

**DETERMINATION OF OXALATE CONTENT OF SELECTED
COMMERCIAL CHOCOLATE CONFECTIONERY
(CHOCOLATE CANDIES)**

BY

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CERTIFICATION

This is to certify that this project work has been written by **AWOYEMI MARY OMOLARA** with Matriculation Number **ND/23/NAD/FT/0043** and has been read and approved as meeting part of requirement for the Award of National Diploma (ND) in the Department of Nutrition and Dietetics, Institute of Applied Sciences IAS), Kwara State Polytechnic, Ilorin, Kwara State

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DEDICATION

This research is work is dedicated to Almighty God the most beneficent the most merciful.

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I want to acknowledge the **Almighty God**, the author and finisher of all things, I want to acknowledge him for the wisdom, knowledge and understanding he grant unto me throughout this journey, for his grace, Mercy and strength May his name be forever praised.

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I will also like to extent my appreciation to all my family and friends. Thank you all for everything you contribute towards the completion of this project

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CHAPTER ONE

1.0 INTRODUCTION

Chocolate is considered the most popular sweet in every section of the age groups of people all over the world. The popularity of chocolate is not just an overnight sensation, it has taken several thousands years from Mayo people in 400 AD, who first planted the cacao tree to the Aztec people, who used cocoa beans as currency. Cocoa beans are the major ingredient of chocolate and related products. In 1875, Daniel Peter developed milk chocolate by using condensed milk powder from henri nestle. A major popular commercialization of chocolate occurred after the production of the first dark chocolate by Rudolph Lindt in Switzerland (1879) by involving the “conching” method; conching enhanced the smoothness of chocolate(1).The world’s annual cocoa production in 2021-22 was around 4.9 million tons. Around 60% of the total cocoa beans are produced in the countries cote d’Ivoire and Ghana, followed by Ecuador with a 7% share (Swiss platform for sustainable cocoa , 2022). Around 43% of the cocoa production are utilized by the chocolate industry. In 2021, revenue generated by the global chocolate confectionery market was US \$ 0.99 trillion (2).The Indian chocolate market was US \$ 2.4 billion and is expected to reach US \$ 4.1 billion in 2028 at a CAGR of 8.8% during 2023 -2028(3). The growth of the cocoa sector is driven by its popularity, extensive appeal in all age groups, and perceived health benefits.

Earlier, the major concern regarding the consumption of chocolate was its high fat and sugar content leading to the development of caries, high blood pressure, coronary artery disease, and diabetes. However, now awareness about the functional and medicinal benefits of chocolate is gaining ground.

Chocolate is a rich source of bioactive compounds that have beneficial effects on heart health, insulin secretion, and brain function among others (4). Research studies are reporting the effect of the use of chocolate on multiple ailments and conditions including aging, high blood pressure, gut microbiota, cancer, depression, diabetes, and Alzheimer's disease among others.

This paper reviews the bioactive compounds in chocolate, the role of chocolate as a functional food, and recent developments in improving the functional properties of the chocolates. It highlights the efficiency of chocolate in the systemic health, metabolism, and immune system beyond its consideration as good mood food.

1.1 PROBLEM STATEMENT

Chocolate confectionaries (ice cream , cookies and candies) is frequently consumed among both young children and adults.

Regular consumption of confectionaries such as ice cream, cookies and candies poses significant health risks , including obesity, dental issues,increased risk of kidney stones formation and other diet related issues, thereby compromising overall health and wellbeing.

1.2. AIM AND OBJECTIVE OF DETERMINING THE OXALATE CONTENT OF SELECTED COMMERCIAL CHOCOLATE CONFECTIONERY (CANDIES)

Aim:

The primary aim of this study is to determine and quantify the content of selected commercial chocolate confectionery products (chocolate candies)

Objectives :

1. QUANTIFICATION OF OXALATE CONTENT : To accurately measure and quantify the oxalate content in selected commercial chocolate confectionery products .

