COLORIMETRIC ASSAY OF CALCIUM PROPIONATE PRESENT IN CORNFLAKES

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SUBMITTED TO

THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE IN UNIVERSITY OF BENIN.

PROJECT SUPERVISED BY

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NOVEMBER, 2025

CERTIFICATION

We hereby certify that this work was carried out by OKODUGHA EHINOMEN ENAKHE in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

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DEDICATION

This work is dedicated to God Almighty, my lovely family and the friends I made along the way

ACKNOWLEDGMENT

"Behind every accomplishment lies the quiet strength of those who refused to let me quit."

First and foremost, I give glory to God, whose mercy and favour has been my constant guide and sustainer. My sincere appreciation goes to my project supervisor, Prof. Cyril Usifoh for his invaluable support, mentorship and guidance. He's a father who would never let his students go astray

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ABSTRACT

Calcium propionate is used as a preservative in baked food as a mould inhibitor. In this work, the quantitative analysis of calcium propionate content of five cornflakes was sampled.

A simple, cost effective, robust and reproducible quantitative method which is based on complexation reaction between calcium propionate and ferric ammonium sulphate was utilized to quantify the amount of calcium propionate in cornflakes samples. A colour complex formation was formed between ferric cations and propionate anions which give a red-brown soluble complex, the complex was allowed to react for 30 mins and viewed at wavelength of maximum absorbance (600nm) subsequently, Absorbance obtained was plotted against concentration (Standard calibration curve). Five different cornflakes were selected, the method above was used to assay calcium propionate in them. The amount of calcium propionate present in each of the cornflakes sample was extrapolated from the graph to obtain the concentration of calcium propionate in the cornflake sampled.

The results showed that four out of the five cornflakes samples experimented contained an amount of calcium propionate above the maximum limit of 2000mg/kg recommended by USFDA. The health implication of exceeding the daily recommended amount of calcium propionate consumption in cornflakes by consumers was exposed. Accordingly, recommendations were made on how all these could be ameliorated to improve on the health of cornflakes consumers in Nigeria

CHAPTER ONE

INTRODUCTION AND LTERATURE REVIEW

LITERATURE REVIEW

1.1 INTRODUCTION

In terms of affordability and accessibility, Bread, Cereal, Patries as well as instant meals like noodles are staple food and in Nigeria due to its evidently cheaper price, and due to the lower purchasing power of the average citizen they are consumed in larger amounts as a meal for the family. However, one of the major challenges in the production of these products is microbial spoilage, particularly mold growth, which significantly reduces shelf life and leads to economic losses as no one would want to buy bread that is already growing mold. To address this issue, food-grade preservatives are commonly incorporated during the baking process to inhibit microbial activity and extend product freshness. Potassium bromate was originally the preservative of choice and it flooded the bakery for its antimicrobial properties till it was banned in Nigeria in 2004 by the National Agency for Food, Drug Administration and Control (NAFDAC) as preservatives in confectioneries and biscuits as a result of its harmful effect on humans.

Calcium Propionate which is widely used as an antifungal agent, has gained prominence in the baking industry due to its effectiveness against mold and rope-forming bacteria, especially in humid climates. The way it works is by interfering with microbial metabolism, thereby preventing the growth of spoilage organisms without adversely affecting yeast performance in dough fermentation. As a food additive, it is approved by major regulatory bodies such as the World Health Organization (WHO), the U.S. Food and Drug Administration (FDA), and Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC).

Despite its efficacy, there have been growing concerns about the potential health effects of calcium propionate, particularly regarding its long-term consumption, possible allergic reactions, such as hyperactivity and consumer preference for preservative-free or "clean-label" products. In light of these concerns, it is essential to monitor the amount of calcium propionate present in these staples

This study aims to investigate the calcium propionate levels in cornflakes of different brands sold commercially in Edo state to ascertain if they all fall within acceptable standards, using a colorimeter and is based on color complex formation, for the determination of calcium propionate.

1.1 LITERATURE REVIEW

1.1.1 FOOD PRESERVATIVES

According to the European food council, Preservatives are food additives that play an important role in making foods last longer or taste better. Specifically, preservatives help to control and prevent the deterioration of food, providing protection against spoilage from micro-organisms (e.g., bacteria, yeast, molds),

Food preservatives are chemical substances added to food to prevent spoilage caused by microorganisms and oxidation. They extend shelf life, maintain nutritional value, and improve consumer safety. Preservatives can be natural (e.g., salt, vinegar, sugar) or synthetic (e.g., benzoates, sorbates, and propionates).

Preservatives can be;

Antimicrobial i.e. inhibit the growth of microorganisms (e.g., sodium benzoate, calcium propionate).

Antioxidant i.e. prevent oxidation of fats and oils (e.g., butylated hydroxyanisole (BHA), tocopherols).

Chelating agents i.e. bind metal ions to inhibit catalysis of oxidation (e.g., EDTA).

In cornflakes the choice of food preservative preferred is usually calcium propionate which prevents mold growth

1.1.2 CALCIUM PROPIONATE

Calcium propionate with the molecular formula $Ca(C_2H_5COO)_2$. is the calcium salt of propionic acid. It is an effective antimicrobial agent against molds, bacteria, and other spoilage organisms. It is commonly used in baked goods, dairy products, animal feeds, and cereal-based foods. In the food industry, calcium propionate is identified as **E282** according to the European food additive numbering system.

