

Thesis  
entitled

**The potential of *Vigna unguiculata* and *Cucurbita maxima* as complementary food ingredients in soup formulations**

submitted

by

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## DECLARATION

I, Mungofa Nyarai of student number 44581998, hereby declare that the dissertation titled “The potential of Vigna unguiculata and Cucurbita maxima as complementary food ingredients in soup formulations”, which I submit for the degree of Doctor of Philosophy in Consumer Science at the university of South Africa, is my own work and has not previously been submitted for a degree at this or any other University. I declare that sources, materials, and results that are not original to this thesis are fully cited and referenced. I declare that during my study I have adhered to the Research Ethics Policy of the University of South Africa and have not acted out of the guidelines (2019/CAES/076) (Appendix 1).

I declare that the content of my dissertation has been submitted through “Turn-it-in” and edited for language before the final submission for examination.



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Date: 23 January 2023

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*“All soup is soul food.”*  
**Bee Wilson**

## **DEDICATION**

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## ABSTRACT

Cowpea and pumpkin have great nutritional potential but remains underutilised. They have diverse health enhancing properties and have potential to be processed into various products both for home and industrial use. In this study, the physical properties, functional properties, rheological properties, proximate composition, total phenolic and consumer acceptability of cowpea-pumpkin composite soup were investigated. Cowpea leaves, cowpea seeds, pumpkin fruit, pumpkin seeds and pumpkin flowers were chosen for this study because of their nutritional value, health promoting properties, their possibility to be incorporated into various culinary preparations like soups and simply because both cowpea and pumpkin are considered sustainable crops. Three soup formulations (Formulation 1-cowpea leaf powder and pumpkin seed powder, Formulation 2 -cowpea seed powder and pumpkin fruit powder and Formulation 3- cowpea leaf powder and pumpkin flower powder) were developed at different levels (5%, 10% and 15%). A control sample (carrot granules, potato powder, onion powder, mushroom powder, ungelatinised corn starch (tasteless), hydrogenated fat, salt, brown sugar and tomato powder) was also formulated for reliability of the results. The viscosity for F2 samples was the highest (1209.7-3116.0 cP) which increased with addition of composite mix at different levels higher than control, F1 and F3 samples (211.0 - 889.0 cP). Control, F1 and F3 samples exhibited a lower rehydration ratio (RR) (7.7-10.7 g/g) compared to F2 samples with a very high RR (11.3-14.7 g/g). F2 had the highest water absorption capacity (WAC) (252.0-263.3 g/g) while F1 and F2 had similar mean values (1.85.7-214.0 g/g) and control had the lowest WAC (65.7 g/g). Significant variations in swelling power were observed among cowpea-pumpkin soup mixes where F1 with 5% composite mix exhibited the lowest mean value (349%) while F3 with 15% composite mix showed the highest mean value (471%). There was no significant difference in the mean values for the solubility index (22.0-49.7%). Higher dispersibility mean values were observed in the control (62.7%) compared to that of the experimental soup samples (F1, F2 and F3) which ranged from 22.7 to 61.3%. Cowpea-pumpkin composite exhibited high protein content with mean values ranging from 22.33 to 28.33 g/100 g at all levels of composite mix which was higher than the control with the lowest mean value of 13.33 g/100 g. The control sample had carbohydrate content of 70.42 g/100 g which was approximately higher than those of F1, F2 and F3 samples which had mean values of 44.06 to 58.00 g/100 g. The Crude fibre had mean values ranging from 5.98 to 17.39 g/100 g, moisture 7.78 to 9.74 g/100 g, fat 1.73-3.10 g/100 g and ash 0.35-0.57 g/100 g. Cowpea leaf powder imparted a dark green colour to the experimental soup samples, and this resulted in an undesirable effect on the overall acceptability of F1 and F3 samples while pumpkin gave an attractive golden-brown colour to the soup in F2 soup samples. The control sample was highly accepted in all sensory attributes

tested in this study. Total Phenolic Content (TPC) varied from 0.25 to 0.63 mg/GAE/100 g. Formulation 3 (cowpea leaf and pumpkin flower) had the highest TPC 0.54 – 0.63 mg/GAE/100 g followed by variations of formulation 2 (cowpea seeds and pumpkin fruit) with 0.32-0.54 mg/GAE/100 g, formulation 1 was the lowest with 0.25-0.32 mg/GAE/100 g. A total of 47 flavonoid compounds belonging to the flavones, chalcones, anthocyanins, flavanols, flavanones were detected in the formulation variations and control samples. The highest number of these flavonoid compounds (42) were detected in Formulation 2 followed by formulation 3 with 28 compounds while the lowest number of compounds was detected in formulation 1 (24).

The findings of this study suggest that cowpea and pumpkin possess physical and functional characteristics for application in product formulations in the food industry. They are important vegetables in people's diets because they are rich in nutrients like protein and carbohydrates, good sources of antioxidants which could be due to the presence of polyphenol compounds. Thus, they can play a pivotal role for addressing the stress of protein-energy malnutrition by complementing less nutritious staple foods.

**Keywords:** Soup, Composite mix, Cowpea, Formulation, Pumpkin, Soup

## OPSOMMING

Cowpea en pampoen het groot voedingspotensiaal, maar blyonderbenut. Hulle het uiteenlopende gesondheidsverbeterende eienskappe en het potensiaal om in verskeie produkte verwerk te word vir beide huishoudelike en industriële gebruik. In hierdie studie is die fisiese eienskappe, funksionele eienskappe, reologiese eienskappe, naaste samestelling, totale fenoliese en verbruikersaanvaarbaarheid van cowpea-pampoen saamgestelde sop ondersoek. Cowpea-blare, cowpea-sade, pampoenvrugte, pampoenpitte en pampoenblomme is vir hierdie studie gekies weens hul voedingswaarde, gesondheidsbevorderende eienskappe, hul moontlikheid om in verskeie kulinêre voorbereidings soos sop ingewerk te word en bloot omdat beide cowpea en pampoen as volhoubaar beskou word gewasse. Drie sopformulerings (Formulasie 1-koei-ertjieblaarpoeier en pampoensaadpoeier, Formulering 2 -koeiertjie-saadpoeier en pampoenvrugpoeier en Formulering 3-koeiertjieblaarpoeier en pampoenblompoeier) is op verskillende vlakke ontwikkel (5%, 10% en 15%). 'n Kontrolemonster (wortelkorrels, aartappelpoeier, uiepoeier, sampioenpoeier, ongegelatineerde mieliestysel (smaakloos), gehidrogeneerde vet, sout, bruinsuiker en tamatiepoeier) is ook geformuleer vir betroubaarheid van die resultate. Viskositeit vir F2-monsters was die hoogste (1209.7-3116.0 cP) wat toeneem het met byvoeging van funksionele saamgestelde mengsel op verskillende vlakke hoër as kontrole, F1 en F3 monsters (211.0-889.0 cP) Kontrole, F1 en F3 monsters het 'n laerrehidrasie verhouding (LV) (7.7-10.7) in vergelyking met F2 getoon monsters met 'n baie hoë RR (11.3-14.7). F2 het die hoogste water absorpsie kapasiteit (WAK) (252.0-263.3g/g) terwyl F1 en F2 soortgelyke gemiddelde waardes (1.85.7-214.0 g/g) gehad het en kontrole die laagste WAK (65.7 g/g). Daar was beduidende variasies in swelkrag waargeneem onder koei-ertjie-pampoen sopmengsels waar F1 met 5% saamgestelde mengsel die laagste gemiddelde waarde (349%) getoon het terwyl F3 met 15% saamgestelde mengsel die hoogste gemiddelde getoon het waarde (471%) Koeiertjie-pampoensop het hoë waardes van swelkrag gehad wat wissel van (349.0-471.0%). Daar was geen betekenisvolle verskil in die gemiddelde waardes vir die oplosbaarheidsindeks (22.0 - 49.7%). Hoër verspreidingsgemiddelde waardes is in die kontrole (62.7%) waargeneem in vergelyking met dié van die eksperimentele sopmonsters (F1, F2 en F3) wat gewissel het van 22.7 tot 61.3%. Cowpea-pampoen-komposiet het hoë proteïënhoud getoon met gemiddelde waardes wat wissel van 22.33-28.33 g/100 g op alle vlakke van funksionele saamgestelde mengsel wat hoër was as die kontrole met die laagste gemiddelde waarde van 13.33 g/100 g. Die kontrolemonster het koolhidraatinhoud van 70.42 g/100 g gehad wat ongeveer hoër was as dié van F1, F2 en F3 monsters wat gemiddelde waardes van 44.06 - 58.00 g/100 g gehad het. Die Ruvesel het



gemiddelde waardes gehad wat wissel van 5.98 – 17.39 g/100 g, vog 7.78-9.74 g/100 g, vet 1.73 – 3.10 g/100 g en as 0.35 – 0.57 g/100 g. Cowpea het 'n donkergroen kleur aan die eksperimentele sopmonsters verleen, en dit het gelei tot 'n ongewenste effek wat die algehele aanvaarbaarheid van F1- en F3-monsters beïnvloed het, terwyl pampoens 'n aantreklike goudbruin kleur aan die sop in F2-sopmonsters gegee het was hoogs aanvaar in alle sensoriese eienskappe wat in hierdie studie getoets is. Hoë aanvaarbaarheid is opgemerk vir kontrolemonster. Totale fenoliese inhoud (TFI) het gewissel van 0.25 tot 0.63 mg/GAE/ml. Formulering 3 (koei-ertjieblaar en pampoensblom) het die hoogste TPC 0.54 – 0.63 mg/GAE/ml gehad, gevolg deur variasies van formulering 2 (koei-ertjiepitte en pampoensvrugte) met 0.32-0.54 mg/GAE/ml, formulering 1 was die laagste met 0.25 – 0.32 mg/GAE/ml. Die hoogste aantal flavonoïedverbindings (42) is in Formulering 2 en Formulering 3 (28) opgespoor terwyl die laagste aantal in Formulering 1 (24) opgespoor is.

Die bevindinge van hierdie studie dui daarop dat koei-ertjies en pampoens fisiese en funksionele eienskappe besit vir toepassing in produk formulering in die voedselindustri. Hulle is belangrike groente in mense se diet omdat hulle ryk is aan voedingstowwe soos proteïene enkoolhidrate, goeie bronne van antioksidante wat te wyte kan wees aan die teenwoordigheid van polifenolverbindings. Hulle kan dus 'n deurslaggewende rol speel om die stres van proteïen-energie wanvoeding aan te spreek deur minder voedsame stapelvoedsel aan te vul.

**Slutelwoorde:** Saamgestelde mengsel, Cowpea, Formulering, Pampoens, Sop

## NAGANWAGO

Cowpea le mokopu di na le bokgoni bjo bogolo bja phepo eupša di dula di sa šomišwe gabotse. Di na le dithoto tša go fapafapana tša go godiša maphelo gomme di na le bokgoni bja go šongwa go ba ditšweletšwa tša go fapafapana bobedi bakeng sa tšhomišo ya ka gae le ya intasteri. Thutong ye, dithoto tša mmele, dithoto tša mošomo, dithoto tša rheological, sebopego sa kgauswi, palomoka ya phenolic le kamogelo ya bareki yamafelo a sopo ya motswako wa cowpea-pumpkin di nyakišišitšwe. Matlakala a cowpea, dipeu tša cowpea, dienywa tša magapu, dipeu tša magapu le matšoba a mokopu di kgethetšwe nyakišišo ye ka lebaka la mohola wa tšona wa phepo, dithoto tša go tšwetša pele maphelo, kgonagalo yatšona ya go tsenywa ka gare ga ditokišetšo tša go fapafapana tša culinary go swana le sopo le fela ka lebaka la gore bobedi cowpea le mokopu di tšewa bjalo ka tše di swarelelago merogo. Ditlhamo tše tharo tša sopo (Sebopego sa 1-phofo yamatlakala a cowpea le phofo ya peu ya mokopu, Sebopego sa 2 phofo ya peu ya peu yakgomo le phofo ya dienywa tša mokopu le Sebopego sa 3- phofo ya matlakala a cowpea le phofo ya matšoba a mokopu) di hlamilwe ka maemo a go fapana (5%, 10% le 15 %). Sampole ya taolo (digranules tša dikharoti, phofo ya ditapola, phofo ya onion, phofoya di-mushroom, setatšhe sa mabele seo se sa gelatinized (se se nago tatso), makhura a hydrogenated, letswai, swikiri ye soothoand phofo ya tamati le yona e ile ya hlangwa bakeng sa go botega ga dipoelo. Viscosity ya F2 samples was the highest (1209.7-3116.0 cP) yeo e oketšegilego ka tlaleletšo ya motswako wa motswako wa mošomo maamong a fapanego a godimo go feta disampole tša taolo, F1 le F3 (211.0-889.0 cP). Disampole tša Taolo, F1 le F3 di bontšhitše tekanyo ya fase ya rehydration (RR) (7.7-10.7) ge e bapetšwa le F2 disampole tše di nago le RR ye e phagamego kudu (11.3-14.7). F2 e be ena le bokgoni bjo bo phagamego bja go monya meets (WAC) (252.0 - 263.3 g/g) mola F1 le F2 di be di na le dikelo tša magareng tše di swanago (1.85.7-214.0 g/g) gomme taolo e be ena le ya fase kudu WAC (65.7 g/g). Go bile le diphapano tše kgolo ka maatla a go ruruga di lemogilwe gare ga metswako ya sopo ya cowpea-pumpkin moo F1 ka 5% ya motswako wa motswako e bontšhitše go boleng bja magareng bja fase kudu (349%) mola F3 ka 15% ya motswako wamotswako e bontšhitše magareng a godimodimo boleng (471%) Sopo ya cowpea-mokopu e be ena le dikelo tše di phagamego tša maatla a go ruruga go tloga go (349.0 - 471.0%). Go be go se na phapano ye kgolo ka dikelo tša magareng tša tšhupamabaka ya go qhibidiga (22.0 - 49.7%). Ditekanyetšo tša magareng tša go phatlalala tša godimo di iletšabonwa ka taolo (62.7%) ge di bapetšwa le tša dišupommu tša sopo yaditeko (F1, F2 le F3) tše di bego di tloga go 22.7 go ya go 61.3%. Motswako wa dierekisi-mokopu o bontšhitše diteng tša godimo tša proteine ka dikelo tša magareng go tloga go 22.33-28.33g/100 g maamong ka moka a motswako wa

motswako wa mošomo wo o bego o le godimo go feta taolo ka boleng bja magareng bja fase bja 13.33 g/100 g. Sampole ya taolo e be ena le diteng tša dikhapohaedreite tša 70.42 g/100 g which e bego e ka bagodimo go feta tšeo tša disampole tša F1, F2 le F3 tšeo di bego di na le dikelo tša magareng tša 44.06 - 58.00 g/100 g. Faeba ya Crude e be ena le dikelo tša magareng go tloga go 5.98-17.39 g/100 g, monola 7.78-9.74 g/100 g, makhura 1.73-3.10 g/100 g le molora 0.35-0.57 g/100 g. Phofa ya matlakala a cowpea e file mmala wo motala wo mohwibidu go dišupommu tša sopo ya diteko, gomme se se feleleditše ka gore se se sa rategego seo se ilegosa ama mafelelo a go amogelega ka kakaretšo ga dišupommu tša F1 le F3 mola mokopu o file mmala wo o kgahlišago wa gauta-sootho go sopo ka disampole tša sopoya F2. Sampole ya taolo e ile ya amogelwa kudu dika ka moka tša dikwi tšeo di lekilwego nyakišišong ye. Go amogelegamo go phagamego go ile gwa lemogwa bakeng sa sampole ya taolo. Palomoka ya Diteng tša Phenolic (TPC) e be e fapana go tšwa go 0.25 go ya go 0.63 mg/GAE/mL. Sebopego sa 3 (letlakala la cowpea le letšoba la mokopu) se be se na le TPC ya godimodimo 0.54 – 0.63 mg/GAE/mL yeo e latetšwego ke diphetogo tša tlhamo ya 2 (dipeu tša marekisi le dienywa tša mokopu) ka 0.32-0.54 mg/GAE/mL, tlhamo ya 1 e be e le ya fase kudu ka 0.25-0.32 mg/GAE/mL. Palo ya godimodimo ya metswako ya flavonoid (42) e lemogilwe ka go Tlhamo 2 le tlhamo 3 (28) mola palo ya fase kudu e lemogilwe ka go tlhamo 1 (24). Dikutollo tša nyakišišo ye di šišinya gore cowpea le mokopu di na le dimelo tša mmele le tša mošomo tša tirišo ka ditlhamong tša ditšweletšwa ka intastering ya dijo. Ke merogo ye bohlokwa ka dijong tša bathoka gobane e humile ka phepo ya go swana le proteine le dikhapohaedreite, methopo ye mebotseya di-antioxidant tšeo di ka bago ka lebaka la go ba gona ga metswako ya polyphenol. Ka gona, di ka kgatha tema e bohlokwa bakeng sa go rarolla kgateletšego yaphepo-mpe yamatla a diprotheine ka go tlaleletša dijo tše di tlwaelegilego tšeo di se nago phepo e ntši.

