PHYSICO-CHEMICAL PROPERTIES AND CONSUMER ACCEPTANCE OF MUFFINS ENRICHED WITH *AMARANTHUS CRUENTUS* LEAF FLOUR

By

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Submitted in partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE IN AGRICULTURE

at the

UNIVERSITY OF SOUTH AFRICA

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March 2023

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I further declare that I submitted the thesis/dissertation to the appropriate originality detection system which is endorsed by Unisa and that it falls within the accepted requirements for originality.

I further declare that I have not previously submitted this work, or part of it, for examination at Unisa for another qualification or at any other higher education institution.

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DEDICATION

This dissertation is dedicated to my God of Mount Sione, my late mother Caroline Nkwana and my late sister Lerato Masibi for providing me with hope and encouragement. My father Pheaha Nkwana, Thato Nkwana and little Kabelo Masibi for their understanding and patience during this study.

ACKNOWLEDGEMENTS

I would like to recognise the following people and institutions for assisting me in concluding this study:

- Prof Frederick Tabit for the input, motivation, encouragement, and immense contribution. Thank you, sir, for your constructive criticism and excellent supervision support throughout this study.
- Dr Martin Maboko, my co-supervisor for his guidance, support, and opportunities for enabling the cultivation of the Amaranth crop at the Agricultural Research Council, Vegetable and Ornamental Plants.
- Mr Silence Chiloane for his utmost support and encouragement
- Mrs Heleen Mokitlana for her support in taking care of the plants and weeding during the week, this could not have been possible without her from the cultivating to harvesting phase.
- Entsika Consulting for the financial assistance towards completing the research study.
- Agricultural Research Council-Vegetable and Ornamental Plants Institute-Roodeplaat for allowing me to utilise their resources to do my research.
- The Department of Research at the University of South Africa for awarding funding required for the research.
- My family and colleagues for the love, encouragement, and utmost support throughout the study.
- FoodBev SETA for the financial assistance towards completing the research study.
- Hlabana Seepe for the results analysis tutorials and inputs in general.
- Beverly Mampholo for the assistance with analysis equipment.

ABSTRACT

Background: The *amaranthus* crop (*Amaranthus spp.)* is an indigenous leafy vegetable identified as a possible solution to address undernutrition in Sub-Saharan Africa. This research will help boost consumption by improving the image of indigenous leafy vegetables such as amaranthus by producing value-added products with good sensory and nutritional properties. The research aims to analyse the physicochemical properties and consumer acceptance of muffins enriched with amaranthus (*Amaranthus cruentus)* leaf flour.

Method: The muffins were made by incorporating wheat and amaranthus flour at a ratio of 90:10, 70:30, 50:50 and 100% wheat flour. The enriched muffins were evaluated for proximate composition, physical attributes, minerals, vitamins, amino acids, and consumer acceptance.

Results: The enriched muffins were significantly higher in dry matter and ash showed an increase, in firmness, lightness, and yellowness compared with the control. The energy, moisture and carbohydrate values for the enriched muffins were significantly lower than the control. The enrichment of amaranthus did not significantly affect the height, but the diameter and weight significantly decreased at 10% enrichment. There was a significant decrease in the acceptance of colour, aroma, texture taste and overall acceptability for 30% and 50% enriched muffins compared to the control.

Conclusion: A 50% enriched muffin with amaranthus had overall improved properties in terms of proximate composition, physical properties, and mineral content.

Recommendation: Amaranthus (*A. cruentus*-Arusha) leaf flour can be explored further to produce other nutritious value-added products with acceptable sensory qualities.

Keywords: Enriched muffins, consumer acceptance, physical properties, mineral content, proximate composition, indigenous leafy vegetables, flour blends

TABLE OF CONTENTS

DECLARATION **2**

DEDICATION **3**

ACKNOWLEDGEMENTS **4**

ABSTRACT **5**

TABLE OF CONTENTS **6**

LIST OF TABLES **9**

LIST OF FIGURES **10**

CHAPTER 1: INTRODUCTION **11**

1.1 BACKGROUND INFORMATION 11

1.2 Problem statement 12

1.3 Motivation for the research 13

1.4 Research aim and objectives 13

1.5 Research hypotheses 13

CHAPTER 2: LITERATURE REVIEW **15**

2.1 Occurrence and distribution of amaranthus 15

2.2 Cultivation and harvesting of amaranthus 15

2.3 The processing techniques of Amaranthus as a vegetable 17

2.4 Nutritional composition of amaranthus 19

2.5 Utilisation of amaranthus 19

2.6 Contribution of amaranthus to household food security 20

2.7 Opportunities and constraints in the commercialisation of amaranthus 21

2.8 Vegetable flour blends and their baked products 21

2.9 Consumer evaluation of vegetable flour blends and their baked products 22

2.10 Identified knowledge gaps evaluated in this thesis 23

CHAPTER 3: RESEARCH METHODOLOGY **24**

3.1 Cultivation Amaranthus 24

3.2 Preparation of flour from amaranthus leaves 25

3.3 Preparation of muffins enriched with the flour of amaranthus leaves. 26

3.4 Proximate composition 27

3.5 Analysis of the physical properties of muffins....29
3.6 Analysis of the mineral content of muffins....31
3.7 Analysis of vitamin C and vitamin E content of muffins....33
3.8 Evaluation of consumer acceptance of muffins....34
3.9 Statistical Analysis....34

CHAPTER 4: RESULTS....36
4.1 PROXIMATE COMPOSITION OF MUFFINS....36
4.2 Physical properties of muffins....38
4.3 Textural properties of muffins....38
4.4 Colour properties of muffins....39
4.5 Amino acids content of muffins....41
4.6 Mineral content of muffins....42
4.7 Vitamin C and E composition of muffins enriched with the flour of A. Cruentus leaves. 43
4.8 Consumer acceptance of muffins....44

CHAPTER 5: DISCUSSION....45
5.1 Proximate composition of enriched muffins with A. Cruentus flour....45
5.1.1 Dry Matter....45
5.1.2 Moisture content....45
5.1.3 Ash content....46
5.1.4 Carbohydrates....46
5.1.5 Fat content....47
5.1.6 Energy Value....47
5.1.7 Protein....47
5.1.8 Dietary fibre....48
5.2 Physical properties of muffins enriched with amaranthus leaf flour....48
5.2.1 Height, diameter, and weight....48
5.2.2 Textural properties....49
5.2.3 Colour properties of muffins....49
5.3 Amino acids composition....50
5.6 Mineral composition of enriched muffins....51
5.7 Vitamin C and E composition of enriched muffins....51
5.8 Consumer acceptance of muffins enriched with A. cruentus flour....52

CHAPTER 6: CONCLUSION AND RECOMMENDATION 54

6.1 Conclusions 54

6.2 Recommendation 55

REFERENCES 56

APPEnDIX A: Ethics approval letter 76

APPENDIX B : CONSUMER SENSORY EVALUATION QUESTIONNAIRE 79

APPENDIX C: CONSUMER ACCEPTABILITY OF AMARANTH/WHEAT FLOUR COMPOSITE MUFFINS. 81

APPENDIX D CONSENT FORM 82

LIST OF TABLES

Table 3.6 Formulation of the preparation of wheat and *Amaranthus cruentus* composite muffins. 27

Table 4.1 Proximate composition of muffins enriched with the flour of amaranthus leaves ..37

Table 4.2 The physical dimension of muffins enriched with the flour of amaranthus amaranth leaves....38

Table 4.3 Textural properties of muffins enriched with the flour of amaranthus leaves.........38

Table 4.4 Colour properties of muffins enriched with flour of amaranthus leaves 41

Table 4.5 Amino acids composition of muffins enriched with flour of amaranthus leaves 41

Table 4.6 Mineral composition of muffins enriched with the flour of amaranthus leaves......43

Table 4.7 Vitamin C and E composition of muffins enriched with the flour of amaranthus leaves....44

Table 4.8 Consumer acceptance of muffins enriched with the flour amaranth leaves 44

LIST OF FIGURES

Figure 2.1 Matured amaranthus *Amaranthus cruentus* (Arusha) plants ready for harvesting.18

Figure 2..2 Harvested *Amaranthus cruentus (Arusha)* leaves at ARC-Roodeplaat-Vegetables and Ornamental Plant Institute.....18

Figure 3.1 Seedlings trays covered with a thin layer of VermIculite growing medium after seedling.....24

Figure 3.2 Five-week-old amaranth seedling ready for transplanting.....25

Figure 3.3 Milling Pulverisette 19 Machine (A), 100% wheat flour (B), 10% amaranthus composite flour (C), 30% amaranthus composite flour (D) and 50% amaranthus composite flour (E).....26

Figure 3.9 Weighing (A) and texture analysis (B) of muffins enriched with the flour of amaranthus leaves.....30

Figure 4.1 100% wheat flour muffin (A), 10% amaranthus composite muffin (B), 30% amaranthus composite muffin (C) and 50% amaranthus composite muffin (D).....40

Figure 4.2 Sensory profile of quality attribute of muffins enriched with A. cruentus flour (A.1 Color; A.2 Aroma; A. 3 Taste; A.4 Texture; A.5 Overall Acceptability). The acceptance was evaluated using a hedonic 9-point scale, where 1 and 9 indicate the lowest and highest intensity.....41

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND INFORMATION

For many centuries, specifically in rural households, indigenous vegetables have been a crucial part of human nutrition (Omotayo et al., 2020). Furthermore, humans rely upon several materials from the earth to survive and maintain their livelihood, including addressing malnutrition and hunger (Omotayo et al., 2020). South Africa has been facing challenges in food supply at the household level as a result of nutrient deficits from zinc, iron, and vitamins A and C (Maseko et al., 2018). Amaranthus species as part of an indigenous leafy vegetation, have been identified as possible solutions to undernutrition (Simon et al., 2020). Amaranthus cultivars contain a substantial amount of vital micronutrients mainly, iron, vitamin C, calcium, folic acid, and β-carotene which are often consumed less by children aged from zero months to four years old in South Africa (Priya et al., 2007). Leafy vegetables' minerals are even far greater than those of other vegetables (Deibler et al., 2021; Kamga et al., 2013), this notion is evident in many domesticated indigenous leafy vegetables such as *Amaranthus spp*. (Pigweed), *Vigna spp*. (Cowpeas), *Solanum spp*, *Cleome gynandra* (Cat's whiskers) and (black nightshade) (Onyango et al., 2009). Like many indigenous leafy vegetables, the consumption of amaranthus is decreasing in many rural communities as a result of being mistakenly contemplated as inferior in nutritional content and taste in contrast to exotic vegetables like Spinach (Weinberger & Msuya, 2004; Uusiku et al., 2010).

The consumer habits for the underutilised and neglected vegetables in rural areas are attributed to local perception of these vegetables considering they are categorised as food for the impoverished, however, other factors that contribute to the downward trend in cultivation include religious and cultural taboos, taste, and smell which gives unpleasant experience to some family members (Adepoju & Aka, 2019). Furthermore, another reason for the trend of reduction in utilisation of indigenous leafy vegetables trends is their unavailability both in the informal and formal markets in rural and urban communities. Aboriginal vegetables are important to people within rural communities because they can serve as a cheap and accessible alternative to conventional leafy vegetables (Ezebilo, 2010). The use of leafy vegetation varies from leafage of annuals and hedges to trees; however, the majority of rural residents depend on leaves collected from the wilderness as the primary source of leafy vegetables (Ntuli, 2019). Several amaranthus cultivars can withstand diverse climate conditions and also possess a high tolerance to pests, diseases, and weeds and can grow spontaneously; hence impoverished rural people rely upon locally accessible leafy vegetables for food and as a source of income (Chatepa & Masamba, 2020). In South Africa, amaranthus species seldom grow as people are convinced that the plant can strive with no cultivation practices needed (Maseko et al., 2018; Maundu et al., 2009; N'Danikou et al., 2010). Indigenous leafy vegetables have been selected as possible alternatives since the base constituent used to produce bakery products is wheat flour, which is very costly to import especially in countries with adverse climates to grow wheat, this contributed to the formulation of flour composites for bakery goods. Composite flour is construed as a composition of different flours derived from legumes and roots etc in addition to wheat flour as part of food fortification (Okoye and Etim, 2021).

Food fortification has been investigated and considered an efficacious feasible strategy for attenuating undernutrition (Qumbisa et al., 2020). The food fortification success intervention is due to the notion that it's not selective and does not focus on the entire populace to alter its eating patterns (Qumbisa et al., 2020). However, the South Africa Food-Based Dietary Guidelines (SAFBDGs) recommends that at least one individual's serving must have starchy food as a basis for the meals, which allows the adequate intake of dietary carbohydrates to fulfil caloric needs (Qumbisa et al., 2020). Fortification research has successfully explored the addition of vegetable powders including spinach (Galla et al., 2017), sweet potato and more (Giri et al., 2022). However, the use of instant noodles fortification on conventional vegetables using amaranthus leaves has not been explored extensively (Qumbisa et al., 2020), which also applies to amaranthus fortification into muffins. The incorporation of vegetables into flour can improve the nutritional value also the overall sensorial characteristics of the ensured baked goods (De LA Borca et al., 2010; Rai et al., 2012). The enrichment and production of baked muffins with acceptable sensory qualities will improve the utilisation and commercialisation potential of these vegetables (Aju & Popoola, 2013; Kwenin et al., 2011; Uusiku et al., 2010).