1.1.3 PROPERTIES

Calcium propionate, chemically represented as $Ca(C_2H_5COO)_2$, is the calcium salt of propionic acid. It has a molar mass of 186.22 g/mol and typically appears as a white crystalline powder with a faint odor similar to that of propionic acid. It is odorless or slightly pungent, depending on purity, and possesses a mildly bitter taste.

The compound is readily soluble in water, forming a neutral to slightly basic solution, and is only sparingly soluble in alcohol. A 1% aqueous solution of calcium propionate generally exhibits a pH ranging between 7 and 9, indicating its mild basicity. It is stable under normal temperature and pressure but decomposes upon strong heating to release propionic acid vapors.

Chemically, calcium propionate is non-hygroscopic, which means it does not easily absorb moisture from the air, making it suitable for dry food applications such as baked goods and cereals. It is compatible with most food ingredients and remains effective across a wide pH range, particularly in slightly acidic to neutral foods. These physical and chemical

characteristics contribute to its versatility and effectiveness as a preservative in the food industry.

1.1.4 ANALYTICAL TECHNIQUES FOR THE IDENTIFICATION OF CALCIUM PROPIONATE

The identification and quantification of calcium propionate in food samples are essential steps in ensuring food safety and compliance with regulatory standards. There have been several analytical techniques that have been developed to determine the presence and concentration of calcium propionate in various food matrices such as bread, biscuits, and breakfast cereals like cornflakes. These methods differ in sensitivity, specificity, cost, and technical requirements. Each with their advantages and drawbacks. The most commonly employed techniques include chromatographic, spectroscopic, and colorimetric methods

1. Chromatographic Techniques

Chromatographic methods are among the most widely employed analytical techniques for the detection and quantification of calcium propionate in food products due to their high accuracy, selectivity, and reproducibility. Both High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) have been successfully applied in the analysis of propionate salts in complex food matrices.

In HPLC analysis, calcium propionate is extracted from the food sample using water or methanol and then injected into a reverse-phase C18 column, where separation occurs based on polarity differences between the analyte and the stationary phase. Detection is usually achieved with a UV detector at wavelengths around 210–230 nm, corresponding to the absorbance maxima of propionic acid derivatives. Quantification is carried out by comparing the sample's chromatographic peak area to that of a standard calibration curve prepared from known concentrations of calcium propionate (Burana-osot et al., 2010).

Gas Chromatography (GC), on the other hand, is mainly used for confirmatory analysis or for volatile derivative forms of propionic acid. The sample is first acidified to liberate propionic acid, which is then extracted, derivatized, and analyzed using a flame ionization detector (FID) or mass spectrometer (GC-MS) (González et al., 2011). GC methods provide excellent sensitivity and specificity but require more complex sample preparation than HPLC.

Both techniques offer quantitative and qualitative determination of calcium propionate, although HPLC is the method of choice when doing routine food preservative analysis due to its ease of operation and minimal sample derivatization requirements.

2. Colorimetric and Spectrophotometric methods

The principle underlying colorimetric and spectrophotometric determination of calcium propionate is based on the appearance or intensity change of color (color shift) that occurs when propionate ions react with specific reagents to form a colored complex. This color change provides a visual and measurable indication of the compound's concentration in a solution. The phenomenon is governed by the Beer–Lambert law, and it is this colour change that is measured by the colorimeter

In the colorimetric assay of calcium propionate, the propionate anion ($CH_3CH_2COO^-$) reacts with ferric ions (Fe^{3+})—commonly supplied by ferric ammonium sulfate—to produce a red-brown ferric–propionate complex. The intensity of this color shift, from pale yellow to reddish-brown, is proportional to the amount of calcium propionate present in the sample. This observable shift provides a convenient and reliable basis for quantitative measurement.

Spectrophotometric methods are particularly advantageous due to their simplicity, precision, and cost-effectiveness, making them suitable for routine analysis in food such as cornflakes. They require only minimal sample preparation and provide rapid results without the need for sophisticated equipment. Significantly less expensive and more adaptable for use in

developing regions or for large-scale monitoring of food safety (Skoog, Holler, & Crouch, 2018).

Disadvantages may however arise due to interferences caused by other colored substances or compounds that absorb light at similar wavelengths. Such interferences can lead to inaccurate readings if not properly controlled. Therefore, careful sample preparation, reagent optimization, and the use of blank controls are essential to improve the accuracy and reproducibility of results (Khopkar, 2014).

1.1.5 FUNCTIONS OF CALCIUM PROPIONATE IN THE FOOD INDUSTRY

Calcium propionate is primarily used in the baking industry as an effective preservative to prevent the growth of molds and certain bacteria that cause spoilage in bread, cakes, and other baked goods. By inhibiting fungal contamination, it helps to extend shelf life and maintain product freshness without altering flavor or texture. In cornflakes, calcium propionate serves a similar function by controlling microbial growth during storage, especially under humid conditions. Its inclusion ensures that these cereal products remain safe, crisp, and appealing over longer periods. Because it is non-toxic, heat-stable, and generally recognized as safe (GRAS) by food regulatory authorities, calcium propionate remains one of the most reliable preservatives in baked and cereal-based food production.