**Mantšu a bohlokwa:** Motsoako o kopaneng, Cowpea, Sebopeho, Mokopu, Sopho

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Review

## Prospective Role of Indigenous Leafy Vegetables as Functional Food Ingredients

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**Abstract:** Indigenous leafy vegetables (ILVs) play a pivotal role in sustaining the lives of many people of low socio-economic status who reside in rural areas of most developing countries. Such ILVs contribute to food security since they withstand harsher weather and soil conditions than their commercial counterparts and supply important nutrients such as dietary fibre, vitamins and minerals. Furthermore, ILVs contain bioactive components such as phenolic compounds, flavonoids, dietary fibre, carotene content and vitamin C that confer health benefits on consumers. Several studies have demonstrated that regular and adequate consumption of vegetables reduces risks of chronic conditions such as diabetes, cancer, metabolic disorders such as obesity in children and adults, as well as cardiovascular disease. However, consumption of ILVs is very low globally as they are associated with unbalanced and poor diets, with being food for the poor and with possibly containing toxic heavy metals. Therefore, this paper reviews the role of ILVs as food security crops, the biodiversity of ILVs, the effects of processing on the bioactivity of ILVs, consumer acceptability of food derived from ILVs, potential toxicity of some ILVs and the potential role ILVs play in the future of eating.

**Keywords:** indigenous leafy vegetables; food security; processing practices; derivative foods; future of eating



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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BHA	Beta hydroxy acid
BHT	Butylated hydroxytoluene
FeCl <sub>2</sub>	Ferrous chloride
HCl	Hydrochloric acid
IFT	Institute of Food Technologists
ILV	Indigenous leafy vegetables
L	litre
m/v	mass/volume
mg	milligram
mL	millilitre
PCA	Principal component analysis
pH	$-\log_{10}c$ , where c is the hydrogen ion concentration in moles/litre
ROS	Reactive oxygen species
rpm	Revolutions per minute
RR	Rehydration ratio
S/N	Serial number
SC	Swelling capacity
SI	Swelling index
SPSS	Statistical Package for the Social Sciences
SST	Soft solids tester
TPC	Total phenolic content
UNISA	University of South Africa
UV-Vis	Ultraviolet - visible
WAC	Water absorption capacity

# CHAPTER 1

## INTRODUCTION

### 1.1 Background to the Study

Vegetables are a key component of a balanced diet and the main drivers in achieving global nutritional security by providing nutrients, vitamins and minerals (Jena et al., 2018). Globally, there are over 7000 plant species of indigenous leafy vegetables (ILVs) cultivated or harvested from the wild for food (Schonfeldt & Pretorius, 2011). The sub-Saharan region is a natural habitat for more than 4000 of these plant species (Muhanjiet al., 2011) and South Africa alone has more than 100 different species of ILVs used for food, including cat's whiskers (*Cleome gynandra*), cowpea (*Vigna unguicula*), wild okra (*Corchorus olitorius*), pumpkin (*Cucurbita maxima*) and lamb's quarters (*Chenopodium album*) (Dweba & Mearns, 2011).

Cowpea and pumpkin are leafy vegetables grown and consumed by people mostly in the Limpopo Province of South Africa (Faber et al., 2010; Hart & Vorster, 2006; Uusiku et al., 2010; Van Rensburg et al., 2007; Vorster et al., 2007). The commonly used parts of cowpea and pumpkin plants are their leaves. However, all parts of these plants are edible, as cowpea also produces nutritious tender green leaves, immature green pods and nearly mature fresh-shelled grains (Wafula et al., 2016; Timko et al., 2007; Mamiroet al., 2011). A flour can be prepared from dry, mature cowpea seeds and processed into various foods such as cakes, bread and couscous and can also be used as a complementary food ingredient (Harouna et al., 2018; Kirigiaet al., 2018). Equally, pumpkins provide nutritious leaves and flowers which may be cooked and consumed as vegetables whilst the pumpkin fruit additionally has seeds which may also be cooked, roasted or milled into powder for incorporation into various food formulations (Dyshlyuk et al., 2017; Rezig et al., 2016; Harouna et al., 2018). Nutritionally, both cowpea and pumpkin are known to contain high levels of nutrients (Oliveira et al., 2016; Gido et al., 2017; Vu & Nguyen, 2017). The high nutritional value of cowpea and pumpkin suggest their potentials in developing nutrient-dense foods including soups which may be used to compliment starchy meals like maize meal porridge.

White maize meal porridge is a commonly consumed food in South Africa with a reported 460 g maize being consumed per person per day in the Eastern Cape Province (Shephard et al., 2002). However, this staple food is deficient in some essential nutrients

(Świątkiewicz&Bojanowski, 2004; Johnson, 2000; Menkiret al., 2008; Nuss & Tanumihardjo, 2011; Jin et al., 2013; Rai et al., 2012). A reduction in the intake of these essential nutrients may lead to health-related conditions but a meta-analysis of available literature indicated no published studies describing product development using various parts of cowpea and pumpkin as complementary sources of such healthy ingredients. Hence, in the current study, soup formulations with ingredients easily harvested from the garden and adopted in a normal kitchen setup were designed as a side dish to accompany maize meal porridge. In exploring the potential of using cowpea and pumpkin as complementary food ingredients, various aspects were studied such as the physical and functional properties of pumpkin and cowpea as well as the presence of health-providing chemicals to determine the suitability of the inclusion of pumpkin and cowpea into soups.

## **1.2 Features of cowpeas and pumpkin plants**

### **1.2.1 Domestic cultivation of cowpea and pumpkin in South Africa**

ILVs, also known as *morogo*, *imifino*, *imfino*, have played a significant role in the livelihoods of rural communities for several centuries. In South Africa, these vegetables have been incorporated in domestic farming setups, particularly in many communities residing in Limpopo and KwaZulu-Natal provinces of South Africa (Faber et al., 2010; Hart & Vorster, 2006; Uusiku et al., 2010; Van Rensburg et al., 2007; Vorster et al., 2007). There are two main groups of ILVs, namely cultivated indigenous vegetables such as pumpkins and cowpeas and uncultivated indigenous vegetables such as blackjack (*Bidens pilosa*) and spider flower (*Cleome* spp.), which grow naturally in cultivated fields, at the roadside and in sandy shade and grasslands (Mavengahama et al., 2013). Of these cultivated ILVs, 20.2% are sold to local markets, 8.3% to township residents, with very few (1.8%) being sold in shops and the majority (69.6%) being sold to roadside passers-by (Mungofa et al., 2018). Figure 1.1 indicates such a roadside venue.

It is important to strengthen people's abilities to cultivate food for themselves, especially ILVs, as opposed to merely depending on government support systems such as social grants. In this way, people in urban areas will be able to maintain healthy balanced diets (Khuzwayo, 2014) particularly as these vegetables offer a significant opportunity for the poorest people living in rural communities to cultivate and trade without requiring a large capital investment (Aju et

al., 2013; Oulai et al., 2014; Adebooye&Opabode, 2004; Uarrotaet al., 2019; Mbhenyane, 2017).

Hence, cultivation of ILVs has the potential to ensure the availability of such vegetables, improve food security, boost income generation at a low cost and alleviate poverty in rural communities (Mungofaet al., 2018).



Figure 1.1 Trading of indigenous leafy vegetables (*morogo*) to passers-by at roadsides in Limpopo Province, South Africa

### 1.2.2 Preparation of soups containing cowpea and pumpkin

Statistics South Africa (Stats SA) reported that food price inflation reached 14.5% in July 2022 compared with 13,3% in June 2021, with the monthly inflation rate for bread and cereal increasing by 2.5% between June and July 2022 with similar large monthly price increases recorded for maize meal (StatsSA, 2022). An increase in food prices has a major effect on food affordability in most low-income households. As a result, consumers should be encouraged to explore alternative sources of food and to try various methods of processing such foods which are affordable, accessible, locally available and underutilized, such as indigenous vegetables, as potential sources of essential nutrients and compounds with health-promoting benefits (Olubiet al., 2021). Processing and cooking of food improve its flavor and color by increasing the bioavailability of food nutrients (Ogunwole, 2021) while, particularly for rural community members, boiling water during the preparing of soup sterilizes water that may have been

obtained from rivers polluted with microbes. Edokpayi et al (2018) reported that residents in rural communities of South Africa have inadequate water supply and resort to collecting water from sources like rivers which are vulnerable to pollution and contaminated by fecal matter. However, lack of knowledge on ILVs restricts the popularity of indigenous vegetables and so an easily prepared and nutritionally dense cowpea and/or pumpkin soup that involves minimal processing combined with low energy utilisation should be explored to benefit the average soup consumer.

### **1.2.3 Physical and functional properties of cowpea and pumpkin**

In order for plant species to be successfully introduced in food applications, they should ideally possess a number of desirable physical and functional properties (Rezig et al., 2016; Khalid & Elharadallou, 2013), critical to the quality of food (Kumari et al., 2021). Physical properties are those aspects such as colour and viscosity (Arifin et al., 2019; Darwish, 2020). Functional properties describe the behaviour of food ingredients during preparation and cooking as well as how they affect the finished food products in terms of how they look, feel and taste (Awuchiet al., 2019). Examples of functional properties are water absorption, swelling capacity, solubility index, dispersibility and rehydration ratio (Emeka-Ike & Chukwuemeka Ike, 2020; Awuchiet al., 2019). Pumpkin and cowpea have recently been reported as novel protein sources with attractive functional and physical properties that make food more appealing (Rezig et al., 2016; Pereira et al., 2020).

### **1.2.4 Health-providing chemicals in cowpea and pumpkin**

All food is made up of chemical substances which are the building blocks of life and nutrient-dense vegetables such as cowpea and pumpkin can contribute to a healthy diet (Khalid & Elharadallou, 2012) as they are rich in phenolic acids (148-1176  $\mu\text{g/g}$ ), flavonol glycosides (27-1060  $\mu\text{g/g}$ ) (Awika & Duodu, 2017), vitamins, beta-carotene, alpha-tocopherol (9-35mg/100g), amino acids (1 - 4 g/100 g), carbohydrates (29 – 39 g/100 g), minerals (8 – 4424 mg/10 g) and fibre (0.9 - 14.26 /100 g) (USDA, 2011; FAO, 2012; Menssen et al., 2017; Ajuru & Nmom, 2017; Campbell et al., 2016; Filho et al., 2017; Chikwendu et al., 2014). They are known to contribute to health by providing anti-cancer, antioxidant, anti-obesity properties and to improve cardio-health (Jeong-Yeon et al., 2016; Mtolo et al., 2017). Pumpkin flowers contain vitamins and minerals and is used in the treatment of the common cold, to enhance immunity, male fertility, eye health and bone formation (Ware, 2018). Thus, the superior chemical

properties in pumpkin and cowpea make them nutritional power houses for good health (Schreinemachers et al., 2018).

### **1.2.5 Qualitative analysis of cowpea and pumpkin soups**

The International Union of Pure and Applied Chemists (IUPAC) provides a general definition of qualitative analysis in which foods are identified or classified based on their chemical or physical properties, such as reactivity, solubility and molecular weight (Doyle et al., 2017). A variety of test methods may be used to evaluate the purity of materials used to produce foodstuffs and their ingredients. In this regard, as suggested by the Association of Official Methods of Analysis (AOAC), the current study used chemical tests to analyse the nutrient content of cowpea and pumpkin soup (AOAC, 2010).

The current study assessed the physical properties of colour and viscosity of the pumpkin and cowpea. The colour of food is one of the most important organoleptic attributes that has a direct effect on how consumers accept and select the food - consumers associate food colour with freshness, flavour and its overall quality (Zhu et al., 2013). Viscosity, on the other hand, is a mechanical property that is used to measure the extent to which a liquid food is thick or thin, characteristic textures of food that deal with the flow and deformation of foods (Zhu et al., 2013).

In addition, functional properties describe the behaviour of ingredients during preparation and cooking as well as how they affect the finished food products in terms of how they look, feel and taste (Awuchi et al., 2019). Swelling power, solubility index, dispersibility, rehydration ratio are examples of functional properties which were assessed in the current study.

Proximate composition of food is an important criterion to assess the overall composition and nutritional status of any ingredient intended for food use (Verma et al 2019). This includes moisture content, ash content, protein content, fibre content, fat content, carbohydrate content, total energy which were assessed in this study.

Consumer acceptability of food involves the use of a sensory evaluation panel who record the extent that they like or dislike the food (Yang & Lee, 2019). The sensory attributes (mouthfeel/texture, appearance, aroma and taste) evaluated in the present study also play a pertinent role in determining the acceptance by the future clientele of the final prepared food product (Hamid et al., 2016).



As indicated, phenolic compounds are plant constituents with biological activity such as redox properties that are known to have antioxidant activity (Aryal et al., 2019). About 8000 plant phenolics occur naturally with half of this number being flavonoids (Sulaiman & Balachandran, 2012) which are a class of low molecular weight phenolic compounds responsible for colour, fragrance and taste of fruits, flowers and seeds (Ekalu & Habila, 2020; Koes et al., 1994).

### **1.3 Problem Statement**

National governments in developing countries as well as non-governmental organizations such as the World Health Organisation (WHO) consider malnourishment as a serious public health challenge (Biebinger & Hurrell., 2008). A significant factor contributing to malnourishment is the tight budget experiences in many low-income households that may prevent the purchase of food such as fruits and vegetables (Akinboade & Adeyefa, 2018). As a result, the diets of many low-income households in South Africa are dominated by starch-based staple foods such as bread, dumplings, potatoes, rice, *pap* (stiff maize porridge) and samp (crushed maize) (Chakona & Shackleton, 2017). Such diets are deficient in certain essential micronutrients that negatively impact the health of children and teenagers by impairing their physical and cognitive development, leading to increased morbidity and mortality (Eicher et al., 2019). Overall, malnourishment may negatively affect the learning capacity and work productivity for individuals, their families and countries as a whole (Biebinger & Hurrell, 2008).

To alleviate malnutrition, there is a need for sustainable feeding strategies that require minimal funding and which provide a diverse diet (Ghosh-Jerathet al., 2016). To this end, there is an abundance of indigenous leafy vegetables (ILVs) that remain underutilized in South Africa (Gupta, 2017; Dhlamini, 2017; Gido et al., 2017) that are nutritionally dense (Schreinemachers et al., 2018). Many of these ILVs are also used for medicinal purposes (Bvenura & Afolayan, 2016).

Thus, there is a need to explore locally available ILVs such as cowpea and pumpkin to act as alternative sources of essential nutrients and diversification of diet that, together with basic vegetable ingredients, may be prepared as scientifically formulated foods with improved nutritional value to support a sustainable strategy directed at alleviating malnutrition.

#### **1.4 Justification for the study**

The current study encourages the inclusion of locally available crops such as cowpea and pumpkin as part of a sustainable strategy towards promoting food security and health in rural South African households. The development and preparation of a soup using different edible parts of cowpea and pumpkin will enable consumers to take advantage of the high nutritional value resulting from combining ILVs that complement each other from a health perspective. Thus, if a part of one of the vegetables is low in a nutrient, that nutrient is adequately provided in the other vegetable. Results from the current study should contribute towards, and support, current, alternative, inexpensive strategies that may be effective in combating micronutrient deficiencies in low-income households. In addition to these critically important food security and health-related features, a unique aspect of the current study became apparent when a preliminary meta-analysis of published literature indicated there were currently no reported research findings regarding the incorporation and analysis of cowpea and pumpkin in soup formulations.

Providing support for a sustainable food security strategy should also contribute towards improving the livelihood of consumers in low-income populations by creating employment through the potential for home-industry preparation and sale of soup formulations. Importantly, the study findings should also raise awareness and understanding regarding the nutritional value and health benefits of food prepared with cowpea and pumpkin and their significant role in reducing the effects of malnutrition. There is no doubt that the study findings are important for not only nutritional and health security but also for safeguarding indigenous knowledge surrounding such ILVs.

#### **1.5 Research hypothesis**

H<sub>1</sub>: The addition of cowpea and pumpkin will improve the functional properties of the soup.

H<sub>0</sub>: The addition of cowpea and pumpkin will not improve the functional properties of the soup.

H<sub>2</sub>: The addition of cowpea and pumpkin will enhance the physical properties of the soup

H<sub>0</sub>: The addition of cowpea and pumpkin will not enhance the physical properties of the soup.

H<sub>3</sub>: The addition of cowpea and pumpkin will highlight the improved proximate composition of the soup.