1.2 Problem statement

The consumption of indigenous leafy vegetables mainly amaranthus is diminishing especially in rural areas, e.g., in South Africa in preference for introduced conventional vegetables (Aphane et al., 2003; Dweba & Mearns, 2011; Jansen van Rensburg et al., 2007). The neglected/underutilised leafy vegetables are disappearing in farmlands as a result of a lack of awareness of the potential wellness and dietary benefits of consuming these vegetables. Inadequate information on indigenous leafy vegetable species has led to the slight neglect of some of the convenient vegetables over the years (Okewole et al., 2018).

Studies have shown that the stigmatisation of indigenous leafy vegetables by the youth can be attributed to a lack of knowledge, upbringing, and sensory properties such as taste, cultural preferences, interurban mangling, age, gender, and social status influence customer acceptability. Commercialisation may influence nutrition through different channels, including income, gender, and availability of own-produced foods within households which in turn enhances market access, not only for rural economic growth but also makes smallholder farmers more nutrition-sensitive to agriculture (Ogutu, et al., 2019).

1.3 Motivation for the research

This research will help boost the consumption and acceptance of indigenous leafy vegetables such as amaranthus by producing value-added products with good sensory and nutritional properties. These value-added products of amaranthus if commercialised boost income generation in rural and urban communities through cultivation and sales of amaranthus as raw material (Aderibigbe et al., 2020; Onyango, 2003). Cultivation and commercialising of indigenous leafy vegetables is important in generating employment in rural communities (Aworh et al., 2015; Chagomoko, et al., 2014).

1.4 Research aim and objectives

This research aimed to characterise the physico-chemical and consumer acceptance of muffins enriched with flours made from amaranthus leaves.

The objectives were:

1. To analyse the effect of amaranthus leaf flour on the proximate and amino acid composition of muffins enriched with amaranthus leaf flour.
2. To determine the effect of amaranthus leaf flour on the physical properties and mineral composition of muffins enriched with amaranthus leaf flour.
3. To investigate the consumer acceptance of muffins enriched with amaranthus leaf flour.

1.5 Research hypotheses

1. The proximate composition of muffins enriched with different amounts of flour made from amaranth (*A. cruentus*-Arusha) leaves will be significantly different at $P \leq 0.05$.
2. The physical properties of muffins enriched with different amounts of flour made from amaranth leaves will be significantly different at $P \leq 0.05$.

3. The mineral composition of muffins enriched with 10, 30, and 50% of amaranth leaves will be significantly different at $P \leq 0.05$.
4. The consumer acceptance of muffins enriched with different amounts of flour made from amaranth leaves flour will be significantly different at $P \leq 0.05$.

CHAPTER 2: LITERATURE REVIEW

2.1 Occurrence and distribution of amaranthus

Leafy vegetables particularly amaranthus are versatile crops that are resistant to drought, pests, and heat and can adapt to harsh weather conditions (Aderibigbe et al, 2020). Although Amaranthus species adapt to diverse conditions, they are reported to do best in well-drained soils, fertile and loose soils with sufficient organic matter content (Stetter, Vidual-Villarejo and Schmid, 2020). The occurrence of amaranthus is different since there are approximately 60-70 species which are extensively cultivated as leafy vegetables in many regions around the world (Achigan-Dako, et al., 2014). The *Amaranthus* genus is categorised into three categories mainly grain, vegetable and weed (Das, 2012; Thapa et al., 2021). The taxonomic composition of amaranthus in the tropical region differs from that in the temperate regions, for instance, *Amaranthus retroflexes* strive and is widely distributed in temperate regions while *Amaranthus dubius* stive in many tropical regions (Sukhorukov et al., 2021).

Amaranthus plant is adapted to ruderal and segetal regions/countries such as Ethiopia, Tunisia, Egypt, Morocco, Botswana, and South Africa in certain areas amaranthus species are regarded as weeds, especially areas where maize is being cultivated (Sukhorukov et al., 2021). Cross-pollination crops, particularly amaranthus have high pollination tendency which results in hybrid seeds that are not desired in many instances (Aderibigbe et al., 2020). Aderibigbe et al., (2020) state that amaranthus is a high-yield vegetable crop among rural and urban communities, for home gardening amaranthus can be grown continually, throughout the year due to its adaptability. Amaranthus can be found growing along roadsides, on grazing fields, and as weeds in agricultural fields and forests (Sahu et al., 2020). According to Joo et al., (2018) bromatological and phyto-technical studies will encourage the cultivation of amaranthus at a larger scale which would result in economic benefits.

2.2 Cultivation and harvesting of amaranthus

In South Africa, amaranthus is seldomly grown since it is perceived to grow without any cultivation practices, however in provinces such as Limpopo and Mpumalanga seeds are broadcasted in cases of cultivation by women (Gerrano et al., 2015). The cultivation of amaranthus with other indigenous leafy vegetables is not well documented with specific directions and guidelines which would encourage research and production (Maseko et al., 2018). According to Ochieng et al., (2019), the average amaranthus seed is approximately 3.0 kg/ha with about 2.3

kg/ha in Kenya and 3.4 kg/ha in Tanzania. The post-adapted cultivation practices include seedbed preparation, seed broadcasting and nursery practices such as seed transplanting, (Ochieng et al., 2019) the practice of transplanting shortens the duration of the crop in the field (see Figure 2.1). Amaranthus seeds can be sown in both narrow and wide rows, with a recommended row spacing of 45 cm with inter-row tillage to accommodate weed control (Pelech, 2021). Amaranthus crops have been found to grow well in nitrogen-rich soils, hence they strive in urban garbage or compost area, however, in situations where fertilizer is required for the cultivation, the recommended nitrogen phosphorus and potassium (NPK) dose should be 10-10-20 at a rate of 400kg/ha (Achigan-Dako et al, 2014). Research studies have indicated that unfavourable factors such as low and high temperatures, over-fertilisation, and lack of oxygen in the soil can affect the survival of amaranthus crops by the time of harvesting, however even after harvesting certain processing techniques can result in nutrient depletion (Nyonje et al., 2021). However, harvesting techniques of vegetable amaranthus differ in different countries but in South Africa leaves are handpicked, (Figure 2.2), alternatively use cutting tools such as a cutter, knife and sickle show harvested *Amaranthus cruentus* (Arusha) leaves picked off at 4-5 weeks after sowing, however in circumstances of multiple harvests, the shoots are harvested first (Pelech, 2021).

2.3 The processing techniques of Amaranthus as a vegetable

There are various processing techniques for indigenous leafy vegetables in many rural communities which include sun-drying after cooking, sun-drying of raw leaves, boiling, steaming, frying and fermentation, this type of processing helps with post-harvest preservation of indigenous leafy vegetables thereby maintaining a constant supply of amaranthus vegetables during hunger periods (Cheptoo et al., 2020; Mahlangu, 2014). The blanching, boiling and steaming techniques are reported to substantially reduce the minerals and vitamin content of vegetables since food preparation water has been reported to reduce the degree of thiamine by 31% and 50% while niacin by 80% (Onyambu et al., 2021). The blanching process can affect texture, colour, flavour, and nutritional quality. Cheptoo et al., (2020) reported that blanching decreases ß-carotene content from week 1 of harvesting however matured leaves contain a higher content of ß-carotene as compared to young leaves. Research studies have shown that processing techniques for indigenous leafy vegetables have an impact on their nutritional quality, as a result, the demand for treated vegetable goods can lead to a reduction in post-harvest and improvements in the nourishment of indigenous leafy vegetables (Tepe et al., 2021). Processing of vegetables can enable producers to provide consumers with access to nutritious food in and out of season due to preservation techniques that are being used such as drying to extend the storage life of vegetables (Tepe et al., 2021). Amaranthus is not the type of vegetable to be consumed raw and it requires going through food preparation methods that differ among consumers, it is reported that the cooking methods induce chemical, physical properties and enzymic modifications in food which affect concentration and bioavailability, phytochemical properties, and physicochemical compounds of nutrients (Idug et al., 2022; Nyonje et al, 2021). Leafy vegetables are contributors to micronutrients in meals since they carry an adequate amount of zinc, calcium, and iron (Maseko et al, 2019).

Figure 2.1 Matured *Amaranthus cruentus* (Arusha) plants ready for harvesting

Figure 2.2 Harvested *Amaranthus cruentus* (Arusha) leaves at ARC-Roodeplaat-Vegetables and Ornamental Plants

2.4 Nutritional composition of amaranthus

The leaf of amaranthus is a good source of nutrients, essential minerals (43.3 mg/100 g Ca, 2.3 mg/100 g Fe, 55.0 mg/100 g Mg, 0.9 mg/100 g Zn), vitamins (43.3 mg/ 100 g vitamin C, 0,2 mg/100 g vitamin B-6) and bioactive compounds (8.91 to 9.93 CE/ 100 g flavonoid), that are vital for human nutrition (Arepally et al., 2023; Hadkar et al., 2023; Sarker et al, 2022; Sishu et al., 2023). Amaranthus leaf is a good source of nutrients such as protein (2.5 g/100 g), and dietary fibres (1.3 g/100 g) and amaranthus is affordable (Jimoh et al., 2018; Nyonje et al., 2021). Consumption of vegetables such as amaranthus has been associated with good health (Adepoju and Aka, 2019), as a result, the World Health Organisation (WHO) suggests that at least an intake of 400 g of fruits and vegetables excluding starchy vegetables such as potatoes are to be consumed per day (WHO, 2013). Furthermore, the recommended dietary guideline for Americans is 5 servings of vegetables per day at an intake of 2000 calories however, one of the five servings should be a green vegetable (Natesh et al., 2017). The recommendation of at least 400 g of vegetables can assist to combat malnutrition in many rural and impoverished communities in Sub-Saharan Africa. In East Africa, amaranthus leaves are occasionally recommended by medical practitioners for patients with anaemia (low red blood cells) (Aderibigbe et al., 2020) because amaranthus possess 13 times more iron, 57 times more vitamin A and 8 times more calcium as compared to other vegetables (Dlamini and Viljoen, 2020).

Amaranthus has been found to play a vital nutritional role in rural communities (Fanzo, 2012). Shayanowako et al., (2021) reported that a 100 g serving of amaranthus contains approximately double the concentration of beta-carotene, iron and amino acids compared to *Brassica oleracea Var*. Furthermore, amaranthus has been found to contribute to the reduction of iron deficiencies among children when consumed in an adequate amount (Alemayehu et al., 2014; Macharia-Mutie et al., 2011). Iron deficiency is prevalent in rural areas although consumption has increased noticeably, especially for crops such as amaranthus, but malnutrition is remaining a major health problem in parts of Africa (Nyonje et al., 2021). Studies have indicated that in some parts of Kenya, 76% of children in 2019 were anaemic, amaranthus vegetables are an excellent source of nutritional iron which can assist in dealing with iron deficiency anaemia.

2.5 Utilisation of amaranthus

The consumption of amaranthus leafy vegetable has medicinal benefits since the leaves contain a substantial amount of calcium, which is important for growth, teeth, muscles and maintenance, among other nutrients found on the leaves is sodium which balances nerve and muscle contraction while magnesium is a vital nutrient component that is in relation with ischemic heart disease (Ntuli, 2019). Leafy vegetables such as amaranthus form part of the daily meals of many people in rural communities especially in Sub-Saharan Africa, Consumption patterns of these vegetables are dependent on factors such as underprivileged background, distance from agricultural markets, period of the year and degree of urbanisation (Dlamini & Viljoen, 2020; Uusiku et al., 2010). Indigenous amaranthus leafy vegetables are frequently prepared and eaten as a relish that accompanies starchy staple food mainly porridge or rice (Chatepa and Masamba, 2020; Masarirambi et al., 2020). During the preparation of indigenous leafy vegetable dishes, ingredients such as shrimp paste, anchovies, coconut oil and dried shrimp are added to enhance palatability and taste depending on preference or availability and cultural groups (Saupi et al., 2020). In countries such as Nigeria, indigenous leafy vegetables such as amaranthus are used as an approach to address and improve food security (Saupi et al., 2020).