1.1.6 PREPARATION OF CALCIUM PROPIONATE

Calcium propionate is prepared by the neutralization of propionic acid with calcium hydroxide or calcium carbonate.

REACTION EQUATION:

[

2CH_3CH_2COOH + Ca(OH)_2 → (CH_3CH_2COO)_2Ca + 2H_2O

]

The product is then filtered, dried, and crystallized. Industrially, propionic acid used in this reaction is produced through fermentation of carbohydrates using *Propionibacterium* species.

1.1.7 MECHANISM OF ACTION

Calcium propionate elicits its effect by inhibiting the growth of molds and some bacteria. In the presence of moisture, calcium propionate dissociates to release propionic acid, which then penetrates microbial cell membranes in its undissociated form. Inside the cell, the acid dissociates, releasing hydrogen ions that lower the intracellular pH, disrupting enzyme activity and inhibiting cellular metabolism.

This mechanism is especially effective against molds such as *Aspergillus*, *Penicillium*, and *Rhizopus* species.

1.2 PROCESS OF CORNFLAKES PRODUCTION IN NIGERIA

The production of cornflakes involves a series of carefully controlled steps designed to convert raw maize grains into a crisp, golden, ready-to-eat breakfast cereal. In Nigeria, as in other parts of the world, the process combines both traditional grain processing techniques and modern food manufacturing technologies to ensure product quality, nutritional value, and shelf stability.

The process begins with the selection and cleaning of maize grains. High-quality yellow maize is chosen for its color, flavor, and nutritional properties. The grains are cleaned thoroughly to remove dust, chaff, stones, and other impurities. After cleaning, the maize is subjected to milling, where it is ground into coarse grits or meal suitable for cooking.

Next, the cooking or hydrothermal treatment stage takes place. The maize grits are cooked under steam pressure with water, salt, sugar, malt extract, and sometimes added vitamins or minerals to improve nutritional content and flavor. During this stage, the starch in the maize gelatinizes, forming a uniform, pliable mass.

After cooking, the mixture is passed through flaking rollers, where it is flattened into thin flakes. The flakes are then dried in hot-air ovens or fluidized bed dryers to remove moisture, followed by toasting at controlled temperatures to achieve the desired golden color, crisp texture, and characteristic toasted flavor.

To enhance product stability and prevent spoilage during storage, preservatives such as calcium propionate may be incorporated either into the initial cooking mixture or as part of a coating process after toasting. This helps protect the product from fungal and bacterial contamination, especially in humid environments common in tropical regions like Nigeria.

Finally, the toasted flakes are cooled, screened, and packaged in moisture-proof materials to maintain freshness and crunchiness. The packaging is designed to prevent air and moisture ingress, extending the shelf life of the product until it reaches consumers.

Overall, the cornflakes production process in Nigeria is a combination of food engineering, chemistry, and preservation science, ensuring that the final product meets safety standards, retains its sensory qualities, and remains microbiologically stable for an extended period.

1.3 JUSTIFICATION FOR THE STUDY

The popularity of cornflakes by all age groups in Nigeria is enormous, the improper or excessive use of preservatives such as calcium propionate poses potential health risks, including gastrointestinal irritation and hyperactivity in children.

Determining the quantitative levels of calcium propionate in cornflakes ensures that they are within acceptable safety limits as specified by food regulatory agencies. This study therefore provides data relevant to public health; consumer protection, food quality assurance, and regulatory compliance. The use of colorimetric methods in this study despite the presence of chromatographic techniques is because of the disadvantages such as cost implication of the complex apparatus needed and it requires trained analysts.

1.4 PROBLEM STATEMENT

Food products that have high nationwide demand such as cornflakes will require the goods to be transported to far places in order to be marketed, poor preservation will lead to spoilage of the product and result in heavy economic losses, hence every producer would want to go the extra mile to keep them fresh. This may lead them putting more than the acceptable preservative level in cornflakes and it may lead to an increase in the side effects such as long-term constipation. This fact is the reason why proper monitoring should be carried out to protect consumers from harmful effects

1.5 HYPOTHESIS

The concentration of calcium propionate in commercial cornflake samples may exceed acceptable safety limits.

1.6 SCOPE OF STUDY

This study focuses on determining the calcium propionate content in selected brands of cornflakes sold in Nigeria using the colorimetric assay technique. It covers:

i. Sample collection from various retail outlets.
ii. Sample preparation and extraction of calcium propionate.
iii. Reaction with ferric ammonium sulfate to form a red-brown complex measurable spectrophotometrically.
iv. Comparison of measured concentrations to regulatory standards.

1.7 AIM OF STUDY

To determine the concentration of calcium propionate in commercially available cornflakes using a colorimetric assay method and to evaluate whether it falls within acceptable safety limits of below 2000mg/kg of calcium propionate in cornflakes

1.8 OBJECTIVES OF STUDY

1. To use colorimetric method for quantifying the calcium propionate in different cornflakes brands.
2. To determine the absorbance of the complex formation in a colorimeter at 600nm.
3. To compare the experimental results with regulatory standards in the United States food and Drugs Administration (USFDA)
4. To assess the public health implications of the preservative levels detected.