H<sub>0</sub>: The addition of cowpea and pumpkin will not highlight the improved proximate composition of the soup.

H<sub>4</sub>: The addition of cowpea and pumpkin will enhance the overall acceptability of the soup.  
H<sub>0</sub>: The addition of cowpea and pumpkin will not enhance the overall acceptability of the soup.

H<sub>5</sub>: The addition of cowpea and pumpkin will improve the total phenolic content, antioxidant activity, and the flavonoids composition of cowpea-pumpkin composite soup

H<sub>0</sub>: The addition of cowpea and pumpkin will not improve the total phenolic content, antioxidant activity, and the flavonoids composition of cowpea-pumpkin composite soup.

## **1.6 Study aim and objectives.**

### **1.6.1 Aim of study**

The aim of the study was to investigate the potential of using cowpea and pumpkin as complementary food ingredients. In achieving this aim, the following study objectives were addressed:

### **1.6.2 Study objectives**

Objective 1: To assess the functional properties of cowpea-pumpkin composite soup.

Objective 2: To assess the physical properties of cowpea-pumpkin composite soup.

Objective 3: To determine the proximate composition of cowpea-pumpkin composite soup.

Objective 4: To evaluate consumer acceptability of cowpea-pumpkin composite soup.

Objective 5: To evaluate the total phenolic content, antioxidant activity and to identify the flavonoids present in cowpea-pumpkin composite soup.

## **1.7 Ethical Considerations**

The research work was conducted in the UNISA Laboratories in Florida under the supervision of Dr D. Beswa. Ethics Approval was obtained from the UNISA Ethics (Reference number 2019/CAES/076) (Appendix 1). To avoid any injuries to other students and staff members, the laboratory code of conduct and the safety rules were followed. The COVID-19 protocols such as maintaining a 2m social distance from other laboratory occupants, wearing a mask and sanitising hands were always observed. Approval to perform the consumer acceptance test in Fetakgomo Tubatse Local Municipality, Limpopo province, South Africa, was obtained from the Municipal Manager (Appendix 2). Prior to the sensory evaluation of the prepared study soups, study participants were informed of the study, told that there was no compensation for

their participation in the study, informed that they that they could leave the study at any time and requested to sign an informed consent form. Plagiarism was avoided, and all published research articles were suitably cited. All research work was kept strictly confidential until published as a completed PhD thesis.

## **1.8 Outline of Thesis**

This thesis is set out in six chapters.

### **Chapter 1: Introduction**

This chapter presents a brief overview of the study with background information on cowpea and pumpkin. This is followed with the research problem, importance of study followed by the research hypothesis, study aims and objectives of the study. It concludes with ethical considerations as well as the outline of the thesis.

### **Chapter 2: Literature review**

This chapter provides a review of the literature surrounding cowpea and pumpkin and their significance to the current study. Firstly, a background provides information of these vegetables followed by their significance as food. A description of the nutritional composition of cowpea and pumpkin follows with health aspects of such foods including their antioxidant potential, total phenolic content and then some of the physical properties of food products developed from cowpea and pumpkin. Finally, this chapter ends with a discussion on the consumer acceptability of cowpea and pumpkin as well as the theory behind techniques used in this study.

### **Chapter 3: Materials and methodology**

This chapter details the methodology applied to address the research objectives of the study. The chapter describes the research materials, preparation of the samples, formulations for preparation of soup and analysis of physical properties of cowpea and pumpkin samples and soup mixes. Furthermore, it details an analysis of functional properties and proximate composition of cowpea and pumpkin samples. A description of the sensory evaluation of soups prepared from cowpea and pumpkin links to consumer acceptability of the soups. Methods that were used to determine the total phenolic content and antioxidant potential of these plants

are discussed followed by a description of the analysis of study data. Lastly, ethical considerations are described.

#### **Chapter 4: Results and discussion**

This chapter presents the results of the study which are then discussed. The results are presented in tables.

#### **Chapter 5: Contributions and limitations of study**

The contributions and limitations of the study are outlined in this chapter.

#### **Chapter 6: General Conclusions and recommendations**

This chapter presents the conclusions drawn from the results according to the study objectives of the study. Furthermore, this chapter discusses the contributions and limitations of the study and suggests recommendations for future research involving cowpea and pumpkin based on the results obtained from the study.

The thesis ends with a list of references of articles cited in the study followed by appendices.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Background

The World Health Organisation (WHO) refers to malnutrition as “deficiencies, excesses or imbalances in a person’s intake of energy and/or nutrients” with nearly two billion adults being overweight, nearly half a billion adults being underweight while around 250 million children suffer from malnutrition (WHO, 2020; Godecke et al., 2018). Of these, more than 2.5 million children die each year from malnutrition and sub-Saharan Africa significantly contributes to this by showing the highest rates of underweight children and mortality due to malnutrition (Naicker, 2015). The increase in obesity due to malnutrition has raised serious health concerns as it is associated with a concurrent increase in non-communicable diseases such as heart disease, stroke, cancer and diabetes (WHO, 2020). Malnutrition has serious repercussions in all its forms on the capability of people to live a full life, work, care for their children, be productive, generate a positive cycle and improve their living conditions (Drimie & Pereira, 2016). Despite governmental efforts to promote food production in an attempt to boost employment, to fortify staple foods and to provide social grants to alleviate the problem of malnutrition (Habib et al., 2015), malnutrition is a persistent problem in South Africa, particularly affecting its children (UNICEF, 2020).

In South Africa, national surveys show that poor people have an inadequate diet that does not meet the daily nutritional requirements as indicated in Table 2.1 (Naicker, 2015). People who are classified as poor typically have limited financial resources and struggle to meet their basic needs, such as food, shelter and healthcare. This can include people who live below the poverty line, have low income or unstable employment, lack access to education and job opportunities and face barriers to accessing essential services (UNICEF, 2020). In addition, South Africa is undergoing a “nutrition transition” which refers to the increased consumption of fats and refined sugars as these become more affordable especially for low-income consumers which are associated with rising rates of overweight, obesity and diet-related heart diseases (Drimie & Pereira, 2016). Poor people live in poor food systems where nutrient dense food like eggs, milk, fruits and vegetables can be very expensive and, thus, making it harder for low-income consumers to diversify away from the nutrient-sparse staple foods (WHO, 2020). The main staple foods consumed around the world (rice, potatoes pap and samp) are nutritionally deficient as they are high in carbohydrates and low in other food nutrients (Amaral et al., 2018) while in South Africa, these staple foods, especially maize-based starchy foods, dominate the

diets of individuals who have a low intake of fruits and vegetables (Ekpa et al., 2018; Ranum et al., 2014). These factors are the main contributors towards nutrient deficiencies in South Africa (Schonfeldtet al., 2010) and the Food and Agriculture Organisation (FAO) and the US Food and Drug Administration (FDA) suggest that an optimal range and concentration of nutrients are needed for optimal growth, health and avoidance of diseases (FAO, 2020; US FDA, 2020). These include the following:

- Dietary fats such as all edible fats and oils which may be produced from plants and animals (FAO, 2020). Besides true fat (triglycerides), crude fat represents materials extractable with ether such as phospholipids, sterols, essential oils and fat-soluble pigments (Ranganna 1986). Fat provides energy and aids in transport of fat-soluble vitamins, insulates and protects internal tissues and contributes to important cell processes (Pamela et al., 2005; Jones et al., 1985).
- Carbohydrates are the main source of energy by providing glucose for the body, particularly the brain cells, to function optimally (Verma et al., 2012). Energy is crucial in maintaining the metabolic rate of the body, growth and synthesis of new tissues, muscular activity and physiological functions (Momanyi et al., 2020)
- Vitamins are organic substances found in natural food stuffs essential for normal growth and activity of the body (FAO, 2020).
- Dietary fibre is an indigestible carbohydrate found in structural components of plants (Sharma et al., 2012). It is the organic residue which remains after a food sample has been treated under standardized laboratory conditions with petroleum spirit, followed by boiling in dilute sulphuric acid, boiling in dilute sodium hydroxide solution or in alcohol. Crude fibre consists largely of cellulose together with lignin (Verma et al., 2019). However, the health benefits of eating fibre-rich diet are immense including prevention of constipation, regulation of blood sugar, protection against heart diseases and prevention of certain form of cancers (Sharma et al., 2012).
- Minerals are found in plants and animals and promote chemical reactions. They form part of many tissues and the minerals important for human health are calcium, phosphorus, potassium, iron, sodium, sulphur, chlorine and magnesium (FAO, 2020).
- Proteins are large molecules made of amino acids. They are found in animal and plant foods and are necessary for growth and development of the body, for body maintenance and repair and replacement of worn out or damaged tissues, to produce metabolic and digestive enzymes and they are an essential constituent of certain hormones (Khattab

& Arntfield 2009; Verma et al., 2012). It is therefore recommended to enhance the protein quality of the diet through easily available and accessible plant protein sources to improve the nutritional status of the low-income group of the population (Khattab & Arntfield 2009). These components of food are listed in Table 2.1.

**Table 2.1:** Recommended dietary daily nutrient requirements for adults

<b>Nutrient</b>	<b>Nutrient density per 1000 kcal</b>	<b>References</b>
Protein	50-56 g/d	FDA, NIH
Fats	78 g	FDA
Saturated fat	<20g	FDA
Carbohydrates	130-275g/d	NIH, FDA
Fibre	28-38 g/d	FDA, NIH
Vitamin A (retinal)	350-900 µg RAE	FDA, NIH
Vitamin D	20 µg- 20µg	FDA, NIH
Vitamin E	3.5-15 mg	FDA, NIH
Vitamin K	20- 120 µg	FDA, NIH
Vitamin C (ascorbic acid)	90-2530 mg	NIH
Biotin	30µg	FDA, NIH
Folate	30 – 400 µg	FDA, NIH
Thiamin	1.2	NIH
Pantothenic Acid	1.2 – 5.0 µg	NIH
Riboflavin	0.6 - 1.3 mg	NIH
Niacin (or equivalent)	6 -16 mg	NIH
Vitamin B <sub>6</sub>	1.0 -1.7 mg	NIH
Vitamin B <sub>12</sub>	0.5 - 2.4 µg	NIH
Iron	18 - 20 mg	FDA, NIH
Zinc	6 -11mg	NIH
Calcium	250 – 1.300 mg	NIH
Iodine	75 -150 µg	NIH
Sodium as NaCl	2300mg-2.5 g	NIH
Magnesium	320 – 420 mg	NIH
Potassium	4700mg	NIH
Phosphorus	700 – 1250 mg	NIH

Source: NIH (<https://ods.od.nih.gov/HealthInformation/nutrientrecommendations.aspx>);FDA (2022)

Note that the ash content of food stuff is the inorganic residue which remains after destruction of organic matter. It is also defined as a measure of the total amount of mineral content or inorganic material that remains after milling (Ejiofor &Kporna, 2019).

Vegetables are essential sources of nutrients needed for healthier diets (Schreinemacherset al., 2018) and the WHO recommends a minimum vegetable intake of 400 g per day to prevent



chronic diseases and to supply needed micronutrients (WHO, 2015). Indigenous vegetables are among the most nutritious vegetables as they are rich in nutrients such as proteins, vitamins, minerals, fibre and are extremely low in fat (Gupta et al., 2017). They are compatible with staple foods to alleviate malnutrition (Maseko et al., 2017). They are sources of high-quality nutrition, are easily accessible, inexpensive for poor people and contain the required nutrients at levels exceeding those found in exotic vegetables (Singh et al., 2013). Increasing their consumption is acknowledged as a strategic intervention for addressing malnutrition (Birolo et al., 2015).

Globally, numerous indigenous vegetables species can be exploited and incorporated into diets to help solve nutrition-related concerns (Bvenura & Sivakumar, 2017). Studies conducted in countries such as Ethiopia, Zimbabwe and Kenya revealed that some poor households rely on indigenous vegetables to maintain an adequate and constant food supply and this has demonstrated that these plants are essential components in local diets (Zhang et al., 2016).

Indigenous leafy vegetables (ILVs) are plant species which are either genuinely native to a particular region or which were introduced to that region to allow their evolution through natural processes or farmer selection (Maseko et al., 2017). In Vietnam, indigenous vegetables can be wild (e.g., spider plant and pigweed) or semi-domesticated such as cowpea and pumpkin (Vu & Nguyen, 2017).

Several studies revealed that ILVs are critical sources of nutrients required for optimal growth, health and avoidance of diseases (Ong & Kim, 2017). They are potent sources of vital nutrients like proteins, carbohydrates, dietary fibres, minerals, and vitamins and can be recommended as a remedy to alleviate malnutrition in South Africa, southern Africa, and the world at large (Pradeepkumar, 2015; Konsam et al., 2016; Ong & Kim., 2017; Nyadanu & Lowor, 2015; Vu & Nguyen, 2017; Gani et al., 2018).

In South Africa, a total of 103 ILV species were reported by various sources from five provinces namely Limpopo (58%), Kwazulu Natal (27%), Eastern Cape (22%), Northwest (27%) and Mpumalanga (3%) (Bvenura & Afolayan, 2015). These vegetables are also known by many other names, including traditional leafy vegetables (TLV), traditional African vegetables (TAV), African leafy vegetables (ALV), indigenous African vegetables (IAV), wild vegetables (WV) and traditional African leafy vegetables (TALV) (Maseko et al., 2017; Bvenura & Sivakumar, 2017; Mokganya & Tshisikhane, 2018). In South Africa, different

ethnic groups have their own names for these vegetables, they are called *imfino* (isiZulu and isiXhosa), *morogo* (Sesotho, Sepedi) or *mihoro* (Tshivenda) (Mavengahama, 2013).

There is strong evidence that “hidden treasures” in the form of ILVs could easily play a huge and positive role in delivering a balanced and healthy diet (Bvenura & Sivakumar, 2017). This can contribute to addressing gaps in nutrition through offering healthy and affordable nutrient-dense alternatives but, despite their nutritive value and potential economic use, the use of ILVs in South Africa remains low (Maseko et al., 2017). This is due to the negative perceptions surrounding their consumption such as their being poverty foods, weeds, or foods for women, children and the weak (Bvenura & Afolayan, 2015).

## **2.2 Cowpea and pumpkin as food**

Encouraging the utilization of nutritious, indigenous leafy vegetables such as cowpea and pumpkin could provide a long-term sustainable food strategy since these crops are locally available and are high in proteins (Menssen et al., 2017; Londonkar & Awanti, 2014; Timko et al., 2007; Ajuru & Nmom, 2017; Habib et al., 2015), minerals such as iron and calcium, as well as vitamins A, B and C (Dhlamini, 2017; Gupta, 2017; Gido et al., 2017; Okonya & Maas, 2014). These crops are also good sources of essential nutrients such as carbohydrates and lipids necessary for maintaining good health (Habib, 2015) and play a crucial role in the health of millions of people in Southern Africa and many other parts of the world (Timko et al., 2007).

These crops have received attention in recent years mainly since their stems, shoots, leaves, fruit and seeds can be eaten to contribute to their health-protective and high nutritional value (Longato et al., 2017; Montesano et al., 2018) and are considered a most important crops and some of the healthiest vegetables in the world (Wamalwa et al., 2016; Kevart 2017).

Cowpea is also known as the orphan crop (i.e., it has little significance and get less attention as a food source) (Timko et al., 2007). It is used in the development of cowpea flour as a protein supplement in biscuits (Campbell, 2016).

Pumpkin is among the crops that are consumed by most ethnic populations of South Africa and Zimbabwe (Van Rensburg et al., 2007). The flowers of pumpkin are also edible both in raw and cooked forms (Shaheen et al., 2017). Apart from their wonderful taste, the bright yellow or orange flower contains essential nutrients, minerals, and vitamins (Jothi et al., 2014). Pumpkin seeds contain oil ranging from 400 to 540 g/kg, this oil is commonly used as

a salad oil (Seymen et al., 2016) and as an ingredient in pumpkin seed cakes (Filbrand, 2012; Batista et al., 2018). Thus, the development of pumpkin flour produces enjoyable organoleptic qualities in snacks and confectionaries of cakes, cookies, scones, and bread (Filbrand, 2012).

Cowpea and Pumpkin plants have many edible parts, this means that the two vegetables can be used as complimentary food ingredients. A complementary food ingredient can be defined as a substance that is added to a food product to enhance its flavour, texture, appearance or nutritional value (WHO, 2020). These vegetables complement each other from a health perspective for example when one part of cowpea is low in a nutrient, that nutrient is adequately provided in pumpkin. Based on the information provided in Table 2.2, cowpea seeds contain the highest amount of carbohydrate (50 – 60 g/100 g) while pumpkin fruit seems to contain the lowest amount of carbohydrates (4.9 - 6.5 g/100 g), therefore, cowpea seeds will contribute a significant amount of carbohydrates in the experimental soup formulations. Furthermore, cowpea seeds have the highest protein content (23.5 g/100 g) while pumpkin fruit has a low amount of (1.0 - 2.4 g/100 g) and cowpea leaves contains a higher amount of fibre (14.3 - 25.1 g/100 g) while pumpkin leaves contain a lower amount of (0.5 - 0.9 g/100 g). This is an example of how the different parts of cowpea and pumpkin can be combined to produce a nutritionally sound composite soup. A composite soup can be defined as a type of soup that is made by combining multiple ingredients and flavours to create a complex and well balanced dish (FDA, 2020). In addition, they can also be used as sources of nutrient supplements in commercial products. However, there is little information on commercial processing of food products using various parts of cowpea and pumpkin plants.