2.6 Contribution of amaranthus to household food security

The FAO detailed food security as a state which exists when all people, always, have physical and economic access to adequate, safe, and nutritious food which meets their dietary needs and preferences, for an effective and healthy life (Drammeh et al., 2019; Kasimba et al., 2017). South Africa is perceived as food nutrition security concerning producing and importing foods for the populace, but food supply is still a threat in some households, to such an extent that Department of Agriculture Forestry and Fisheries (DAFF) developed agricultural projects within communities as a means of making household revenue and creating jobs since it is reported that approximately 4.5 million households survive below the poverty line (Ngema et al., 2018). Many households in rural communities meet their nutritional requirements by consuming unconventional leafy vegetables with water rice, during winter seasons, in Odisha, Eastern India, the kitchens are filled with the aroma of leafy vegetables in winter seasons (Sahu et al., 2020). Amaranthus vegetable is widely consumed in many rural communities throughout the world, particularly in Africa, India, and other Asian countries, North and South America. Amaranthus leaves are the most consumed part of the plant and possess a high amount of protein, vitamins, minerals, and dietary fibre (Narwade & Pinto, 2018). Furthermore, while the consumption of amaranth provides dietary and nutritional benefits, income generated from the sales of amaranth can be used to supplement or complement the income of poor households in rural communities (Mahlangu, 2014). Widespread of these crops consumption in urban areas may lead to increased demand which in turn increases income generation by rural farmers through sales thereby improving their household income and standard of living (Schippers, 2000; Schonfeldt & Pretorius, 2011). Home gardening of aboriginal leafy vegetables can also help in improving food supply, dietary diversity, and earning revenue (Ferdous et al., 2016).

2.7 Opportunities and constraints in the commercialisation of amaranthus

In recent years, a rise in consumption of indigenous leafy vegetables has been reported in several African countries (Imathiu, 2021), but the increase in consumption has not contributed to commercialisation due to a lack of scientifically documented information such as specific guidelines on production, processing, marketing, nutritional composition, and distribution (Maseko et al., 2017). Poor seed quality is restricting leafy vegetable production as producers still depend on retained seeds since there are identified gaps in the market for formal seed retailers (Shayanowako et al., 2021). However, studies have shown that economic benefits can be achieved from attempts to focus on the best agronomic practices in amaranthus cultivation to increase production (Alemayehu et al., 2014). The economic perspective in which small-scale farmers have habitually operated has drastically shifted due to urbanisation and newly developed food systems such as primary production of food, non-food, storage, postharvest, handling techniques, transportation, processing techniques, distribution channels, marketing avenues, disposal techniques and consumption to the growing population (Badiane and Jayne, 2021. The shift was especially in cities which, are reshaping African farmers' access to the market (Badiane and Jayne, 2021). According to Shiundu and Oniango (2007), in countries such as Tanzania, Nigeria, and Ethiopia, the market allocation of leafy vegetables in contrast with other vegetable crops has increased tremendously in urban markets which in turn positively increased consumption in rural areas. Furthermore, due to urbanisation and industrialisation, land is becoming scarce which will be a challenge for underutilised leafy vegetables such as amaranthus, but the cultivation of underutilised leafy vegetables will contribute to economic growth by providing a vital source of income in modern society (Sahu et al., 2020).

2.8 Vegetable flour blends and their baked products

Many studies have found that substituting or incorporating cereal and vegetable-based flour into wheat flour can enhance the nutritional value of baked goods and their sensory characteristics (Rai et al., 2012; Singh et al., 2008; Sudha et al., 2005). The production of gluten-free bread using amaranthus flour has been found to enhance the nutritional, sensory, and physical properties of the baked bread (Alvarez-Jubete et al., 2010; De LA Barca et al., 2010; Rahaie et al., 2012). Use of locally available amaranthus flour for biscuit production can help reduce dependence on imported wheat flour even though the incorporation of indigenous vegetable flour at different substitution levels can compromise the physical-chemical, and sensorial characteristics of the final products (Gimenez et al., 2012; Okpala & Egwu, 2015). Furthermore, indigenous leafy vegetables can be blended with cereal to make pasta products with increased dietary fibre and protein content (Gimenez, 2012). According to some literature sources, amaranthus flour possesses exceptional nutritional value and has a low viscosity. When amaranthus is added to fortify with oats products, the by-products can reduce cholesterol and postprandial serum glucose levels (Inglett, Chen, Liu, 2015). Oats and amaranthus flour mix well, as amaranthus grain cultivars are restricted in some of the essential amino acids mainly threonine and leucine. Amaranthus-oats composite cookies have improved nutritional value, water-holding capacity, and gluten-free quality, the cookies are said to be acceptable in texture, colour, and flavour (Inglett, Chen and Liu, 2015). According to Odunlande (2017), amaranthus leaf powder blends well with wheat flour hence amaranthus-wheat composite instant noodles were produced by Qumbisa et al., (2021). The study proposed that instant noodle sachets be made available from amaranthus powder and will complement noodles which will improve flavour, colour, and aroma. The study also indicated that promotional campaigns and awareness are needed to improve the quality of amaranthus leaf powder instant noodles.

2.9 Consumer evaluation of vegetable flour blends and their baked products

Consumer evaluation is reported as an important parameter for analysing consumer requirements (Nasir et al., 2020). Many studies have been conducted on consumer evaluation of vegetable flour blends and their baked product to establish consumer acceptability. It is often reported in the literature that consumer acceptability evaluation is based on a 9-point hedonic scale that measures sensorial characteristics such as aroma, taste, look, colouring, texture, and overall permissibility of the bi-product (Nasir et al., 2020). According to Chauhan et al., (2016), amaranthus cookies with 60% amaranthus incorporation were accepted meanwhile taste decreased significantly due to the aftertaste and the bitterness of amaranthus flour. According to Isah et al., (2020), 20 untrained panellists were recruited to access the sensory characteristics of Bambaranut-soybean-carrot composite biscuits. The study found that there was a compelling difference ($p>0.05$) in taste, smell, look and overall acceptability and the addition of Bambara groundnut-soybean-carrot flour decreased the taste and smell value of the composite biscuit. The studies of amaranthus-based wheat flour bread show that the average rating for bread colour decreased when amaranthus flour was added, and the results went further to show that the overall palatability of the composite bread was good at 10% incorporation with regards to physical traits (Nasir et al., 2020).

2.10 Identified knowledge gaps evaluated in this thesis

There are three main gaps identified concerning amaranthus leafy vegetable, mainly lack of consumption and poor image, poor cultivation practices and lack of commercialisation, and how amaranthus can be explored further to produce value-added products considering there is limited literature in terms of food fortification avenues. Amaranthus is known to contain a substantial amount of nutrients such as calcium, iron, folic acid, and β-carotene (Priya et al., 2007), which would come in handy when it comes to addressing nutrient deficiencies. Although amaranthus is known to be a good source of nutrients such as dietary fibre and protein (Nyonje et al., 2021), there is inadequate information proven in studies to support the notion, which would have resulted in boosting consumption and commercialisation of amaranthus among the consumers. Furthermore, if food fortification was explored using amaranthus, literature would be available to support the nutritional composition of amaranthus which would in turn improve its utilisation. In this research study, amaranthus fortification will be explored to characterise the physico-chemical and consumer acceptance of muffins enriched with flour from amaranthus leaves. Amaranthus-enriched muffins will be examined to determine proximate and amino acid composition, physical properties and mineral composition and consumer acceptance of amaranthus-enriched muffins.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Cultivation Amaranthus

Amaranthus cruentus seeds (Arusha) used in the research were obtained from the seed store of the Agricultural Research Council, Vegetable and Ornamental Plants, South Africa. Seedling trays were utilised to sow the seed at a 200 pore per cavity, then poured with Hygromix (Hygrotech Pty. Limited) and then, covered with a thin layer of vermiculite growing medium (Figure 3.1). Transplanting of 5-week-old seedlings was conducted on raised beds 20 cm high and 1.2 m broad at a planting spacing of 30 x 25 cm and the beds were 1m (Figure 3.2). The topsoil at 30 cm was sandy clay loam consisting of 25% clay, 6% silt, and 69% sand, with a total pH of 7.04.

Figure 3.1 Seedling trays covered with a thin layer of vermiculite growing medium after seeding.

Figure 3.2 Five-week-old amaranth seedling ready for transplanting

Plants were irrigated with drip irrigation at a splashing degree of 2 $L{\cdot}h^{-1}$ with a speed of 150 kPa which supplied a total of 450 mm of water throughout the season. Amaranthus was considered mature when they reached a height of 0.6 m from 4-5 weeks after sowing (Achigan-Dako et al., 2014) (Figure 2.1). Amaranthus leaves were harvested from mature amaranthus plants and used to process the amaranthus flour.

3.2 Preparation of flour from amaranthus leaves

The flour of amaranthus leaves was produced according to the method described by Chauhan et al. (2016). Fresh amaranthus leaves were cleaned a couple of times thoroughly until the soil particles were removed and then dehydrated in a Labotec Digital EcoTherm Industrial hot air oven from a high temperature of 250°C to a low temperature of 50 °C for 24 hours. Following dehydration, the leaves were pulverised using Fritsch cutting Mill Pulverisette 19 (Figure 3.3 (A). The flour was sifted with a 60-mesh sieve with 0,25 pore size (Stedman, model 20x12) (Borneo & Aquirre, 2008) and stored at 4°C in 5 medium airtight zip lock bags.

Figure 3.3 Milling Pulverisette 19 milling machine (A), 100% Wheat Flour (B), 10% Amaranthus composite flour (C), 30% Amaranthus composite four (D) and 50% Amaranthus composite Flour (E)

3.3 Preparation of muffins enriched with the flour of amaranthus leaves.

Wheat flour and amaranthus leaf flour were uniformly combined in the following proportions: 100:0, 90:10, 70:30 and 50:50 to obtain a composite flour (Mokhele et al., 2020) (Figure 3.3 (A, B, C and D). The composite flours were kept in resealable plastic bags at a storage temperature of 4°C before being used (Okpala and Chinyelu, 2011). The ingredients used for making the muffins were purchased at Checkers Hyper Waterfall, Midrand, South Africa. The ingredients comprised 200 g flour, 12.1 g sugar, 0.1 g salt, 1.5 g baking powder, 27.7 g skim milk, 44.0 g eggs and 14.3 g canola oil (Table 3.1). The muffins were baked as described by Struck (2016). The dry and liquid ingredients were combined separately and then combined with an electric mixer. Paper muffin cups were greased with butter, the mixture dough was scooped and poured into paper muffin cups, then put in a baking tray and baked at 180 °C for 24 minutes (Miwe, Arntein, Germany). The muffins were left to cool for 5 minutes, packaged in small-sized airtight resealable plastic bags and then kept at 4 °C until examined.

Table 3.6 Formulation of the preparation of wheat and muffins enriched with the flour of amaranthus leaves.

Sample	Wheat flour (%)	Amaranth flour (%)	Sugar (g)	Canola oil (g)	Salt (g)	Skim milk (g)	Baking powder (g)	Eggs (g)
W-100	**100**	**0**	**12.1**	**14.3**	**0.1**	**27.7**	**1.5**	**44.0**
AA-10	**90**	**10**	**12.1**	**14.3**	**0.1**	**27.7**	**1.5**	**44.0**
AA-30	**70**	**30**	**12.1**	**14.3**	**0.1**	**27.7**	**1.5**	**44.0**
AA -50	**50**	**50**	**12.1**	**14.3**	**0.1**	**27.7**	**1.5**	**44.0**

W-100 = 100% wheat flour; AA-10, AA-30 and AA 50 represent muffins enriched with 10%, 30% and 50% amaranthus leaves flour, respectively.

3.4 Proximate composition

Muffins derived by incorporating amaranthus and wheat flour were analysed with the Association of Official Analytical Chemists (AOAC) 2005 methods, to establish their proximate composition.

Ash content: The ash content of the muffins was determined by standard method AOAC No 936.07 (AOAC 2005). In this method 2.0 g of dried muffins were pre-weighed, and inserted in a muffle furnace which had been pre-ignited and temperature at 600℃ 120 minutes. The crucible having ash was left to reach room temperature and weight loss was determined as ash content percentage.