1.9 COLORIMETRY

Colorimetry is a quantitative analytical technique used to determine the concentration of colored compounds in solution. It is based on the measurement of light absorbance by a solution at a specific wavelength. The degree of color intensity is directly proportional to the concentration of the analyte according to Beer–Lambert's Law. In the colorimetric assay of calcium propionate, the preservative reacts with ferric ions (Fe^{3+}), typically from ferric ammonium sulfate, to form a red-brown ferric–propionate complex. The development of this color indicates the presence of propionate ions, and the intensity of the color shift corresponds to the concentration of calcium propionate in the sample. The absorbance of the complex is then measured using a colorimeter or spectrophotometer at a specific 600 nm—where maximum absorption occurs.

To perform the assay, the cornflakes sample is first homogenized and extracted using deionized water, ensuring the complete dissolution of the preservative. The extract is filtered to remove particulates, and a measured aliquot is treated with ferric ammonium sulfate reagent. After allowing adequate time for color development, the absorbance of the resulting solution is measured using a colorimeter or UV–visible spectrophotometer against a reagent blank. A calibration curve is established using standard solutions of known calcium

propionate concentrations, allowing the preservative content of the sample to be calculated by interpolation.

1.9.1 PRINCIPLES OF COLORIMETER

The colorimeter operates on Beer–Lambert's Law, which states that the absorbance (A) of light by a solution is directly proportional to the concentration (C) of the absorbing substance and the path length (l):

$A = \varepsilon lc$

Where:

- A = Absorbance
- ε = Molar absorptivity
- l = Path length (cm)
- c = Concentration (mol/L)

1.9.2 USES OF COLORIMETER

i. Determination of concentration of colored compounds.
ii. Measurement of enzyme activity in biological assays.
iii. Analysis of food additives, nutrients, and preservatives.
iv. Water and wastewater analysis.
v. Pharmaceutical and biochemical studies.

1.9.3 ADVANTAGES OF A COLORIMETER

i. Simple and easy to use.
ii. Cost-effective compared to spectrophotometers.
iii. Provides rapid results.
iv. Suitable for field and laboratory analysis.
v. Requires small sample volume.

1.9.4 DISADVANTAGES OF A COLORIMETER

i. Limited to visible light range (400–700 nm). It cannot measure Infrared.

ii. Lower accuracy and sensitivity compared to UV–Vis spectrophotometers.

iii. Cannot analyze colorless or non-chromophoric compounds directly.

iv. Affected by sample turbidity and stray light.

1.9.5 APPLICATION OF A COLORIMETER

Colorimetry has a wide range of applications across various scientific and industrial fields due to its simplicity, accuracy, and reliability in quantitative analysis. In food analysis, colorimeters are extensively used to determine the concentration of additives and preservatives such as calcium propionate in baked products and cereals. The method is based on the formation of a colored complex that allows for precise quantification of the analyte by measuring its absorbance. This technique ensures that the preservative content in food products remains within acceptable safety limits (Oyeleke et al., 2012).

In clinical and biochemical laboratories, colorimeters are employed to measure the concentration of biomolecules such as glucose, cholesterol, and proteins in biological fluids, where the color intensity correlates with analyte concentration (Kumar et al., 2020). They are also used in environmental monitoring to assess water quality by detecting pollutants such as nitrates, phosphates, and heavy metals (Skoog et al., 2017). Additionally, in industrial chemistry, colorimetry assists in the quality control of dyes, paints, and pharmaceuticals, ensuring product consistency and regulatory compliance.

The versatility of colorimetry extends to kinetic studies, where it is used to monitor the progress of chemical reactions by measuring changes in absorbance over time. Its cost-effectiveness, ease of operation, and ability to provide rapid and reproducible results make it a valuable tool in both research and routine analytical work.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

i. Volumetric flasks (25ml, 50ml, 100ml, 250ml),

ii. Glass funnel,

iii. Analytical weighing balance,

iv. Filter paper,

v. Masking tape,

vi. 10ml Measuring cylinder,

vii. Cotton wool,

viii. Glass rod,

ix. Spatula.

2.2 INSTRUMENT USED

Colorimetric Visible Spectrophotometer Labtech-722 (Four compartments).

2.3 CHEMICAL AND REAGENTS

x. Calcium propionate,

xi. Ferric Ammonium sulphate,

xii. Deionized water.

2.4 SAMPLE COLLECTIONS

Five brands of cornflakes samples were randomly selected and used for this study.

2.5 PREPARATION OF STANDARD STOCK SOLUTION OF CALCIUM PROPIONATE

Calcium propionate solution of concentration of 2mg/ml was prepared by dissolving 200mg of calcium propionate with 100ml of deionized water in 250ml volumetric flask. The solution obtained was labeled as stock solution of calcium propionate.