## **2.3 Nutritional composition of cowpea and pumpkin**

### **2.3.1 Proximate composition**

Consumers should not eat food only to satisfy hunger but must also eat food that provides the necessary nutrients to maintain good health. It is therefore important to know the overall composition and nutritional status of any ingredients intended for human consumption. The proximate composition of food includes moisture, ash, lipid, protein and carbohydrate content and is an important criterion to assess the overall composition and nutritional status of any ingredient intended for food use (Verma et al., 2019). To ensure a healthy diet, the FAO recommends a daily intake of 20 – 25 g of protein, 16 – 39 g of fats, 140 – 190 g of carbohydrates and 8 - 20g of fibre (FAO, 2012). An example of a balanced menu might be a

dinner that includes one trout fillet, one cup of green beans, one cup of brown rice, one small garden salad with two tablespoons of salad dressing, one glass of low fat milk and sparkling water with a lemon or lime slice. The meal contains all the required macronutrients: 634 calories, 27 g protein, 78 g carbohydrates, 13 g fat (Lim, 2018; US Department of Health, 2020).

Cowpea and pumpkin have a high nutritional value (Harouna et al., 2018; Chen et al., 2017). They are valuable and excellent sources of protein (21- 25 g/100 g) which are cheaper than animal protein (poultry, meat or fish) for the rural and urban poor (Harouna et al., 2018; Adewale et al., 2016; Habib et al., 2015; Longato et al., 2017; Ogradowska et al., 2017; Chikwendu et al., 2014; Menssen, 2017). It is well documented that animal-based proteins are comparably high in terms of quality than plant-based proteins, this is due to higher levels of their essential amino acid and are generally considered to be complete protein sources of essential amino acids required by humans (Kerksick et al., 2021; Hertzler et al., 2020) with superior levels of digestibility (Kerksick et al., 2021). Nevertheless, the sources of animal protein are highly priced and mostly unaffordable to low-income households. Therefore, frequent consumption of diets with cowpea and pumpkin could have a significant contribution of protein in the diets of consumers in low-income households (Timko et al., 2007).

The protein content of cowpea seeds ranges from 20.3% to 39.4% and these seeds are also high in B-complex vitamins, vitamin C, vitamin E and carotenoids (Saarvedra et al., 2015; Gonçalves et al., 2016). The oil content of pumpkin ranges between 31.5% and 51% (Rezig et al., 2016; Saarvedra et al., 2015) and pumpkin also has a high vitamin E and linoleic acid content (Badr et al., 2011) as well as carotenoids such as  $\beta$ -carotene, lutein, and zeaxanthin (Song et al., 2017). In addition, pumpkin seeds are high in protein (24% to 36.5%) and are also a good source of zinc and polyunsaturated fatty acids (Kim et al., 2012) and nutraceutically bioactive compounds (Montesano et al., 2018).

Pumpkin and cowpea are also inexpensive and excellent sources of complex carbohydrates which includes starch, oligosaccharides and crude fibre which ranges from 25 – 32 g/100 g (Habib et al., 2015; Longato et al., 2017; Ogradowska et al., 2017; Campbell et al., 2016; Filho et al., 2017; Ebere et al., 2017). Several researchers also found that pumpkin (seeds) and cowpea are important sources of minerals, and being rich in fats and fibre, they can be used as attractive sources of fat and fibre (Ogradowska, 2017; Habib et al., 2015; Ajuru & Nmomo, 2017; Campbell et al., 2016; Filho et al., 2017; Chikwendu et al., 2014). Pumpkin fruit and

cowpea seeds also contain higher amounts of moisture and ash content which can improve mineral content of food products (Aukkanit & Rrakit, 2016; Biama et al., 2020).

Therefore, these two vegetables play a crucial role in the lives of millions of people in the world by providing them with a major source of dietary protein that nutritionally complements low protein cereal and crop staples. The proximate composition of various parts of cowpea and pumpkin is illustrated in Table 2.2 below.

**Table 2.2.** Proximate composition of cowpea and pumpkin (dry weight)

Nutrient (g/100g)	CL	CS	PL	PS	PFr	PFl	References
Carbohydrates	39.1	60.3	9.3	12.6	4.9	3.3	Ebere et al. (2017); Ngozi et al. (2014); Menssen (2017); USDA (2011)
Protein	17.9	23.5	4.82	30.2	2.36	0.34	Ebere et al. (2017); Ngozi et al. (2014); USDA (2011)
Ash	14.80	14.8	2.3	4.9	1.5	0.6	Chikwendu et al. (2014); Ngozi et al. (2014); USDA (2011)
Fibre	14.26	10.7	13.7	3.7	0.9	0.0	Chikwendu et al. (2014); USDA (2011); Ngozi et al. (2014); Ebere et al. (2017)
Fat	1.31	2.07	1.5		0.9	0.2	Chikwendu et al. (2014); USDA (2011); Ngozi et al. (2014)
Moisture	13.0	11.0	68.2	5.3	89.6	31.40	Ebere et al. (2017); USDA (2011); Ngozi et al. (2014); Menssen (2017)

**Note:** A general reference for these data is USDA (2011). Abbreviations include cowpea leaves as CL; cowpea seeds as CS; pumpkin leaves as PL; pumpkin seeds as PS; Pumpkin fruits as PFr and pumpkin flowers as PFl.

### 2.3.2 Mineral composition

Minerals are vital in human nutrition for overall physical and mental health as well as for the maintenance of acid-base balance (Kalsoom et al., 2021). They affect the utilisation of dietary vitamins and are integral parts of bones, teeth, soft tissues, muscles, blood and nerve cells (Kulaitieneetal., 2014). An appropriate intake of minerals is necessary for humans to meet their metabolic needs, hence avoiding a wide range of associated health problems (Santos et al., 2016).

Pumpkin and cowpea seeds are also important sources of minerals such as iron at 8.27 mg/10 g (cowpea seeds) to 8.82 mg/10 g (pumpkin seeds), as well as zinc at 337 mg/10 g to 781 mg/100 g), contain very high levels of potassium (1233 – 4424 mg/100 g) and magnesium (184 – 592 mg/100 g) and other essential minerals (Ajuru & Nmom, 2017; Campbell et al., 2016; Filho et al., 2017; Chikwendu et al.,2014). The addition of cowpea and pumpkin leaf relish appears to improve the intake of nutrients such as zinc and iron in children’s diets (Vilakati et al., 2016). Pumpkin and cowpea contain moderately high levels of Ca, Na, Mn, Fe, Zn and Cu, the elements which make cowpea and pumpkin valuable for food formulations (Ajuru &

Nmom, 2017). The distribution of minerals in various parts of pumpkin and cowpea is illustrated in Table 2.3 below.

**Table 2.3.** Mineral composition of cowpea and pumpkin plants

Nutrient (mg/100 g)	CL	CS	PL	PS	PFr	PFl	References
Ca	39	594.2	15.21	9.78	21	12.87	Elinge et al. (2012); Aremu et al. (2006); USDA (2011)
Fe	77	2.5	0.87	3.75	0.8	0.23	Elinge et al. (2012); USDA (2011)
Cu	0.1	0.5	0.05	2.17	0.2	-	Elinge et al. (2012); Aremu et al. (2006); USDA (2011)
P	3.8	80	40.56	47.68	30	16.17	Elinge et al. (2012); Aremu et al. (2006); USDA (2011)
K	1380	357	170.04	273.24	340	57.09	USDA (2011); Aremu et al. (2006); Elinge et al. (2012)
Na	-	65	4.29	170.35	1	1.65	Aremu et al. (2006); USDA (2011); Elinge et al. (2012);
Mn	-	12.4	0.135	0.06	0.1	-	Aremu et al. (2006); USDA (2011); Elinge et al. (2012)
Mg	-	53	14.82	67.41	12	7.92	USDA (2011); Elinge et al. (2012)
Zn	-	337	0.08	14.4	0.3	-	Aremu et al. (2006); USDA (2011); Elinge et al. (2012)
Se	-	-	0.35	9.4	0.3	0.23	USDA (2011)

**Note:** Abbreviations include cowpea leaves as CL; cowpea seeds as CS; pumpkin leaves as PL; pumpkin seeds as PS; Pumpkin fruits as PFr and pumpkin flowers as PFl.

### 2.3.3 Fatty acid composition

Fatty acids are lipids that are a major component of many plants, foods and medicines including pumpkins and cowpea. They are important dietary sources of energy and are important structural components of cells (Chen, 2012). Among the multiple roles of fatty acids, they have structural functions as constituents of phospholipids which are the building blocks of cell membranes, as part neutral lipids fatty acids serving as storage materials in cells and as fatty acid derivatives that are involved in cell signalling (Carvalho & Caramujo, 2018).

Thus, fatty acids are nutritionally important and can be introduced in the diet easily by consuming indigenous vegetables such as pumpkin and cowpea (Habib et al., 2015; Longato, 2017; Ogrodowska et al., 2017). As illustrated in Table 2.4 below, these two vegetables contain

significant minimum levels of recommended daily intake of saturated fatty acid such as lauric and stearic acid. Senekal et al. (2019) stated that the amount of saturated fat consumed should be as low as possible with nutritionally adequate diet. It can be concluded that the fatty acid content of cowpea is lower than in pumpkin hence a mix of cowpea and pumpkin would average out to a reasonable level of fatty acid intake.

**Table 2.4.** Fatty acid composition of cowpea and pumpkin

<b>Nutrient (Fatty acids)</b>	<b>CL (g/100 g)</b>	<b>CS (g/100 g)</b>	<b>PL (mg/100 g)</b>	<b>PS (mg/100 g)</b>	<b>PFr (mg/100 g)</b>	<b>PFI (mg/100 g)</b>	<b>References</b>
Saturated	14.0	0.138	0.081	29.0	20.2	0.12	Antova et al. (2014); FDA (2019); Prommaban et al. (2021); Rueda et al. (2014)
Monounsaturated	6.9	0.044	0.02	31.40	24.7	0.03	Antova et al. (2014); FDA (2019); Prommaban et al. (2021); Rueda et al. (2014)
Polyunsaturated	25.2	0.225	0.009	39.24	5.1	0.01	Antova et al. (2014); FDA (2019); Prommaban et al. (2021); Rueda et al. (2014)

**Note:** Abbreviations include cowpea leaves as CL; cowpea seeds as CS; pumpkin leaves as PL; pumpkin seeds as PS; Pumpkin fruits as PFr and pumpkin flowers as PFI.

### 2.3.4 Vitamin composition

Vitamins are indispensable to maintaining various functions of physiological importance such as muscle contractility, nerve transmission, blood coagulation, digestive processes and acid base balance (Hardisson, 2001). Vitamin C, for example, is a powerful water-soluble antioxidant that protects cells and cellular components from free radicals by donating electrons that allow the regeneration of other antioxidants such as Vitamin E (tocopherol) (Kim et al., 2012). Pumpkin and cowpea are excellent sources of vitamins and, therefore, a high intake of these vegetables provides various benefits to improve overall health.

Pumpkin and cowpea contain many vitamins especially vitamin E (up to 35 mg/100 g) and vitamin C (up to 9 mg/100 g) (Menssen et al., 2017; Ajuru & Nmom, 2017; Goncalves et al., 2016; Timko et al., 2007; Chikwendu et al., 2014; Asare et al., 2013). A daily dietary intake of pumpkin and cowpea will not only help in solving hunger problems but will also be beneficial in minimising the risk of chronic health problems and treatment of common colds, in

maintaining eye health, in bone formation, proper growth and protection from disease (Kirigiaet al., 2018; Amin et al., 2019). The vitamin composition of cowpea and pumpkin is presented in Table 2.5 below.

**Table 2.5.** Vitamin Composition of Cowpea (*Vigna unguiculata*) and pumpkin (*Cucurbita maxima*) per 100 g

Vitamins	CS	PS	PL	PFr	PFI	References
B1	0.00076	0.034	0.4	0.05	0.014	Abebe (2022); Batool et al. (2022); USDA (2019)
B2	0.00019	0.0052	0.1	0.11	0.025	Abebe (2022); Batool et al. (2022); USDA (2019)
B3	0.00314	0.286	0.4	0.6	0.028	Abebe (2022); Batool et al. (2022); USDA (2019)
B5	0.411	0.056	0.0	0.298	-	USDA (2019); Batool et al. (2022); Abebe (2022)
B6	0.00041	0.0037	0.1	0.061	-	Abebe (2022); Batool et al. (2022); USDA (2019)
B9	208	9	14	16	19.47	USDA (2019)
C	0.00169	0.3	4.3	9.0	9.2	Abebe (2022); Batool et al. (2022); USDA (2019)
E	0.28	35.1	-	1.06	19.47	USDA (2019)
K	1.7	-	-	0.001	-	USDA (2019); Batool et al. (2022)
A	0.0002	0.019	37-38	0.426	32.0	Abebe (2022); Batool et al. (2022); USDA (2019)

Note: With the exception of vitamin B9 whose measured availability unit is  $\mu\text{g}/100\text{ g}$ , the unit for the remainder of these vitamins is  $\text{mg}/100\text{ g}$ . Abbreviations include cowpea seeds as CS; pumpkin leaves as PL; pumpkin seeds as PS; Pumpkin fruits as PFr and pumpkin flowers as PFI

### 2.3.5 Amino acids

Proteins are made up from 20 different building blocks of amino acids and play many critical roles in the body including cellular structure, hormones and acting as neurotransmitters. Thus, it is crucial to eat high quality protein foods that contain essential amino acids (Dash, 2017).

While all 20 amino acids are important for health, only nine are classified as essential - histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Jayathilake et al., 2018). Fortunately, pumpkin and cowpea are rich in amino acids essential for the many metabolic processes in the human body (Goncalves et al., 2016). The WHO and FAO recommend food that has the best protein category which must have an essential amino acid/ total amino acids ratio above 40%. Both cowpea and pumpkin contain amino acids values that are higher than the set limits of the WHO and FAO, which suggest that these vegetables are good sources of free amino acids. Histidine (1 – 3 g/100 g), Lysine (2-4 g/100 g) and Arginine (1- 2.66 g/100 g) are found to be the most concentrated essential amino acids in cowpea and pumpkin. This is illustrated in Table 2.5 below.



**Table 2.6.** Amino acids composition of cowpea and pumpkin (g per 100 g)

Amino acids	CS	PS	PL	PFr	References
Tryptophan	0.1	15.3	0.016	0.1	USDA (2019); Glew et al. (2006)
Threonine	22.9	18.4	0.061	0.0	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Histidine	20.0	13.8	0.020	0.0	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Isoleucine	30.8	23.0	0.061	0.1	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Lysine	28.0	22.0	0.078	0.1	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Methionine	17.1	12.4	0.021	0.0	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Cystine	4.0	6.73	0.012	0.0	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Phenylalanine	37.0	31.4	0.067	0.0	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Tyrosine	26.3	22.1	0.061	0.1	USDA (2019); Glew et al. (2006)
Valine	31.2	28.2	0.071	0.0	Aremu et al. (2006); Glew et al. (2006); USDA (2019)
Arginine	34.6	93.2	0.085	1.1	Aremu et al. (2006); Glew et al. (2006); USDA (2019)

**Note:** The general reference is USDA, 2019). Abbreviations include cowpea seeds as CS; pumpkin leaves as PL; pumpkin seeds as PS; Pumpkin fruits as PFr.

## 2.4 Antioxidant and total phenolic content of products developed from cowpea and pumpkin

Antioxidants are a wide group of chemical compounds capable of inhibiting or delaying the oxidation of lipids and other biomolecules and thus reducing damage to body cells caused by such oxidation (Amin et al., 2018). An antioxidant is, thus, a molecule that may prevent or slow the oxidation of many other molecules. Oxidation reactions produce free radicals, thereby starting chain reactions which contribute to cell damage while antioxidants stop such chain reactions by eliminating free radicals by being oxidized themselves (Oliveira et al., 2016). They are an essential part of the defence system of the body and assist in coping with oxidative stress associated with reactive oxygen species (Zia-UI-Haq et al., 2013). The antioxidant potential of many different plants is associated with the presence of phenolic components such as phenolic acid in them hence there is a growing interest in plant-based extracts due to their potential in prevention of diseases and promotion of consumer health (Sopan et al., 2014).