The ash content was calculated using Eq.1

$$\% \text{ Ash content} = \frac{Mass\ of\ ash\ (g)}{Mass\ of\ sample\ (g)} \times 100$$

Moisture content: The contentment of moisture was measured by standard Method 993.26 (AOAC, 2005). 5 grams of each muffin part was placed in a bowl and dried in the drying oven for 120 minutes. It was timed from when the oven reached a temperature of 130°C after the bowl was placed in the oven. The bowl was removed after 120 minutes, left to cool inside a desiccator, and later re-weighed. The bowl was placed back in the 30-minute interval until it reached constant weight. Moisture content was calculated using Eq. 2

$$\% \, Moisture = \frac{M1 - M2}{M1 - M2} \times 100$$

M1 = mass Weight of the bowl and fresh sample, M =Sample mass weight And M2 = mass Weight of the bowl and dried sample

Fat content: The muffins' fat content was determined by the standard, AOAC 2003.05 (AOAC, 2005). The fat content was extracted using ether petroleum at 0.5 gram of each sample, which was weighed into a thimble at which fat was extracted into a flask with hexane for 8 hours using Siphon (100°C). The flask with the extracted fat was left to reach room temperature in a desiccator and later weighed. The fat was calculated using Eq. 3

$$\% \, Fat = \frac{Mass \; of \; flask \; after \; drying - Initial \; mass \; of \; flask}{Mass} \times 100$$

Crude protein content: Protein content was determined using the Kjeldahl nitrogen determination method (AOAC No. 2001.11 (AOAC 2005). 2.0 g of the dry muffin was placed into a Kjeldahl flask and 25 mL of HS 2 O 4 (98%) mixture. The mixture was poured into a volumetric flask that is 100 mL adjusted the size to note the distilled water and titrated against a blank. Titres from repeated samples were documented then percent nitrogen was evaluated. Per cent nitrogen (%N) was turned into crude protein by a factor of 6.25.

$$\% \, N = \frac{(mL \; standard \; acid - mL \; blank) \; X \; N \; of \; acid \; X \; 1.4007}{Weight \; of \; sample \; in \; grams} \times 100$$

Therefore, the percentage protein content was evaluated by the following formula:

$$\% \, Protein = 6.25 \times \% \, N$$

Crude Fibre content: Crude fibre was determined using Fibertec, 2010 based on AOAC 978.10. The fitted crucible was pre-dried at 130 +/-2 °C for 30 minutes. A sample of 1.000 g was weighed into a crucible and placed into a Fibertec hot extraction unit. 150 mL of 1.25% H_2O_4 was added into each column containing the sample, to prevent foaming 2 drops of n-octanol were added. The heater was turned to max, and when the reagent started to boil, the heather temperature was adjusted to moderate, after the reagent boiled for 30 minutes. The heater was switched off, the heated sample was washed thrice with hot deionised water. After washing, 150 mL of preheated 1.25% NaOH solution was poured into reagent 2, and the sample was boiled for an additional 30 minutes. The sample was washed again with hot deionised water. The crucible was transferred inside a cold extraction unit where 25 mL of acetone was added. The extraction unit was placed in a vacuum position to filter the acetone, the process was conducted thrice. The crucible was placed elsewhere and left to cool down until the acetone had vaporised. The crucible was dried for 120 minutes at a temperature of 130 ℃, allowed to cool in a desiccator and measured at an accuracy of 0.1mg. The sample was ash in the crucible for 180 minutes at 525+/-15 ℃, then cooled in a desiccator and weighed accurately to 0.1mg. The crude fibre was calculated using Eq. 4

$$\% \; Crude \; Fibre = \frac{W_2-(W^3+C)}{W_1} \times 100$$

Carbohydrate content: Carbohydrate percentage was determined by difference.

Thus: $\% \; Carbohydrates = 100 - (Protein \; + Fat + Ash + Fibre)$

Energy value: Muffin energy values were evaluated by the Atwater calorie conversion factor. In this method, estimation criteria were used with the notion that a single sample gram of protein, carbohydrates, including fat results in 17 kilojoules for both protein and carbohydrate and 37 kilojoules for fat. The results were outlined in kilojoules (kJ) (Mokhele et al., 2020).

3.5 Analysis of the physical properties of muffins

Texture analysis: The texture of the muffins was measured with the HD Plus Texture Analyzer (Stable Micro System, Surrey United Kingdom), with a provision of 5 kg load cell. To get the data, a Texture Profile Analysis (TPA) procedure was utilized, while the results were evaluated with 6.1.9.0 exponent software (Stable Micro System, Surrey United Kingdom). Each muffin was evaluated, and the evaluation ratings were achieved following what was outlined by Rodrigues et al. (2022). The paper cups of the muffins were removed, and each muffin sample was put through two 50% of initial compression, with a 75 mm flat-end aluminium cylindrical probe (p/75) at an acceleration speed of 1mm/s pre-test rate of 3 mm/s and 5 mm/s post-test rate of 5 mm/s at a 5-second interval of the two cycles. The parameters evaluated were firmness (g), cohesiveness, elasticity (g) and brittleness (g) (Figure 3.9 (B)).

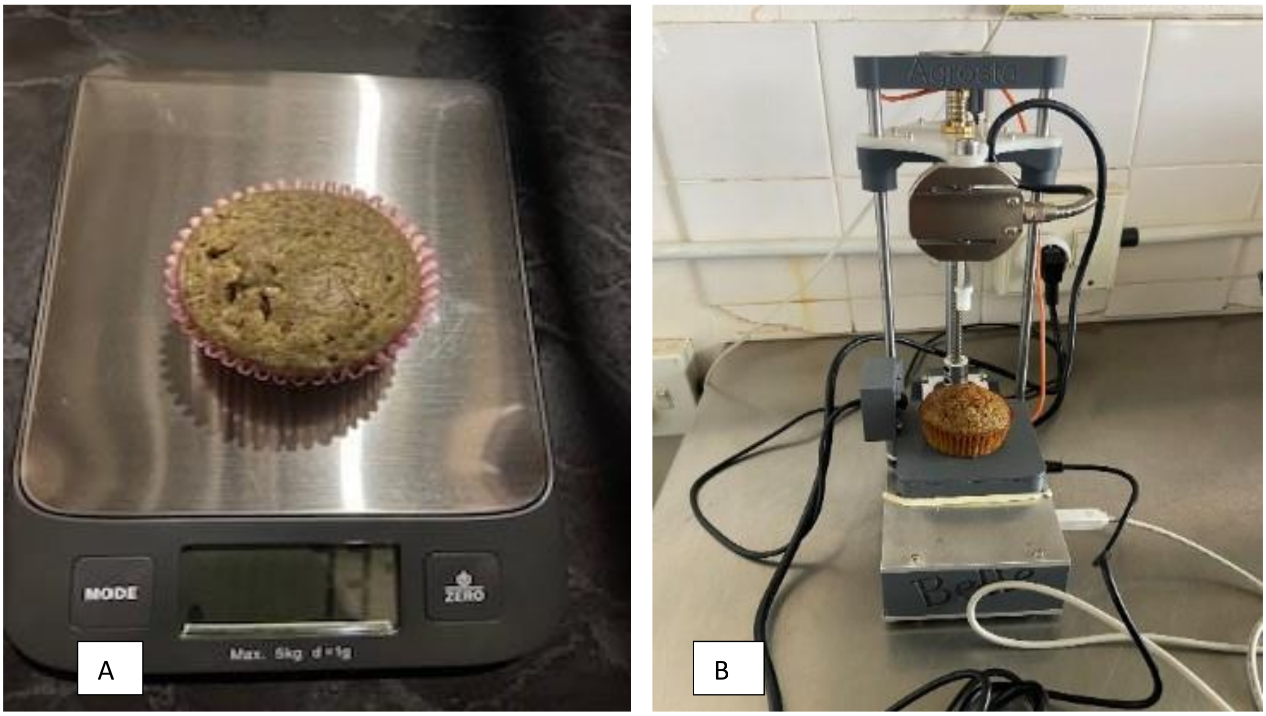


Figure 3.9 Weighing (A) and texture analysis (B) of muffins enriched with the flour of amaranthus leaves.

Colour: The colour of the muffin was evaluated using a Minolta CR-400 chromameter (Minolta, Osaka, Japan). The standard white tile was calibrated to operate the chromameter. In the Commission Internationale de I'Elcairage (CIE) colour system, a* positive value outlined the state of red colour, b* positive values outlined the state of yellow colour while lightness was outlined by L* value. The readings were indicated as coordinates of the CIE lab system with the use of D65 illuminant, The calibrated instruments were read on a pattern with white tile. Evaluations were recorded from the top and bottom surfaces of the muffins with a sum of three replications for each sample.

Amino acids composition: modified method AOAC 982.30 (AOAC, 2005) was used to determine the amino acids composition. The muffin samples had fat content and were moist, then derivatised. For amino acids evaluation, the derivatised sample solution was poured into a vial measured at 1ml volume, then administered into gas chromatography (HP 6890 powered with HP [1206] software). The hydrogen was utilized as a gas transporter with a velocity of 1.0 ml/minute column at 1.0 ml x 0.25 um dimension. The starting oven temperature was 110℃, increased at 27℃/minutes to 320℃ and kept for five minutes. The quantity of amino acids within the muffin sample was evaluated from the net weight and approximate area of each peak (representing each amino acid mainly histidine, threonine, methionine, valine, isoleucine, leucine, tryptophan, phenylalanine, lysine, alanine, glutamic acid, glycine, serine, tyrosine, proline, HO-Proline, cysteine, arginine, and aspartic acid) and was indicated as g/100 g protein.

3.6 Analysis of the mineral content of muffins

The mineral composition of the muffins value was determined by the method of the No 985.35 and 967,21 (AOAC 2005), for each element mainly iron (Fe) calcium (Ca), nickel (Ni), magnesium (Mg), copper (Cu), potassium (K), sodium (Na), phosphorus (P), zinc (Zn) and manganese (Mn). The ash was dissolved in diluted hydrochloric acid and the resulting 'solution was used for analysis.

Calcium (Ca) determination: Calcium content was measured by a complex isometric titration procedure, in which muffin extract of 20 ml was placed into a conical flask and the various masking agents mainly hydroxy tannin, hydrochloride and potassium cyanide were added. A pinch of indicator-ferrochrome black was added and mixed well, and a permanent blue colour was observed, then the ratings were noted down. The calcium content was calculated using the below formula:

$$Calcium\ (mg/100g) = \frac{Tv\ X\ 0.4008\ X\ 100}{Volume\ of\ sample}$$

Magnesium (Mg) determination: After placing a sample 10 mL inside a 250 mL conical flask, 25 nL of ammonia buffer solution was poured into the conical flask and mixed to ensure the mixture was mixed well. A pinch of T black indicator was also added into the mixture, then titrated with EDTA 0.02 N until the mixture colour changed from red wine to blue colour. The magnesium content was calculated using Eq. 5

$$Magnesuim\ (mg/100g) = \frac{Tv\ X\ 0.2432\ X\ 100}{Volume\ of\ sample\ used}$$

Potassium (K) determination: The concentration of potassium (ppm) was measured with a UV-Spectrophotometer at a wavelength of 766.5 nm, and then the concentration was calculated using Eq. 6

$$Pottasium\ (mg/100g) = \frac{(Concentration\ (ppm)\ X\ Dilution\ Factor\ X\ 1000}{Weight\ of\ sample}$$

Sodium (Na) determination: The concentration of sodium (ppm) was analysed using the atomic absorption spectrophotometer at a wavelength of 243 nm, then the concentration was calculated using Eq. 7

$$Sodium\ (Na)\ (mg/100g) = \frac{(Concentration\ (ppm)\ X\ Dilution\ Factor\ X\ 1000}{Weight\ of\ sample}$$

Phosphorus (P) determined: A 20 mL sample solution was transferred to a 100 mL volumetric flask. The solution was neutralised with ammonia and nitric acid solution (1:2). 20 mL of vanadate molybdate reagent was added and then diluted to a mark. The mixture was left for 10 minutes, then the reading on the absorbance was 470 nm in the ultraviolet region. The phosphorus concentration was calculated using Eq. 8

$$Phosphorus\ (P)\ (mg/100g) = \frac{(Concentration\ (ppm)\ X\ Dilution\ Factor\ X\ 1000}{Weight\ of\ sample}$$

Copper (Cu) determination: The concentrations of copper (ppm) were analysed using an atomic absorption spectrophotometer at a wavelength of 244 nm, and then the concentration was calculated using Eq. 9

$$Cupper\ (Cu)\ (mg/100g) = \frac{(Concentration\ (ppm)\ X\ Dilution\ Factor\ X\ 1000}{Weight\ of\ sample}$$

Iron (Fe) determination: A 5 mL sample was placed into a test tube and various components were added to it, such as hydroquinol acetone buffer and 0.1 pyridine. The solution was mixed properly and left for 24 hours for the colour to develop. A spectrophotometer was used to measure the absorbance of 530 nm. The mixture was calculated using Eq. 10

$$Iron\ (Fe)\ (mg/100g) = \frac{(Concentration\ (ppm)\ X\ Dilution\ Factor\ X\ 1000}{Weight\ of\ sample}$$

Zinc (Zn) determinants: The AOAC method 985.35 was used to determine zinc content (AOAC, 2005). 1 g of muffin sample was turned to ash and weight. The ash weight was processed with 5 ml of 6 N HCI, and then placed to dry on a hot plate, a mixture of 15 mL of 3 N HCI was poured into 5 mL of 6 N HCI, and then heated until it boiled on a hot plate. The mixture was left to be cooled down, then later passed through a Whatman No 1 filter paper in a 50 mL volumetric flask, and then later poured into de-ionised water to evaluate zinc. The sample standard was oxidized air-acetylene as a form of atomization energy. A measurement of absorbencies on both samples was conducted at 213.9 nm, 248.4 nm, and 422.7 nm. The sample zinc concentration was evaluated from a standard graph and expressed as mg/100 g.