2.6 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 properly labeled. This were prepared using these formulae:

$M_1V_1=M_2V_2$

M_1= Molarity the initial concentration from the stock

V_1= Initial volume/ concentrated solution

M_2= Molarity of final / diluted solution

V_2= Volume of final / diluted solution

Table 1: Preparation of 50ml standard concentrations of calcium propionate solutions

Concentration prepared (mg/ml)	Volume of stock (ml)	Volume of Deionized water (ml)	Total volume (ml)
0.2	5	45	50
0.3	7.5	42.5	50
0.4	10	40	50
0.5	12.5	37.5	50
0.6	15	35	50
0.7	17.5	32.5	50
0.8	20	30	50

2.7 PREPARATION OF FERRIC AMMONIUM SULPHATE TEST SOLUTION

Test solution of Ferric ammonium sulphate (0.08g/ml) was prepared by dissolving 2g of Ferric ammonium sulphate in 25ml of deionized water.

2.8 PREPARATION OF STANDARD CALIBRATION CURVE

Several standard concentrations of calcium propionate solution of (0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8mg/ml) prepared were used to prepare standard calibration curve. The absorbance was obtained by transferring 2.0ml of 0.2mg/ml standard calcium propionate concentration into a cleaned 10ml sample test tubes containing 0.2ml of ferric ammonium sulphate test solution. The colorimeter was cleaned and dust free and then put-on and allowed to equilibrate for 30 mins. Filters of different wavelengths (580, 600 and 620nm) of the colorimeter were used for determining absorbance. The filter that gave the best absorbance was selected for study and was found to be 600nm. The samples tube was cleaned after each determination, the colorimeter was zeroed with a blank solution containing 1-2 drops of ferric ammonium sulphate and 2.0ml of deionized water after which absorbance value of each of the standard concentrations (0.2 to 0.8mg/ml) was determined at 600nm. The results obtained were used to determine the standard calibration curve.

2.9 COLLECTION OF SAMPLES USED

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

2.9.1 EXTRACTION OF CALCIUM PROPIONATE FROM 10G CORNFLAKES SAMPLES

Each cornflakes ample was weighed to know the net weight of the cornflakes aside the stated weight thereafter 10g of cornflakes sample was accurately weighed and was reduced to small size then placed into 250ml ml beaker 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 amount of calcium propionate in the colorimeter using 600nm wavelength. The same procedures were used for other cornflakes samples to obtain their absorbance.

2.9.2 DETERMINATION OF CALCIUM PROPIONATE

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

CHAPTER THREE

RESULTS

The 5 cornflakes samples had their weight measured on a weighing balance, then recorded. The 5 cornflakes samples had their absorbance measured in triplicates, and the average determined and recorded, after which their respective concentrations were extrapolated using the standard caliberation curve (fig 1)

THE STANDARD CALIBERATION CURVE

Several standard concentrations of calcium propionate solution of (0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8mg/ml) prepared were used to prepare standard calibration curve. The absorbance was obtained by transferring 2.0ml of 0.2mg/ml standard calcium propionate concentration into a cleaned 10ml sample test tubes containing 0.2ml of ferric ammonium sulphate test solution. The colorimeter was cleaned and dust free and then put-on and allowed to equilibrate for 30 mins. Filters of different wavelengths (580, 600 and 620nm) of the colorimeter were used for determining absorbance. The filter that gave the best absorbance was selected for study and was found to be 600nm. The samples tube was cleaned after each determination, the colorimeter was zeroed with a blank solution containing 1-2 drops of ferric ammonium sulphate and 2.0ml of deionized water after which absorbance value of each of the standard concentrations (0.2 to 0.8mg/ml) was determined at 600nm. The results obtained were used to determine the standard calibration curve.

Table 2: Absorbance measured for each concentration at 600nm

Concentration (mg/ml)	Absorbance at 600nm
0.2	0.006
0.3	0.106
0.4	0.261
0.5	0.398
0.6	0.509
0.7	0.659
0.8	0.758

Figure 1 shows the calibration plot for the standard concentration within the range of 0.2 to 0.8mg/ml and its absorbance.

