit the growth and survival of microorganisms that cause juices spoilage and transported to liver where it is filtered and expelled in urine [25]. Safety limit used in food concentration of preservative is limited by FDA to 0.13% by weight and it causes side effect when consumed in large doses [39].In combination with ascorbic acid or vitamin C, Sodium benzoate and potassium sorbate may form benzene, a known carcigen. Heat, light and shell life can affect the rate at which benzene is formed [32].

2.3.4. Thickeners and Stabilizers

A variety of organic compounds form the group of thickeners stabilizers that used for increasing the viscosity of foodstuffs and for maintaining the physicochemical state of foo and d and also might have the ability to retain or enhance the natural color of food - color. They include guar gum, gum Arabic, karaya gum, gum ghathi, tragacanth gum; locust bean gum, gelatin, baking soda, monocalcium phosphate and calcium carbonate are being used as thickening agents **[**40**]**.

2.3.5 Color Additives

The colouring matter in food may be natural and synthetic colours. It has to be separated from food before identification can be done. Natural colours consist of chlorophyll, carotenes, cantaxanthene, riboflavin, annatto, saffron, turmeric, curcumin, caramel etc. Synthetic colours are of importance as they are widely used in different foods, margarine, cheese and soft drinks [41].

2.3.6 Flavouring agents

Flavouring agents are added to food to improve aroma or taste make up the greatest number of additives used in foods. Flovouring agents are also known artificial sweeteners which increase the sweetness. There are hundreds of varieties of flavourings used in a wide variety of foods, from confectionery and soft drinks to cereal, cake, and yoghurt. Natural flavouring agents include nut, fruit and spice blends, as well as those derived from vegetables and wine. In addition, there are flavourings that imitate natural flavours.They are used in trace amounts to impart a characteristic flavour. Menthol, vanillin and monosodium glutamate are of interest as they are extensively used in various foods. Menthol is used mainly to flavour confectionery and panmasala. Vanillin is extensively used in ice creams and monosodium glutamate to enhance flavour of meat and soups [42].

2.4 Processing technology

The technology to obtain fruit juices varies depending on many factors, such as the nature of the raw material, final product desired, size of plant. In general terms, the process to obtain fruit juices involves the following steps [43].

2.4.1 Preparation of the fruit juices

The process starts with the selection of mature, undamaged fruits. Any fruits that are mouldy or under-ripe should be sorted and removed. Wash the fruit in clean water. It may be necessary to chlorinate the water by adding 1 table spoon of bleach to 5 L of water. Peel the fruit and remove stones or seeds. If necessary, chop the fruit into pieces that will fit into the liquidiser or pulper. Sound fruit freshly harvested from the field or taken from refrigerated or frozen storage. Thorough washing removes dirt and foreign objects and may be followed by a sanitation step to decrease the load of contaminants. Sorting to remove decayed and mouldy fruit is also necessary to make sure the final juice will not have a high microbial load, undesirable flavours, or mycotoxins contamination. If necessary, other operations (e.g. trimming or pitting) are applied to remove stone seeds, stems or calyx. After that, fruit is disintegrated mechanically or thermally by heating (at about 80 ºC) or by freezing (less than -5ºC). The yield can be increased by enzymatic pectin degradation (particularly of stone fruits and of berries) or by applying procedures such as electropermeabilization [44].

2.4.2 Juice extraction

There are several methods to extract juice depending on the type of fruit you use. Separation of the juice is achieved by different methods such as pressing, diffusion, centrifugal procedures, and reverse-osmosis. The type of equipment applied depends on the fruit species, production line, and economy of scale. The most widely used solution is pressing. This step should be done as rapidly as possible so as to minimize its oxidation by naturally present enzymes [45].

2.4.3 Clarification

Extracted fruit juices are usually turbid, due to insoluble plant particles (fibres, cellulose, hemicellulose, protopectin, starch, and lipids) and colloid macromolecules (pectin, proteins, soluble-starch fractions, certain polyphenols, and their oxidized or condensed derivatives). Clarified juices are real solutions that should not contain dissolved colloid substances, and these compounds therefore have to be eliminated. Juice clarification can be performed by physico-chemical methods, mechanical procedures, and their combination. The former involves treatment with enzymes, mostly pectinolytic, and, if necessary, removal of starch and polyphenols using gelatine, alone or together with colloidal silicic acid or tannin, or polyvinylpyrrolidone. Finally, proteins are removed by adsorption on bentonite **[**46**].**

2.4.4. Batch preparation

When the juice or pulp has been collected, it is necessary to prepare the batch according to the chosen recipe. This is very much a matter of choice and judgement, and must be done carefully to suit local tastes. Fruit squashes would normally contain about 25% fruit material mixed with a sugar syrup to give a final sugar concentration of about 40% , another popular product is fruit nectar, which is a sweet mixture of fruit pulp, sugar and water which is consumed on a 'one shot' basis. Essentially, these consist of a 30% mix of fruit pulp and sugar syrup to give a final sugar level of about 12-14%. All fruits contain sugar, usually around 8-10%. The actual levels vary from fruit to fruit and with the stage of ripeness of the fruit. They also vary within the same fruit grown in different parts of the world. The addition of sugar to the fruit pulp to achieve the recommended levels for preservation must take into account the amount of sugar already present in the juice. In all cases, sugar should be added to the fruit juice as sugar syrup. The syrup should be filtered through a muslin cloth prior to mixing to remove particles of dirt which are always present; this gives a clearer, higher quality product [47].