Manganese (Mn) determinants: The solution obtained from dry ashing the sample at 500℃, dissolving it in 10% HCI (25 mL) and 5% (2 mL) lanthanum chloride, boiled, filtered, and brought up to standard with deionised water, was used to determine manganese content. Manganese was determined with buck Scientific model 210 VGP Atomic Absorption Spectrometer (AAS). Three replication readings for the sample were done. A calibration curve of absorbance against concentration of manganese under investigation was constructed and the concentration was finally determined from the calibration curve of its standard by interpolation.

3.7 Analysis of vitamin C and vitamin E content of muffins

Vitamin C determinants: The ascorbic acid was measured utilizing method No 967.21 (AOAC 2005), by weighing 10g of the sample and adding 100 ml of water. The sample was filtered, and a clear solution was obtained, a small flask was then used to prepare the solution. The 10 ml of clear solution was added to the flask, along with a blank sample, both the blank and the samples were then subjected to a mixture of indophenols, which produced a faint pink colour. The vitamin C content was calculated using Eq. 11

$$Vitamin\ C\ (mg/100g) = \frac{(20\ X\ (V_1 - V_2)\ X\ C}{Weight\ of\ sample}$$

Vitamin E determination: Tocopheryl acetate determination in ethanol by mean if colourimetry was employed in the form of absorbance measurements using a spectrophotometer The analysis was based on the sulpho-phospho-vanilin (SPV) method. 100 µl sample was heated for 10 min at 100 °C to remove solvent. The residue was digested with 250 µl concentrated sulphuric acid (99%) for 10 min at 100 °C. Five millilitres of the SPV reagent, i.e. a mixture of 3 g vanillin, 0.5 g Millipore water and 2 litres of concentrated phosphoric acid (85%), were added and mixed vigorously to allow the formation of a pink adduct. The mixture was then incubated at ambient temperature for 30 min for complete colour formation. The absorbance of the resulting solutions was determined at λmax = 525 nm. A standard curve was drawn by diluting tocopheryl acetate in ethanol at a series of concentrations up to 0.5%. The blank test remained colourless, and average experimental values were reported from triplicates with the standard deviations remaining within ±0.04.

3.8 Evaluation of consumer acceptance of muffins

A total of 20 persons were recruited from Entsika Consulting Services and Agricultural Research Council Vegetable and Ornamental Plants (ARC-VOP), the recruited participant was assessed using questionnaires (Appendix B) to set up a panel that is not trained. The selection criteria were for people who eat muffins no less than one time a week and have no food allergies. During the sessions, communication was shared with participants informing them that they are allowed to quit the testing evaluations at any time they wish, therefore, a request to complete and then sign a consent form before tasting the muffin samples was conducted. A total of four muffin types (what flour muffins and the three enriched muffins) at 90:10, 70:30 and 50:50 formulations were coded with a four-digit number code. The muffin sample was bestowed to each member of the panel and room temperature water was supplied to clean the mouth during the testing sessions. Two testing sessions were conducted on 12 July 2021 (Figure 3.16) and the other on 20 October 2021. Utilising a 9-point hedonic scale each muffin sample was evaluated for overall acceptability which focused on aroma, texture, taste, and colour. The ranges on the 9 acceptability structure levels ranged starting at nine indicating (line extremely), eight (very much like), seven (moderately like), six (slightly like), five (neither dislike nor like), four (slightly dislike), three (moderately dislike), two (very much dislike), while one was (extremely dislike). The ratings provided by the panel for overall acceptability were based on each criterion under evaluation. The sensorial evaluation forms had questions and scales displayed (Chauhan et al., 2016).

3.9 Statistical Analysis

The STATISTICA-8 software was used to analyse data. The differences in each dependent variable between the four factors (100% wheat muffin, 10%, 30% and 50% enriched muffins) were evaluated using one-way analysis of variance (ANOVA) and means with significant differences were identified using Duncan's Multiple Range Test (DMRT). The dependent variables were dry matter, moisture, ash, carbohydrates, fat, energy, protein, dietary fibre, diameter, height, weight, firmness, elasticity, brittleness lightness, redness, yellowness, essential and non-essential amino acids, calcium, magnesium, potassium, sodium, phosphorus, zinc, manganese, iron, nickel, copper, vitamin C and A, sensory characteristics colour, aroma, taste, texture, and overall acceptability. Data were reported as mean ± standard deviation and statistical significance was set at $p \leq 0.05$.

CHAPTER 4: RESULTS

4.1 PROXIMATE COMPOSITION OF MUFFINS

The dry matter of all the enriched muffins was significantly higher than that of (control). An increase in the percentage of enrichment with amaranthus flour did not progressively increase the dry matter content of the enriched muffins significantly (Table 4.1). The moisture content of all the enriched muffins was significantly lower than that of the control muffins. An increase in the percentage of enrichment with Amaranthus flour did not give rise to any significant progressive change in the moisture content of the enriched muffins at 10% and 30% enrichment, unlike the 50% enriched muffins where there was a significant increase in the moisture content (Table 4.1). The ash content of all the enriched muffins was significantly higher than those of the control. A content was significantly high on enriched muffins with 10% amaranthus wheat flour (Table 4.1). An increase in the percentage of enrichment with amaranthus flour did not give rise to any significant change in the ash content.

The carbohydrate content of all the enriched muffins was significantly lower than those of the control. An increase in the percentage enrichment with amaranthus flour gives rise to a significant progressive reduction in the carbohydrate content only at 50% enrichment (Table 4.1). There was no significant difference in the fat content of enriched muffins and the control. An increase in the percentage enrichment with amaranthus flour did not give rise to any significant changes in the fat content of enriched muffins (Table 4.1). The energy content of muffins enriched with amaranth flour was significantly lower than that of the control. An increase in the percentage of enrichment with amaranthus flour gave rise to a significant reduction in the energy content only at 50% enrichment (Table 4.1). An increase in the percentage of enrichments with amaranthus flour gave rise to a significant reduction in the protein content only at 50% enrichment (Table 4.1). There was no significant difference in protein content between the control and the 10 and 30% enriched muffins. An increase in the percentage of enrichments with amaranthus flour gave rise to a significant increase in the dietary fibre content only after 30% when compared to the control and the 10 and 30% enriched muffins (Table 4.1).

Table 4.1 Proximate composition of muffins enriched with the flour of amaranthus leaves

Treatment	Dry Matter (g/100g)	Moisture (g/100g)	Ash (g/100g)	Carbohydrate (g/100g)	Fat (g/100g)	Energy (g/100g)	Protein (g/100g)	Dietary Fibre (g/100g)
WF-100	61.96^{c}±0.44	38.49^{a}±0.01	1.50^{c}±0.10	64.42^{a}±1.50	12.85^{a}±0.18	1323.50^{a}±24.50	7.15^{a} ±0.35	5.25^{b}±0.24
AA-10	64.80^{b}±0.60	32.41^{bc}±0.01	4.12^{a}±0.11	55.35^{b}±1.09	11.88^{a}±0.32	1309.50 ^{b}±8.50	6.77 ^{a}±0.25	4.40^{c}±0.12
AA-30	66.76^{ab}±0.44	32.61^{b}±1.07	3.47^{b}±0.12	52.58^{b}±0.25	12.03^{a}±0.45	1302,50^{b}±8.32	7.95^{a}±0.30	5.72 ^{b}±0.39
AA-50	68.29^{a}±0.72	34.25^{b}±0.35	3.79^{b} ±0.13	49.82^{c}±0.32	12.19^{a}±0.61	1295.50^{c}±5.50	4.90 ^{b}±0.12	6.86^{a}±0.21

WF100 = Wheat flour (control), AA 10 = *Amaranthus cruentus* enriched at 10%, AA 30 = *A. cruentus* enriched at 30%, AA 50 = *A. cruentus* enriched at 50%. Figures are outlined as Mean ± Standard deviation. This means within the same column not having a similar superscript vary ($p \leq 0.05$) while values with a similar superscript do not vary ($p > 0.05$)

4.2 Physical properties of muffins

Enrichment did not significantly affect the height. The diameter and weight were significantly reduced at 10% enrichment. The increase in the level of enrichment for diameter and weight was not significantly different from that of the control (Table 4.2).

Table 4.2 The physical dimension of muffins enriched with the flour of amaranthus leaves (n=02)

Treatment	Diameter (cm)	Height (cm)	Weight (g/muffin)
W-100	$6.70^{a}\pm0.20$	$5.70^{a}\pm0.20$	$100.50^{a}\pm4.50$
AA-10	$6.00^{b}\pm0.00$	$4.50^{a}\pm0.39$	$80.50^{b}\pm7.50$
AA-30	$6.05^{b}\pm0.05$	$5.00^{a}\pm0.50$	$84.50^{ab}\pm1.50$
AA-50	$6.50^{ab}\pm0.10$	$5.60^{a}\pm0.10$	$88.50^{ab}\pm0.50$

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Figures are outlined as Mean ± Standard deviation. This means within the same column not having a similar superscript vary ($p\leq0.05$) while values with a similar superscript do not vary ($p>0.05$)

4.3 Textural properties of muffins

The firmness of enriched muffins was significantly higher than those of the control while only the 50% enriched muffins had significantly lower brittleness when compared to the control (Table 4.3). An increase in the enrichment with amaranthus flour gave rise to a significant progressive decrease in the firmness of the muffins. There was no significant difference in the brittleness of 10 and 30% enriched muffins and the control. An increase in the percentage of amaranthus flour during enrichment did not give rise to any significant difference in the cohesiveness and elasticity of enriched muffins (Table 4.3).

Table 4.3 Textural properties of muffins enriched with the flour of amaranthus leaves

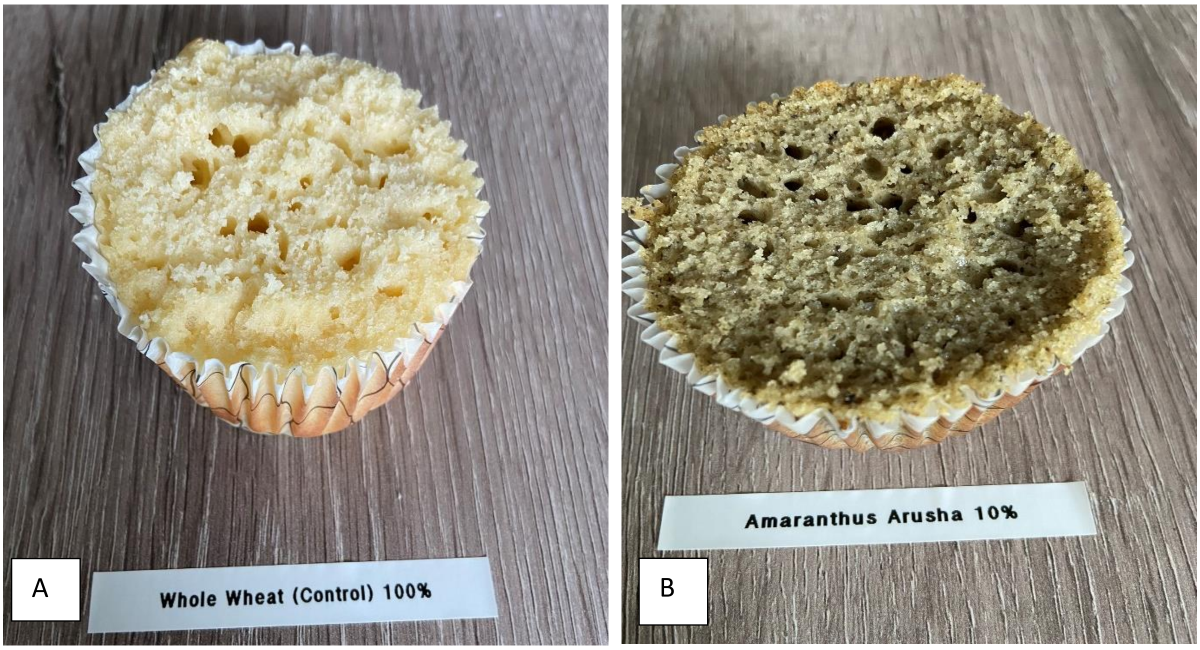
Treatment	Firmness (g)	Cohesiveness	Elasticity (g)	Brittleness (g)

W-100	183.00^{d} ±2.00	0^{a}	0.50^{a}±0.50	96.30^{a}±0.10
AA-10	**370.00^{a} ±5.00**	0^{a}	0.51^{a}±0.05	96.35^{a}±0.05
AA-30	**285.50^{b}±1.50**	0^{a}	0.50^{a}±0.50	96.15^{a}±0.05
AA-50	**203.50 ^{c}±1.50**	0^{a}	0.50^{a}±0.50	**94.70^{b}±0.10**

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Figures are outlined as Mean ± Standard deviation. The means within the same column not having a similar superscript vary ($p \leq 0.05$) while values with a similar superscript do not vary ($p > 0.05$)

4.4 Colour properties of muffins

The lightness and yellowness of muffins enriched with amaranthus flour increased significantly compared to the control. The lightness decreased significantly from 10, 30 and 50% respectively while the yellowness increased significantly from 10, 30 and 50% enrichments. The redness of enriched muffins was significantly higher than those of the control only after 50% enrichment and significantly lower at 10% enrichment (Table 4.4).