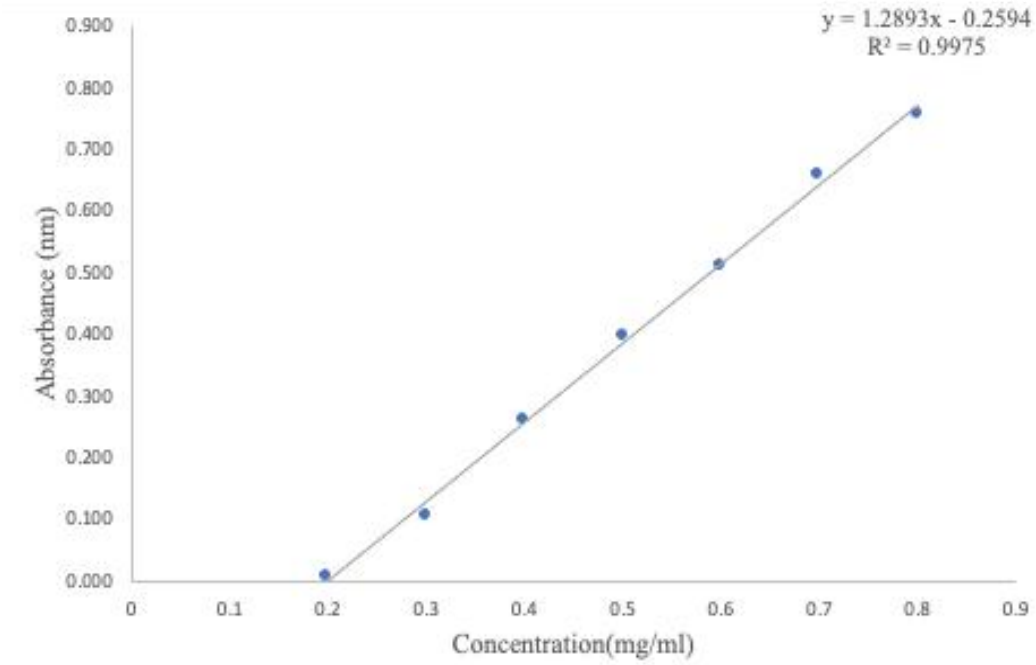

Figure 1: Graph of Absorbance against Concentration (mg/ml) using 600nm filter

Table 3 shows the calculated weight of each sample

	Total weight (g)
A	31.7593
B	31.2505
C	27.4351
D	29.236
E	28.531

Table 4: Concentration of calcium propionate (mg/ml) in each Cornflakes sample obtained using 600nm wavelength

The absorbance readings for the 5 cornflakes samples at 600nm are as follows;

Sample	1st reading	2nd reading	3rd reading	Average value
A	0.230	0.227	0.232	0.229
B	0.352	0.331	0.347	0.343
C	0.401	0.339	0.338	0.359
D	0.556	0.522	0.541	0.539
E	0.325	0.341	0.341	0.325

Table 5: Extrapolated concentration (mg/ml) of calcium propionate from the graph

Sample	Average Absorbance (nm)	Extrapolated Concentration (mg/ml)
A	0.229	0.3788
B	0.343	0.4672
C	0.359	0.4796
D	0.539	0.6912
E	0.325	0.4533

CALCULATIONS

Calculation for the amount of Calcium propionate in the 2ml placed in the corvette using the formula