2.4.5 Pasteurization

Pasteurisation is a relatively mild form of heat treatment, generally at a temperature lower than the boiling point of water. Because it is a mild heat treatment, pasteurisation causes minimal changes in the taste, colour and nutritive value of a food. Foods (fruit and vegetable juices and purees) are generally pasteurised to reduce enzyme and microbial activity and thereby increase the shelf life. Pasteurisation extends the storage life of bottled fruits and juices by several months [48]. The severity of heat treatment and the resulting extension of shelf life are mostly determined by the pH of the food [6]. In low acid foods (pH>4.5) the main purpose is to destroy pathogenic bacteria. In foods with pH below 4.5, the main purpose is to destroy spoilage micro-organisms and prevent enzyme activity [6]. The benefit of pasteurising in containers is that the risk of contamination of the product after packaging is greatly reduced. The main factors that influence pasteurisation of a food are as follows: temperature and time, acidity of the products and air remaining in the containers [48].

The juice is pasteurized to control spoilage microorganisms and to inactivate enzymes that occur naturally in fruit. In the traditional way, liquids are heated to 82-85 ºC, filled at this temperature, and then pasteurized in water bath at 84-88 ºC for 15-40 minutes depending on the size of the packaging container. After the heat treatment, products are cooled to room temperature. With aseptic filling technology, the liquid is pasteurized at 94-112 ºC for 30-60 seconds in a closed, flow-through system. Then the fluid is cooled to 25-30 ºC and filled into containers that have already been sterilized. The aseptic filling-closing system is a closed unit under overpressure, which hinders the post-contamination of heat-treated products [48].

2.4.6 Filling

In all cases, the products should be hot-filled into clean, sterilised bottles. A stainless steel bucket, drilled to accept a small outlet tap, is very effective bottle filler. The output can be doubled quite simply by fitting a second tap on the other side of the bucket [49]. Fruit-based beverages are filled into glass and plastic bottles or carton boxes made of combined layers [46].

2.4.7 Quality parameters

The essential physical, chemical, organoleptic and nutritional characteristics of the fruit from which it comes. Since product selection by consumers greatly depends on these characteristics, systemic activities are necessary to obtain juice of the highest quality. Moreover, the final product needs to be characterized to ensure that it fulfil the pertinent regulations as well as consumer expectances. Among the physicochemical features, the soluble solids content is the first parameter characterizing a fruit juice. These solids are mostly sugars naturally occurring in fruits (i.e. glucose, fructose and sucrose) and organic acids in second place. The soluble solids content is determined by means of the refractive index and is generally expressed as Brix. However, this measurement is only an estimate which considers that everything in solution has the same refractive index as sucrose. Furthermore, it is common to check the levels of the individual sugars in the product to assess its authenticity and quality [50].

2.5 Nutritional values of fruit juices

Fresh juices are important in human nutrition far beyond their use as refreshing sources of liquid. They have water content similar to that of the fruits themselves, the removal of the plant cell wall material having only a minor effect overall. Since the clarification step removes all the plant cell wall polysaccharides (dietary fibre), clear juices are very low in non-starch polysaccharides and the carbohydrates present are limited to mixtures of the free sugars which are characteristic of the fruit and the processing conditions [51].

2.6 Importance of fruit juices

Like whole fruits and vegetables, these products are rich in potassium, magnesium, folate, vitamin A and vitamin C. They also contain other bioavailable plant components, such as polyphenols -compounds that have strong antioxidant and antinflammatory activity [52]. Overall, fruit juices can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits. However, it should be noted that changes occur, particularly to the minor components of juice during storage [53].

2.7 Common methods for analysis

Many analytical methods have been reported for the determination of the contents of sodium benzoate and potassium sorbate from the processed packed fruit juices. The majority of the methods rely on objective chemical or physical processes and various instrumentation techniques are HPLC [54] and UV-Vis spectrophotometer method [55].

2.7.1 High performance liquid chromatography

This method is based on the principle that a sample is composed of a mixture of components, which are separated when the mixture passes through two phases: a mobile (liquid or gaseous) and a stationary (solid, liquid or gel). It is used for the qualitative and quantitative analysis and the components are separated and analyzed according the properties of a given solution. Chromatography is a physical and chemical method of separating, identification and quantification of different components of samples. The interaction of the matrices components with the mix of both phases is influenced by different intermolecular forces including ionic, dipole, non-polar and effects of specific affinity or solubility and the absorbent material, named as stationary phase the component to be analyzed must have solubility with the mobile phase, and different compounds have different retention time sodium benzoate and potassium sorbate in processed packed fruit juices [54]. This method is the most frequently and accurate techniques used for the determination of the contents of preservatives in processed packed fruit juices and is preferred technique because of it’s rapidly, high accuracy and reproducibility, but this technique is very expensive and required high performance of skilled to apply to determine the concentration of preservatives in processed packed fruit juices [54].

2.7.2 UV-Vis spectrophotometer method

The UV-Vis spectrophotometer method is widely used to measure sodium benzoate and potassium sorbate contents in processed packed fruit juices. Many studies have discussed the advantages and disadvantage of using routinely this method to quantify the contents of sodium benzoate and potassium sorbate and most of them seems to agree that they are easy to perform, low cost, rapid and applicable routinely in the most laboratories. These methods are still considered useful for a quick and prior screening of numerous samples [55].

3. MATERIALS AND METHODS

3.1 Study area and period

The study was conduct in south west Ethiopia, Oromia Region, Jimma city located 348 km away from Addis Ababa, Capital city of Ethiopia. 3D mango, 7Star mango, Alang, Yoyo, Rani and Raubi commercially available were purchased from local market of Jimma city.The study was conducted in the analytical chemistry laboratory at Jimma University. The study was carried out from August 2021 until October 2021.

3.2 Chemicals and materials

3.2.1 Chemicals

Laboratory chemicals and reagents that used in this study were analytical grade. Hydrochloric acid (Wodehouse Road, Jahangir Villa, India), petroleum ether (Wodehouse Road, Jahangir Villa, India, sodium benzoate (Guangdoing guarghua chemical factory, China) and potassium sorbate (China) standard were used.