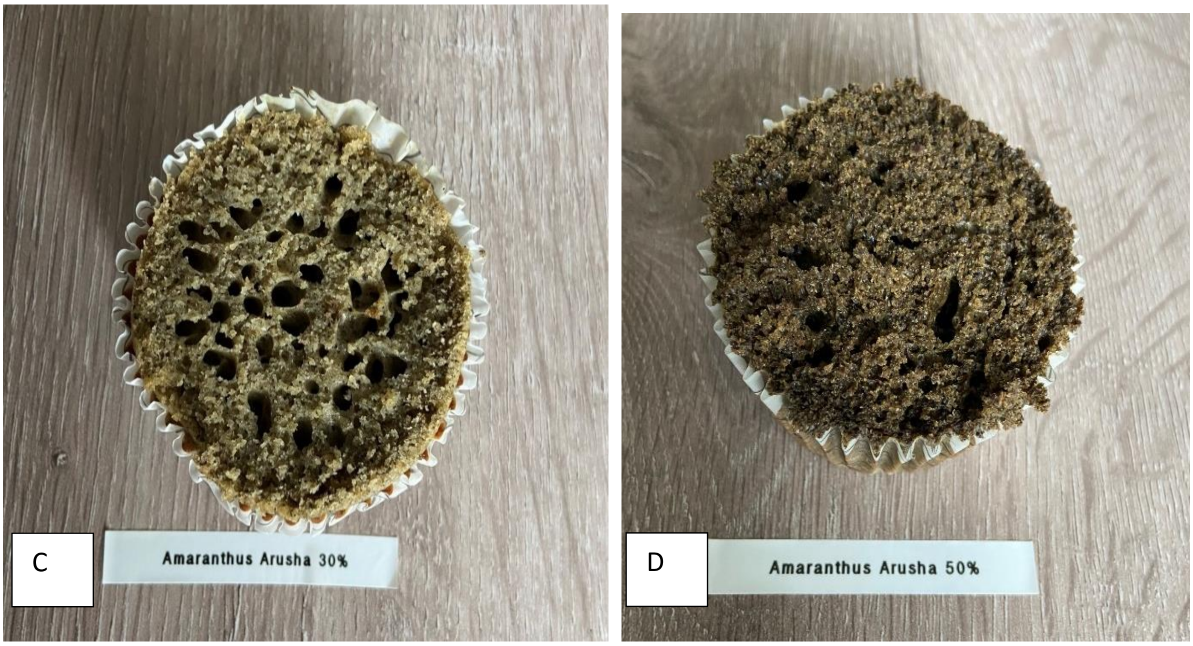


Figure 4.1 100% wheat flour muffin (A), 10% amaranth composite muffin (B), 30% amaranthus composite muffin (C) and 50% amaranthus composite muffin (D).

Table 4.4 Colour properties of muffins enriched with a flour of amaranthus leaves

Treatment	Lightness (L*)	Redness (a*)	Yellowness (b*)
W-100	28.06[d] ±0.25	3.73[b]±0.05	2.71[d] ±0.10
AA-10	58.56[a] ±1.63	0.00[c] ±0.00	9.68[c] ±0.75
AA-30	36.46.[b] ±0.54	3.16[b] ±0.04	13.70[b] ±1.51
AA-50	32.04[c] ±0.49	8.93[a] ±0.52	27.63[a] ±0.65

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Figures are outlined as Mean ± Standard deviation. This means within the same column not having a similar superscript vary ($p \leq 0.05$) while values with a similar superscript do not vary ($p > 0.05$)

4.5 Amino acids content of muffins

Regarding the essential amino acid, there was no significant difference in the histidine, threonine, methionine, valine, isoleucine, leucine, tryptophan, and phenylalanine content of the enriched muffins compared to the control (Table 4.5). The content of lysine significantly increased from 10%, and 30% enrichment compared to those of the control.

Regarding the non-essential amino acid, there was no significant difference in the glycine, tyrosine, HO-proline, and Cysteine of the enriched muffins compared to those of the control (Table 4.5). The content of alanine, serine, arginine, and aspartic acid significantly increased from 10% to 30% to 50% enrichment compared to those of the control. However, glutamic and proline were high in wheat muffins compared to enriched muffins, except for 10% enriched muffins which had the highest glutamic acid (Table 4.5).

Table 4.5 Amino acids composition of muffins enriched with Amaranthus cruentus flour

Amino acids (g/100g)	W-100	AA-10	AA-30	AA-50
		Essential amino acids		
Histidine	0.14[a]±0.04	0.13[a]±0.01	**0.11[a]±0.00**	**0.13[a]±0.01**
Threonine	0.27[a]±0.01	0.33[a]±0.01	**0.32[a]±0.02**	**0.33[a]±0.01**
Methionine	0.15[a]±0.01	0.17[a]±0.01	**0.15[a]±0.01**	**0.18[a]±0.02**

Valine	0.39^{a} ±0.05	**0.41^{a}±0.02**	**0.45^{a}±0.03**	**0.47^{a}±0.04**
Isoleucine	0.31^{a} ±0.03	**0.33^{a}±0.01**	**0.36^{a}±0.02**	**0.38^{a}±0.03**
Leucine	0.52^{a}±0.03	0.59^{a}±0.02	**0.57^{a}±0.01**	**0.59^{a}±0.02**
Tryptophan	0.12^{a}±0.00	0,10^{a}±0.01	0.12^{a}±0.00	0.12^{a}±0.00
Phenylalanine	0.35^{a}±0.03	**0.40^{a}±0.03**	**0.42^{a}±0.05**	**0.43^{a}±0.07**
Lysine	0.37^{b} ±0.02	0.44^{a}±0.03	**0.48^{a}±0.03**	**0.46^{a}±0.01**
Non-Essential amino acids				
Alanine	0.22^{b}±0.03	0.35^{a}±0.02	**0.37^{a}±0.02**	**0.34^{a}±0.01**
Glutamic Acid	1.51^{ab}±0.04	1.60^{a}±0.01	1.43^{bc}±0.02	1.40^{c}±0.01
Glycine	0.21^{a}±0.02	0.30^{a}±0.02	**0.28^{a}±0.02**	**0.30^{a}±0.02**
Serine	0.42^{b}±0.00	**0.46^{a}±0.00**	**0.46^{a}±0.00**	**0.46^{a}±0.00**
Tyrosine	0.34^{a}±0.02	**0.39^{a}±0.01**	**0.42^{a}±0.02**	**0.39^{a}±0.02**
Proline	0.50^{a}±0.01	**0.48^{b}±0.01**	**0.47^{b}±0.01**	**0.46^{b}±0.01**
HO-Proline	0.01^{a}±0.00	0.01^{a}±0.00	0.01^{a}±0.00	0.01^{a}±0.00
Cysteine	0.18^{a}±0.01	0.10 ^{a} ±0.01	0.21^{a}±0.03	0.21^{a}±0.00
Arginine	0.41^{b}±0.00	**0.50^{a}±0.01**	**0.50^{a}±0.01**	**0.51^{a}±0.00**
Aspartic Acid	0.49^{c}±0.02	0.59^{b}±0.01	**0.62^{a}±0.00**	**0.66^{a}±0.01**

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Figures are outlined as Mean ± Standard deviation. The means within the same row not having a similar superscript vary ($p \leq 0.05$) while values with a similar superscript do not vary ($p > 0.05$)

4.6 Mineral content of muffins

The concentration of Ca, Mg, K, Na, P and Zn significantly increased in enriched muffins progressively from 10%, 30% to 50% enrichment when compared to the control. The concentration of Cu, Mn and Fe in the enriched muffins significantly increased only at 50% enrichment when compared to the control and the 10% and 30% enriched muffins. The concentration of Ni significantly increased only at 30% enrichment but stayed the same at 50% enrichment (Table 4.6).

Table 4.6 Mineral composition of muffins enriched with the flour of A. Cruentus leaves

Mineral	W-100	AA-10	AA-30	AA-50
Ca (g/100g)	262,67 [d]± 13,0	**511,33 [c]±30,0**	**682,00 [b]±48,1**	**1468,67 [a] ± 132,8**
Mg (g/100g)	42,93 [d] ± 1,6	**124,60 [c]±3,5**	**188,27 [b] ±11,0**	**468,00 [a] ±38,2**
K (g/100g)	177,53 [d] ±7,9	**343,33 [c]±19.0**	**473,33 [b] ±29,3**	**1202,00 [a] ±117,2**
Na (g/100g)	374,67 [d]±17,9	**412,67 [c]±20,3**	**434,00 [b] ±22,7**	**621,33 [a] ±57,8**
P (g/100g)	347,33 [d] ±14,4	**405,33 [c]±26,3**	**461,33 [b]±35,7**	**556,00 [a]±26,0**
Zn (mg/100g)	3,66 [d] ±0,3	**1,99 [c] ±0,1**	**2,04 [b]±0,2**	**3,08 [a] ±0,3**
Cu (mg/100g)	1,77 [b] ±0,1	1,75 [b] ±0,1	1,86 [b] ±0,3	**2,06 [a] ±0,0**
Mn (mg/100g)	1,46 [b] ±0,1	0,94 [c] ±0,1	1,10 [b] ±0,1	**2,97 [a] ±0,3**
Fe (mg/100g)	5,61 [c] ±0,1	13,27[b] ±0,9	13,81[b] ±0,8	**27,33 [a] ±2,0**
Ni (mg/100g)	0,70 [b] ±0,1	0,55 [c] ±0,1	**0,78 [a] ±0,1**	**0,78 [a] ±0,0**

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Figures are outlined as Mean ± Standard deviation. The means within the same row not having a similar superscript vary ($p \leq 0.05$) while values with a similar superscript do not vary ($p > 0.05$)

4.7 Vitamin C and E composition of muffins enriched with the flour of A. Cruentus leaves.

The was a significant increase in the concentration of vitamin C in enriched muffins compared to those of the control but the increase was highest at 10% enrichment and lowest at 50% enrichment.

The was a significant increase in the concentration of vitamin E in the enriched muffins compared to those of the control but the increase was lowest at 10% enrichment and highest at 50% enrichment (Table 4.7).

Table 4.7 Vitamin C and E composition of muffins enriched with the flour of amaranthus leaves

Vitamins (mg/100g)	W-100	AA-10	AA-30	AA-50
Vitamin C	51.71 ^{c}± 1.63	**65.81^{a} ±1.70**	**61.96^{b} ±0.13**	**60.24^{b} ±1.25**
Vitamin E	36.69 ^{c}± 0.18	**41.72 ^{b} ±0.32**	**43,29^{b} ±0.25**	**44.87 ^{a} ±0,27**

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Values are outlined as Mean ± Standard deviation. Means within the same row not having a common superscript differ (p≤0.05) while values with a common superscript do not differ (p>0.05).

4.8 Consumer acceptance of muffins

There was a significant decrease in the colour, aroma, taste, texture, and overall acceptability of the 30% and 50% enriched muffins compared to those of the control. The acceptance of colour and aroma significantly decreased from 10% enrichment (Table 4.8).

Table 4.8 Consumer acceptability of muffins enriched with Amaranthus Cruentus leaf flour

Treatment	Colour	Aroma	Taste	Texture	Overall Acceptability
W-100	7.75^{a}±0.34	7.70^{a}±0.29	7.65^{a}±0.37	7.70^{a}±0.27	7.70^{a}±0.26
AA-10	**6.75^{b}±0.37**	**5.75 ^{b}±0.42**	7.05^{a}±0.41	7.15^{a}±0.27	**6.75^{b}±0.44**
AA-30	**5.90 ^{c}±0.50**	**5.65^{b}±0.43**	**6.30^{b}±0.44**	**6.85^{b}±0.30**	**6.15^{b}±0.35**
AA-50	**5.10 ^{c}±0.64**	**4.95 ^{c}±0.62**	**4.35 ^{c}±0.61**	**5.90 ^{c}±0.49**	**4.40 ^{c}±0.55**

WF100 = Wheat flour (control), AA 10 = Amaranthus cruentus enriched at 10%, AA 30 = A. cruentus enriched at 30%, AA 50 = A. cruentus enriched at 50%. Figures are outlined as Mean ± Standard deviation. The mean within the same column not having a similar superscript varies (p≤0.05) while values with a similar superscript do not vary (p>0.05). Hedonic scale: nine = extremely like", eight = "too much", seven = "slightly like", six = moderately like", five = "like in neither dislike nor like (in between)", four = "slightly dislike", three = "moderately dislike", two = "very much dislike" while one = "extremely dislike".

CHAPTER 5: DISCUSSION

5.1 Proximate composition of enriched muffins with A. Cruentus flour

5.1.1 Dry Matter

The dry matter of all the enriched muffins was significantly higher than that of wheat flour muffins (control). The increase in dry matter content of the enriched muffins is due to available nutrients in amaranth flour compared to wheat flour. Dry matter presents the total nutrients within foodstuff which includes fat, carbohydrates, protein, vitamins, and minerals. The dry matter is described as the portion of the food remaining after all water has been removed. Dry matter content is associated with excellent food preparation qualities and extended shelf-life (Vuylsteke et al., 1997). Changes in cooking quality are related to changes in dry matter or non-starch components, considering the changes that occur in the characteristics of the starch such as swelling power, solubility, and gelatinization range rather than changes in the total starch content (Safo-Kantank and Osei-Minta, 1996). The high dry matter of enriched muffins is the same as the findings indicated by Biernacka et al (2022) that the dry matter content was high in leek-enriched pasta due to its high nutrient content.

5.1.2 Moisture content

The moisture of all the enriched muffins was significantly lower than that of wheat flour muffins (control). The low moisture content can be due to the lowering content of starch which disrupts the gluten network allowing water vapour to escape during baking (Milicevic et al., 2020; Roze et al., 2021). Additionally, the outcome of the study indicates that more fibre started to retain moisture at 50% enrichment, the moisture retention at 50% enrichment is because fibre fast tracks dough expansion time, considering non-starch polysaccharides need adequate time to absorb water before the dough reaches absolute uniformity (Onyango et al., 2022). The moisture represents the quantity of available water in food material and high moisture content signifies a short shelf life because of an increased microbial spoilage risk due to the presence of moisture (Thongram et al., 2016). Consequently, low moisture content enhances storage stability by preventing the growth of mould and reducing biochemical reactions (Awuchi, 2019). In addition, food products having a moisture content of 14% and above are prone to contamination by microorganisms especially mould and fungi (Manely, 2000). The result of the study corresponds to those outlined by Falsafi et al (2022) that the moisture value of RS4 formulated cookies was significantly lower in contrast to wheat flour cookies, this is due to the rigid structure of RS4 at elevated levels of crosslinking which minimises the capability to retain water molecules.

5.1.3 Ash content

The ash of all the enriched muffins was significantly higher than those of control, this could be attributed to the increase in mineral content due to the addition of amaranthus leaves flour (Twinomuhwezi et al., 2020). Higher ash content directly affects the muffin quality considering amaranthus flour is high in minerals (Nasir et al., 2020). Ash is the total inorganic residue left following the complete food oxidation of organic matter that provides a measure of total minerals in food (Ismail, 2017). The ash content influences the physicochemical properties of foodstuff changing the nutritional quality and water activity (Awuchi, 2019; Igual and Martinez-Monzo, 2022; Twinomuhwezi et al., 2020). The microbiological stability of foodstuffs can be enhanced by high mineral content considering that minerals can retard microbial growth (Twinomuhwezi et al., 2020) and affect the texture, taste, and appearance of food products. These results are the same as those of Zula et al (2020) in which bread supplemented with amaranthus had a significantly higher ash value compared to bread made with 100% wheat flour due to the increase in mineral contents.

5.1.4 Carbohydrates

The carbohydrate of all the enriched muffins was significantly lower than those of wheat flour muffins (control). The low carbohydrates could be due to the low content of carbohydrates in the added amaranthus flour compared to wheat flour (Hawa et al., 2018). A low level of carbohydrate in the form of starch is not desirable for gelatinization and product texture development (Malavi et al., 2022). Carbohydrates are described as macromolecules which include a group of sugar, starch, and cellulose which provide energy when consumed (Thongram et al., 2016). Carbohydrates are inevitable and crucial elements of day-to-day life; they add up to the bulk of our daily meals. (Twinomuhwezi et al., 2020). The benefits of increased carbohydrates of the enriched flours would make it a good source of energy in breakfast formulations and enriched can be used to combat protein-energy malnutrition (Awuchi, 2019). The results of the study are the same as those obtained by Hawa et al (2018) where carbohydrates were found to decrease with the addition of red teff and okara flour to cookies, the reduction was attributed to okara not being a source of good carbohydrates when compared to wheat and teff. All the enriched flours produced muffins with low carbohydrate content which would be beneficial to those who need low carbohydrate food leading to intensified health for overweight and obese persons (Hawa et al., 2018).

5.1.5 Fat content

There was no significant difference between the fat content of enriched muffins and control, this could be due to the very low-fat content in amaranthus leaves and wheat flour (Nyonje et al., 2021; Qumbisa et al., 2020). Furthermore, the findings of the study are contrary to the findings obtained when wheat flour was supplemented with seeds (Delgado-Garcia et al., 2022). According to Begum et al (2018), the addition of fat in baked products imparts tenderness resulting in pleasant palatability and it helps in gas retention by creating more airtight gluten. The content of fat, flour and sugar are important ingredients which affect the mechanical, structural, and textural properties etc of bakery products (Renzyaeva, 2013). Fat is a top caloric content when compared to protein and carbohydrates (Melese and Keyata, 2022). In human health, the high fat content intake has a negative effect as it can lead to the development of life-threatening chronic diseases such as diabetes, obesity, high blood pressure, heart conditions etc (Milicevic et al., 2020).

5.1.6 Energy Value

The energy content of muffins enriched with amaranthus flour was significantly lower than the control. The low energy content was due to the reduction in the amount of starch as a result of the enrichment (Laguna et al., 2011). In nutrition, starch plays a major role in supplying energy that enables the body to perform its functions (Liu, 2005). Energy in terms of food is indicated as energy released from carbohydrates, fats, protein, and other organic compounds (Jiang et al., 2014). The energy released by foods is a critical parameter in nutrition because numerous chronic diseases such as diabetes, cardiovascular disease and obesity have been associated with excess energy intake (Jiang et al., 2014). The results of the study support the findings by Zula et al (2020) that the gross energy of the bread significantly decreased when the percentage of amaranthus was increased, and the decrease could be due to the decrease in carbohydrates as amaranthus concentration is increased.

5.1.7 Protein

An increase in the percentage of enrichments with amaranthus flour gave rise to a significant reduction in the protein content only after 50% enrichment. The reduction in protein content is due to the low protein content of amaranthus leaves compared to wheat flour (control). Protein is important in the human diet because it aids in tissue maintenance, repair of muscles and maintenance of host defence mechanism (Rattan, 2019). A low protein diet in children restricts growth and results in low weight, and low Body Mass Index (BMI) at the infant stage to 6 years meanwhile, low protein in adults results in illnesses, infections, swelling legs, slow wound healing and high blood pressure during pregnancy which results in preeclampsia (Braun et al., 2016; Leal, 2022). Protein also contributes towards texture, and sensorial properties of food (Awuchi et al., 2019; Hui et al., 2006).

5.1.8 Dietary fibre

An increase in the percentage of enrichments with amaranthus flour gave rise to a significant increase in the dietary fibre content only for the 50% enriched muffins when compared to the control and the 10 and 30% enriched muffins. The increase in dietary fibre can be attributed to the high fibre content of amaranthus, up to 20,6 g/100 g compared to that of wheat (Nascimento et al., 2014; Tamsen et al., 2028). Dietary fibre is defined as the amount of non-digestive constituent of a foodstuff. Most pastries are rich in carbohydrates, fat, and calories, yet have less fibre content therefore, the addition of dietary fibre in enriched muffins is desirable (Mishra and Chandra, 2012). Fibre promotes digestion and has several positive outcomes on human health, such as mitigating the risk of diverticulum constipation, obesity, colon cancer, heart disease, and others (Raczyk et al., 2021). Mishra and Chandra (2012) reported that the fibre content of biscuits made from soy flour and rice bran had increased fibre content with an increase in soy and rice bran flour.

5.2 Physical properties of muffins enriched with amaranthus leaf flour

5.2.1 Height, diameter, and weight

Height: There was no significant difference in the height of the enriched muffins and wheat flour muffins (control), this could be ascribed to the inclusion of amaranthus flour that decreases the formation of gluten network (Nasir et al., 2020). During baking, the dough imperfectly developed gluten network failed to retain the leavened gas which resulted in a product with a lower volume and height due to low-fat content, which influenced the interfacial material from fat that did not produce muffins with fine structure (Kadir et al., 2022). The results are similar to those obtained by Akter et al (2023), whereby, there was no significant difference in the height of potato peel cake due to the high crude fibre percentage in potato peel flour.

Diameter: There was no significant difference in the diameter of the enriched muffins and control, this could be ascribed to the inclusion of amaranthus flour that decreases the formation of the gluten network (Akter et al., 2023; Nasir et al., 2020). The results are similar to those reported by Raihan and Saini (2017) that no significant difference was obtained among the diameter of the various cookies produced from wheat flour and other composite flours (amaranth, sorghum, and oats) at different formulations, this could be attributed to competition of ingredients and flour which fail to absorb water during dough mixing.

Weight: The weight of the Muffin was significantly reduced at 10% enrichment as a result of the low level of gluten present in the enriched muffins, which generated less carbon dioxide ($C0_2$) absorption in the dough during the baking process. As a result, the fermentation of protein, carbohydrates and fat was diluted and influenced the muffin resulting in less weight (Onoja et al., 2011). The outcome of the study is similar to those outlined by Koh et al (2023) that the reduction in weight of the cookies is due to moisture loss from the dough during the baking process as a result of partial replacement of rice flour with green tea leaf powder which had a low level of gluten.

5.2.2 Textural properties

The firmness of enriched muffins was significantly higher than that of control. This is because of the influence of high fibre in amaranthus leaf powder (Boff et al., 2022). Firmness is the strength required to bite, which is a vital characteristic for acceptance of a product (Boff et al., 2022; Ureta et al., 2014). Consumers prefer products with lower firmness since they indicate higher crispness properties as compared to high firmness which indicates a rigid structure which is undesirable (Melese and Keyata, 2022). Consequently, muffins with lower firmness are associated with freshness by consumers (Guine, 2022). The 50% enrichment is recommended because it is the enrichment with a low firmness (Guine, 2022). Only the 50% enriched muffins had a significantly lower brittleness compared to the control. This is because fibre in amaranthus flour will interrupt the gluten network and reduction in starch to form a high-fibre network thereby reducing the brittleness (Boff et al., 2022). Brittleness is defined as the capability of objects to fracture when subdued to stress such as the distance travelled by a blade through the muffin before it fractures (Conte et al., 2021). The enrichment did not give rise to any significant difference in the cohesiveness and elasticity of enriched muffins. This is because the stickiness and elasticity of the amaranthus flour and wheat flour dough were not different. The results are similar to those obtained by Gomes et al (2022) that there was no significant difference between muffins made with different flour at different ratios of resilience and elasticity.

5.2.3 Colour properties of muffins

The lightness and yellowness of muffins enriched with amaranthus flour increased significantly compared to those of wheat flour muffins while the redness increased only at 50%. The increase of lightness and yellowness could be attributed to anthoxanthin (flavonoid) which is white or pale yellowish, found in plant cell sap. (Vaclavik et al., 2008). Colour and appearance are mainly the first considerations that consumer’s minds pay attention to (Youssef et al., 2022). In addition, food colour is very persuasive considering it has the potential to alter how consumers discern taste and food quality, and also provide some indicator of the quality in terms of the main ingredients (Okin et al., 2021). The colour of the muffins suggests the presence of pigments known to occur in food, such as anthocyanins and anthoxanthin etc (Boff et al., 2022; Vaclavik et al., 2008).

5.3 Amino acids composition

The concentration of essential amino acids such as histidine, threonine, methionine, leucine, and lysine significantly increased from 30% to 50% enrichment while that of valine, isoleucine and phenylalanine significantly increased from 10% to 50% enrichment, compared to those of wheat flour muffins. Similarly, the concentration of non-essential amino acids such as alanine, glycine, and aspartic acid significantly increased from 30% to 50% enrichment while that of serine, tyrosine proline and arginine significantly increased from 10% to 50% enrichment, compared to those of wheat flour muffins. The increase could be attributed to amaranthus flour having high concentrations of these amino acids such as leucine, phenylalanine, and lysine (Gebriel et al., 2020). Amino acids are the building blocks of protein and serve as intermediate compounds in various metabolic pathways (Kari et al., 2022). The findings of the current study are the same as those of Liu et al. (2022) in which the amino acids composition of amaranth composite cookies was found to be significantly higher in isoleucine, lysine, and threonine when compared to wheat flour cookies. The consumption of enriched muffins can boost the uptake of essential amino acids that the body requires to function optimally (Kari et al., 2022). Essential amino acids are those amino acids that the human body is unable to generate and must be obtained from external sources while non-essential amino acids are described as the ones the body can generate Kadey (2021). Food supplements with non-essential amino acids such as serine are significant since serine performs a vital role in cell signalling while glycine adds toward metabolic regulation and promotes protein synthesis in the human body (Kari et al, 2022). The 50% enrichment is recommended in terms of providing a high concentration of amino acids.