$Y = 1.2893x + 0.2594$

Where y = Absorbance

X = concentration

Then, make x the subject of the formula

X = y + 0.2594 ÷ 1.2893

For

Sample A

Y = 0.229

X = 0.229 + 0.2594 ÷ 1.2893

X = 0.3788mg

Therefore 0.3788mg of calcium propionate was contained in 2ml

To calculate for the total calcium propionate in 10g

10g of cornflakes was dissolved in 100ml

2ml of the dissolved sample contains 0.3788mg

Therefore 100ml = 100 x 0.3788 ÷ 2

= 18.94 mg

Therefore 18.94mg of calcium propionate is contained in 10g of the Sample A

The weight of total Sample A = 31.7593g

If 10 contains 18.94 mg of calcium propionate

Therefore 31.7593g = 18.94 x 31.7593 ÷10

=60.15mg

The standard amount of calcium propionate is 2mg/g

31.7593g if cornflakes contains 60.15mg of calcium propionate

Therefore 1g contains= 60.15 ÷ 31.7593

= 1.89mg

Sample A has acceptable standards of calcium propionate

Sample B

Y = 0.343

X = 0.343 + 0.2594 ÷ 1.2893

X = 0.4672mg

Therefore 0.4672mg of calcium propionate was contained in 2ml

To calculate for the total calcium propionate in 10g

10g of cornflakes was dissolved in 100ml

2ml of the dissolved sample contains 0.4672mg

Therefore 100ml = 100 x 0.4672 ÷ 2

= 23.36 mg

Therefore 23.36mg of calcium propionate is contained in 10g of the Sample B

The weight of total Sample B sample = 31.2505g

If 10 contains 23.36 mg of calcium propionate

Therefore 31.2505g = 23.36 x 31.7593 ÷10

=73.00mg

The standard amount of calcium propionate is 2mg/g

31.2505g if cornflakes contains 73.00mg of calcium propionate

Therefore 1g contains= 73.00 ÷ 31.2505

= 2.34mg

Sample B

Does not have acceptable standards of calcium propionate

Sample C

Y = 0.359

X = 0.359 + 0.2594 ÷ 1.2893

X= 0.4796mg

Therefore 0.4796mg of calcium propionate was contained in 2ml

To calculate for the total calcium propionate in 10g

10g of cornflakes was dissolved in 100ml

2ml of the dissolved sample contains 0.4796mg

Therefore 100ml = 100 x 0.4796 ÷ 2

= 23.98 mg

Therefore 23.98mg of calcium propionate is contained in 10g of the Sample C

The weight of total C = 27.4351g

If 10 contains 23.98 mg of calcium propionate

Therefore 27.4351g = 23.98 x 27.4351 ÷10

=65.79mg

The standard amount of calcium propionate is 2mg/g

27.4351g of cornflakes contains 65.79mg of calcium propionate

Therefore 1g contains= 65.79 ÷ 27.4351

= 2.40mg

Sample C

Does not have acceptable standards of calcium propionate

Sample D

Y = 0.539

X = 0.539 + 0.2594 ÷ 1.2893

X= 0.6192mg

Therefore 0.6192mg of calcium propionate was contained in 2ml

To calculate for the total calcium propionate in 10g

10g of cornflakes was dissolved in 100ml

2ml of the dissolved sample contains 0.6192mg

Therefore 100ml = 100 x 0.6192 ÷ 2

= 30.96 mg

Therefore 30.96mg of calcium propionate is contained in 10g of the Sample D

The weight of total Sample D = 29.336g

If 10 contains 30.96 mg of. calcium propionate

Therefore 29.36 = 30.96 x 29.36 ÷10

=90.89mg

The standard amount of calcium propionate is 2mg/g

29.36 if cornflakes contains 90.89mg of calcium propionate

Therefore 1g contains= 90.89 ÷ 29.36

= 3.09mg

Sample D

Does not have acceptable standards of calcium propionate

Sample E

Y = 0.343

X = 0.325 + 0.2594 ÷ 1.2893

X= 0.4533mg

Therefore 0.4533mg of calcium propionate was contained in 2ml

To calculate for the total calcium propionate in 10g

10g of cornflakes was dissolved in 100ml

2ml of the dissolved sample contains 0.4533mg

Therefore 100ml = 100 x 0.4533 ÷ 2

= 22.665mg

Therefore 22.665mg of calcium propionate is contained in 10g of Sample E

The weight of Sample E = 28.531

If 10 contains 22.665 mg of calcium propionate

Therefore 28.531g = 22.665 x 28.531 ÷10

=64.66mg

The standard amount of calcium propionate is 2mg/g

28.531 if cornflakes contains 64.66mg of calcium propionate

Therefore 1g contains

ns= 64.66 ÷ 28.531

= 2.26mg

Sample E

Cornflakes does not have acceptable standards of calcium propionate

Table 6: Amount of calcium propionate in different weights of the sample

Sample	Amount of calcium Propionate contained in 1g(mg/g)	Amount of calcium Propionate contained in 10g(mg/10g)	Total contained in the sample
A	1.89	18.94	60.15
B	2.34	23.36	73.00
C	2.40	23.36	65.79
D	3.09	30.96	90.89
E	2.26	22.67	64.66

CHAPTER FOUR

DISCUSSION

The determination of calcium propionate content in bread using a colorimetric assay based on complex formation with ferric ammonium sulfate (FAS) proved to be an effective analytical approach. This method relies on the ability of ferric ions (Fe^{3+}) to form a red-brown colored complex with propionate anions, allowing quantification through absorbance measurement at 600 nm.

This method enabled clear differentiation between samples that complied with or exceeded the USFDA limit of 2000 mg/kg for calcium propionate in food products. Samples with higher absorbance values corresponded to higher propionate content and were easily identified. .

This study was carried out to determine the concentration of calcium propionate in five different cornflakes samples using a colorimetric assay method. Calcium propionate is a widely used antimicrobial preservative in cereal and baked products, known for its effectiveness against spoilage fungi such as *Aspergillus* and *Rhizopus* species. However, its concentration in food products must be carefully monitored because while insufficient quantities may lead to microbial spoilage, excessive levels can pose potential health risks. According to international food safety standards (FAO/WHO, 2020; FDA, 2018), the recommended safe limit for calcium propionate in cereal-based foods is approximately 2 mg per gram of product.

The experimental results showed varying concentrations of calcium propionate among the five cornflakes samples: Sample A (1.80 mg/g), Sample B (2.34 mg/g), Sample C (2.40 mg/g), Sample D (3.09 mg/g), and Sample E (2.26 mg/g). Based on these findings, Samples B, C, and E had calcium propionate concentrations slightly above the recommended limit considering minor analytical and production variations. Sample A, with a concentration of

1.80 mg/g, fell within the recommended threshold, indicating ideal preservation but may be met with a higher susceptibility to microbial degradation during storage. Conversely, Sample D contained 3.09 mg/g, which by far exceeded the permissible limit, indicating overuse of the preservative and suggests a lack of strict quality control in production.

The deviations from the permissible standards such as those observed in Sample D are particularly concerning because prolonged consumption of foods with elevated calcium propionate levels has been linked to behavioral and neurological effects, including irritability, restlessness, and hyperactivity in children (Choudhary & Bandopadhyay, 2016). These risks become even more significant considering that cornflakes are a highly consumed breakfast product, and individuals of all ages, especially children, often consume multiple sachets (3–5 or more) at a time. Consequently, the cumulative intake of calcium propionate in such individuals could far exceed the acceptable daily intake (ADI), thereby increasing the likelihood of adverse health outcomes over time. This highlights the need for strict monitoring of preservative levels in ready-to-eat foods to prevent potential toxicological effects associated with chronic exposure.

The observed differences in preservative concentrations across the samples may be attributed to variations in manufacturing practices, ingredient formulation, and quality assurance protocols among producers. While slight deviations from standard levels may not pose immediate danger, consistent overuse, as seen in Sample D, raises concerns regarding regulatory compliance and consumer safety.