3.2.2 Apparatus and instruments

UV-Vis spectrophotometer (JENWAY 6705, England), volumetric flasks, beakers, filter paper, separator funnel, measuring cylinder, quart curvet, wash bottle, pipette, analytical balance, litmus paper and conical flask and micropipette were used.

3.3 Sampling and Sample Preparation

The six selected processed packed fruit juice samples commercially available were purchased from market of Jimma city and used in this study. The samples information and codes are given in Table 5 below.

Table 5. Fruit juice samples information and codes

Brand name	Packing volume (mL)	Expire date	Code
3D Mango	500	16/9/2022	W1
7Star Mango	350	27/09/2022	W2
Alang Mango	350	24/06/2021	W3
Yoyo	550	9/09/2022	W4
Rani	100	23/07/2022	W5
Raubi	125	10/2/2022	W6

The samples were prepared by mixing 10 mL from each sample with 90 mL of distilled water in 100 mL volumetric flask and immediately filtered the mixture through filter paper. From each filtrate 5 mL were taken, and then 0.4 mL of hydrochloric acid (6 M HCl) was added. The preservatives were extracted with 45 mL of petroleum ether in a separate 100 mL volumetric flask and diluted up to the mark with distilled water.

3.4 Determination Method (Analysis)

The quantitative determination for sodium benzoate and potassium sorbate in six different samples processed packed fruit juices were carried out by UV-vis spectrophotometer at 227 nm and 250 nm wavelength respectively. The procedures were adapted from the Association of Official Analytical Chemists (A. O. A. C*)* Official Methods Analysis and an International Organization for Standardization (ISO) guidelines [56**].**

3.5 Preparation of standard solution of sodium benzoate and potassium sorbate

3.5.1 Preparation of standard solution

Standard solutions of sodium benzoate and potassium sorbate were prepared by dissolving 100 mg of sodium benzoate and potassium sorbate, separately in 100 mL distilled water (100 mg/0.1L) in two different volumetric flasks to give 1000 mg/L of sodium benzoate and potassium sorbate.

3.5.2 Preparation of working solutions of sodium benzoate and potassium sorbate

In a series 10, 20, 30, 40 and 50 mg/L (1 mL, 2 mL, 3 mL, 4 mL and 5 mL) of diluted standard solution of sodium benzoate and potassium sorbate solutions were introduced into different 100 mL volumetric flasks, then 0.4 mL of hydrochloric acid (6 M) was added to each standard solutions and were extracted with 45 mL of petroleum ether and diluted up to the mark with distilled water. Then, absorbance of standard samples sodium benzoate and potassium sorbate were read at 227 and 250 nm, respectively.

3.6 Method Validation

Validation of analytical method can be tested by different parameters [57]. In this work, linearity, precision, LOD, LOQ and recovery tests were used to check the validity of the method.

3.6.1 Linearity

Sodium benzoate and potassium sorbate standards were prepared to test the linearity of the method. The absorbance of each standard was recorded by using UV-Vis spectrometer. The obtained data were processed by using Microsoft offices excel and absorbance versus concentration graphs was plotted using Origin 6 software. Then, the regression coefficient (R^2) was calculated.

3.6.2 Precision

The precision of an analytical procedure is usually expressed as the variance, relative standard deviation and percentage relative standard deviation of series of measurements and the precision of the results was evaluated by percentage relative deviation of the results of each sample in triplicates.

$$\%RSD = \frac{SD}{X} \times 100$$

,

Where, SD is standard deviation and x is mean.

3.6.3 Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be done by three times standard deviation of blank divided by the slope (LOD=3SD/S); where SD is the standard deviation of the response and S is the slope of the calibration curve.

3.6.4 Limit of Quantification (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. LOQ were calculated ten times standard deviation of blank divided by slope LOQ=10SD/S.

3.6.5 Recovery test

Recovery is the fraction of the analyte determined after addition of a known amount of the analyte to a sample. The recovery test was carried out by spiking known concentration of standard solution of sodium benzoate and potassium sorbate in selected processed packed fruit juices. Then, the absorbance of solution before spiked and after spiked was measured by UV-Vis spectrometer. Spiking method was used to test the accuracy of the method. The percent of standard sodium benzoate and potassium sorbate recovered from the solution were calculated by using the following formula.

$$\% \text{ Recovery} = \left(\frac{\text{C spiked sample} - \text{C unspiked sample}}{\text{C added (Spiked)}}\right) \text{x}100$$

3.7 Statistical Analysis

Linear regression analysis of calibration curve was used to calculate unknown concentration, sensitivity, correlation coefficients and standard deviation. Average concentration and standard deviations of triplicate measurement for the sample were reported. Single factor Analysis of variance (ANOVA) was used to test the level of significance difference at $\alpha = 0.05$ [58].

4. RESULTS AND DISCUSSION

4.1 Calibration curves for sodium benzoate and potassium sorbate

A five point calibration graph was drawn by plotting absorbance versus concentration. A linear graph of an acceptable correlation coefficient with (R^2= 0.998 and 0.997) were obtained for both sodium benzoate and potassium sorbate as shown in Fig. 1 and Fig. 2. The sepectra of both sodium benzoate and potassium sorbate also shown in fig 3 and 4 respectivelly.

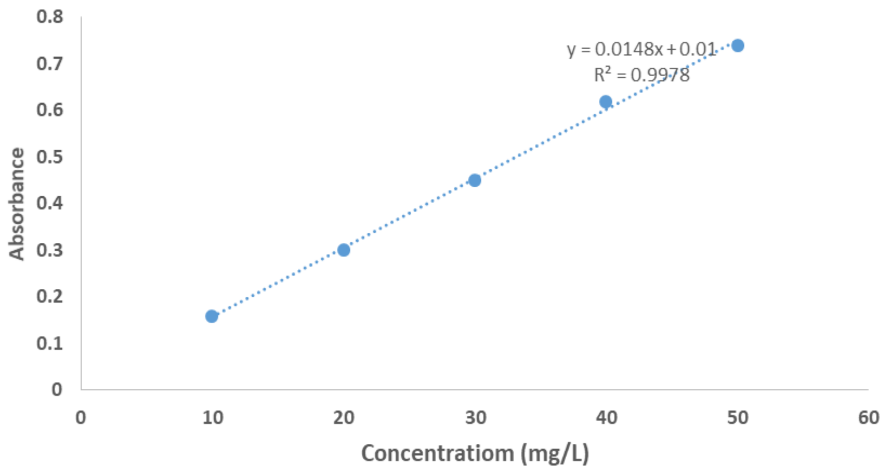

Fig. 1**.** Calibration curve for Sodium benzoate at 227nm

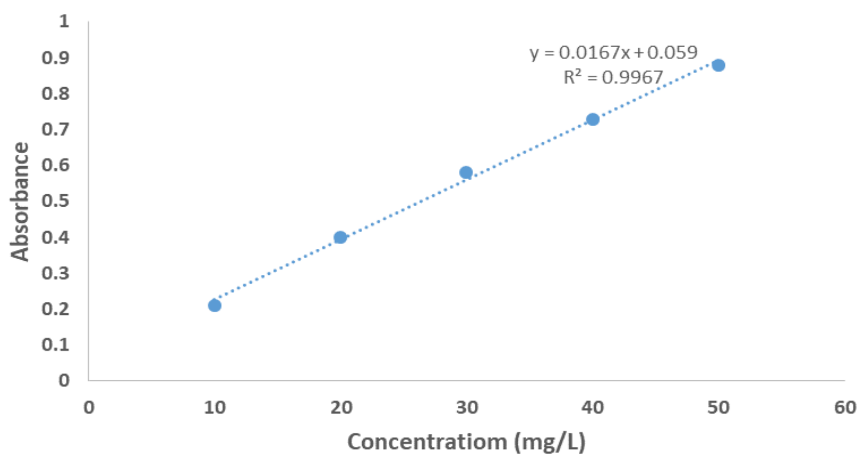

Fig. 2. Calibration curve for potassium sorbate at 250 nm

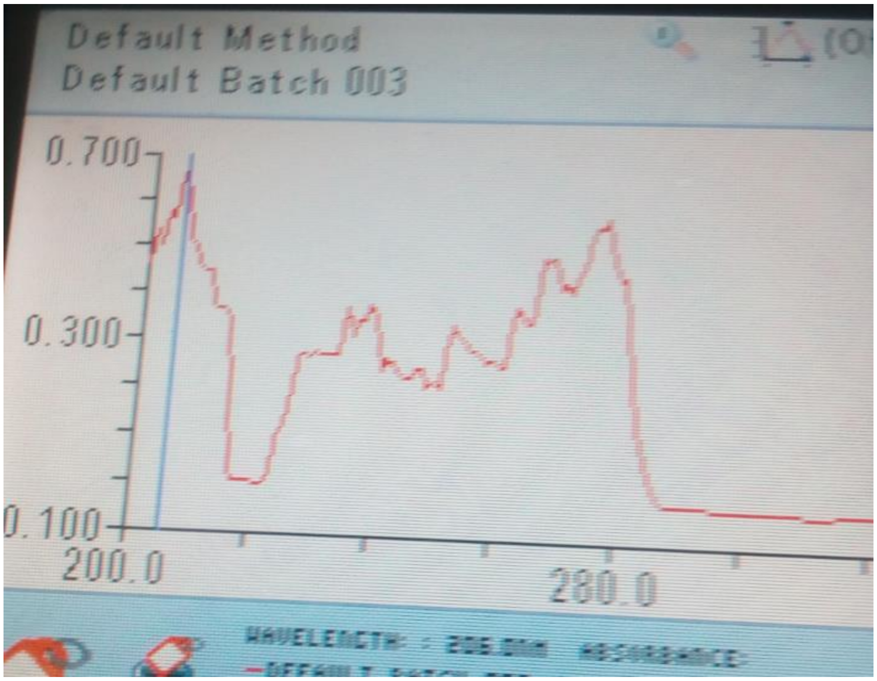

Fig.3 spectra for Sodium benzoate

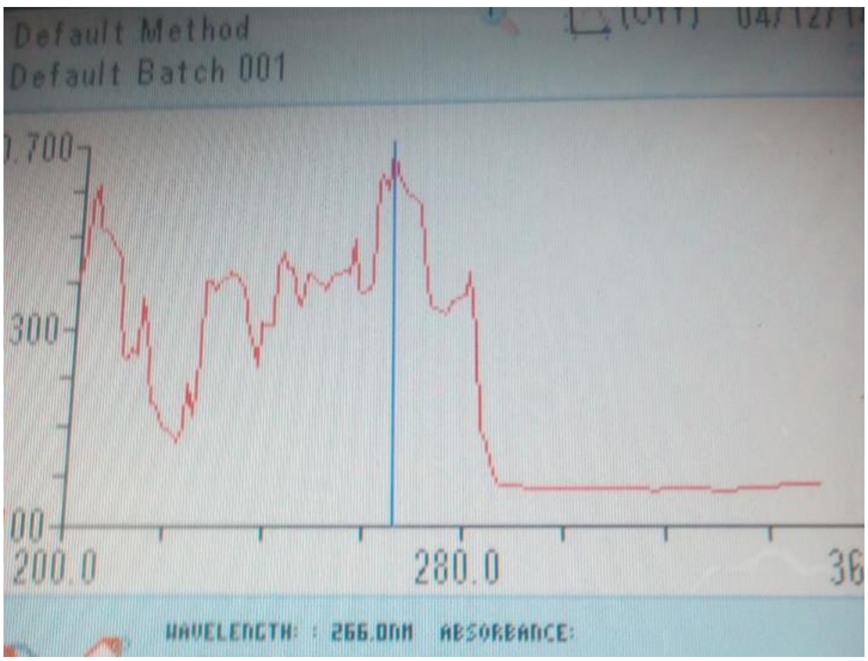

Fig.4. Spectra for Potassium sorbate

4.2 Method validation

4.2.1 Precision

Precision was tested to evaluate consistency of the instrument response for a given analyte and the repetitiveness of concentrations of different juice samples. The precision of the samples was expressed in percent of relative standard deviation (% RSD). The precision of an analytical method is also a criterion that provides information regarding the random error or dispersion of measurements [57]. The obtained %RSD was in the range of 4.90 to 9.98 and 5.57 to 10.34 for sodium benzoate and potassium sorbate, respectively with three replicate determinations of the sample concentration.

4.2.2 Limit of detection and quantification

Limit of detection is the lowest concentration of analyte that can be detected confidently and limit of quantification is the lowest concentration level at which the measurement is quantitatively detected. In this study, limit of detection and limit of quantification of the method were calculated three times standard deviation and ten times stander deviation of blank divided by the slope calculated from calibration curve respectively [57]. The limit of detection of the method for sodium benzoate and potassium sorbate was 2.38 mg/L and 1.96 mg/L, respectively. The limit of quantification of the method for sodium benzoate and potassium sorbate was 7.94 mg/L and 6.54 mg/L, respectively (table 6).

Table 6. Results of linearity, limits of detection (LOD) and quantification (LOQ) from calibrations curves for sodium benzoate and potassium sorbate

Parameter	Sodium benzoate standard	Potassium sorbate standard
Con in mg/L	10-50	10-50
Slope	0.014	0.0167
y-intercept	0.01	0.059
R^2	0.998	0.997
LOD in mg/L	2.38	1.96

LOQ in mg/L	7.93	6.54

4.2.3 Accuracy

The accuracy of the quantification was assessed in a recovery study. The recovery of Sodium benzoate and potassium sorbate in spiked samples was calculated to study the effect of matrix on the determination of Sodium benzoate and potassium sorbate [57]. The recovery studies were carried out at three concentrations of 15 mg/L was added to each concentration of unknown 3D Mango, 7Star Mango, Alang, Yoyo, Rani and Raubi of samples. For this three portions of pre-analyzed six different fruit juice samples were spiked with 15 mg/L in three replicates. The recoveries of Sodium benzoate and potassium sorbate from the fruit juice samples were evaluated on the basis of the comparison of the theoretical concentration level of the spiked solutions with the observed concentration gave acceptable and good percent recoveries found in the range of **87**% to **104**% are shows in Table 7.

Table 7. Results of recovery test for sodium benzoate and potassium sorbate (%)

Spiked Analyte (15 mg/L)	**Percent Recovery (%)**					
	3DMango	7Star	Alang	Yoyo	Rani	Raubi
Sodium benzoate	95.1	93.8	101	99	91	97
Potassium sorbate	87	96	104	92.6	90	94

4.3 Level of preservatives in different packed juices

In this study, six different commercially available packed juices were selected and quantified the levels of sodium benzoate and potassium sorbate preservatives. The concentration of sodium benzoate and potassium sorbate in all analyzed selected processed packed juices samples were found to be different among different packed juices and their contents of level were not exceeded the maximum permissible levels of sodium benzoate and potassium sorbate in packed juice [9] as shown in Table 8.

Table 8. Concentrations of sodium benzoate and potassium sorbate in selected packed juice (in mg/L)*

Sample	Sodium Benzoate (M±SD)	Potassium Sorbate (M±SD)
3D Mango	30.25 ± 1.40	34.68 ± 0.66
7Star Mango	37.00 ± 1.63	29.8 ± 0.52
Alang	43.03 ± 1.92	27.14 ± 1.01
Yoyo	19.49 ± 0.87	23.15 ±1.36
Rani	25.15 ± 0.28	39.00 ± 0.38
Raubi	38.66 ± 1.74	43.68 ± 0.62

*All concentrations of sodium benzoate and potassium sorbate were in 5 mL sample

Table 8 gives the content of sodium benzoate and potassium sorbate in six selected packed fruit juices. The result showed that the content of sodium benzoate was the highest in Alang whereas Rani was the lowest. The contents of sodium benzoate in all selected packed juices were in the range of maximum permitted level of 130 mg/L [9]. The results in table 8 also showed that the contents of potassium sorbate was highest in Raubi while the lowest was observed in Yoyo.The contents of potassium sorbate in all selected packed juices were in the range of maximum permitted level [9]. The contents of sodium benzoate were higher than the contents of potassium sorbate in 7Star mango and Alang and the contents of potassium sorbate were higher than the contents of sodium benzoate in the 3D mango, Yoyo, Rani and Raubi juices. The contents of sodium benzoate and potassium sorbate obtained in this study were in the range of similar previous works those obtanied by both HPLC and UV-Vis spectrometer methods [59, 60]. The contents of sodium benzoate obtained by HPLC from the previous study were close to the contents of sodium benzoate that obtained in the present study.The contents both sodium benzoate and potassium sorbate obtained in the present and previous study were less that of the maximum permitted level and hence, the contents of both sodium benzoate and potassium sorbate were obtained in all these juices were in the range of maximum permitted levels of 130 mg/L and 200 mg /L respectively [9].

4.4. Comparison of the present study with previous studies

Table 9 showed comparison of the present study results with previous studies. The contents of sodium benzoate and potassium sorbate could be determined by using both HPLC and UV-Vis spectrophotometer [54, 55]. The present study showed that the contents of sodium benzoate in mango juices were in range of the previous studies those were deternimed by HPLC method [54]. The contents of sodium benzoate obtained in mango juices in the previous study by UV-Vis spectrophotometer were higher than the contents of sodium benzoate that obtained in the present study, but the contents of sodium benzoate obtained in the present study were in the range of the previous study by HPLC method [60, 61]. The contents of potassium sorbate in mango fruit juices in the case of the present study were less than that of the previous studies that were determined by using HPLC and UV-Vis spectrophotometer method [59-61]. The contents of sodium benzoate and potassium sorbate were obtained by the UV- Vis spectrophotometer in the previous study were higher than the contents of both sodium benzoate and potassium sorbate obtained in the present study [59]. In both previous and present study the contents of both sodium benzoate and potassium sorbate obtained were within the maximum permitted level [9]. The contents of both sodium benzoate and potassium sorbate in mango juices in the present and previous studies are showed in table 9. The variation of the contents of sodium benzoate and potassium sorbate in the present and previous studies may vary due to methods used, processing of juices, ratio of amount added, chemical composition, chemicals used for extraction and quality attributes of preservatives during the manufacturing these preservatives in industry.

Table 9.Comparison of the present study with other study

Juices Sample	present study		previous study		Method	Reference
	sodium benzoate (mg/L)	potassium sorbate (mg/L)	sodium benzoate (mg/L)	potassium sorbate (mg/L)		
Mango	30.25±1.4	34.68 ±0.66	75.6	-	UV	[59]
Mango	30.25±1.4	34.68 ±0.66	83.52	108.8	UV	[59]
Mango	30.25±1.4	-	35.11±1.94	-	HPLC	[60]
Mango	30.25±1.4	-	28.04±0.52	-	HPLC	[60]
Mango	30.25±1.4	29.8±0.52	47.53±1.61	98.5	HPLC	[61]
Mango	30.25±1.4	29.8±0.52	46.71±0.49	-	HPLC	[61]
Mango	30.25±1.4	29.8±0.52	46.56±0.31	-	HPLC	[61]

4.4. Statistical Evaluation

Statistical analyses of the results were made to verify whether there were significant differences in concentrations of the packed fruit juices. In the present study, all the samples were analyzed in triplicates and the significance of variation within sample and between samples have been studied using ANOVA [58]. At 95% confidence level, no significance difference was observed in the levels of between samples ($p > 0.05$) and in between the level of preservatives in the sample at ($p>0.05$), Table 10.

Table 10. ANOVA result

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	415.5823417	5	83.11647	1.506062	0.332065	5.050329
Columns	1.248075	1	1.248075	0.022615	0.886341	6.607891
Error	275.939675	5	55.18794			
Total	692.7700917	11				

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study was carried out to determine the concentration of sodium benzoate and potassium sorbate in six different selected packed fruit juices commercially available in Jimma city. Dermination of the contents preservatives in various packed juices need more attention in order to develop good quality products and ensure juice safety. UV- Vis spectrophotometer method has been used and validated for the determination of the contents of two preservatives in six different selected packed juices. The experimental results revealed that this method fulfil good linearity, recoveries and low LODs for analysis and determining of the presence of the studied preservatives in fruit juices. The contents of potassium sorbate obtained in 7Star and Alang selected packed juices were lower than in the contents of sodium benzoate except in Raubi juice. The highest contents of sodium benzoate and potassium sorbate were obtained in the Alang and Raubi juice respectively. The contents of sodium benzoate and potassium sorbate obtained in selected packed fruit juices were in the maximum permissible levels approved by FDA.

5.2 Recommendation

It is recommended that to conduct further research periodically to quantify the levels of preservatives in different commercially available juices due to most of the packed juices are imported from abroad. Even if the current study result showed the level were lower than the permitted max level, the national FDA must follow and check the safety and quality of all packed juices before distribute to the costumers because children and adults may consume more packed juices per day, which possibly increase the consumption of the preservative and may exceed the permissible level. Consequently, they can expose for cancer, dental caries, obesity and diabetes.

6. REFERENCES

1. Zielinski AA, Haminiuk CW, Nunes CA, Schnitzler E, Ruth SM, Granato D. Chemical composition, sensory properties, provenance, and bioactivity of fruit juices as assessed by chemometrics: a critical review and guideline.Comprehensive reviews in food science and food safety. **2014**; 13(3):300-16.

2. Dueñas M, Muñoz-González I, Cueva C, Jiménez-Girón A, Sánchez-Patán F, Santos-Buelga C,A survey of modulation of gut microbiota by dietary polyphenols.BioMed research international; **2015**; 02:26-30

3. Tfouni AS, Toledo MC. Estimates of the mean per capita daily intake of benzoic and sorbic acids in Brazil.Food Addit. Contam.**2002**, 19(7):647-654.

4. Thomas OE, Adegoke OA. Toxicity of food colours and additives: A review. Afr. J. Biotechnol. **2015**, 9(36):900-914.

5. Martyn, DM, Mcnulty, BA, Nugget, AP, Gibney MJ.Food additives and preschool children. Proc. Nutr. Soc. **2013**, 72(1):109-116.