5.6 Mineral composition of enriched muffins

The concentration of Ca, Mg, K, Na, P and Zn increased significantly in enriched muffins progressively from 10%, 30% to 50% enrichment when compared to control. The concentration of Cu, Mn and Fe in the enriched muffins significantly increased only at 50% enrichment. The increase in minerals can be attributed to the blended amaranthus leaf flour considering amaranthus has higher mineral content in contrast to wheat flour (Mishra and Chandra, 2012). Minerals are inorganic elements that are essentially in the body to carry out important functions such as transmitting nerve impulses, bone building and growth of cells and tissues in the body (Gharibzahedi and Jafari, 2017; Zoroddu et al., 2019). Minerals are classified as macro and micro according to the amount present and required in the body to maintain health. Macro minerals include magnesium, calcium, phosphorus, potassium sodium, chlorine, and sulphur while micro minerals include zinc, iron, copper, and manganese (Zoroddu et al., 2019). Amaranthus cultivars are reported to contain high minerals such as Ca, Mg, P, Zn, Fe, K, Cu, Zn and Mn (Beswa et al, 2016). In addition, amaranthus leaves are reported to contain calcium two times that of milk which might help reduce the risk of osteoporosis and other calcium deficiencies (Sushil and Suneeta, 2018). Humans require more than 22 mineral elements including zinc, iron, calcium etc. that can be supplied through various diets (Oyetayo, 2023).

5.7 Vitamin C and E composition of enriched muffins

There was a remarkable increment in the concentration of vitamin C and E in enriched muffins compared to that of control. The increase in vitamin C and E could be a result of the high levels of vitamin C and E in amaranthus leaves (Narwade and Pinto, 2018). Vitamins are defined as a wide group of organic compounds required for maintaining human health which can be obtained from diet (Oblong and Jansen, 2022). Vitamins support human health by inducing immune activation at the optimal level (Kocabas, 2022). Vitamin C is known for its antioxidant activities that neutralise free radicals that can cause heart, cancer, and other diseases (Chambial et al., 2013). The results of the study support the findings by Igile et al. (2013) that indigenous vegetables are potentially good sources of vitamins, and consumption of foods with high vitamin C (Kocabas, 2022). Vitamin E is a group of fat-soluble compounds that include tocotrienols and four tocopherols (alpha, beta, gamma, and delta) found in food (Frances and Johnson, 2022; Lee and Han, 2018). Vitamin E is known for being effective in the prevention of various disease complications, which can be attributed to its function as an antioxidant that helps lower inflammation in the eyes and reduces the risk of heart attacks (Rizvi et al., 2014).

5.8 Consumer acceptance of muffins enriched with A. cruentus flour.

There was a significant decrease in colour, aroma, taste, texture, and overall acceptability of the 10%, 30% and 50% enriched muffins compared to those of control, but taste and texture acceptability were not significantly different. The significant decrease in colour acceptance by the panellist could be due to the colour of plant pigments in the amaranthus flour which consumers are not acquainted with when compared to the lighter colour of the traditional muffins (control) (Nasir et al., 2020; Zula et al., 2020). Although the panellist accepted 100% wheat flour muffins, the 10% enrichment was the most accepted in terms of taste and texture. The significant decrease in taste and aroma acceptability could be due to the leafy flavour and taste of amaranthus in the enriched muffins (Sampson and Assuah, 2016). Product aroma is a sensation perceived through the nose and most products attract consumers as a result of their aroma. The results are similar to those reported by Sampson and Assuah (2016), where there was a significant decrease in acceptance of aroma at 30% and 50% of muffins enriched with unpolished brown rice compared to wheat muffins. The significant decrease in the acceptance of taste by the panellist could be due to the taste of phytochemicals such as leaf pigment (chlorophyll, betacyanin and carotenoids), phenolic, flavonoids and vitamin C present in amaranth leaves (Sarker et al., 2020). Taste is an indicator of palatability and influences the overall acceptance of the product (Javed et al., 2022). The result of the current study is similar to those obtained by Ukeyima et al (2019), where the acceptability taste of the cookies decreased when the substitution with soybean was increased.

The significant decrease in texture acceptability could be due to the interference of fibres in amaranth that interfere with the structure of the product (Boff et al., 2022). The low-fat content of the enriched muffins resulted in a chewy texture and low moisture content as compared to wheat flour muffins (Zoulias et al., 2002). The findings of the study are similar to those reported by Okin et al., (2021) where cookies made from wheat flour (control) had the highest texture rating as compared to composite cookies. The overall acceptability of enriched muffins was significantly low from 10% enrichment but there was no significant difference between those of the 10 and 30% enrichment. The overall acceptability is significant for priming consumers' acceptability of a product (Fiorentini et al., 2022). The muffins enriched with amaranthus were perceived as unconventional when compared to wheat flour muffins (Fiorentini et al., 2022). The results of the study are supported by those of Beswa et al (2016) where an increase in the amount of amaranthus leaf flour in snacks decreased the overall likeness significantly.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusions

For the proximate composition of the muffins, the dry matter, and ash were significantly higher in enriched muffins as compared to the control. There was no significant difference in the fat content of the enriched muffin and the control. The energy, moisture and carbohydrate values for the enriched muffins were significantly lower than those of the control. In terms of physical properties, supplementation with amaranthus leaf the enrichment of amaranth did not significantly affect the height and diameter, but the weight of the muffin was significantly reduced at 10% enrichment. For textural properties, the firmness of the enriched muffins was significantly higher than that of the control while 50% enrichment resulted in lower brittleness as compared to the control. The enrichment did not give rise to any significant difference in terms of the cohesiveness and elasticity of the muffins. The lightness (Hunter L value) and yellowness (Hunter b value) of the enriched muffin were significantly higher than those of the control.

The amaranthus-enriched muffins have enhanced nutritional composition in terms of amino acids, minerals, and vitamin content compared to wheat flour muffins (control). The concentration of Ca, Mg, K, Na, P and Zn significantly increased in enriched muffins progressively from 10, 30 to 50% enrichment when compared with those of wheat flour muffins (control). There was a significant increase in the concentration of vitamin C and vitamin in enriched muffins compared to those of wheat flour muffins. In terms of consumer acceptance, there was a significant reduction in colour, aroma, texture taste and overall acceptability for 30% and 50% enriched muffins compared to those of wheat flour muffins (the control). Consumers moderately liked the 10% enriched muffin. The study demonstrates that amaranthus (*Amaranthus Cruentus-Arusha*) leaf flour can be used to produce muffins with better physico-chemical and acceptable consumer attributes.

6.2 Recommendation

Based on the findings of the study, the researcher recommends the following:

- The 10% enrichment is recommended to produce muffins with high nutritional content to boost the consumption of amaranthus.
- The 10% enrichment can be studied further in terms of how the sensory properties can be improved for youth consumption which would eliminate poor stigmatisation of amaranthus leafy vegetables.
- The amaranthus *cruentus* can be researched further to investigate the potential of commercialisation which is important in addressing constraints in terms of cultivation and economic potential.

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APPEnDIX A: Ethics approval letter

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 11/05/2021

Dear Ms Nkwana

NHREC Registration # : REC-170616-051
REC Reference # : 2017/CAES/023
Name : Ms AM Nkwana
Student #: 49371908

Decision: Ethics Approval Confirmation after First Review from 01/02/2017 to completion

Researcher(s): Ms AM Nkwana
49371908@mylife.unisa.ac.za

Supervisor (s): Prof FT Tabit
tabitft@unisa.ac.za; 011-471-2080

Working title of research:

Properties of the flour blends and baked muffins products of amaranth

Qualification: MSc Agriculture

Thank you for the submission of your yearly progress report to the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is confirmed to continue for the originally approved period, **subject to submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for next progress report: 30 April 2022

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*The **low risk application** was originally **reviewed** by the UNISA-CAES Health Research Ethics Committee on 01 February 2017 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2017/CAES/023** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

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Tel: (011) 471-3649

APPENDIX B : CONSUMER SENSORY EVALUATION QUESTIONNAIRE

Section 1: **Socio-biographic details of respondents**

Name: ____

Race: ____

Gender:

____

Age____

Tell:

____

Alternative Tell: ____

E-mail address: ____

Section 2: Screening questions

1. Regular buyers of muffins Yes/No
2. Specify types of muffins
3. How often do you consume muffins?

At least once every two weeks	
At least once every three weeks	
At least once a month	
More than once a month	
Never	Closed

4. **What is your work status**?

	Unemployed
	Do not work – student
	Do not work – housewife
	Work part time (8-29 hours per week)
	Work full time (30+ hours per week)

5. **Which industry do you work in?**

CLOSE	Market research industry
CLOSE	Advertising company
	Other

6. **Which industry does your family or siblings work in?**

CLOSE	Market research industry
CLOSE	Advertising company
	Other

7. **Which industry does your family or siblings work in?**

CLOSE	Market research industry
CLOSE	Food industry
	Other

8. **Are you good in English?**

	Yes
CLOSE	No

Section 2: Muffin taste Time Schedule

1. Muffin Tasting invitation.

Time	
Date	
Address	

APPENDIX C: CONSUMER ACCEPTABILITY OF AMARANTH/WHEAT FLOUR COMPOSITE MUFFINS.

Score card for consumer testing of muffins

Name…………………………………………………………………………….

Date…………………………………………………………………………….

Set No…………………………………………………………………………

Session number Code……………………………………………………….

Age…………………………………………………………………………….

South African *Yes/No*

Please rinse take a sip of water before and in between tasting the samples.

Please evaluate this amaranth/wheat flour composite muffin for the selected characteristics in the order given from **LEFT** to **RIGHT**

Please make a cross on the line in the box which indicates how well you like it.

Product code: ………………………

Hedonic scale	Colour	Aroma	Taste	Texture	Overall acceptability
Like extremely	_____	____	____	____	______
Like very much	_____	____	____	____	______
Like moderately	_____	____	____	____	______
Like slightly	_____	____	____	____	______
Neither like nor dislike	_____	____	____	____	______
Dislike slightly	-------	-------	-------	--------	---------
Dislike moderately	_____	____	____	____	______
Dislike very much	_____	____	____	____	______
Dislike extremely	_____	____	____	____	______

Thank you for your response. Please complete the questionnaire.

APPENDIX D CONSENT FORM

TITLE OF RESEARCH PROJECT

PROPERTIES OF THE FLOUR BLENDS AND BAKED MUFFINS PRODUCTS OF AMARNTH.

Dear Mr/Mrs/Miss/Ms ________________________________ Date....…/...…..... /20......

NATURE AND PURPOSE OF THE STUDY

This aim of this study is to determine the sensory properties and consumer acceptability of amaranth/wheat flour composite muffins...

RESEARCH PROCESS

The researcher will interview people who patronise and eat muffins at least once a month regarding the acceptability of different amaranth muffin composites. Your socio-demographic information such as gender, age, marital status, and mode of income generation will also be recorded.

CONFIDENTIALITY

Your ratings and assessments of any of the research instruments as well as your opinions are viewed as strictly confidential, and only members of the research team will have access to the information.
No data published in dissertations and journals will contain any information by means of which you may be identified. Your anonymity is therefore ensured.

WITHDRAWAL CLAUSE

I understand that I may withdraw from the study at any time. I therefore participate voluntarily until such time as I request otherwise.

POTENTIAL BENEFITS OF THE STUDY

Amaranthus is a traditional staple root crop in South Africa and is currently underutilised. Therefore, developing amaranth composite muffins will add value to amaranth, improve its image and encourage farmers to grow more amaranth which will result to income generation and ensuring food security in the rural communities. Furthermore, a nutrient rich amaranth/wheat flour composite muffin will improve the utilisation as well as the commercial value of the amaranth plants.

INFORMATION (contact information of your supervisors)

If there is any question concerning this study contact the following: DR Frederick Tabit, 0114712080, Department of Life and Consumer Sciences, University of South Africa?

CONSENT

I, the undersigned, ………………………………………………………………… (Full name) have read the above information relating to the project and have also heard the verbal version and declare that I understand it. I have been afforded the opportunity to discuss relevant aspects of the project with the project leader, and hereby declare that I agree voluntarily to participate in the project.

I indemnify the university and any employee or student at the university against any liability that I may incur during the course of this project.

I further undertake to make no claim against the university in respect of damages to my person or reputation that may be incurred because of the project/trial or through the fault of other participants, unless resulting from negligence on the part of the university, its employees, or students.

I have received a signed copy of this consent form.

Signature of participant: ....

Signed at ………………………………… on ……………………………………………

WITNESSES

1 ....

2 ....