Overall, this study underscores that while calcium propionate remains an effective and essential preservative for maintaining product stability and shelf life, its use must be quantitatively controlled. Continuous regulatory supervision, routine laboratory testing, and public awareness are necessary to ensure that preservative concentrations remain within safe

limits, thereby safeguarding public health — especially among children, who are more vulnerable to the effects of excessive additive intake

Special care must also be taken when carrying out this assay like the use of deionized water as the solvent rather than distilled water. The use of deionized water in the preparation of reagents and during sample extraction was essential to avoid interference from extraneous ions such as calcium, magnesium, sulfate, or chloride, which are commonly present in tap water. These ions could either participate in side reactions or alter the ionic strength of the solution, potentially affecting the complexation process and skewing the absorbance readings. Deionised water ensures purity, reproducibility, and accuracy of the assay by minimizing contamination. It is also essential for calibration

In spectrophotometry, even minor ionic contamination can shift the baseline or alter the blank, affecting all measurements. deionized water ensures that the standard curve and samples are measured accurately. However, it is important to note that proper control of pH and reagent concentration is crucial for reproducibility, as ferric ions can hydrolyze or form other complexes under inappropriate conditions.

CHAPTER FIVE

CONCLUSION

The findings from this study revealed that while calcium propionate remains an effective and widely accepted preservative in cornflakes production, its concentration varies among commercially available brands. Only one sample (sample A) was below the 2mg/g threshold. Out of the five samples analyzed, three (Samples B, C, and E) contained calcium propionate levels slightly above the recommended limit, while one (Sample D) exceeded the permissible range significantly, indicating possible overuse of the preservative.

In conclusion, Due to the variations and inconsistencies in formulation there is need for strict quality control and adherence to regulatory standards during manufacturing. As well as proper enforcement of these regulatory standards

RECOMMENDATIONS

1. Regulatory agencies such as NAFDAC and SON should intensify monitoring and enforcement of preservative use in cereal-based products to ensure compliance with safety standards.
2. Manufacturers should adopt precise formulation techniques and conduct routine preservative analysis using validated analytical methods such as colorimetry or spectrophotometry.
3. Public awareness programs should be implemented to educate consumers on the possible health risks associated with excessive consumption of preserved foods.
4. Further research should be carried out to evaluate the long-term effects of calcium propionate exposure, particularly in children and other sensitive populations.

Maintaining a balance between effective preservation and public safety is essential to ensure that cornflakes and similar food products remain both safe and nutritious for regular consumption.

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APPENDIX

Manufacturing Information of CORNFLAKES Samples

Sample A

Location collected: Uselu

CORNFLAKES Name: Nasco

NAFDAC Reg No: 08- 0381

Production Date:20/09/25

Expiry Date:19/09/26

Batch No: 1 20 H3N

Net Weight: 30g

Ingredients: maize, grits, sugar, cereal, malted extract, salt, antioxidant INS 320), VITAMINS (A,B1 B2, B6 B12 folic acid)

Allergy information: NASCO CORNFLAKES can trigger allergies in people with sensitivities to wheat (gluten), milk, oats, and potentially so crosscheck ingredients.

Manufacturer Address: Made in Nigeria by Nasco Foods Limited, Yakubu gowon way, P.o box716 Plateau State Nigeria.

For Enquiries: marketing@nasco.net

Sample B

Location collected: Uselu

CORNFLAKES Name: NASCO STRAWBERRY

NAFDAC Reg No: A8-107224

Production Date: 13/04/25

Expiry Date: 12/04/26

Batch No: 113 H9

Net Weight: 30g

Ingredients: maize, glucose sugar, cereal malt extract, salt, Iron), VITAMINS

Allergy information: crosscheck ingredients

Manufacturer Address: No 1. old airport road, P.O Box 716, Jos, Nigeria

For Enquiries: info@nasco.net

Sample C

Location collected: Uselu

CORNFLAKES Name: Kelloggs

NAFDAC Reg No: A8-4114

Production Date: 28/02/25

Expiry Date: 27/02/26

Batch No: 1B 28 02 25

Net Weight: 32g

Ingredients: maize, grits, sugar, cereal malted extract, salt, antioxidant INS 320), VITAMINS

Allergy information: crosscheck ingredients

Manufacturer Address: 44 Jimoh Odutola street, Iganmu Lagos, Nigeria

For Enquiries: conttact@kelloggtolaram.com

Sample D

Location collected: Uselu

CORNFLAKES Name: Infinity

NAFDAC Reg No: B1-3511

Production Date: not available

Expiry Date: not available

Batch No: not available

Net Weight: 200g

Ingredients: wheat flour, vegetable oil (palm oil), salt, and sodium bicarbonate.

Allergy information: Individuals with wheat or gluten allergy should avoid cream crackers.

Allergy: Manufacturer Address: Imowoh Road, Ijebu – ode Ogun state, Nigeria

For Enquiries: customercare@infinitysnacks.com

Sample E

Location collected: Ikpoba okha

CORNFLAKES Name: Frosties

NAFDAC Reg No: OB-7591

Production Date: 14/11/24

Expiry Date: 13/11/25

Batch No: 8A 18:15 253

Net Weight: 30g

Ingredients: maize, grits,sugar, cereal malted extract, salt, antioxidant INS 320), VITAMINS

Allergy information: check ingredients

Manufacturer Address: 44 Jimoh Odutola street, Iganmu Lagos, Nigeria

For Enquiries: conttact@kelloggtolaram.com

LIST OF ABBREVIATIONS

Abbreviation	Description
USFDA	United States Food and Drug Administration
CP	Calcium Propionate
Fe	Ferric
NAFDAC	National Agency For Food Administration and Control
Aw	Water activity
WHO	World Health Organization
GMP	Good Manufacturing Practice
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography