COLORIMETRIC ASSAY OF CALCIUM PROPIONATE LEVELS IN COMMERCIALLY AVAILABLE BISCUITS

BY

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SUPERVISED BY

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DEDICATION

I dedicate this project to God Almighty for seeing me through it all. All Glory, Honour and Adoration be unto Him.

ACKNOWLEDGMENT

My profound gratitude goes to the Almighty God for His love, care, and for granting me the opportunity to study in this prestigious citadel of learning, the University of Benin, Benin City. I thank God Immensely for preserving my life and seeing me through it all. Glory, honor, and adoration be unto His name.

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I say a big thank you and God bless you all.

TABLE OF CONTENTS

ABSTRACT

Food preservation is an important aspect of extending shelf life and ensuring food safety. Calcium propionate is a widely used preservative that inhibits mold growth and extends the shelf life of baked products such as biscuits.

Concerns have been raised about the potential health implications of the intake of excessive amounts of calcium propionate including its neurological as well as metabolic effects. This study focuses on the colorimetric assay of calcium propionate in commercially produced biscuits to determine its concentration and evaluate compliance with permissible food additive limits.

In this analysis, a colorimetric method was employed, based on the reaction of propionate anions with ferric cations to produce a reddish-brown complex. A UV–visible spectrophotometer was used to measure the absorbance of the resultant solution at a wavelength of 600nm and the concentration was determined from a standard calibration curve.

Five different brands of biscuits were randomly selected and analyzed to determine their calcium propionate content. The results obtained showed all tested biscuit samples contained calcium propionate levels exceeding the internationally permissible limit of 2mg/g or 2000mg/kg according to the US Food and Drug Administration's (USFDA) recommendations.Among the analyzed samples, the lowest recorded concentration was 0.6634mg/ml, with the highest recorded concentration at 1.4716mg/ml.

The presence of this high calcium propionate content poses significant health implications, some of which are irritability, restlessness and other metabolic disturbances. Accordingly recommendations were made on how these concerns could be ameliorated to improve the health of biscuit consumers in Nigeria.

CHAPTER ONE

INTRODUCTION

In light of their nutritional qualities and potential for use in feeding programs and crisis scenarios like earthquakes, interest in bakery goods is increasing daily. Numerous studies on cereal science and technology shed light on the research methods used in the examination of baked goods. Biscuits are a flexible snack that holds a prominent position in the food business among bakery items because of their appealing qualities, which include a longer shelf life, a variety of tastes and textures, and wider consumption. Because they are inexpensive and have a lengthy shelf life, biscuits are a particularly popular ready-to-eat dish. Biscuits are essentially composed of flour, fat, sugar, water, milk, salt, and artificial yeast as a raising agent. Because of its adaptable structure, they have a good chance of incorporating nutrients and functionality.

Due to their versatility and adaptability, biscuits have been a staple of convenience meals for generations..

In a world of baked goods, biscuits have carved out a unique niche, celebrated for their delightful crunch and diverse flavors. However, a less glamorous aspect of biscuit production is preservative usage.

Preservatives are chemicals that are applied to food items to increase their shelf life, prevent spoilage, and maintain optimal taste and texture. In this article, we will explore the various types of preservatives commonly used in biscuits and the baking industry.

The primary reason for incorporating preservatives in biscuits is to ensure their safety and longevity. Without these additives, biscuits can quickly become stale or moldy, resulting in food

wastage. Additionally, preservatives help maintain the texture and taste of biscuits over time. For manufacturers, these additives are crucial for quality control and consistency, allowing them to meet consumer expectations and maintain their brand reputation.

Preservatives are categorized into two main types natural and artificial. Natural preservatives, derived from plant or animal sources, are gaining popularity due to the growing demand for clean labels and healthier eating options. Common natural preservatives include salt, sugar, vinegar, and certain essential oils. On the other hand, artificial preservatives, synthesized in laboratories, are frequently used because they are often more effective and cost-efficient.

Some Common Preservatives in Biscuits are: Sodium benzoate, Potassium Sorbate, BHT (Butylated Hydroxytoluene), Sorbic Acid and Calcium Propionate. Of all these preservatives stated, Calcium propionate is the preservative of primary concern.

The preservative Calcium propionate (E282) is mostly used in baked goods to improve shelf life by preventing the growth of bacteria and mould. It is a naturally occurring calcium salt of propionic acid that is also utilized in processed meats, animal feed, and some cheeses. Although it is generally accepted that it is safe to consume at prescribed amounts, there is disagreement over its use, and high doses may alter flavour or even cause health problems.

.Like benzoate, propionate stop microorganisms from generating the energy they require. Propionate, however, does not need an acidic environment, in contrast to benzoate. As a mould inhibitor, calcium propionate is utilized in baked goods. Bakers view mould contamination as a major issue, and typical baking environments provide conditions that are almost ideal for the growth of mould. In a double-blind, placebo-controlled crossover trial, children were given either calcium propionate or a placebo through daily bread. A statistically significant difference

was seen between the percentage of children whose behaviour "worsened" with challenge (52%) and the percentage whose behaviour "improved" with challenge (19%), even though there was no significant difference by two measures.When propanoic acid was infused directly into rodents' brains, it produced reversible behavior changes (e.g. hyperactivity, dystonia, social impairment, reservation) and brain changes (e.g. innate inflammation, glutathione depletion) partially mimicking human autism.

1.1 Literature Review

Food additives are permitted for a variety of applications. Some improve the nutritional content of specific meals, while others enhance their taste, texture, consistency, or colour. Preservatives are food additives that help preserve food nutritious and appetising while it is being transported to markets that may be hundreds of kilometres away from where it was grown or produced. Preservatives also help to extend the shelf life of foods in the household. Propionates are approved preservatives in carefully limited quantities in a variety of baked goods. Bread, biscuits, cakes, pastries, and other flour products can contain propionate. Sodium propionate (SP), calcium propionate (CP), and potassium propionate (PP) are sodium, calcium, and potassium salts of propionic acid.

1.2 Biscuit Making Process in Nigeria

1.2.1 Ingredients Selection and Preparation

The first step in the biscuit making process is selecting and preparing the ingredients. The key components of biscuits typically include flour, fat, liquid, and leavening agents. Each of these

ingredients performs an important part in defining the texture, flavour, and overall quality of the biscuits.

Flour is the primary ingredient in most biscuit recipes, providing structure and texture are added to the finished product. There are various flour options available, including all-purpose flour, cake flour, and self-raising flour. Type of flour chosen will impact the final texture and consistency of the biscuits.

The fat component, such as butter, shortening, or lard, is essential for creating a tender and flaky texture in the biscuits. The fat is typically cut into the dry ingredients using a pastry blender or two forks until the mixture resembles coarse crumbs.

To bring the dough together, liquids like milk, buttermilk, or cream are mixed in with the dry ingredients,the type and amount of liquid used will affect the moisture content and flavor of the biscuits

Leavening agents, such as baking powder or baking soda, are essential for the biscuits to rise and become light and fluffy. The type and quantity of leavening agents used are crucial to producing the proper texture and height in the finished biscuits.

Once the ingredients are selected, they must be prepared according to the specific recipe being used. This may involve sifting the dry ingredients, cutting the fat into the flour mixture, and carefully measuring and mixing the liquids and leavening agents. The goal of this step is to ensure that all the ingredients are properly combined and ready for the next stage of the biscuit making process.

1.2.2 Dough Formation and Shaping

After the ingredients have been selected and prepared, the next step in the biscuit making process is forming and shaping the dough. This step requires precision and attention to detail to achieve the desired texture and appearance of the biscuits.

Once the wet and dry ingredients are combined, the dough is typically turned out onto a lightly floured surface and gently kneaded until it comes together. Overworking the dough can result in tough and dense biscuits, so it's important to handle the dough with care.

Once the dough is formed, it is then rolled out to a specific thickness, usually around 1/2 inch to 3/4 inch, depending on the desired final product. A rolling pin is used to achieve an even thickness, and a biscuit cutter or sharp knife is used to cut out the individual biscuits.

Shaping the biscuits can vary depending on the desired outcome. For traditional round biscuits, a biscuit cutter is used to create uniform shapes. Alternatively, the dough can be gently shaped and cut into squares or triangles for a rustic appearance. Regardless of the shape chosen, it's important to handle the dough as little as possible to avoid toughening the biscuits.

After shaping the dough, the biscuits are placed on a baking sheet coated with parchment paper or a silicone baking mat. It is critical to provide enough space between each biscuit to ensure consistent baking and expansion during the cooking process.

1.2.3 Baking and Cooling

Once the biscuits are shaped and arranged on the baking sheet, they are ready to be baked. The baking time and temperature may differ based on the recipe and type of biscuits used, but most biscuits are baked in a hot oven (usually 425-450F) for 10-15 minutes.

The heat used in baking allows the leavening chemicals to react, causing the biscuits to rise and become light and flaky. The fat in the biscuits melts during baking, forming pockets of air that contribute to their soft texture.

The biscuits must be regularly monitored during the baking process to avoid overcooking or becoming too black.When the biscuits are golden brown and puffy, take them from the oven and leave to cool on the baking sheet for a few minutes.

After the initial chilling phase, the biscuits are transferred to a wire rack and allowed to cool entirely. This step is critical for enabling steam to escape and keeping the biscuits from getting soggy. Once totally cooled, the biscuits are ready to eat or store for later.

1.2.4 Quality Control and Testing

Quality control and testing are critical throughout the biscuit manufacturing process to guarantee that the finished product satisfies the desired texture, flavour, and appearance criteria. This step involves carefully inspecting the ingredients, monitoring the dough formation, and evaluating the baking results.

Quality control begins with inspecting the ingredients for freshness and quality. This may involve checking the expiration dates of the flour, leavening agents, and other perishable ingredients. Any ingredients that appear to be stale or past their prime should be discarded and replaced with fresh supplies.

As the dough is being prepared, it's important to periodically test the texture and consistency to ensure that it meets the desired specifications. This may involve adjusting the amount of liquid or flour used to achieve the perfect balance of tenderness and structure in the biscuits.

Once the biscuits are baked and cooled, they are carefully evaluated for flavor, texture, and appearance. This may involve tasting the biscuits to ensure they have the desired flavor profile and cutting them open to inspect the interior texture. Any biscuits that do not meet the established criteria for quality are either discarded or used for testing and feedback purposes.

In addition to visual and sensory testing, a sample of the biscuits may be sent to a laboratory for further analysis, such as moisture content, crumb structure, and shelf stability. This data is used to make any necessary adjustments to the biscuit making process to ensure consistent quality in the final product.

1.2.5 Packaging and Distribution

The final step in the biscuit making process is packaging and distribution. Once the biscuits have been baked, cooled, and evaluated for quality, they are ready to be packaged for sale or distribution to consumers.

Packaging for biscuits can vary widely depending on the intended market and consumer preferences. In some cases, biscuits may be individually wrapped in plastic or foil for single-serve convenience. Alternatively, they may be packaged in resalable bags or boxes for multi-serve consumption.

Regardless of the packaging format, it's important to ensure that the biscuits are properly protected from moisture, air, and other potential sources of contamination. This may involve using quality packaging materials, such as barrier films or laminates, to maintain the freshness and integrity of the biscuits.

Once packaged, the biscuits are labelled with important information, such as the product name, contents, nutritional information, and expiration date. This information is crucial for consumers to make informed purchasing decisions and ensure the safe consumption of the product.

Finally, the packaged biscuits are distributed to retailers, wholesalers, or directly to consumers through various channels, such as supermarkets, convenience stores, or online marketplaces. It's essential to ensure that the biscuits are handled and transported with care to prevent damage and maintain their quality until they reach the end consumer.

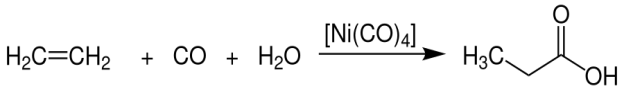
1.3 Calcium Propionate as a food preservative

Calcium propionate (E282) is a popular preservative in the baking sector, notably for bread, due to its ability to inhibit mould and bacterial growth. Although calcium propionate and sodium propionate are both efficient antimicrobial agents, calcium propionate is the most often used preservative in bread production around the world. Studies on the genotoxic effects of sodium propionate, calcium propionate, and potassium propionate on the root meristem cells of Allium cepa revealed that SP, CP, and PP, which are extensively employed in the food business, had clear chromotoxic properties. As a result, it is critical to exercise caution when utilizing these chemicals as food additives, as excessive use might cause health issues such as gastrointestinal irritation and possibly behavioural impacts in children. To ensure food safety and consumer health, accurate and reliable analytical methods for determining propionate in foodstuffs of varied matrices must be developed. Regulatory bodies, notably the WHO Joint Expert Committee on Food Additives (JECFA), have established tolerable daily intake. (ADI) limits, making the analytical detection and quantification of this compound in food products a critical area of food safety research.

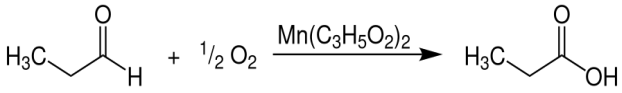
In order to evaluate food safety several analytical methods have been reported for quantitative determination of Calcium propionate in different matrices, including gas chromatography, capillary electrophoresis, colorimetry and high performance liquid chromatography with UV detection (HPLC–UV).

1.3.2 Chemistry of Propionic Acid

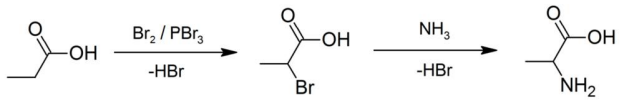
Propionic acid (also known as propanoic acid) is a naturally occurring carboxylic acid with the chemical formula CH3CH2CO2H, derived from the Greek words prōtos (meaning "first") and píōn (meaning "fat"). It is a liquid with a strong and unpleasant odour that resembles body odour. Propionate or propanoates refer to the anions CH3CH2CO2− and the salts and esters of propionic acid. Propionic acid is used as a preservative in both animal feed and human food, accounting for over half of global output. It also serves as an intermediary in the manufacture of other compounds, particularly polymers. Propionic acid has physical properties that fall in between those of smaller carboxylic acids (formic and acetic acids) and larger fatty acids.It is miscible with water, but can be extracted from it by adding salt. It is made up of hydrogen-bonded pairs of molecules in both liquid and vapour form, just as acetic and formic acids. In industry, propionic acid is primarily generated by the hydrocarboxylation of ethylene utilising nickel carbonyl as a catalyst:



It is also formed via the aerobic oxidation of propionaldehyde. In the presence of cobalt or manganese salts (manganese propionate is most usually used), this reaction occurs rapidly at temperatures as mild as 40-50 °C:

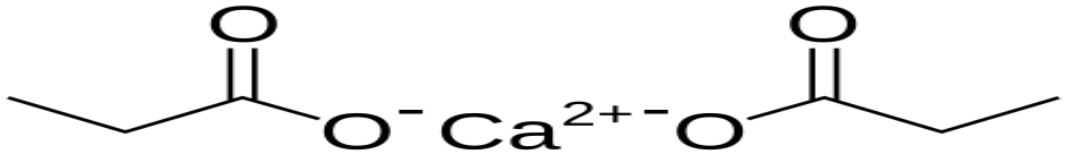


Propionic acid exhibits the general features of carboxylic acids, including the ability to create amide, ester, anhydride, and chloride derivatives. The Hell-Volhard-Zelinsky reaction catalyses the α-halogenation of a carboxylic acid with bromine, resulting in the formation of 2-bromopropanoic acid (CH3CHBrCOOH). This substance was used to produce a racemic mixture of alanine via ammonolysis.



1.3.2 Chemistry of Calcium Propionate

Calcium propionate, an organic salt with the chemical formula C6H10CaO4, has a molecular weight of 186.22 g/mol. It's also known as Calcium Propaonate. Calcium propionate is easily soluble in both water and ethanol and hydrolyzed into Ca++ and propionic acid. The structure of calcium propionate is shown below:



The physical and chemical properties of calcium propionate are represented below:

-SYNONYMS: Calcium propanoate, INS No. 282

-DEFINITION

Chemical name: Calcium propionate

Chemical formula:C6H10CaO4

Structural formula:(CH3CH2COO-)2 Ca++

Formula weight:186.22

Assay: Not less than 98,0% on the dried basis

-DESCRIPTION

White crystals, powder or granules with not more than a faint odor of propionic acid

-FUNCTIONAL USES

Preservative, antimould and antirope agent

CHARACTERISTICS

-IDENTIFICATION

Solubility: Freely soluble in water, soluble in ethanol

Positive test for calcium: Passes test

Positive test for propionate: Warm the sample with sulfuric acid. The propionic acid evolved may be recognized by its odor.

Positive test for alkali salt of organic acid: Ignite the sample at a relatively low temperature. The alkaline organic acid residue effervesces with acid.

-PURITY

Loss on drying: Not more than 4% (105°. 2h)

pH:7.5 - 10.5 (1 in 10 sol)

1.3.3 Synthesis of Calcium Propionate

Calcium propionate is primarily produced via the neutralisation of propionic acid with calcium hydroxide or calcium carbonate.This reaction is usually carried out at room temperature. By controlling the reaction conditions and subsequent processing steps, a calcium propionate product with higher purity can be obtained. The reactions supporting this are as follows:

Core reactions (balanced)

1. Neutralization with calcium hydroxide (common lab/industrial route):

2 C2H5COOH + Ca(OH)2 → Ca(C2H5COO)2 + 2 H2O

2. Neutralization with calcium carbonate (produces CO_2):

2 C2H5COOH + CaCO3 → Ca(C2H5COO)2 + H2O + CO2↑

3. Double displacement (salt metathesis):

2 NaC2H5COO + CaCl2 → Ca(C2H5COO)2 + 2 NaCl

(useful if sodium propionate is on hand — remove NaCl by selective solubility/crystallization)

1.3.4 Mode of Action of Calcium Propionate

Calcium propionate functions as a preservative by breaking down to propionic acid, which damages microbial cell membranes, inhibits enzyme activity, and disrupts cell reproduction. It

also has a metabolic function, acting as a glucose precursor in ruminants and supporting energy balance by inhibiting hepatic lipid oxidation and ketone body production.

1.3.5 Application of Calcium propionate

They contain application of Calcium Propionate in the food industry is mainly reflected on its use as a preservative. The following is the detailed application of Calcium Propionate in the food industry:

-Baking Industry

Calcium propionate is most commonly used in the baking business. It is routinely applied to bread and other baked items to prevent mould formation and keep them fresh. Calcium propionate prevents mould spores from growing, ensuring that baked goods are safe to consume for an extended period of time.

-Dairy Products

Calcium propionate is used in dairy products like cheese and yoghurt to inhibit the growth of unwanted microbes. Its preservative characteristics help dairy formulas remain stable on the shelf, maintaining product quality and consumer safety.

-Animal Feed

Calcium propionate is used as a feed additive to prevent spoilage and maintain feed integrity. By inhibiting mould growth and microbial proliferation, it improves feed safety and nutritional quality, thereby supporting livestock and poultry health and productivity.**-Plant-Based Diets**

With the growing popularity of plant-based diets, calcium propionate has found a place in plant-based food products. It is used as a preservative in vegan and vegetarian alternatives to typical dairy and meat products, extending their shelf life while maintaining quality and taste.

-Processed Foods

Convenience meals, cereals, and snacks are just a few of the processed foods that frequently include calcium propionate. By lowering the possibility of microbial contamination during storage and distribution, its preservation qualities support the stability and safety of packaged goods.

1.4 Statement of Problem

The statement of the problem regarding calcium propionate involves a conflict between its widely accepted safety as a food preservative and potential concerns about its metabolic effects, particularly insulin resistance, and neurological side effects in susceptible individuals, as well as potential negative effects in sensitive animal populations. While generally recognized as safe, some studies suggest a need for more investigation of its long-term impacts on human health and animal welfare, leading to questions about its role in conditions like insulin resistance and irritability, especially at high doses or in sensitive populations.

Metabolic and neurological concerns in humans

Insulin resistance: Some studies suggest calcium propionate consumption is linked to increased insulin and glucagon production, potentially contributing to insulin resistance, a type 2 diabetes risk factor. But this link requires further investigation to determine if it is a direct cause-and-effect relationship in humans.

Neurological effects: A small study on children found that daily consumption of calcium propionate-containing bread was associated with irritability, restlessness, poor attention, and sleep issues. More research is needed to confirm this, but it raises a potential problem for sensitive children.

Migraine headaches: Some evidence suggests a link between propionate and migraine headaches in susceptible individuals.

Concerns in animal populations

Dairy cows: Supplementation in dairy cows has been shown to improve milk production but also to decrease antioxidant capacity and affect various serum metabolites. The long-term effects of these metabolic changes need to be understood.

Chemical and industrial concerns

Decomposition: When heated to decomposition, calcium propionate can emit acrid smoke and irritating fumes.

Reactivity: It can irritate the respiratory system and is incompatible with strong oxidizing agents.

1.5 Justification of Study

A study of calcium propionate is justified by its crucial role as a preservative in food and feed, its safety profile for preventing mold and bacterial growth, and its specific applications in animal

nutrition and human health. Research continues to explore its effects on satiety, metabolic processes, and animal performance, particularly in the the dairy sector.

1.6 Scope of Study

The scope of the study is to analyze the amount of Calcium propionate in different biscuit samples in Nigeria.

1.7 Aim of Study

To determine the amount of Calcium propionate by the Colorimetric analysis method using biscuit samples and to ensure that Calcium propionate is used safely within recommended limits.

1.8 Objectives of the Study

-To determine the calcium propionate content of some commercially available biscuits using the colorimetric method based on the principle of the complexation reaction between ferric ammonium sulfate and propionate ions to give a reddish-brown complex.

-To assess whether the detected levels comply with food safety standards given as 2000mg/kg or 2mg/g according to the US Food and Drug Administration's (USFDA) recommendations.

1.9 Colorimetry

Colorimetry is defined as "the science and technology used to quantify and describe physically the human colour perception" . Its concern in reducing spectra to the physical correlates of colour perception sets it apart from spectrophotometry. The two primary applications of colorimetry, the science and technology of measuring and characterizing colour, are the scientific quantification and description of human colour perception and the objective measurement of the

concentration of coloured compounds in a solution. It is a method in chemistry that uses a colorimeter to detect the amount of light absorbed at a particular wavelength in order to calculate the concentration of coloured compounds in a solution.

In chemistry

1.9.1 Principle: It relies on the Beer-Lambert Law, which states that the amount of light absorbed by a solution is directly proportional to the concentration of the solute in the solution. This can be represented as shown below;

A = £CL

Where; A=Absorbance/optical density of the reaction

£=molar absorptivity

C=concentration of the solution

L=length of light path

Colorimetry falls under absorption photometry, one of several types of analytical methods based on the photometry principle. A popular analytical method for quantitative colour measurement, colorimetry is used to determine the quantity of a coloured chemical in a sample solution, such as water or biological samples, at the visible spectrum of light (380–780 nm). One device that uses this method is the colorimeter. Another name for it is an absorptiometer. A substance must be colorful or should have property of forming chromogens through the addition of reagents which will absorb light according to their color intensity to be measured. The concentration of the coloured ingredient determines how intense the colour is.The majority of the analytical methods currently employed in our clinical laboratory are based on photometric principles,

which quantify light that has been absorbed, transmitted, or emitted. Spectrophotometry is the measurement of intensity at various wavelengths over the entire electromagnetic spectrum.

1.9.2 Method

A colorimeter shines a beam of light through a sample. The amount of light that passes through is measured, and this is compared to the amount of light that passed through a known standard to ascertain the unknown sample's concentration.

1.9.3 Application

It is used in various fields, including:

-Medicine: To measure the amount of hemoglobin in a blood sample or biochemical composition of fluids.

-Food and Beverage: To quantify the color of products like beer, sugar, and vegetable products.

Environmental testing: To test for the presence of chemicals like cyanide, iron, and fluorine in water.

Industries: Used in the printing, textile, and paint industries to ensure color consistency.

In human color perception

Principle: It quantifies and describes the way humans perceive color.

Method: This involves creating color spaces and defining color values based on human eye receptors (trichromatic vision).

Applications:

Optometry: Used to determine if colored lenses can help with visual stress.

Digital displays: Used to evaluate the color contrast and brightness of screens on devices like computers and mobile phones.

1.9.4 Disadvantages of Colorimetry

The main disadvantages of colorimetry include its limited range to the visible spectrum (400–700nm), making it unsuitable for analyzing colorless compounds or measuring in the UV/IR regions. It also struggles with specific color types like fluorescent or metallic, cannot identify metamerism, and is prone to interference from turbidity, bubbles, and fingerprints in the sample. Accuracy depends heavily on proper calibration and sample preparation, and it offers less versatility for research and complex color formulation compared to a spectrophotometer.

CHAPTER TWO

MATERIALS AND METHOD

2.0 Materials And Method

2.1 Materials

2.1.1 Equipment and Apparatus used

-25ml volumetric flasks

-50ml volumetric flasks

-250ml volumetric flasks

-Plastic funnels

-Portable electronic scale

-Masking tape

-100ml measuring cylinder

-Tissue paper

-Cotton wool

-Weighing paper

-Glass stirrer

-Colorimetric Visible Spectrophotometer Labtech-722(four compartments)

-Glass cuvette

-Test tubes

-Test tubes rack

-Rubber pipettes

-Spatula

2.1.2 Reagents used

-Calcium propionate

-Ferric ammonium sulphate

-Deionized water

2.1.3 Samples used

Five brands of commonly consumed biscuit products were randomly selected and used for this study.

-Sample A(Coaster Biscuit)

-Sample B(Parle-G Biscuit)

-Sample C(Cream Crackers Biscuit)

-Sample D(Chic-Choc Biscuit)

-Sample E(Noreos Biscuit)

2.2 Method

2.2.1 Preparation of Standard stock solution of Calcium Propionate

Calcium propionate standard stock solution with a concentration of 2mg/ml was prepared by dissolving 200mg of calcium propionate in a 250ml volumetric flask with sufficient deionized water to produce a stock solution. More deionized water was added to make up to the100ml mark. The solution obtained was labeled as the stock solution of Calcium Propionate.

2.2.2 Preparation of Standard Calcium Propionate solution

From the stock solution, further dilution of the calcium propionate stock solution was prepared with deionized water to obtain 50ml of standard concentration of 0.2mg/ml to 0.8mg/ml of calcium propionate and they were labeled appropriately. This was prepared using the formula: M1V1 =M2V2

M1= Molarity of the initial/concentrated solution

V1= Volume of the initial/concentrated solution

M2= Molarity of the final/diluted solution

V2= Volume of the final/diluted solution

Table 2.1: Preparation of the 50ml standard concentration of Calcium Propionate solution

Concentration prepared(mg/ml)	Volume of stock (ml)	Volume of deionized water(ml)	Total volume (ml)
0.2	5	45	50
0.4	10	40	50
0.6	15	35	50
0.8	20	30	50

2.2.3 Preparation of Ferric ammonium sulphate test solution

Ferric ammonium sulphate solution was prepared by weighing 2.0g of standard ferric ammonium sulfate and dissolved using deionized water up to the 25ml volume mark.

2.2.4 Preparation of Standard Calibration Curve

Several standard concentration of calcium propionate solution of(0.2,0.4,0.6,0.8mg/ml) prepared were used to prepare the standard calibration curve. The absorbance was obtained by pipettes 2ml of 0.2mg/ml standard calcium propionate concentration into a clean 10ml sample test tube containing 0.2ml of ferric ammonium sulfate solution. The colorimeter was turned on and allowed to equilibrate for 30 mins. The filter that gave the best absorbance was obtained at a wavelength of 600nm. The samples tube were cleaned after each determination and the colorimeter was zeroed with a blank solution containing 2 drops of ferric ammonium sulfate and 2ml of deionized water after which the absorbance values of each standard concentration(0.2-

0.8mg/ml) was determined. The results obtained were used to determine the standard calibration curve.

2.3 Collection of samples used

Five different brands of biscuit samples were randomly selected in Benin city. The biscuit samples that were selected had the manufacturing information such as biscuit names, ingredients, mailing address, manufacturing date, expiration date, NAFDAC Registration no, nutritional information and other pertinent information which were documented.

Manufacturing Information of Biscuit Samples

Sample A

Brand Name: Coaster Biscuit

NAFDAC Reg No: 01-0089

Production Date:07/2025

Expiry Date:04/26

Batch No:03D25M

Net Weight:40g

Ingredients: Wheat flour, Sugar, Vegetable fat, Invert syrup, Ammonium bicarbonate, Sodium bicarbonate, Sodium methylbisulphite, Soya lecithin, Flavour(Vanilla)

Allergy Information: Contains gluten and soya

Nutritional Information:

Nutritional Information	Amount per 100g
Sodium as salt	0.77g
Fat	8.97g
Carbohydrates	60.33g
Protein	7.75g

Manufacturers Address

Produced by: Prime Bisco Nigeria Ltd; Plot 14,Block B, Ilasamaja Industrial scheme, Apapa-Oshodi Expressway, Lagos, Nigeria.

Tel: 08172654592

Sample B

Brand Name: Parle-G Biscuit

NAFDAC Reg No: 01-9330

Production Date:08/2025

Expiry Date:07/26

Batch No:P128B

Net Weight:65g

Ingredients: Wheat flour, Sugar, Hydrogenated Vegetable oil, Invert syrup, Ammonium bicarbonate, Sodium bicarbonate, Sodium methylbisulphite, Soya lecithin, Flavour(Vanilla),Whey powder, Salt, Emulsifier of vegetable origin(di-acetyl tartaric acid esters of mono- and di-glyceride)

Allergy Information: Contains wheat(gluten) and milk ingredient

Nutritional Information:

Nutritional Information	**Amount per 100g**
Energy	451 kcal
Fat	12.5g
Carbohydrates	77.7g
Protein	7.0g

Manufacturers Address

Produced by: P1-Pardee Foods Nigeria Ltd, Lynson Chemical Avenue, Off KM 38 Lagos-Abeokuta Express Way, Sango-Ota, Ogun State, Nigeria.

Tel: 07055219015

Sample C

Brand Name: Cream Crackers Biscuit

NAFDAC Reg No: 01-0547

Production Date:07/2025

Expiry Date:04/26

Batch No:04D2L2M

Net Weight:35g

Ingredients: Wheat flour, Sugar, Vegetable fat, Invert syrup, Ammonium bicarbonate, Sodium bicarbonate, Sodium methylbisulphite, Soya lecithin, Flavour(Vanilla)

Allergy Information: Contains gluten

Nutritional Information:

Nutritional Information	**Amount per 100g**
Sodium as salt	1.67g
Fat	9.32g
Carbohydrates	75.69g
Protein	9.70g
Energy	425.44 kcal

Manufacturers Address

Produced by: Prime Bisco Nigeria Ltd; Plot 14,Block B, Ilasamaja Industrial scheme, Apapa-Oshodi Expressway, Lagos, Nigeria.

Tel: 08172654592

Sample D

Brand Name: Chic-Choc Biscuit

NAFDAC Reg No: 08-0191

Production Date:08/2025

Expiry Date:08/26

Batch No:FT-3763

Net Weight:32g

Ingredients: Wheat flour, Sugar, Vegetable fat, Invert syrup, Milk solids, Corn starch, Salt, Ammonium bicarbonate, Sodium bicarbonate, Sodium methylbisulphite, Soya lecithin, Flavour (Vanilla), Strawberry flavour, Permitted food colour (E129), Dough conditioner (E223), Citric acid

Allergy Information: Contains Wheat gluten, milk protein and soya

Nutritional Information:

Nutritional Information	**Amount per 100g**
Energy value	484 kcal
Fat	19.3g
Carbohydrates	70.9g
Protein	6.5g

Manufacturers Address

Produced by: Ok Foods Ltd; Plot 6,Block E, Oshodi Industrial scheme, Apapa-Oshodi Expressway, Lagos, Nigeria.

Tel: 07045914591

Sample E

Brand Name: Noreos Biscuit

NAFDAC Reg No: 06-3011

Production date:07/2025

Expiry date:11/2026

Batch No:BT-4874

Net Weight: 23g

Ingredients: Wheat flour, Sugar, Vegetable fat, Invert syrup, Milk solids, Corn starch,Salt, Ammonium bicarbonate, Sodium bicarbonate, Sodium methylbisulphite, Soya lecithin, Flavour (Vanilla),Strawberry flavour, Permitted food colour (E129),Dough conditioner (E223),Citric acid

Allergy Information: Contains Wheat gluten, milk protein and soya

Nutritional Information:

Nutritional Information	Amount per 100g
Energy value	473 kcal
Fat	18.2g
Carbohydrates	71.0g
Protein	6.4g

Manufacturers Address

Produced by: Hansbro Food Ltd; 79/94 Abeokuta Expressway, Dopemu, Lagos, Nigeria.

Tel: 07079012347

2.3.2 Extraction of Calcium Propionate from 10g biscuit samples

Each biscuit sample was weighed to know the net weight of the biscuit. 10g of biscuit sample was accurately weighed using the electronic weighing scale. It was then broken into smaller bits then placed into 250ml conical flask and 100ml of deionized water was added to it, the mixture was allowed to stand for 2 hours with proper stirring using glass rod at interval of 30mins before filtering. The filtrate obtained from the mixture was used to test for the presence and absorbance of calcium propionate in the spectrophotometer using 600nm wavelength. The same procedures were used for other biscuit samples to obtain their absorbance.

2.3.3 Testing for Calcium Propionate

2ml of biscuit sample filtrate was measured and transferred into a sample tube containing 0.2ml ferric ammonium sulphate test solution with the aid of a rubber pipette. The reaction was carried out in triplicate and allowed to stand for 30 mins each and then inserted into the colorimeter having four compartments. The absorbance was obtained with the predetermined filter (600nm). The experiment was repeated for each of the biscuit samples and the results were recorded appropriately.

CHAPTER THREE

RESULTS

The five different biscuits samples tested produced absorbance values which were used to determine their respective concentrations by extrapolation using the standard calibration curve. The concentration of each biscuit sample was then used to determine the amount of calcium propionate in the biscuits.

Samples A,B,C,D and E contained calcium propionate content of 3.32mg/g, 5.43mg/g, 6.08mg/g, 7.20mg/g and 7.36mg/g respectively.

The figure below shows the calibration plot for the standard concentration within the range of 0.2 to 0.8mg/ml and its absorbance

Table 3.1:Result of the different concentration of standard Calcium Propionate solutions

Concentration(mg/ml)	Absorbance at 600nm
0.2	0.08
0.4	0.255
0.6	0.510
0.8	0.775

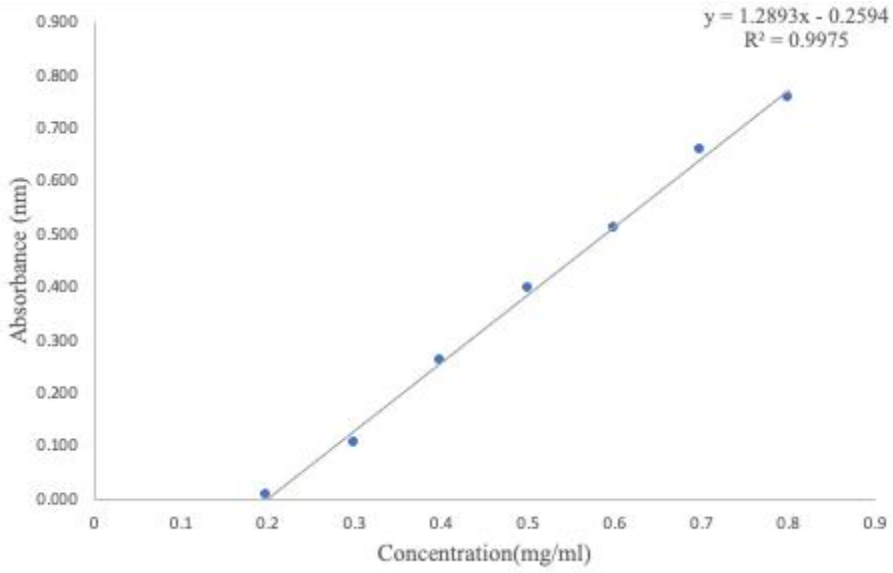


Figure 1: Graph of Absorbance against Concentration (mg/ml) using 600nm filter

Table 3.2:Calculated Average Absorbance of Calcium Propionate in the biscuit samples obtained using 600nm wavelength

Sample Code	1st Absorbance reading	2nd Absorbance reading	3rd Absorbance reading	Average Absorbance reading
A	0.595	0.596	0.597	0.596
B	1.142	1.143	1.141	1.142
C	1.310	1.308	1.309	1.309
D	1.596	1.597	1.598	1.597
E	1.638	1.639	1.637	1.638

Table 3.3:Calculated Concentration of Calcium Propionate(mg/ml) in the biscuit samples using 600nm wavelength

Sample Code	Average Absorbance(nm)	Calculated Concentration(mg/ml)
A	0.596	0.6634
B	1.142	1.0869
C	1.309	1.2164
D	1.597	1.4398
E	1.638	1.4716

Table 3.4:Amount of Calcium Propionate(mg) contained in each brand of biscuit sample

Sample Code	Weight in 10g(mg/10g)	Weight in 1g(mg/g)	Amount per Sample Weight(mg)	Sample Weight(g)
A	33.17	3.32	130.67	39.3584
B	54.35	5.43	353.92	65.1794
C	60.82	6.08	212.13	34.89
D	71.99	7.20	255.67	35.51
E	73.58	7.36	164.42	22.34

Key:

A= Coaster Biscuit

B= Parle-G Biscuit

C= Cream Crackers Biscuit

D= Chic-Choc Biscuit

E= Noreos Biscuit

Formula For Calculating Mean Average

$$\mathbf{X = \frac{X1 + X2 + X3}{n}}$$

where;

X= Mean average

X1,X2,X3= Individual sample

n= number of observations

Using Sample A

X1= 0.595, X2= 0.596, X3= 0.597

$$X = \frac{0.595 + 0.596 + 0.597}{3}$$

$$X = \frac{1.788}{3}$$

X= 0.596

The sample calculations were carried out for samples B to E respectively

Equation for calculating the actual concentration of Calcium Propionate content per 10g of each biscuit sample

Using the formula y= 1.2893x - 0.2594, the concentration for each sample was then calculated.

Where y= Absorbance, x= Concentration

For Sample A(Coaster Biscuit)

Absorbance value(y)= 0.596

0.596= 1.2893x - 0.2594

x= (0.596 +0.2594)/1.2893 = 0.6634mg/ml

Hence;

2ml contains 0.6634mg of calcium propionate

100ml will contain x mg of calcium propionate

x= (0.6634×100)/2 =33.17mg

Total mass of Biscuit = 39.3584g

10g of biscuit sample contains 33.17mg of calcium propionate

39.3584g of biscuit sample will contain x mg of calcium propionate

x= (33.17×39.3584)/10 = 130.55mg

Therefore in 1g, x= 130.55mg/39.3584g = 3.32mg/g

Compared to the standard of 2mg/g, the Biscuit sample contains a high level of Calcium propionate.

For Sample B(Parle-G Biscuit)

Absorbance value(y)= 1.142

1.142= 1.2893x - 0.2594

x= (1.142+0.2594)/1.2893 = 1.0869mg/ml

Hence;

2ml contains 1.0869mg of calcium propionate

100ml contains x mg of calcium propionate

x= (1.0869 ×100)/2 = 54.345mg

Total mass of Biscuit = 65.1794g

10g of biscuit sample contains 54.345mg of calcium propionate

65.1794g of biscuit sample will contain x mg of calcium propionate

x= (54.345 ×65.1794)/10 = 354.22mg

Therefore in 1g, x= 354.22mg/65.1794g = 5.43mg/g

Compared to the standard of 2mg/g, the Biscuit sample contains a high level of Calcium propionate.

For Sample C(Cream Crackers Biscuit)

Absorbance value(y)= 1 309

1.309= 1.2893x - 0.2594

x= (1.309+0.2594)/1.2893= 1.2164mg/ml

Hence;

2ml contains 1.2164mg of calcium propionate

100ml will contain x mg of calcium propionate

x= (1.2164×100)/2 =60.82mg

Total mass of Biscuit = 34.8874g

10g of biscuit sample contains 60.82mg of calcium propionate

34.8874g of biscuit sample will contain x mg of calcium propionate

x= (60.82×34.8874)/10 = 212.18mg

Therefore in 1g, x= 212.18mg/34.8874g= 6.08mg/g

Compared to the standard of 2mg/g, the Biscuit sample contains a high level of Calcium propionate.

For Sample D(Chic-Choc Biscuit)

Absorbance value(y)= 1.597

1.597= 1.2893x- 0.2594

x= (1.597+0 2594)/1.2893 = 1.4398mg/ml

Hence;

2ml contains 1.4398mg of calcium propionate

100ml will contain x mg of calcium propionate

x= (1.4398×100)/2 = 71.99mg

Total mass of Biscuit = 35.5137g

10g of biscuit sample contains 71.99mg

35.5137g of biscuit sample will contain x mg of calcium propionate

x= (71.99×35.5137)/10 = 255.66mg

Therefore in 1g, x= 255.66mg/35.5137g= 7.20mg/g

Compared to the standard of 2mg/g, the Biscuit sample contains a high level of Calcium propionate.

For Sample E(Noreos Biscuit)

Absorbance value(y)= 1.638

1.638= 1 2893x - 0.2594

x= (1.638+0.2594)/1.2893= 1.4716mg/ml

Hence;

2ml contains 1.4716mg of calcium propionate

100ml will contain x mg of calcium propionate

x=(1.4716×100)/2 = 73.58mg

Total mass of Biscuit = 22.3356g

10g of biscuit sample contains 73.58mg

22.3356g of biscuit sample will contain x mg of calcium propionate

x= (73.58×22.3356)/10= 164.34mg/g

Therefore in 1g,x= 164.34mg/22.3356g = 7.36mg/g

Compared to the standard of 2mg/g, the Biscuit sample contains a high level of Calcium propionate.

To calculate the amount of Calcium propionate in the total weight of the biscuit

Total weight of Sample A=39.36g

Weight of Calcium propionate in 1g of sample A=3.32mg

Total weight of Calcium propionate in 39.36g of sample A=39.36g × 3.32mg

Total weight of calcium propionate in sample A=130.67mg/total weight of Sample A

The sample calculations were carried out for samples B,C,D and E respectively

CHAPTER FOUR

DISCUSSION

Food preservation is a crucial aspect of ensuring food safety and extending shelf life. Calcium propionate, a widely used antimicrobial agent, is commonly added to baked foods such as bread, biscuits, cakes and other flour products to prevent mold and bacterial growth. However, increasing concerns have been raised about the potential health implications of consuming excessive amounts of calcium propionate, including metabolic and neurological effects. This study employs a colorimetric analysis method to quantitatively determine the concentration of calcium propionate in commonly consumed bread brands.

The methodology is based on the principle of complexation between ferric ammonium sulfate and propionate anions, forming a reddish-brown complex that is measurable by colorimetric methods at a wavelength of 600nm. The equation of the reaction is given as:

$$Fe^{3+}(aq) + 3CH_3CH_2COO^-(aq) \rightleftharpoons Fe(CH_3CH_2COO)_3.$$

Five(5) different brands of bread were randomly selected and analyzed to assess their calcium propionate content. The results revealed that all tested bread samples contained calcium propionate levels with samples A,B,C,D and E having masses of 3.32mg/g,5.43mg/g and 6.08mg/g,7.20mg/g and 7.36mg/g respectively, all of which exceed the internationally permitted limit of 2mg/g, as set by regulatory food safety agencies. The study also established a direct correlation between increasing concentration and absorbance values, confirming the reliability of the colorimetric method for determining calcium propionate levels.

The result obtained from the study indicates the quantity of calcium propionate contained in some commonly consumed biscuit products contain a high amount of Calcium propionate which significantly extend the shelf-life of baked goods. However, the presence of calcium propionate in quantities higher than the regulatory limit raises for significant public health concerns. Studies suggest that prolonged exposure to high doses of this preservative may be linked to adverse effects such as Gastrointestinal effects such as irritability, disruption of gut flora. High levels of calcium propionate has raised Neurological concerns especially in children seen as hyperactivity, attention deficits, and potential metabolic and endocrine disturbances such as insulin resistance and glucose dysregulation.

Due to the low monetary value of these said biscuits, of which some can be gotten for as low as #100,most parents see it as a easy form of snacks and children may be given as many as 2,3,4 or 5,hence massively exceeding the daily recommended limit of 2mg/g(a case of excessive consumption).This will significantly impair the long term neurological function of such children which is seen as hyperactivity and attention deficits. This is aside the potential gastrointestinal effects as well as metabolic and endocrine disturbances which have been said to be observed in some individuals.

CHAPTER FIVE

CONCLUSION

From the results shown above, it can be inferred that Samples A,B,C,D and E all had masses at levels above the permitted limit hence infers that they are unsafe for human consumption and would require adequate monitoring

The findings of this study highlight the need for stringent regulatory oversight to monitor and enforce permissible levels of calcium propionate in food products. Manufacturers should ensure compliance with established food safety standards, while consumers should be aware of the potential risks associated with excessive preservative intake and opt for preservative-free or naturally preserved alternatives when possible.

This research underscores the importance of continuous quality control in the food industry, promoting consumer safety through proper labeling, awareness campaigns, and alternative preservation methods.

5.2 RECOMMENDATIONS

Based on the findings and observations obtained from the analysis, the following recommendations are made regarding the use of calcium propionate:

-Compliance with Regulatory Standards

The use of calcium propionate should conform to established food safety standards such as those set by the Codex Alimentarius Commission, the Food and Drug Administration (FDA), and the National Agency for Food and Drug Administration and Control (NAFDAC).

-Health and Safety Considerations

Although calcium propionate is generally regarded as safe, excessive consumption may cause mild irritation or metabolic imbalances in sensitive individuals. Therefore, its use should be carefully monitored to ensure that the amount present in finished products does not exceed recommended limits. Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) systems should be implemented during production to maintain safety and consistency.

-Quality Assurance

Routine quality control testing should be conducted to verify the concentration and effectiveness of calcium propionate in food products. Analytical techniques such as colorimetry, spectrophotometry, or chromatography can be employed to ensure product safety and compliance with standards.

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