2. COMPARISON OF OXALATE CONTENT : To compare the oxalate content among different types of chocolate products

3. IDENTIFICATION OF HIGH OXALATE PRODUCTS : To identify which commercial chocolate confectionery products contain high levels of oxalates, potentially posing a risk to individuals with kidney stones or other oxalate -related

health issues.

4. EVALUATION OF LABELING AND REGULATORY COMPLIANCE : To assess whether the selected commercial chocolate confectionery products comply with relevant labeling regulations and guidelines regarding oxalate content.

5. PROVIDING CONSUMERS GUIDANCE : To provide consumers, particularly those with oxalate - related health concerns, with accurate and reliable information about the oxalate content of popular commercial chocolate confectionery products.

1.3. JUSTIFICATION OF THE STUDY

1. *Public Health Concerns*: Oxalates are a key component in the formation of kidney stones, and excessive consumption of oxalate-rich foods can increase the risk of kidney stone formation. This study will provide valuable information on the oxalate content of commercial chocolate candies, enabling consumers to make informed choices about their diet.

2. *Knowledge Gap*: Despite the potential health implications, there is a lack of accurate and reliable data on the oxalate content of commercial chocolate candies. This study will address this knowledge gap, providing new insights and data on the oxalate content of these products.

3. *Regulatory Compliance*: Food safety regulations and guidelines require manufacturers to provide accurate labeling information about the oxalate content of their products. This study will support regulatory compliance by providing manufacturers with accurate data on the oxalate content of their products.

4. *Consumer Awareness*: This study will provide consumers with accurate information about the oxalate content of commercial chocolate candies, enabling them to make informed choices about their diet and lifestyle.

5. *Contribution to Existing Literature*: This study will contribute to the existing body of research on oxalate content in food products, providing new insights and data for future studies.

1.4 SCOPE OF DETERMINING OXALATE CONTENT OF CHOCOLATE CANDIES

1. PRODUCTS SELECTION : The study will focus on selected commercial chocolate candies including:

- * Snick candy

- * Full bar candy

- * Mars candy

- * Linker candy

- * Trixy candy

- * Bence candy

2. OXALATE ANALYSIS: The study will involve the quantitative analysis of oxalate content in the selected chocolate candies using standardized methods, such as ;

- * High performance liquid chromatography (HPLC)

- * Enzymes assays

- * Spectrophotometry

3. SAMPLE PREPARATION : samples of each chocolate candies will be prepared according to standardized protocols including;

- * Homogenization

- * Extraction

- * Filtration

4. DATA ANALYSIS : The oxalate content data will be analyze statistically : -

- * Determine the mean oxalate content of each candy type

- * Compare the oxalate content of each candy type

- * Identify any correlations between oxalate content and candy characteristics (e.g ; cocoa content, sugar content)

5. REGULAR COMPLIANCE : The study will assess whether the selected chocolate candies comply with relevant regulations and guidelines regarding oxalate content

1.5 RELEVANCE OF THE STUDY

1. *Public Health Significance*: The study's findings on the oxalate content of chocolate candies will inform consumers, particularly those with kidney stone issues or other oxalate-related health concerns.

2. *Food Safety and Regulation*: The study's results will contribute to ensuring compliance with food safety regulations and guidelines related to oxalate content in food products.

3. *Consumer Awareness and Education*: The study will provide valuable information to consumers, enabling them to make informed choices about their diet and lifestyle.

4. *Product Development and Reformulation*: The study's findings will inform manufacturers about the oxalate content of their products, guiding them in developing new products or reformulating existing ones to reduce oxalate content.

5. *Contribution to Existing Literature*: The study will contribute to the existing body of research on oxalate content in food products, providing new insights and data for future studies.

CHAPTER 2

2.0.LITERATURE REVIEW

This literature review examines the health implications of dietary oxalate consumption, focusing on its role in kidney stone formation and associated health risks. Oxalates, naturally occurring compounds in many foods, particularly vegetables, have been linked to increased calcium oxalate stone formation, with studies indicating a direct correlation between high dietary oxalate intake and the prevalence of kidney stones. The review highlights key dietary factors contributing to stone development, including calcium intake, the influence of gut microbiota, and the impact of various food types, such as high oxalate vegetables and sugary beverages. It also discusses socioeconomic disparities in kidney stone prevalence and emphasizes the importance of personalized dietary interventions, including limiting oxalate rich foods and incorporating adequate calcium intake to mitigate urinary oxalate excretion.

2.1. BIOLOGY OF COCOA

Cocoa is a dried and fully fermented cocoa bean product from *Theobroma cacao*, an evergreen tree in the family malvaceae. The name signifies "Food of God" in Greek. There are two main groups of cocoa beans, purple seeded forastero, the most widely used cocoa bean, and criollo, the lesser used mild flavoured high-quality cocoa (sein et Al., 2009) 5. The cocoa plant is a branching tree with simple, pointed (lanceolate) leave measuring up to 61cm (24 in) long and 10 cm (4 in) wide. The tree produces clusters of pale-yellow flowers each with five petals and sepals. The cocoa pods can be green-white, yellow, purplish or red in color each of which contains 20-50 seeds, the cocoa beans are arranged in five distinct rows. The plant has its origin in the upper Amazon region of south America (plant village ,2022)6.

2.2. COMPONENTS OF COCOA

Cocoa beans have a composition that includes lipids, carbohydrates, proteins, minerals and bioactive components with rich functional properties. The main component of cocoa beans is lipid fraction, the cocoa butter approximately 50%, mainly constituted by neural lipids, with a predominant fraction of triglyceride molecules. Oleic acid, stearic acid and palmitic acid are the main constituents of cocoa butter. Protein fraction constitutes 10% to 15% of the dry weight of cocoa seeds, and it is composed of 52% and 43% of albumin and globulin fractions, respectively. Other proteins, such as glutelins and prolamins, are present in lower concentrations. Cocoa beans are also rich in carbohydrates 31%, fiber 16%, and minerals (shahanas et al. ,2019)7. Cocoa beans contain stimulant substances, such as theobromine, caffeine, and theophylline, named purinic alkaloids, which affect the central nervous system (Bertazzo et al., 2013) 8.

2.3. PROCESSING OF CHOCOLATE

Cocoa fruit at the harvesting stage contains about 30-40 seeds. The seeds are covered by a mucilaginous pulp which is removed by yeast and bacteria during fermentation. The fermentation step

is essential for the development of chocolate flavor and lasts from three to seven days. This step also produces aroma precursors. Fermentation is followed by drying where the moisture content is reduced to 5% to 7% at 45°C to 60°C and shelf life is increased. Dried cocoa beans or nibs are then broken to reduce the size of the kernel and then roasted at around 120°C to 150°C to develop the chocolate flavor. The nibs are then ground to cocoa liquor.

The dark chocolate is made by mixing cocoa liquor, sugar, Cocoa butter and emulsifiers. To produce milk chocolate, milk, and other ingredients are added and refined to reduce the particle size of solids.

After refining the moisture undergoes a conching operation wherein the chocolate mass is agitated at a high temperature of around 50°C. Conching is followed by tempering, which consists of heating, cooling, and mixing (Dimattia et al., 2017; Shafi et al., 2018) (9,10).

2.4 TYPES OF CHOCOLATE

There are several types of chocolate with types including dark chocolate, white chocolate and milk chocolate. Among these types of chocolate, dark chocolate has gained some form of prominence because of its health benefits. Dark chocolate became part of the delicacies of the South Americans about 3000 years ago as history will have it and it was later shipped to Europe by Christopher Columbus (11).

The composition of white and milk chocolate includes cocoa butter, milk, and sugar and low amounts of cocoa bean solid (<10% of total weight) respectively (Corti et al., 2010) (12). Notably, the westerner views dark chocolate as one with a higher content of cocoa and vice versa for milk chocolate also referred to as sweet chocolate.

2.5 BENEFITS OF CHOCOLATE CONSUMPTION

Despite the harmful effects high consumption of chocolate causes, moderate consumption of the same has proven to provide health benefits (13).

One of the health benefits of consumption of chocolate focused on is cardiovascular health (14). Through several pathways, consumers of chocolate may be protected from cardiovascular diseases because of the flavonoids component. Flavonoids may also have other influences by acting as antioxidant, anti platelet and anti inflammatory agent (15). Other latent risk factors of cardiovascular diseases such as hypercholesterolemia, hypertension may be improved by flavonoids as well as improvement on endothelial functions (16). Apart from the cardiovascular health benefits consumption of chocolate may have, it also may have neurological health benefits and benign other ailments (15).

2.6 FUNCTIONAL PROPERTIES OF CHOCOLATE

The different functional properties of chocolate are as follows:

2.6.1 CHOCOLATE AS AN ANTIOXIDANT

The utilization of natural antioxidants was obligated as a rational strategy to combat stress - related diseases. Plant polyphenols are considered to be important dietary antioxidants, and dietary intake of these compounds can be up to 1200mg per day. Flavonoids, phenolic acids, and procyanidins are considered the main polyphenol classes and they all possess strong antioxidant potential confirmed in chemical based assays (Srdic- Rajic & Ristic, 2016)(17). Single servings of cocoa and cocoa products are found to contain more phenolic antioxidants than most other foods.

2.6.2 CHOCOLATE AS CARDIO PROTECTIVE

Sakaki et al (2019) (18) highlighted the polyphenols protection role against cardiovascular heart disease by acting as antioxidants and impairing the development of atherosclerosis. Polyphenol- rich diets have been shown in several clinical trials to be useful in the prevention and treatment of CVDs due to their antioxidants, antiinflammatory, anti platelet, and other pleiotropic effect (Behl et al., 2020)(19).

2.6.3 CHOCOLATE FOR ALZHEIMER'S DISEASE (AD)

Alzheimer's disease is a neurodegenerative disorder in which gradual cognitive impairment leading to dementia is observed. Clinical and epidemiological findings suggest a protective effect of flavonoids and polyphenols against neurodegenerative disease are supported by data obtained in animal model (Calderaro et al., 2022)(20)

2.6.4 CHOCOLATE AS A GOOD MOOD FOOD

Positive mood can be created in various ways but if food can make mood positive then it is just like icing on the cake. Chocolate is considered a good mood food due to the presence of numerous compounds, that act on the brain and produce a sense of delight that no other food matches (Shine et al., 2022)(21).

2.6.5 CHOCOLATE AS ANTIDIABETIC

Antioxidant effects of cocoa polyphenols directly influence insulin resistance and, in turn, minimize the risk for diabetes. Insulin sensitivity can also be improved by the vasodilatory effects of cocoa (Shah et al., 2017)(22). Precise intake of dark chocolate enhances insulin sensitivity in healthy as well as glucose intolerant hypertensive people (Ackar et al., 2013; Grassibet al., 2005)(23).

2.7 EFFECT OF PROCESSING ON FUNCTIONAL PROPERTIES OF CHOCOLATE

During the drying of cocoa beans, the brown polymers are synthesized as a result of oxidation of polyphenols, thus helping to form new flavor compound and reduce bitterness (Goya et al., 2022)(24). High temperature processing may lead to the loss of some important features of beans like texture, color, and also some bioactive compounds such as polyphenols. So, it is important to cautiously select the relevant roasting process.

2.8. HEALTH IMPLICATIONS OF OXALATE CONSUMPTION

Oxalate, Which are naturally occurring organic compounds present in many foods, have attracted considerably interest because of their potential health effects.

High dietary oxalate intake has been associated with an increased risk of kidney stone formation, particularly calcium oxalate stones.

Kidney stone formation related to diet is influenced primarily by three factors: the consumption of oxalate, the intake of calcium and other divalent cations, and the intestinal handling of oxalate.

However, according to (Massey, 2003) there are ten foods forbidden for people prone to kidney diseases. They are spinach, rhubarb, beets, nuts, chocolate, concentrated brans, legumes, regular tea, parsley and berries. A better understanding of the mechanisms and risk factors involved in kidney stone formation could help in creating innovative prevention strategies.

CHAPTER 3

3.0 EXPERIMENTAL

3.1 REAGENT & APPARATUS

3.1.1 REAGENTS

I. KMnO_4

II. H_2SO_4

3.1.2 APPARATUS & EQUIPMENTS

I. Volumetric flask

II. Chronical flask

III. Electronic balance

IV. Standard weight

V. Filter paper

VI. Funnel

VII. Measuring cylinder

3.1.3 SAMPLES USED:

I. Snickers candy

II. Mars candy

III. Foll bar chocolate candy

IV. Linkers candy

V. Trixy candy

VI. Bence candy

3.2 COLLECTION OF SAMPLES:

The chocolate candies samples were purchased from retail sellers and shopping malls with Ilorin metropolis.

Each samples was ensured to be sealed at the time of purchase and were kept at freezing temperatures till the time of analysis.

3.3 PREPARATION OF SAMPLES

Each samples of cookies was pulverized with a clean dry ceramic mortar and pestle.

The ice cream was each weighed while frozen once the pack was unsealed without any other treatment.

3.4. PREPARATION OF SOLUTION

3.4.1. 0.5M SULPHURIC ACID SOLUTION

54.35ml of concentrated sulphuric acid (stock solution) was carefully measured into a small quantity of water in a 2L standard (volumetric) flask . The content of the flask was mixed by swirling the flask without splashing the content . More distilled water was added to make up to the mark . The standard flask was stoppered and inverted severally to mix properly . This gives approximately 0.5m H_2SO_4 solution .

3.4.2. 0.025M POTASSIUM PERMANGANATE SOLUTION (KMnO_4)

7.90g of potassium permanganate crystals, and dissolved in a small amount of distilled . The solution was quantitatively transferred into a 2L volumetric flask and topped off with more distilled water . The solution was transferred into an amber bottle (2.5L) and tightly stoppered . This KMnO_4 solution was used for the redox titration to determine the oxalate

3.4.3. PREPARATION OF 0.1M OXALIC ACID SOLUTION

9.03g of oxalic acid crystals were accurately weighed into a clean dry beaker , small quantity of distilled water was added to dissolve the crystals. The solution was quantitatively transferred into a clean 1L standard flask and made up to the mark with more distilled water . The content of the flask was

inverted severally to properly mix the solution. The oxalic acid solution was used in standardizing the KMnO_4 solution .

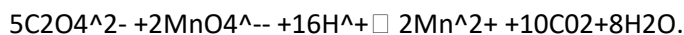
3.5. DIGESTION OF SAMPLES

5g of each powdered sample was carefully weighed and 50ml of 0.5m sulphuric acid solution added, swirled and boiled for 15 minutes to free the oxalate in the sample . The solution was filtered and 10ml of each filtrate was diluted to 100ml with distilled water . This was used for the titrimetric analysis .

3.6. STANDARDIZATION OF KMnO_4 SOLUTION

10ml of the 0.1m oxalic acid solution was pipetted into a clean 250ml Erlenmeyer flask . 15ml of 0.5m H_2SO_4 was added to $60 \pm 5^\circ\text{C}$.

The content was titrated hot against KMnO_4 solution from a burette to a faint Pink end point . The titration was repeated to obtain 2 or 3 concordant titres . By mole concept the exact concentration of the KMnO_4 solution was obtained using the equation of reaction below .



3.7 DETERMINATION OF OXALATE CONTENT

10ml of each of the diluted solution of each samples was pipetted into a clean Erlenmeyer flask . 15ml of 0.5m H_2SO_4 was added and the content heated to $60 \pm 5^\circ\text{C}$. The hot solution was immediately titrated hot against the standardized KMnO_4 solution . The titration was repeated to obtain at least two (2) concordant titres from each samples .

The oxalate content was calculated by employing the same equation of reaction used during standardization.

CHAPTER 4

CALCULATION ON DETERMINATION OF OXALATE CONTENT IN CHOCOLATE CANDIES

PRODUCTS / OXALATE CONTENT (mg/g)

Mars candy

1.82

Snick candy

1.35

Linker candy

1.52

Full bar candy

1.76

Bence candy

8.32

Trixy candy

1.08

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