6. Lennerz BS, Vafai SB, Delaney NF, Clish AB, Deik AA, Pierce KA, Ludwig DS, Mootha VK.Effect of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Mol. Genet.Metab. **2015**, 114(1):73-79

7. Akintonwa A, Awodele O, Emeka PM, Osajare O. The mutagenic potentials of potassium bromate and some commonly used food additives. Afr. J. Biotechnol. **2006**, (8):1004-1006

8. My burgh N.G, Harkedold, Heller K.J and Ashurtz principle of the consumption soft drinks strategies American journal of beverage **2001**,(39) 271-280.

9. Food Safety and Standards Authority of India (FSSAI).Food safety and standards (food products standards and food additives) regulations. New Delhi: Int Law Book Company; **2011**, p. 449-97.

10. Wen, Y., Wang, Y., & Feng Y.Q. A simple and rapid method for simultaneous determination of benzoic and sorbic acids in food using in-tube solid-phase micro extraction coupled with high-performance liquid chromatography. Analytical Bio analytical Chemistry. 2007; 388 (8):1779-1787.

11. Kokoski CJ, Henry SH, Lin CS, Ekelman KB Methods used in safety evaluation. In: Branen A, Davidson PM, Salminen S (eds.), Food Additives New York: Marcel Dekker, **1990**, pp. 579-616.

12. Cardoso LAC, Kanno KYF, Karp SG. Microbial production of carotenoids–A review. Afr. J. Biotechnol, **2017**; 16(4):139-146.

13. Haen D the paradox of E-Numbers: Ethical, Aesthetic, and Cultural Concerns in the Dutch Discourse on Food Additives. Journal of Agricultural Environment and Ethics, **2014**; 27(1): 27-42.

14. Wilson B, Bahna S adverse reactions to food additives. Annals of Allergy Asthma Immunology; **2005**; 95(6): 499-507.

15. Young E Prevalence of intolerance to food additives. Environmental Toxicology and Pharmacology; **1997**, 4(1-2): 111-114

16. Zhang G, Ma Y. Spectroscopic studies on the interaction of sodium benzoate, a food preservative, with calf thymus DNA. Food Chem. **2013**; 141(1):41-47.

17. Sultana, T., J. Rana, S.R. Chakraborty, K.K. Das, T. Rahman and R. Noor, Microbiological analysis of common preservatives used in food items and demonstration of their in vitro anti-bacterial activity. Asian Pac. J. Trop. Dis., **2014**, 4(6): 452-456.

18. Di Sotto A, Maffei F, Hrelia P, Di Giacomo S, Pagano E, Borrelli F, Mazzanti G). Genotoxicity assessment of some cosmetic and food additives. Regul Toxicol Pharmacol. **2014**, 68:16-22.

19. El-Ziney MG. GC-MS analysis of benzoate and sorbate in Saudi dairy and food products with estimation of daily exposure. J Food Technol. **2009**, 7(4):127-34.

20. Ren L, Meng M, Wang P, Xu Z, Eremin SA, Zhao J, Yin Y, Xi R. Determination of sodium benzoate in food products by fluorescence polarization immunoassay. **2014**, 121:136-143.

21. Yavav A, Kumar A, Das M, Tripathi A Sodium benzoate, a food preservative, affects the functional and activation status of splenocytes at non cytotoxic dose. Food Chem. Toxicol. **2016**, 88:40-47.

22. Tsay HJ, Wang YH, Chen WL, and Huang MY, Chen YH Treatment with sodium benzoate leads to malformation of zebrafish larvae. Neurotoxicol. Teratol. **2007**, 29(5):562-569.

23. Pylypiw HM, Grether MTJ Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods, J. Chrom. A. **2000**, 883: 299-304.

24. Kücükcetin A, fiik B, Cicek A, Certel M. Determination of sodium benzoate and potassium sorbate in yoghurt and white pickled cheese by HPLC. Milchwissenschaft, **2004**, 59: 420-422.

25. French, V.M., R.A. Cooper and P.C. Molan, Antibacterial activity of honey against coagulase-negative Staphylococci. J. Antimicrob.Chemother. **2005**; 56(1): 228-231.

26. Viuda-Martos, M., Y.Ruiz-Navajas, J. Fernandez-Lopez and J.A. Perez-Alvarez, Functional properties of honey, propolis, and royal jelly. J. Food Sci., 2**008**; 73(9):117-124.

27. Sharma, P.C., S. Jain, G. Yadav and A. Sharma. Natural peservatives: Current insights and Applications. Der Pharmacia Sinica, **2010** 1(3): 95-108.

28. Can N.O., Arli, G., & Lafci, Y. A novel RP-HPLC method for simultaneous determination of potassium sorbate and sodium benzoate in soft drinks using C18-bonded monolithic silica column. Journal of Separation Science. **2011**; 34 (16-17): 2214-2222.

29. Clague, J.A & Fellers, C.R.” Relation of benzoic acid content and other constituents of cranberries to keeping quality”.Plant Physiology **1998** (9): 631-636

30. Guynot ME, Ramos AJ, Sanchis V, Marin S. Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5-5.5). Int J Food Microbiol **2005**; 101:161-8.

31. FDA,"Data on benzene in soft drinks and other beverages,"United states Food and Drug Administration. thttp://www.cfsan.fda.gov/~dms/benzene.html 16 (Accessed on 2nd June, 2006).

32. Heyman, Melvin B.; Abrams, Steven A. "Fruit Juice in Infants, Children, and Adolescents: Current Recommendations".**2017**; 139:6.

33. "Feeding Your Baby and Toddler (Birth to Age Two): Your Child: University of Michigan Health System". Med.umich.edu. Retrieved **2014**, 8-25.

34. American Academy of Pediatrics Committee on Nutrition."The use and misuse of fruit juice in pediatrics". **2001**,107 (5): 1210–3.

35. Muraki, Isao; Imamura, Fumiaki; Manson, Joann E.; Hu, Frank B.; Willett, Walter C.; van Dam, Rob M.; Sun, Qi. "Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohortstudies".**2013**, 13: 121-127.

36. Pongsavee M. Effect of sodium benzoate preservative on micronucleus induction, chomosome break, and Ala40Thr superoxide dismutase gene mutation in lymphocytes, Biomed. Res. Int. **2015**:1-5.

37. Gören C, Bilsel G, Simsek A, Bilsel M, Akcadaq F, Topal K, Ozqen H HPLC and LC–MS/MS methods for determination of sodium benzoate and potassium sorbate in food and beverages: Performances of local accredited laboratories via proficiency tests in Turkey. Food Chem. **2015**, 175:273-279.

38. T founi SAV, Toledo MCF.Determination of benzoic acid and sorbic acids in Brazilian food. Food Control **2002**; 13:125-9.

39. Horwitz W. AOAC Official method 960.38 benzoic acid in non-solid food and beverages. 17thed. Gaithersburg, Md: AOAC Int; **2000**: 192-205.

40. Kitano K, Fukukawa T, Ohtsuji Y, Masuda T, Yamaguchi H. Mutagenicity and DNA–damaging activity caused by decomposed products of potassium sorbate reacting with ascorbic acid in the presence of Fe salt. Food Chem Toxicol **2002**; 40:1589-94.

41. Saad B, Bari MF, Saleh MI, Ahmad K, Talib MK. Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in food stuffs Using high-performance liquid chromatography. **2005**; 1073:393-7.

42. Isabel MF, Eual IM, Paula B, Margarida AF. Simultaneous determination of benzoic and sorbic acids in quince jam by HPLC. Food Res Int **2001**; 33:113-7.

43. Martínez, L.Hrens, C. Gertz, Bestimmung, Manufacturing fruit beverages and concentrates of fruits and fruit processing **1990**, 92(3), 107–109.

44. Ubi, B.E., Honda, C., Bessho, H., Kondo, S., Wada, M., Kobayashi, S., and Moriguchi, T. "Expression Analysis of Anthocyanin Biosynthetic Genes in Apple Skin: Effect of UV-B and Temperature". **2006**; 170:571–578.

45. Orlowska, M., Koutchma, T., Grapperhaus, M., Gallagher, J., Schaefer, R., and Defelice, C "Continuous and Pulsed Ultraviolet Light for Non-thermal Treatment of Liquid Foods. Part 1: Effects on Quality of Fructose Solution, APPLE Juice and Milk." Food and Bioprocess Technology. **2012**; 10:779-8.

46. Stevens, C., Khan V.A., Lu J.Y., Wilson C.L., Pusey P.L., Kabwe M.K., Igwegbe E.C.K., Chalutz E., and Droby S. The germicidal and hormetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. Crop Protection, **1998**; 17: 75–84.

47. Lemoine, M.L, Civello, P.M., Martínez, G.A, and Chaves, A.R. "Influence of Postharvest UV-C Treatment on Refrigerated Storage of Minimally Processed Broccoli (Brassica oleracea var. Italica)." Journal of the Science of Food and Agriculture.**2007**; 87:1132–1139.

48. Koutchma, T "Advances in UV Light Technology for Non-thermal Processing of Liquid Foods."Food and Bioprocess Technology **2007**; 2:138–155.

49. Anonymous. 2000. “Kinetics of microbial inactivation for alternative food processing technologies.”InstituteofFoodTechnologists.J.FoodSci.Supplement http//vm.cfsan.fda.gov/~comm/ift-pref.html. (Accessed on 7 Februry, 2012).

50. Kaewsuksaeng, S, Urano, Y, Aiamla-or, S, Shigyo, M, and Yamauchi, N. “Effect of UV-B Irradiation on Chlorophyll-degrading Enzyme Activities and Postharvest Quality in Stored Lime (Citrus latifolia Tan.) Fruit.”Postharvest Biology and Technology. **2011**; 61:124–130.

51. Southgate, D.A.T., Johnson, I.T., & Fenwick, G.R. Nutritional value and safety of processed fruit juices. In: Production and Packaging of Non-Carbonated Fruit juices and Fruit Beverages. Second. **1995,** 108-112.

52. Brauns, F, Massatu, H.R.Health aspects of juice consumption. In: Liquid Fruit Market. AIJN (European Fruit Juice Association). **2014**: 318-31.

53. Horai, K. Kushida, T. Ishizaki, Ashurst, P.R. Chemistry and Technology of Soft Drinks and Fruit Juices.**1999**:135-171.

54. Dawei Wen, C. L. A Universal HPLC Method for the Determination of preservatives in fruit juices. **2005**, 53, 6624-6629.

55. Sasikala, S Analysis of Total Phenol in Developed Nutraceutical by UV-VIS Spectrophotometry.International Journal of Science and Research, **2015**, 6, 391.

56. Puttemans, L. Dryon, D.L Massart, Journal of the Association of Official Analytical Chemists, **1984**, 67, 880–885.

57. Food Safety and Standards Authority of India (FSSAI).Food safety and standards (food products standards and food additives) regulations. New Delhi: Int Law Book Company; **2011**. p. 449-97.

58. Miller. JN and JC. Miller Statistics and Chemometrics for Analytical Chemistry 6th ed., Pearson, Harlow, England, 2010.

59. Marjan Mohboubifor and ZahraSohani the analysis of sodium benzoate and potassium sorbate UV method. **2010**; 13(2):5-6.

60. Jr, H.M.P. and Grether, M.T...Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods, J. Chromatogr. A, **2006**,168, 219-241.

61. S.F.Song, S.Zhang and D. J. HPLC and UV method for analysis of sodium benzoate and potassium sorbate in juice. **2011**:21, p.1369.