Epidemiological studies have reported that the consumption of phenolic antioxidant rich foods affect human health by inhibiting / delaying the activity of reactive oxygen species thus limiting their harmful effect and reducing the risk of many diseases including cardiovascular diseases, cancers and neurodegenerative diseases, ageing and diabetes. Hence, the antioxidative activity of the compounds consumed every day is important for health (Kulczynski et al., 2020; Zhou et al 2013; Mala and Kurian, 2016). Antioxidants may soothe inflammations, viral infections and they are said to prevent age related eye diseases (Gramza-Michalowska et al., 2017).

They are an important part of the defense system of the human body and help to cope with oxidative stress caused by reactive oxygen species (Zia-Ui-Haq et al., 2013). These compounds are characterized by the ability to scavenge (neutralize) reactive oxygen species (ROS), including hydroperoxide radicals, superoxide anion radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals (Oroian & Esrliche, 2015; Nathan and Cunningham-Bussel., 2013). Antioxidants are not only important to maintain good health, but they are also widely used in food technology, they inhibit oxidation and limit the degradation of phytosterols and fats and they also preserve the high colour of food products (Liu et al., 2015; Gramza-Michalowska et al., 2017; Kulczynski et al., 2020).

Antioxidants can be natural or synthetic. Synthetic antioxidants like butylated hydroxytoluene, butylated hydroxy anisole and tertiary butylhydroquinone are restricted to be used because of their carcinogenic effects. Research provided evidence of adverse effects of these synthetic antioxidants (Yesilgurt et al., 2008; Mala and Kurian, 2016). Therefore, interest of scientists in natural antioxidants, especially of plant origin has grown up because of their beneficial health effects. They have attracted the attention of researchers because they play a very important role in human health by protecting the body tissues from oxidative stress (Hussain et al., 2021; Chiou et al., 2007). The following compounds are listed as strong antioxidants: carotenoids (e.g beta-carotene, lycopene, astaxanthin), tocopherols (e.g alpha-tocopherol, gamma-tocopherol), flavonoids (e.g anthocyanins, flavanols, flavonones, isoflavones) phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids), stilbenes, some vitamins (vitamin C), coenzyme Q10, sulphur compounds (e.g., allicin), mineral components (e.g., selenium, zinc) (Kulczynski et al., 2020; Evans and Lawrenson, 2017; Pala et al., 2018).

Antioxidants play a very important role in different ways during different types of chronic diseases, in the body of living by protecting the cells from oxidation and scavenging the free radicals produced in the body (Skandrani et al., 2010). They inhibit the oxidizing chain reactions in the living body (Hussain et al., 2021). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Sopan et al., 2014). A high antioxidative capacity is attributed to fruits and vegetables, so far the antioxidative properties of various plant materials have been tested (Kulczynski et al., 2020).

Fruits and vegetables are believed to have protective mechanism due to the presence of phenolic antioxidants. Phenolic compounds are the most important group of bioactive

compounds which also have the potential to protect the body against chronic diseases (Mtolo et al., 2017). They act as scavengers of radicals, reducing agents and metal ion chelators (Zhao et al., 2014). Dietary phenolics are mainly found in crops and through their free radical-scavenging capacity, have proven to show high levels of antioxidant activity (Apea-Bah et al., 2016; Amin et al., 2018). Considering the bioactivity of these substances and their presence in a variety of vegetables, dietary phenolics are viewed as natural antioxidants and the vegetable sources that it contains as functional foods (Amin et al., 2018).

Phenolic compounds from natural sources have attracted great attention during the last two decades, because they are famous powerful chain breaking antioxidants that play an important role in protecting the body tissues against oxidative stress, and contribute to human health (Zlabur et al., 2017; Barba et al 2012; Chiou et al., 2007; Apeah-Bah et al., 2017; Sopan et al., 2014). They possess many hydroxyl groups which have very strong radical scavenging activity (Sopan et al., 2014). Polyphenols present in fruits and vegetables and also in products made from these plants for example cocoa and beverages are important naturally occurring antioxidants (Aoudi et al., 2011). In addition, many of the vitamins (vitamin c) and mineral, which in most cases are abundantly found in fruits and vegetables, are strong antioxidants (Barba et al., 2012).

Cowpea and pumpkin are versatile vegetables having identical position among all vegetables due to their different parts which are consumable, each possessing outstanding phytochemicals applicable in treatment and prevention of medical disorders (Sharma et al., 2020). The different parts of cowpea and pumpkin are nutrient dense sources of protein, carbohydrates, dietary fibre along with minerals and vitamins (Yadav et al., 2018; Sombie et al., 2018) besides these nutrients they serve as rich sources of biologically active compounds which include dietary phenolics such as phenolic acids, anthocyanins, flavonoids, proanthocyanins, flavones tocopherols and tocotrienols, compounds that promote health by virtue of their antioxidant properties (Asif et al., 2017; Bardaa et al., 2016; Apea-Bah et al., 2017; Sopan et al., 2013; Kulczynski et al., 2020). Various researchers have clearly reflected the antioxidant properties of these biologically active compounds in these vegetables by means of various antioxidant parameters like total phenolic content and ferric reducing antioxidant power (Yadav et al., 2018).

Bochnak and Swieca (2020) reported that pumpkin powder is a good source of potentially bio accessible phenolics and antioxidant capacities which might play an important role in pre-

diabetics, diabetics and individuals with vascular injury (Mala and Kurian, 2016). Cowpea and pumpkin have proven to be beneficial in working against oxidative damage associated with cardiovascular diseases and other lifestyle diseases. To date, there have been only few studies on the antioxidative activity of pumpkin and cowpea but not a combination of the two vegetables.

#### **2.4.1 Pumpkin (*Cucurbita maxima*)**

Previous studies investigated the antioxidant activity of pumpkin flours, broccoli, carrots, asparagus, red pepper and kale and a significantly higher antioxidant activity was observed in pumpkin flours because of the increased levels of phenolic compounds (Oliveira et al., 2016; Dinu et al., 2016). Thus, pumpkin has received much attention recently as being able to provide exceptional protection against chronic conditions such as hypertension and cancer mainly because of such its anti-inflammatory and antioxidant attributes (Bardaa et al., 2016). Eating pumpkin seeds as a snack can help prevent the most common types of kidney stones and are beneficial in the treatment of roundworms, sore chests, tapeworms, intestinal parasites, fever, bronchitis and enlarged prostates (Ajuru & Nmom, 2017). Furthermore, the addition of pumpkin seeds has also proved to increase the antioxidant properties of raw burgers (Longato et al., 2017).

A study of the oxidative stability characteristics of pumpkin seed oil would enhance the development and appreciation (valorization) of such essential oil particularly in the food production industry (Bardaa et al., 2016). This oil has many pharmaceutical uses as it is rich in several bioactive components such as sterols, squalene, unsaturated fatty acids, tocopherols, and carotenoid pigments that show antitumor, antimicrobial, antioxidant, antihypertensive and anti-diabetic activity (Li et al., 2016). However, pumpkin as a plant is usually viewed as the basis of medicines and functional foods because pumpkin products have a variety of biological activities mainly because of the presence of natural complexes of fatty acids, minerals, soluble and insoluble fibre, vitamins, antioxidants and other bio-regulators (Rabrenovic et al., 2014; Dyshlyuket et al., 2017). Important for the current study, however, is that previous research has shown that pumpkin pulp possesses strong antioxidant action that promotes the use of pumpkin flour as a vital component in the formulation of various food products other than in bakery (Dyshlyuket et al., 2017).

#### **2.4.2 Cowpea (*Vigna unguiculata*)**

Cowpea is also considered an excellent source of dietary phenolics, especially flavan-3-ols, flavonols and flavonoids that safeguard body cells from radical-induced oxidative damage (Apea-Bah et al., 2017). From a nutritional perspective, epidemiological studies have shown a correlation between the consumption of phenolic acid-rich foods and a decline in several chronic diseases (Zia-UI-Haq et al., 2013).

Cowpea is famous for its medicinal properties and extracts of this plant is used in several naturopathic, homeopathic and allopathic remedies (Londonkar&Awanti, 2014). Evidence suggests that a cowpea diet may offer benefits in treating diabetes, cancer, cardiovascular disease and has significant anti-inflammatory effects (Awika & Duodu, 2017). In addition, a mixture of cowpea seed powder and oil may be used to treat stubborn boils; eating cowpea seeds has a diuretic effect and is effective against stomach worms; is used to treat amenorrhea; painful menstruation, epilepsy and chest pains, while bilharzia and blood in urine can also be treated by consuming cooked cowpea roots and seeds (Londonkar & Awanti, 2014). The proteins in cowpea also demonstrate important cholesterol lowering and antioxidant effects (Shevkani et al., 2015).

The literature indicates the potential of cowpea and pumpkin to not only provide functional foods that minimise oxidative stress-related conditions but also to improve the quality of food (Amin et al., 2018).

#### **2.5 Physical properties of products developed from cowpea and pumpkin**

Physical properties of food are those properties that lend themselves to description and quantification by physical rather than chemical means (Berk, 2018). Examples of physical properties include viscosity and colour. Viscosity is the internal friction of a liquid or its ability to resist flow (Darwish, 2020). However, colour is the most important attribute that directly affects consumer preference for any product (Arifin et al., 2019). Producing food products with an attractive colour is one of the main goals in the food manufacturing industry and pumpkin flour is popular due to its highly desirable deep yellow-orange colour (Kiharason et al., 2017). Cakes made with pumpkin flour have a very soft and moist texture, appear golden brown in colour, have a savoury taste, look very appetizing and increase one's appetite (Borro & Gemora, 2016). The addition of pumpkin puree to pies also produces products with a colour that was highly appetizing (Islam et al., 2014).

Several reports indicated that the addition of pumpkin powder in the manufacturing of bread leads not only to desirable organoleptic qualities but also to an increase in the volume of the final baked product (Manjula & Suneetha, 2014; Dyshlyuket al., 2017). Starch is a very useful polymer in the food production and pumpkin starch exhibits the highest viscosity value and is well known for their lower value of gelatinization temperatures, highest value of hardness, cohesiveness, and chewiness (Roznowska, 2017). Adding pumpkin is linked to desirable changes in cooking attributes of chicken burgers, suggesting that texture and juiciness of products may be significantly enhanced (Longatoet al., 2017). Furthermore, products made from pumpkin have proved to be optimal in appearance (Kiharasonet al., 2017). Pumpkin seeds contain 400-540g/kg oil which is mainly used as salad oil. However, the use as pumpkin oil in cooking remains limited mainly due to its strong aroma, dark greenish colour and foaming characteristics (Seymen et al., 2016; Santos et al., 2016; Filbrandt, 2012).

On the other hand, higher swelling during baking and a higher peak viscosity are a result of greater quantities of starch in cowpea (Jeong & Chung, 2018). Thus, muffins prepared with cowpea flour show a higher volume and increased height by increasing batter visco-elasticity and accelerating stability of air bubbles (Shevkani et al., 2015). Dough containing cowpea is significantly harder as shown in increased hardness of crumbs and decreased loaf volume in bread made with cowpea flour (Campbell et al., 2016). Biscuits supplemented with cowpea have a poor colour which is considered unappealing (Hama-Ba et al., 2018). Cowpea flour also yields greater hardness in muffins, but this does not affect the textural properties of cowpea products (Jeong & Chung, 2018). Some of these factors contribute to the limited exploitation of cowpea products in food markets (Campbell, 2016).

## **2.6 Consumer acceptance of cowpea and pumpkin**

In spite of being consumed as food, indigenous leafy vegetables (ILVs) have been generally regarded as weeds (Mavengahama, 2013), low value vegetables (Kidane et al., 2015) and are restricted to certain areas or communities (Konsamet al., 2016). Lower utilization levels can be accredited to the perception that ILVs are food for hard times and for the poor (Dhlamini, 2017). They are also considered cheap and suitable for children as well as the weak for consumption in times of drought, all factors contributing to the low levels of their utilisation (Gido et al., 2017; Maseko et al., 2017; Bvenura & Afolayan, 2016; Neugart et al., 2017). The many advantages and economic benefits of ILVs may not be fully realized unless and until

these false and negative perceptions surrounding these vegetables change (Bvenura & Afolayan, 2016).

Most muffins containing cowpea flour have a relatively reduced taste and flavour while biscuits supplemented with cowpea were less attractive as to smell, taste and poor colour and are considered unpleasant (Hama-Ba et al., 2018). The coarseness introduced by cowpea flour also affected mouth feel and taste of cowpea porridge and cowpea fortified weaning foods causing a reduction in acceptability of cowpea products (Tortoe et al., 2014). The beany flavour in products formulated from cowpea is related to the presence in cowpea of lipoxygenases that reduce the acceptability of products containing cowpea (Tortoe et al., 2014; Jeong & Chung, 2018).

However, products made from pumpkin are highly acceptable due to its desirable flavour and sweetness, but caution must be exercised as the addition of too much pumpkin flour may decrease its likeability level (Kiharasonet al., 2017). Fortifying yoghurt using pumpkin pulp is linked with a statistically crucial effect on sensory parameters such as flavour and overall consumer choices required that the yoghurt be highly acceptable (Barakat et al., 2017). In a study to determine the sensory acceptability of mashed pumpkin included in ice-cream, the mashed pumpkin was highly acceptable as a vegetarian ice-cream in the market without even mentioning its nutritional benefits (Moreno et al., 2015). Furthermore, pies formulated from pumpkin had the highest flavour and highest overall acceptability compared with other formulated pies. This indicates that pumpkin can be used in bakery and confectionery industries to increase market value because pumpkin-containing products are highly acceptable (Islam et al., 2014). Cakes made of pumpkin look very appetizing, tasted savoury and had a very moist and soft texture. Thus, in terms of general acceptability, these cakes were favourably comparable (Borro & Gemora, 2016). Several reports indicate that the addition of pumpkin powder in bread production leads not only to desirable organoleptic qualities of the bread, but also increases the volume of the product (Manjula & Suneetha, 2014).

Therefore, it can be concluded that a combination of cowpea and pumpkin would provide complementary inputs to food products to improve their palatability and health provision.

## **2.7. Theory behind study techniques**

Understanding the way food materials respond to physical and chemical treatments allows for optimum design of food equipment and processes to ensure food quality and safety (Wilhem

et al., 2004). In this study, physical and functional properties of cowpea-pumpkin soup, their proximate composition, total phenolic and antioxidant compounds were determined.

## **2.7.1 Physical properties**

### *2.7.1.1. Colour*

The quantification of colour in the present study was carried out using a colorimeter, a light sensitive device that determines the transmittance and absorption of light which is passed through a sample component. This method quantifies colour by measuring the three primary colour components of light as seen by the human eye i.e., red, green and blue (Giese, 2003). This measures colour much the same as the human eye and the colour indices used are L\* (lightness; a\* and b\* for chromaticity).

### *2.7.1.2. Viscosity*

A rheometer was used to determine the viscosity of cowpea-pumpkin soup formulations to provide insights into their stability and appearance. This method is based on measuring the torque exerted on the shaft of impeller as a function of its rotational speed in the test solution. The rheological behaviour is determined under constant temperature conditions. The greater the viscosity of the solution, the greater the torque exerted on the impeller (Abbas et al., 2010).

## **2.7.2 Determination of proximate composition of soup samples**

Food is a complex mixture of moisture, crude fat, crude protein, ash, crude fibre, and total carbohydrates and their determination is termed proximate analysis (Aurand et al., 1987). In this regard, AOAC methods were used to determine the proximate composition of cowpea-pumpkin composite soup samples. These methods are a comprehensive and reliable collection of chemical and microbiological methods and consensus standards which undergo rigorous, systematic scientific scrutiny to ensure that results are credible and defensible and can be used with confidence (Mohanta, 2016).

### *2.7.2.1. Protein*

The Kjeldahl distillation apparatus was used to determine the protein content of samples of soup formulations. Since proteins are some of the only molecules in food that contain nitrogen, the Kjeldahl method is based on the measurement of the protein content of a soup formulation indirectly by quantifying the nitrogen content of a soup sample (Krotz et al., 2008).



#### *2.7.2.2. Carbohydrate*

Acid hydrolysis is used to determine total carbohydrate. This is based on subjecting the samples to digestion by takadiastase under such conditions as are necessary for the breakdown of starch, dextrins and maltose to glucose (Weinmann, 1947).

#### *2.7.2.3. Moisture content*

Moisture content is determined using the thermogravimetric approach. This method is based on loss on drying, this involves heating the sample, drying and the weight loss during evaporation of moisture is recorded (Nielsen, 2006).

#### *2.7.2.4. Ash*

The dry ashing technique is used to determine ash content in food. This method is based on burning off the organic matter and to determine the inorganic matter which remains. This method involves removing the water present and to char the sample thoroughly and then ashing at 550°C in a muffle furnace (AOAC, 1980). The ash content is calculated by dividing the weight after ashing with original weight sample (weight before ashing) and multiplied by 100.

#### *2.7.2.5. Fats*

The Gas chromatograph procedure is used to analyse the fat content. This involves releasing triglycerides and other molecules containing fatty acids from the samples using an acid base. The triglycerides are then extracted into a mixture of ethyl and petroleum ether. The fatty acids are then esterified to form fatty acid methyl esters. Total fat is calculated as the sum of individual fatty acids expressed as their respective triglyceride equivalents (AOAC, 2016).

### **2.7.3. Functional properties**

Functional properties improve mouth feel and flavour retention which makes it a very important property in food formulations (Emeka-Ike & Chukwuemeka, 2020). Considering the promising opportunities that protein fractions from cowpea and pumpkin could open for the development of novel, efficient and cost-effective products, it was necessary to determine if they possess functional properties suitable for food applications and consumer acceptability. Functional properties of cowpea-pumpkin composite soup examined in the current study include water absorption capacity, rehydration, dispersibility, swelling power and solubility.

#### *2.7.3.1. Water absorption capacity*

Water absorption capacity (WAC), also called water absorption, is the amount of water (moisture) taken up by food/powder to achieve the desired consistency needed to create quality food products (Awuchi et al., 2019). This is based on adding water or an aqueous solution enough to cover the sample (1g sample and 15ml of water). This is followed by centrifuging and quantification of the water retained by the pelleted material in the centrifuge tube (Damoradan et al., 2010).

#### *2.7.3.2. Rehydration*

Rehydration measures the extent to which food products presents the characteristics of texture and increased volume that render them suitable for consumption after being reconstituted. This is based on soaking the samples in water to wet the sample. It allows penetration of the water into the pores, absorption on the surface of the matrix, diffusion into the solid matrix and equilibration (Berk, 2009).

#### *2.7.3.3. Swelling power*

Swelling power measures the volume (ml) taken up during swelling of food material under specific conditions and is determined based upon the volume of water or swelling agent added to the sample (Awuchi et al., 2019). This was done by heating a mixture of cowpea-pumpkin in a water bath, centrifuge then measuring the sediment.

#### *2.7.3.4. Solubility*

Solubility in foods is both a chemical and a functional property that measures the ability of a given food substance to dissolve in a solvent, usually water or oil. Solubility involves soluble component of the soup powder while dispersibility involves insoluble or clumped components of your soup powder.

The extent of solubility of a food substance in a specific solvent is commonly measured as the saturation concentration, in which addition of more solute does not increase concentration of the solution and rather starts to precipitate the excess quantity of solute (Awuchi et al., 2019). This was measured using an insoluble index, after mixing and centrifuging the mixture the undissolvable content is measured.

#### *2.7.3.5. Dispersability*

Dispersibility is generally defined as a rapid, uniform distribution of a powder into solution enough to cover the food without forming lumps. This is based on separating agglomerated particles from each other generating a new interface between the inner surface of liquid dispersion medium and the surface of the dispersed particles using a test tube (Wootta & Beckjr, 2013).

### **2.7.4 Total Phenolic content and antioxidant potential**

#### *2.7.4.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay*

Shalaby & Shanab (2013) reported that the DPPH antioxidant assay is based on the measurement of the scavenging capacity of antioxidants towards a stable free radical  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH; C<sub>18</sub>H<sub>12</sub>N<sub>5</sub>O<sub>6</sub>, M = 394.33). The delocalisation of the free hydrogen gives DPPH its deep violet colour, with an absorption in ethanol solution at around 520 nm and its reduced form in the presence of antioxidants has a reduced violet colour (Kedare & Singh, 2011).

#### *2.7.4.2. 2,2'-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) antioxidant assay*

The 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS<sup>•+</sup>) is a stable free radical used to estimate the total antioxidant capacity (TAC) of natural products. The ABTS method is based on spectrophotometric monitoring of the decay of this radical cation following oxidation of ABTS caused by the addition of an antioxidant present in a sample. The ABTS strongly absorbs at longer wave lengths such as 415, 645, 734, and 815 nm but 734 nm is used by most investigators (Rajurkar & Hande, 2011).

#### *2.7.5. Sensory evaluation*

Sensory evaluation is a method uses a sensory panel to measure sensory characteristics of food that can be quantified which include aroma, appearance, flavour, texture and taste. This method differentiates between products based on their sensory characteristics and determines quantitative description of all the sensory attributes (Stone & Sidel, 1993; IFT, 2007). The panel uses an instrument called a hedonic scale to provide data comparable in nature to the instrument output.

Furthermore, sensory evaluation in these early stages of product development can help to pinpoint the imperative sensory characteristics driving acceptability of food (Sharif et al.,

2017). It was important to evaluate the acceptability of cowpea-pumpkin soup to evaluate the level of liking of the soup hence a panel was recruited to evaluate the soup.

The next chapter describes the methods used in detail.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Research materials

The vegetables used in this study were planted in Burgersfort, Limpopo Province, South Africa and harvested manually during the 2019 and 2020 cropping seasons. Raw soup materials such as dry carrot granules, potato powder, onion powder, mushroom powder, ungelatinised corn starch, salt, brown sugar and tomato powder was procured locally from Burgersfort supermarkets (Pick n Pay, Shoprite and Karachi Corner). Laboratory chemicals were provided by the Food Science Laboratory, University of South Africa, Florida Campus, South Africa. These were purchased from Sigma Aldrich, Modderfontein, Johannesburg, South Africa.



Figure 3.1. A. Photograph of pumpkin leaves. Photograph taken by candidate. B. Photograph of cowpea leaves. Adopted from Boari et al. (2022).

#### 3.2 Preparation of samples

##### 3.2.1 Pumpkin flowers

Preparation of the pumpkin flowers was conducted as described by Pongjanta (2006). Briefly, pumpkin flowers were sorted before being washed. The pumpkin flowers were blanched as follows, enough water to fully submerge the flowers was boiled (100°C) in a pot. The flowers were immersed in the boiled water for two minutes. The flowers were drained, cooled and oven-drying at 60°C until completely dry. Then they were milled, screened through a clean

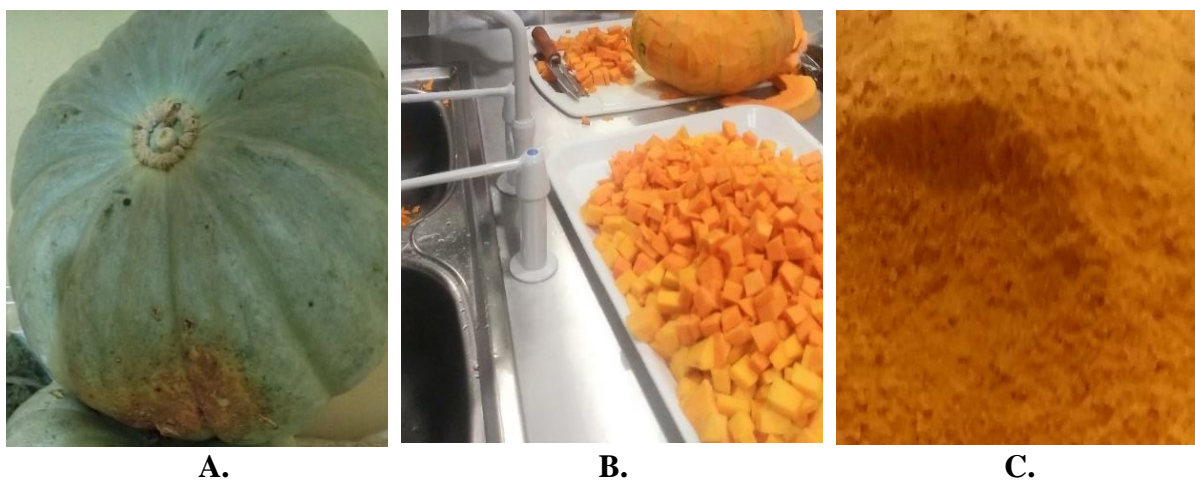
mesh sieve with 2 mm spaces before being packed in low density polyethylene bags and stored at 4°C in a refrigerator until use.



**Figure 3.2.** A. Pumpkin flowers. B. Powdered, dried pumpkin flowers. Photographs taken by candidate.

### 3.2.2 Pumpkin fruit

The preparation of the pumpkin fruit was as described by Różyło et al. (2014) and Pongjanta et al. (2006). Briefly, the fruit was cleaned, peeled, seeds were removed, and the flesh was cut into 2 cm<sup>3</sup> pieces. These pieces were soaked in 0.1% (w/v) citric acid for 15 min to prevent oxidation, before being drained, crushed and pulped. The pulp (flesh residue) was screened through a 0.35mm sieve and spread on stainless steel trays. The flesh residue was then oven-dried at 65°C for 8 hours. The dried pulp was milled, screened using a 2mm mesh sieve before being packaged in polyethylene bags and stored in a refrigerator at 4°C.



**Figure 3.3.** Preparation of pumpkin fruit powder. A. Pumpkin fruit. B. Diced pumpkin fruit. C. Powdered, dried pumpkin fruit. Photographs taken by candidate.



### 3.2.3 Pumpkin seeds

The pumpkin seeds were prepared as described by Rezig et al. (2016). Briefly, the seeds were cleaned and oven-dried at 80°C for 6 hours before being de-hulled, milled and screened by passage through a 2 mm mesh sieve. The powder was packed in polyethylene bags and stored at a low temperature (4°C) until use.



**Figure 3.4.**A. Photograph of pumpkin seeds. B. Photograph of powdered pumpkin seeds.

### 3.2.4 Cowpea leaves

The preparation of the leaves was conducted as described by Idris (2011). Briefly, this involved sorting the cowpea leaves, followed by their washing. The leaves were blanched as follows, enough water to fully submerge the flowers was boiled (100°C) in a pot. The flowers were immersed in the boiled water for 2 min. They were drained, cooled and oven-dried at 60°C until they reached a constant weight. The dry leaves were milled, screened (2 mm mesh sieve), packed in polyethylene bags and stored at 4°C until use.



**Figure 3.5.**A. Fresh cowpea leaves. B. Powdered cowpea leaves. Photographs taken by candidate.

### 3.2.5 Cowpea seeds

Cowpea seeds were sorted, cleaned and soaked in tap water (1:10 v/v) at 25°C for 24 hr. They were then drained, de-hulled and boiled in fresh tap water for 30 min. The boiled seeds were cooled and oven-dried at 130°C for 6 hr. The dried seeds were milled, screened (2 mm mesh sieve), packed in polyethylene bags and stored in a refrigerator until use.



**Figure 3.6.** A -Cowpea seeds; B - Powdered cowpea seeds. Photographs taken by candidate.

### 3.3 Formulations for preparation of soup

Three (3) soup formulations and a control that did not contain either cowpea or pumpkin were developed as a novel feature of this study. Composite mixes that consisted of a mixture of cowpea leaf powder and pumpkin seed powder in a ratio of 1:1 (Mix A), or cowpea seed powder and pumpkin fruit powder in a ratio of 1:1 (Mix B) or cowpea leaf powder and pumpkin flower powder in a ratio of 1:1 (Mix C) were prepared separately. The amounts of ingredients were calculated based on a batch size of 100 g (Table 1).

**Table 3.1.** Formulation of soup mixes based on 100 g batch size.

Ingredients	Control	F1 (5%)	F1 (10%)	F1 (15%)	F2 (5%)	F2 (10%)	F2 (15%)	F3 (5%)	F3 (10%)	F3 (15%)
Dry carrots	10	10	10	10	10	10	10	10	10	10
Potato powder	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75
Onion powder	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mushroom powder	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Ungelatinised corn starch	15	15	15	15	15	15	15	15	15	15
hydrogenated fat	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Salt	3	3	3	3	3	3	3	3	3	3
Brown sugar	2	2	2	2	2	2	2	2	2	2
Tomato powder	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Composite mix A	-	5	10	15	-	-	-	-	-	-
Composite mix B	-	-	-	-	5	10	15	-	-	-
Composite mix C	-	-	-	-	-	-	-	5	10	15
<b>Total</b>	<b>61.75</b>	<b>66.75</b>	<b>71.75</b>	<b>76.75</b>	<b>66.75</b>	<b>71.75</b>	<b>76.75</b>	<b>66.75</b>	<b>71.75</b>	<b>76.75</b>





**Figure 3.7.** Soup recipes formulated in Table 3.1

Note: Control = 100% CIMix+ 0% composite mix. F1 (5%) = formulation 1 with 5% (w/w) of cowpea leaf powder and pumpkin seeds powder at a ratio 1:1; F1 (10%) = formulation 1 with 10% (w/w) of cowpea leaf powder and pumpkin seeds powder at a ratio 1:1; F1 (15%) = formulation with 15% (w/w) of cowpea leaf powder and pumpkin seeds powder at a ratio 1:1; F2 (5%) = formulation 2 with 5% (w/w) of cowpea seeds powder and pumpkin fruit powder at ratio 1:1; F2 (10%) = formulation 2 with 10% (w/w) of cowpea seeds powder and pumpkin fruit powder at ratio 1:1; F2 (15%) = formulation 2 with 15% (w/w) of cowpea seeds powder and pumpkin fruit powder at ratio 1:1; F3 (5%) = formulation 3 with 5% (w/w) of cowpea leaf powder and pumpkin flower powder at ratio 1:1; F3 (10%) = formulation 3 with 10% (w/w) of cowpea leaf powder and pumpkin flower powder at ratio 1:1; F3 (15%) = formulation 3 with 15% (w/w) of cowpea leaf powder and pumpkin flower powder at ratio 1:1.

### **3.4 Analysis of functional properties of cowpea and pumpkin samples**

#### **3.4.1 Water absorption capacity**

The water absorption capacity was determined using the method described by Adegunwa et al. (2012). Briefly, 15 ml of distilled water was added to 1 g of the formulated soup mix in a pre-weighed centrifuge tube. The tube and its content were agitated on a IKA orbital shaker (KS 130 Basic shaker, Lasec laboratories) for 2 min and then centrifuged (Sorvall GLC-1 centrifuge (Model 06470, USA) for 20min at 4000 g. The clear supernatant was decanted and the mass of each centrifuge tube containing the sediment was measured. The quantity of water bound by the sample was determined by subtracting the mass of the centrifuge tube plus the mass of the soup mix from the mass of the tube plus the soup mix sediment. The difference between these masses was then calculated as the volume of water bound per 100 g of dry soup powder.

#### **3.4.2 Swelling power and solubility index**

The method of Adegunwa et al. (2012) was used to determine the solubility and swelling power. Briefly, 1g of soup powder was weighed into each of four pre-weighed 50 ml centrifuge tubes and gently mixed with 50 ml of distilled water. Respective soup slurries were then heated in a water bath at 70, 80, 90 or 100°C for 15 min. To prevent the soup from clumping, each slurry was stirred gently during heating. After 15 min, the tube with the paste was centrifuged at 3000 rpm for 10 minutes using a centrifuge (Sorvall GLC-1 centrifuge (Model 06470, USA). The supernatant was decanted promptly after centrifuging. The weight of the sediment was measured and recorded. The content of moisture in each sediment was determined relative to the dry matter as follows:

$$\text{Swelling power} = \frac{\text{Weight of wet mass sediment}}{\text{Weight of dry matter in the gel}} \times 100$$

$$\text{Solubility index (\%)} = \text{weight of dry solid after drying} \times 100$$

#### **3.4.3 Dispersibility**

Dispersibility was determined using the method described by Kulkarni et al. (1991). Samples were prepared in triplicate of 10 g of each soup mix. Each sample was measured into a 100 ml measuring cylinder. Distilled water was then added to the measuring cylinder to make up to 100ml of the mixture. The measuring cylinder was sealed with parafilm, and the mixture was physically shaken vigorously for 5 minutes and left aside to settle for 3 hours. The volume of

the settled particles was measured and recorded and subtracted from 100 ml. The difference was reported as percentage dispersibility.

#### **3.4.4 Rehydration ratio**

The rehydration ratio provides details of the mass of rehydrated and drained food to the mass of the original material (Severini et al., 2005). Its measurement was done using the method described by Krokida and Marinos-Kouris (2003). Exactly 2 g of each of the formulated cowpea-pumpkin soup powders was mixed with 20 ml distilled water in a glass beaker and immersed in a water bath at (40, 60 and 80°C, respectively, while agitated at a constant speed of 100 rpm. After 10 min, the samples were removed from the water bath, then blot dried using tissue paper (to remove the extra solution) and weighed. The rehydration ratio is the ratio of weight of rehydrated samples relative to the dry weight of the sample.

### **3.5. Determination of physical properties of cowpea and pumpkin samples, and soup mixes**

#### **3.5.1 Colour measurement**

A pre-calibrated Hunter Lab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to measure sample colour. About 20 g of the sample were measured into a clean dry glass sample cup, placed in a sample port, covered and results were recorded. The readings were recorded as Hunter Lab values, whereby: L\* (100=white; 0=black) indicated lightness; a\* measures chromaticity (i.e., the quality of colour), with negative values showing greenness and positive values indicating redness; and b\* measures chromaticity, with negative values indicating blueness and positive values showing yellowness.

#### **3.5.2 Viscosity**

*Sample Preparation:* Exactly 10 g of different soup composition was weighed and dispersed in 100 ml of distilled water. The mixture was stirred with heating until it reached boiling point and started thickening and then continued to boil for a further 5-10 minutes with constant stirring. However, it was noticeable that once you stop stirring the mixture had a non-uniform composition. Soups were then cooled to room temperature before analysis was started. The measurements were performed on the Brookfield Ametek RST Rheometer (Type: RSTSST, S/N: 7123080) with CCT-25 spindle (S/N: 0400174), software used was Rheo3000, v2.0. Method Parameters: time 60s; Speed: 0-1300rpm; Reading points: 60; No temperature control.

### 3.6 Proximate composition

A proximate composition analysis of the samples including determination of crude fat, moisture content, crude fibre, and crude protein of the formulations was determined according to AOAC (2000) methods. Carbohydrate content was calculated by subtracting the percentage sum of moisture, crude fibre, crude protein, crude fat and ash from 100%, while energy was determined using combustion calorimetry (Jeong & Chung, 2018). The samples were analysed in triplicate and the methods are described in detail in the following sections.

#### 3.6.1 Moisture content

The moisture content was determined using methods described by the AOAC (2010). Briefly, this involved drying two grams of each sample to constant weight at 105°C in a pre-weighed crucible for five hours (AOAC, 2010). The samples were then cooled for 10 minutes, weighed and the percentage moisture content was calculated as:

$$\text{moisture content (\%)} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Where  $W_1$  = Initial weight of empty crucible,  
 $W_2$  = Weight of crucible + sample before drying and  
 $W_3$  = final weight of crucible + sample after drying.

#### 3.6.2 Crude protein

The micro Kjeldahl method described by AOAC (2010) was used. Two grams (2g) of the samples were mixed with 10 ml of concentrated sulphuric acid ( $H_2SO_4$ ) in a heating tube. One tablet of selenium catalyst was added to each tube, and the mixture heated inside a fume cupboard. The digest was then transferred into a 100 ml volumetric flask and made up to volume with distilled water. A portion of the digest (10 ml) was mixed with 10 mL 45% NaOH solution and poured into a Kjeldahl distillation apparatus (Buchi Digestion and Distillation Protein Analyser, Switzerland). The mixture was distilled and the distillate collected into 4% boric acid solution containing 3 drops of methyl orange indicator. Each distillate (50 ml) was collected and titrated using 0.1 M Hydrochloric acid (HCl). Percentage nitrogen was first calculated as:

$$\% N = [100 \times W \times N \times 14 \times V_f] \times T / 100 \times V_a;$$

where  $W$  = weight of the sample.  
 $N$  = the normality of the nitrate (0.1N).  
 $V_f$  = total volume of the digest (100 mL).

*T = the Titre value and*  
*Va = the aliquot volume distilled.*

Crude protein was calculated as:

$$\text{protein (\%)} = \text{N (\%)} \times 6.25$$

where N (%) is the percentage nitrogen and 6.25 is the conversion factor.

### **3.6.3 Ash content**

Exactly 2 g samples in a pre-weighed crucible were placed in a muffle furnace for 8 h at 600°C, then cooled after which the crucible was removed and reweighed (AOAC, 2010). Percentage ash was calculated as follows:

$$\text{Ash content (\%)} = \frac{(\text{ash weight} + \text{crucible}) - \text{crucible weight}}{(\text{sample weight} + \text{crucible}) - \text{crucible weight}} \times 100$$

### **3.6.4 Crude fibre**

Two grams of each sample was weighed into a flask and 100 ml of 0.25 N H<sub>2</sub>SO<sub>4</sub> was added to it at room temperature. The mixture was heated to boil and refluxed for 60 minutes from onset of boiling. The mixture was filtered. To the residue was added 100 ml of 0.31 N NaOH and further heated for 1 h. The mixture obtained was filtered using a fibre sieve cloth and 10 mL of acetone was added to the filtrate. The obtained residue was washed with hot water and transferred to a crucible. The samples were dried at 105°C for 2 hours and allowed to cool in a desiccator. After cooling, the samples were weighed in triplicate (AOAC, 2010). The crude fibre was calculated using the following equation:

$$\text{Crude fibre content (\%)} = \frac{\text{weight after drying}}{\text{weight of sample}} \times 100$$

### **3.6.5 Crude fat**

The crude fat of samples was determined using the methods described by the AOAC (2010) using a Soxhlet apparatus and extraction using petroleum ether. Ground samples of 2 g were weighed into pre-weighed thimbles. This was then extracted for 8 h in a heating mantle and

dried at room temperature. The thimbles and sample were reweighed and the difference in weight was expressed as percentage crude fat using the following formula:

$$\text{Crude fat content (\%)} = \frac{(\text{weight of beaker} - \text{weight of fat sample}) - \text{weight of beaker}}{\text{weight of sample}} \times 100$$

### **3.6.6 Carbohydrate content**

Total carbohydrate was calculated by subtracting the percentage sum of moisture, crude fibre, crude protein, crude fat and ash from 100% according to methods described in the AOAC (2010).

$$\text{Total carbohydrate content (\%)} = 100 - (\text{moisture} + \text{ash} + \text{fat} + \text{fibre} + \text{protein})$$

### **3.7 Consumer acceptability of soup**

Approval was obtained from the Municipal Manager (Appendix 2 and 3) to perform the consumer acceptance test in FetakgomoTubatse Local Municipality, Limpopo Province, South Africa. To evaluate the various vegetable soups, a panel of seventy (n=70) untrained consumers who were over the age of 18 years were selected randomly based on availability as determined during their recruitment. The consumers were all selected from a rural environment, the main beneficiaries of the results and recommendations from this study, who preferred an informal environment in which to conduct the sensory evaluation. Due to the Covid 19 pandemic and lockdown restrictions the sensory evaluation was conducted outside the laboratory. Since a sensory evaluation lab could not be procured, a noise and odour-free testing venue compatible with a professional sensory evaluation laboratory was provided at a convenient area for the target population. To avoid distractions and allow people to focus on the evaluation, the environment was very quiet, no talking was allowed, no music, no eating or drinking was permitted. To comply with COVID-19 rules and regulations, social distancing was observed by seating the panellists one metre apart as well as to prevent them from influencing each other. The consumer panel comprised males (n=29) and females (n=41) who confirmed that they were not allergic to the ingredients used to formulate the soup recipe. The soup samples were labelled and presented in a randomized order as described by Granato et al. (2012). The panel evaluated a spoonful (25–30 mL) of hot soup samples (intransparent plastic cups) for visual appearance, odour, mouthful/texture, taste, and overall acceptability using a 9-point hedonic

scale (9 = liked very extremely, 8 = liked very much, 7 = liked moderately, 6 = liked slightly, 5 = neither liked or disliked, 4 = disliked slightly, 3 = disliked moderately, 2 = disliked very much, and 1 = disliked extremely) (Meilgaard et al., 2007; Stone & Sidel, 2004). The panel was provided with tap water in separate containers to rinse their mouths and plates before and between tastings. The panellists wrote their response directly onto a questionnaire which was provided to each panellist (Appendix 2).

### **3.8 Total phenolic content and antioxidant potential**

#### **3.8.1 Sample extraction**

The phenolic compounds from each cowpea and pumpkin soup powder sample were extracted using the method described by Waterman & Mole (1994). This was done using acidified methanol (1% HCl in methanol). Each sample was extracted with 30 ml solvent in three phases as follows: 10 ml solvent was added to 0.3 g of the sample in a conical flask and completely covered with aluminium foil. Each sample was stirred for 2 h, transferred to 40 ml plastic centrifuge tubes, centrifuged at 3500 rpm for 10 min (25°C) and decanted, retaining the supernatant. The sample residue was rinsed again with 10 ml of the solvent stirred for 20 min centrifuged again as above and decanted, keeping the supernatant. This step was repeated as described. The supernatants were combined and stored in a glass bottle covered with aluminium foil and kept in the cold room until analysed.

### **3.8.2 Total phenolic content (TPC)**

The Folin-Ciocalteu assay was used to determine the total phenolic content (TPC) of cowpea and pumpkin soup mixes. Gallic acid was used as the standard for this assay. To perform the assay, 0.5 ml of extracts were mixed with 10 ml of distilled water in a volumetric flask. Then, 2.5 ml of Folin-Ciocalteu reagent was added to the flask, followed by shaking and incubating in the dark. After 2 minutes, 7.5 ml of sodium carbonate solution (20 g/100 ml) was added to each flask. The contents were mixed and made up to volume with deionized water. The flask was stoppered and thoroughly mixed by inverting several times. Following the addition of sodium carbonate, the flask was allowed to stand for 2 hours before measuring the absorbance at 760 nm using a UV/Vis spectrophotometer. A reagent blank was also prepared using distilled water. The concentration of total phenolic compounds in the extract was expressed as milligrams of gallic acid equivalent (GAE) per 100 g sample (mg GAE/100 g). All samples were analysed in triplicate.

### **3.8.3 Antioxidant activity (ABTS)**

The antioxidant activity was determined using the method described by Awika et al. (2003). The solution of ABTS radical cation ( $ABTS^{\cdot+}$ ) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate ( $K_2S_2O_8$ ) using deionised water. The solution was allowed to react for at least 12 hours in the dark at room temperature before use. A phosphate buffer solution (pH 7.4) was prepared by mixing 0.2 M  $Na_2HPO_4$  (40.5 ml), 0.2 M  $Na_2H_2PO_4$  (9.5 ml) and 0.877g NaCl and filling up to 100 ml with deionised water. The buffer solution (pH 7.4) can be adjusted using 1 M HCl or 1 M NaOH. A working solution was prepared by adding 2.5 ml of  $ABTS^{\cdot+}$  solution to phosphate buffer solution (72.5 ml).

#### ***Sample analysis:***

The working solution (2.9 ml) was added to methanolic extracts (0.1 ml) or Trolox standard (0.1ml) in each test tube and mixed. The test tubes were allowed to stand 30 min for both Trolox standard and the samples. The absorbance of the standards and samples was measured at 734 nm. The results were expressed as  $\mu$ M Trolox equivalent/100 mg sample.

### **3.8.4 Antioxidant activity (DPPH assay)**

The DPPH assay was conducted using the method described by Awika et al. (2003). This involved the preparation of the following solutions:



*DPPH stock solution:*

A total of 12 mg (0.0012 g) of DPPH was weighed into a 50 ml volumetric flask and made up to volume with methanol. Note that it dissolves rather slowly and should be shaken for at least 20 minutes to make sure all DPPH particles are dissolved. This solution should remain stable for use for at least 3 weeks when stored at -20°C.

*DPPH working solution:*

A 10 ml volume of DPPH stock solution was pipetted into each 100 ml measuring cylinder and diluted with 50 ml methanol to obtain an absorbance of approximately 1.1 OD units at 515 nm. If too low, this solution could be adjusted with DPPH stock solution. Methanol was used to zero the spectrophotometer.

*1000 µM Trolox Stock Solution:*

Exactly 25 mg (0.0025 g) Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was weighed into a 100 ml volumetric flask and made to the mark with methanol. Trolox serial dilutions (0 – 1000 µM) were prepared and a standard curve drawn. To marked sealable tubes prepared in duplicate, 150 µl of Trolox standard and 2850 µl of DPPH working solution was added. These were covered with foil and shaken for 15 minutes. Absorbance was measured at 515 nm.

*Sample analysis:*

A volume of 2850 µl of DPPH working solution was added to 150 µl sample extract in a sealable tube. This was prepared in duplicate. It was shaken for 60 minutes (usually for 6 hours) and absorbance was measured at 515 nm. A blank was included in every sample. Absorbance reading should be between 0.2 and 1.1. If absorbance is less than 0.2 additional dilution of sample extract is necessary. NB: to be on the safe side, absorbance should be checked after the first 15-30 minutes, and make sure it is at least 0.5.

### **3.8.5 Flavonoids profiling**

#### **3.8.5.1. Sample preparation for flavonoid profiling**

To prepare the samples for metabolite profiling, 1 g of each soup mix was weighed and placed in centrifuge tubes. Then, 10 mL of 80% aqueous methanol was added and mixed well. The tubes were placed in an ultrasonic bath (Scientech 704, Labotech, South Africa) and allowed

to sonicate for 1 hr at 4°C. This was followed by centrifugating the tubes at 3500 rpm for 5 min at 4°C using an Eppendorf 5702R centrifuge (Merck South Africa). The supernatant, was carefully transferred to a round bottom flask and subsequently concentrated using a rotavapor under vacuum at 40°C. The extract was dried, and to reconstitute it, 1 mL of high-quality methanol suitable for chromatography was added. The reconstituted extract was filtered into dark amber vials for analysis. The whole extraction process was repeated three times for each sample.

### 3.8.5.2. Sample analysis

Following a method described by Adebo et al. (2019), the LECO Pegasus GC-HRTOF-MS system (LECO Corporation, St Joseph, MI, USA) was calibrated using perfluorotributylamine (PFTBA) as a mass calibration compound, with 11 masses utilised for pre-analysis calibration: CF<sub>3</sub> (m/z 68.9952), C<sub>2</sub>F<sub>4</sub> (m/z 99.9936), C<sub>2</sub>F<sub>4</sub>N (113.9967), C<sub>2</sub>F<sub>5</sub> (m/z 130.9920) C<sub>3</sub>F<sub>6</sub> (m/z 149.9904), C<sub>4</sub>F<sub>9</sub> (m/z 218.9856), C<sub>5</sub>F<sub>10</sub>N (m/z 263.9871), C<sub>8</sub>F<sub>16</sub>N (m/z 413.9775), C<sub>9</sub>F<sub>18</sub>N (m/z 463.9743), and C<sub>9</sub>F<sub>20</sub>N (m/z 501.9711). The observed intensity and resolution were 41,392 and 40,200, respectively, with a mass accuracy root mean square (RMS) of less than 1 ppm. Sample analysis was done with the GC-HRTOF-MS system, equipped with an Agilent 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) operating in high-resolution, a Gerstel MPS multipurpose autosampler (Gerstel Inc. Germany) using a Rxi®-5 ms column (30m×0.25mm ID×0.25 μm) (Restek, Bellefonte, USA). The samples were injected in a spitless mode by introducing one microliter of each sample using helium as a carrier gas, flowing consistently at a rate of 1 mL/min. The temperature of the inlet and transfer line was kept at 250 and 225°C, respectively. The initial oven temperature was set at 70°C and held for 0.5 min. The temperature was then increased at a rate of 10°C per minute until it reached 150°C. It was maintained at this temperature for 2 min. Afterwards, the temperature was further increased at a rate of 10°C per minute until it reached 330°C. It was then held at this temperature for 3 min to allow the column to bake out. A recommended rate of 13 spectra/s, with an m/z range of 30–1000, was used for MS data acquisition. The electron ionization was adjusted to 70 electron volts (eV), and the temperature of the ion source was kept at 250°C. Additionally, the system utilized a recommended extraction frequency of 1.5 kilohertz (kHz). To guarantee precision, the sample extracts from three replicates were analysed twice, resulting in a total of six analytical injections for each sample. The gathered GC-HRTOF-MS dataset was changed into mzML format using the LECO ChromaTOF-HRT software. Afterwards, it

was processed using the XCMS open-source tool, which included peak picking and alignment. The outcome was a peak list containing 6270 variables, including corrected peak retention times (in minutes), mass-to-charge ratios (m/z), and integrated peak areas.

### **3.9 Data analysis**

The statistical analysis was conducted utilizing the Statistical Package for the Social Sciences (SPSS) version 28.0 (IBM, New York, USA). A one-way analysis of variance (ANOVA) was executed to evaluate significant differences ( $p < 0.05$ ) among the means. The outcomes were presented as the mean values  $\pm$  standard deviation of three replicates, and the mean comparison was performed using Duncan's multiple range test (Duncan, 1995). Data for viscosity, functional properties, crude protein and carbohydrates were analysed using Principal Component Analysis (PCA) to determine variations between soup formulations based on loading of their attributes.

### **3.10 Ethical considerations**

Experiments were carried out in the UNISA Laboratories under the supervision of Dr D Beswa and Dr JJ Sibanyoni. Approval to perform the consumer acceptance test in the FetakgomoTubatse Local Municipality, Limpopo Province, South Africa, was obtained from the Municipal Manager (Appendix 2). Each panellist who participated in the evaluation of consumer acceptability of the soup was provided with a consent form (appendix 3) which was read and explained to ensure that all the participants understand the purpose of the study. Then, the panel members were asked to complete and sign their consent forms to indicate their consent to participate in the study and to confirm that they understood the purpose of the study. The consumer panel members were informed that participation in the study was voluntary and that they were free to withdraw from the study at any stage and that their personal information would be kept confidential. In addition, participants were informed that there was no recompense associated with their participation in the study. Ethics approval was obtained from the UNISA Ethics Committee (appendix 1) Reference number 2019/CAES/076. The laboratory rules on safety were strictly followed so as to avoid any injuries or harm to personnel. Plagiarism was by all means possible avoided, and all citations were done in the form of interpretation and reconstruction of public works. Confidentiality in all research work was adhered to until the completion of the PhD thesis.

## CHAPTER 4

### RESULTS AND DISCUSSIONS

#### 4.1. Effect of cowpea powder and pumpkin powder on the functional properties of cowpea-pumpkin composite soup

Food components provide a variety of functions and play a significant role in the development of new food products. Functional properties describe the behaviour of ingredients during preparation and cooking, as well as how they affect the finished products in terms of appearance, feel (mouth and hand) and taste (Awuchi et al., 2019). These properties are very important in food formulations as they improve mouth feel and flavour retention (Emeka-Ike & Chukwuemeka, 2020). Ingredients used in food contribute variously to the functional property of food. An example of this is starch that is responsible for gelatinization, browning and flavouring of food. When foods containing starch are exposed to dry heat, dextrinization occurs leading to the formation of dextrans, which imparts a brown colour to the food to make it sweeter (Chinaza et al., 2019). Results of functional properties of cowpea-pumpkin composite soup are presented in Table 4.1.

**Table 4.1.** Functional properties of cowpea-pumpkin soup mixes

Soup mix	RR (g/g)	WAC (g/g)	SP (%)	SI (%)	Dispersibility (%)
Control	7.7 <sup>a</sup> ± 0.6	65.7 <sup>a</sup> ± 1.5	437.0 <sup>e</sup> ± 2.0	27.3 <sup>ab</sup> ± 2.5	62.7 <sup>c</sup> ± 1.5
F1 (5%)	8.0 <sup>ab</sup> ± 1.0	214.0 <sup>e</sup> ± 1.7	349.0 <sup>a</sup> ± 2.0	41.3 <sup>d</sup> ± 4.0	57.7 <sup>d</sup> ± 2.1
F1 (10%)	8.7 <sup>abc</sup> ± 0.6	185.7 <sup>b</sup> ± 1.5	354.0 <sup>b</sup> ± 2.0	49.7 <sup>e</sup> ± 5.5	61.3 <sup>de</sup> ± 2.1
F1 (15%)	8.7 <sup>abc</sup> ± 0.6	194.3 <sup>c</sup> ± 1.5	445.0 <sup>f</sup> ± 2.0	22.0 <sup>a</sup> ± 2.0	59.0 <sup>de</sup> ± 1.0
<b>F2 (5%)</b>	<b>12.3<sup>de</sup> ± 1.5</b>	<b>252.0<sup>f</sup> ± 1.0</b>	<b>464.0<sup>h</sup> ± 1.7</b>	<b>36.0<sup>cd</sup> ± 0.0</b>	<b>47.0<sup>c</sup> ± 2.0</b>
<b>F2 (10%)</b>	<b>11.3<sup>cd</sup> ± 0.6</b>	<b>252.0<sup>f</sup> ± 2.0</b>	<b>445.3<sup>f</sup> ± 1.5</b>	<b>25.0<sup>a</sup> ± 0.0</b>	<b>39.3<sup>b</sup> ± 1.5</b>
<b>F2 (15%)</b>	<b>14.7<sup>e</sup> ± 1.5</b>	<b>263.3<sup>g</sup> ± 2.0</b>	<b>451.7<sup>g</sup> ± 1.2</b>	<b>38.3<sup>cd</sup> ± 0.6</b>	<b>22.7<sup>a</sup> ± 2.1</b>
F3 (5%)	8.0 <sup>ab</sup> ± 1.0	206.3 <sup>d</sup> ± 1.5	364.7 <sup>c</sup> ± 1.5	33.7 <sup>bc</sup> ± 0.6	56.7 <sup>d</sup> ± 1.5
F3 (10%)	10.0 <sup>abcd</sup> ± 1.0	185.7 <sup>b</sup> ± 1.5	423.3 <sup>d</sup> ± 1.5	28.3 <sup>ab</sup> ± 0.6	51.7 <sup>c</sup> ± 1.5
F3 (15%)	10.7 <sup>bcd</sup> ± 0.6	189.3 <sup>b</sup> ± 1.5	471.0 <sup>i</sup> ± 1.0	41.7 <sup>d</sup> ± 0.6	49.3 <sup>c</sup> ± 1.5

Mean ± Standard deviation. Mean values followed by different letters in a column are significantly different  $p < 0.05$  (LSD). Note: F1- Formulation 1; F2- Formulation 2; F3- formulation 3; WAC – water absorption capacity; SP – swelling power; SI – solubility index; RR- rehydration ratio

#### **4.1.1. Water Absorption Capacity**

The water absorption capacity (WAC), also called water holding capacity, is the amount of water (moisture) taken up by food/powder to achieve a desirable consistency and to create a quality food product (Awuchi et al., 2019). The higher the moisture content of food, the lower the water absorption capacity (Sapirstein et al., 2018). The WAC for the experimental soup samples was significantly higher (185.7 – 263.3 g/g) than that of the control sample (65.7 g/g). Among the formulation variations, it appears that the addition of cowpea leaf powder and pumpkin seed powder had a significant effect on the WAC – an inverse effect was noted whereby a reduction in the WAC from 214 to 185.7 g/g was associated with an increase in cowpea leaf powder and pumpkin seeds powder. The decrease in WAC may be attributed to the low protein content in cowpea leaf powder. Proteins are both hydrophobic and hydrophilic in nature and so can interact with the water in foods (Awuchi et al., 2019) and according to Khalid & Elharadallou (2013), a low protein content contributes to a reduced water absorption. A similar trend was observed for the variations of formulation 3 where the WAC decreased from 206.3 to 185.7 g/g.

However, variations for formulation 2 followed an opposing trend where the WAC increased from 252 to 263.3 g/g. As observed by Aukkanit & Rrakit (2016) and Biama et al. (2020), a decrease in the WAC of formulation 3 variations was probably due to the higher moisture content of cowpea seeds and pumpkin fruits. In a study conducted by Aukkanit & Rrakit, (2016) to investigate the influence of the addition of dried pumpkin fruit powder on chemical, physical, cooking and sensory characteristics of noodles, it was reported that pumpkin contains high levels of water so that noodles together with 30 g dried pumpkin powder was highest regarding water absorption. In a study conducted by Biama (2020) to investigate the nutritional and technological characteristics of new cowpea varieties grown in Eastern Kenya, it was also reported that cowpea seeds have increased levels of absorbed water which also reduce cooking time. On the other hand, the increasing WAC values for formulation 2 variations may be linked to the higher protein content of cowpea seeds. The WAC was reported to increase with increasing protein content, and a high moisture content was observed in a protein isolates from dehulled, defatted cowpea (*Vigna unguiculata* L) seeds (Khalid et al., 2012).

Several researchers have reported that cowpea and pumpkin are potent sources of vital nutrients such as proteins and carbohydrates (Pradeepkumar, 2015; Konsam et al., 2016; Nyadanu & Lowor, 2015; Ong & Kim., 2017; Gani et al., 2018).

The high-water absorption capacity could also be due to the high carbohydrate content observed in the soup powder samples. According to Emeka-Ike & Chukwuemeka (2020) and Adejuyitan (2009), a high-water absorption capacity could be attributed to increased levels of carbohydrates present in food. Similar results were also obtained by Sharma & Lakhawat (2017), who also obtained a WAC of 189 g/g in pumpkin seed powder. The results are also compatible to those obtained by Kumari et al. (2021), with a WAC for pumpkin seed flour ranging from 178.00 g/g to 296.10 g/g. The results of this study are also in line with other studies that reported an increase in the WAC in pumpkin flour (Akintade et al., 2019). However, it is reported that very low or excessive water absorption can negatively affect the quality of food products (Awuchi et al 2019) hence the moderate WAC results obtained in this study are important in the consistency of the soup (Iwe et al., 2016) and makes cowpea and pumpkin acceptable for soup formulations.

#### **4.1.2. Swelling Power**

The swelling capacity (SP) is the volume in millilitre taken up by the swelling of one gram (1 g) of food material under specific conditions. Its determination is based upon the addition water or a swelling agent (Awuchi et al., 2019). The SP or SI is considered a quality measure in some food products as it is an indication of the non-covalent bonding between molecules of starch granules and is also one of the factors in determining the  $\alpha$ -amylose and amylopectin ratios (Iwe et al., 2016). The results from the current study regarding the determination of swelling power are shown in Table 4.2 and indicate that the swelling power for the experimental samples was significantly high - with mean values ranging from 349% to 471% – while the control sample showed an intermediate mean value of 437%. Among the variations of formulation 1, it appears that the addition of cowpea leaf powder and pumpkin seeds powder had a significant effect on swelling power as shown by the upward trend where the swelling power increased (from 349.0% - 445.0%) with an increase in cowpea leaf powder and pumpkin seed powder. A similar trend was also observed for the variations of formulation 3 where the swelling power increased from 364.7% – 471.0% in parallel with an increase in cowpea leaf powder and pumpkin flower. According to Surech & Samsher (2013), a high starch content increases swelling power (index) of foods and the increase in swelling power was probably due to high amounts of starch reported in all the samples.

Similar results were also observed by Moutaleb et al. (2017), who noted that an increase in sweet potato in blends resulted in an increase in the swelling power of the final product. The

results of the present study were also compatible with the previous findings of Nyam et al. (2013), who also noted higher swelling power contributed by pumpkin seeds. Furthermore, swelling power results of the present study are desirable for soup formulation with digestion rate suitable for dietary demands. Deng et al. (2020) stated that swelling power affects the digestion rate by enhancing acid diffusion and modulating partitioning of pepsin at the food-gastric fluid interface and thereby the total amount of pepsin in the food particle.

#### **4.1.3. Solubility Index**

Food solubility is a chemical and functional property referring to the ability of a given food substance to dissolve in a solvent, usually water or oil. This is commonly measured as the saturation concentration, in which addition of more solute does not increase the concentration of the solution and rather starts to precipitate excess solute (Awuchi et al., 2019). According to Zayas, (1997), solubility is the amount of proteins in a sample that dissolves in a sample which means it is that proportion of nitrogen in a protein product which is in the soluble state under specific conditions. Solubility is an important prerequisite particularly when protein is useful as a functional ingredient in foods (Rezig et al., 2016).

Results of the current study (Table 4.1) revealed that solubility for the experimental samples varied from 22.0 % to 49.7 % compared to the control sample (27.3 %). The solubility was found to be significantly higher in Formulation 1, variations 5% and 10% of cowpea leaf and pumpkin seed and in Formulation 3, variation 15% of cowpea leaf powder and pumpkin flower. The samples had mean values\* ranging from 41.3 % to 49.7 %. The higher rate of solubility observed could be due to the amino acid content in cowpea leaf powder in the samples. Amino acids are generally soluble in water due to the ionic attraction between the amino acid charges being replaced by strong attraction between polar water molecules and Zwitterions (Rezig et al., 2016). Generally, the level of amino acids in cowpea and pumpkin is low, so that, for example, tryptophan in cowpea, pumpkin seed, pumpkin flower and pumpkin fruit ranges from 0.01 mg/100 g to 0.095 mg/100 g (Ilesanmi et al., 2016; USDA, 2019; Glew et al., 2006).

The addition of cowpea leaf powder and pumpkin powder in variations of all the soup formulations showed a significant effect on their solubility so that an increase of cowpea leaf and pumpkin seed in formulation 1 led to a decrease in the soup solubility (from 49.7 - 22.0%). A similar trend was also observed for the variation of Formulation 2 where the addition of cowpea seeds and pumpkin fruit at 10% decreased solubility from 36.0% – 25.0%. The trend was also noted in Formulation 3 variations where the solubility decreased from 33.7% - 28.3%

following the addition of cowpea leaf and pumpkin flower. Conversely, solubility of the control sample (27.3 %) was rather lower than Formulation 1 (5% and 10%), Formulation 2 (5% and 15%) and formulation 3 varieties. The trend in formulation 2 and 3 varieties was not followed by 10% probably due to the effect of pumpkin fruit and pumpkin flower in the samples. The low solubility of the control sample could be due to the absence of cowpea and pumpkin during formulation because they contain starch granules responsible for solubility. It might also be due to the presence of lipids in the control sample that may reduce water absorption capacity of foods which can lead to reduced swelling capacity and consequently reduced solubility (Oppong et al., 2015). The high solubility of cowpea and pumpkin provide further support for their promising candidacy for future application in commercial soup products. Similar observations were reported by Rezig et al. (2016) who recorded solubility of 41.4% and 43.7% in protein fractions from pumpkin seeds.

The results revealed that Formulation 1 with 15% of cowpea leaf powder and pumpkin seed powder exhibited the lowest solubility of 22.0%. An increase to 15% cowpea leaf and pumpkin seed powder in the composite mix was noted to induce a decrease in the solubility of that soup formulation. A similar trend was observed for Formulation 2 with 10% of cowpea seed and pumpkin fruit powder (25.0 %) and Formulation 3 with 10% of cowpea leaf and pumpkin flower powder (28.3 %) where the addition of composite mix to 10% led to a decrease in the solubility of the samples.

In conclusion, the high solubility results of cowpea-pumpkin in the present study are highly desirable for soup formulation because it may increase its digestibility. According to Oppong et al. (2015), high solubility of food shows high digestibility of the food which may indicate excellent use for food formulation.

#### ***4.1.4. Dispersibility***

The dispersibility of a mixture in water indicates its reconstitutability and in food formulations, the higher the dispersibility of a food mixture, the better (Modawi, 2006). The data for dispersibility of cowpea-pumpkin soup presented in Table 4.1 varied from 22.7% to 49.3. The control sample without cowpea and pumpkin powder in the formulation, had a significantly high dispersibility of 62.7%. The addition of pumpkin seed, fruit, flower and cowpea leaf and seed powder had a significant effect on the dispersibility of the formulations. Among the variations of formulation 1, it appears that the dispersibility increased with addition of cowpea



leaf powder and pumpkin seed powder as shown by an increased from 57.7% -61.3%. Conversely, a downward trend was noted among the variations of formulation 2 where dispersibility decreased from 47.0 % to 22.7 % with the addition of cowpea seed powder and pumpkin fruit powder. A similar trend was also observed in variations of formulation 3 where the dispersibility decreased from 56.7 % to 49.3 % with an increase in cowpea leaf powder and pumpkin flower powder. It has been reported that dispersibility increases with increasing particle size distribution and decreases with an increasing proportion of fine particles below 90  $\mu$ m (Fang et al., 2008). Hence the dispersibility results obtained in this study are desirable for a soup because they indicate that the particles are not fine which could result in lumps or slurry neither are they too large. It can be concluded that the addition of pumpkin and cowpea decreases the dispersibility of the food products.

#### **4.1.5. Rehydration Ratio**

Rehydration is a diffusion process, during which water moves from the outside of the cells into the interior, and the rehydration capacities of samples depend on the dehydration method that is used (Severini et al., 2005). Rehydration is a way to analyse dried products where a high rehydration ratio value means that the dried product has a good quality because the pores allow water to re-enter the cells (Noomhorm, 2007). It is an important physical property of dried pumpkin, because it reflects the intrinsic property and molecular structure of the dried product. The data for the rehydration ratio of the experimental samples ranged from 8.0 to 14.7 g/g compared to the control sample that was the lowest (7.7) (Table 4.1).

The addition of cowpea pumpkin powder had a significant effect on the RR of the formulations as shown by an increase in the RR after their addition to the soup mix. The RR for the control sample was significantly lower than the variations in Formulations 1, 2 and 3. The lower rehydration values are evidence for product shrinkage caused by prolonged drying resulting in irreversible physico-chemical changes (Singh & Pandey, 2011). Among the variations of formulation 1, it appears that the RR increased from 8.0 to 8.7 g/g along with an increase in cowpea leaf and pumpkin seed powder. A similar trend was also observed for the variation of formulation 3 where the rehydration ratio increased from 8.0 – 10.7 g/g with an increase in cowpea leaf powder and pumpkin flower. The upward trend was also observed among the variation of formulation 3 where the RR increased from 12.3 to 14.7 g/g with an increase in cowpea seed powder and pumpkin fruit. The increase in rehydration ratio may be attributed to the high content of starch present in the samples, as reported by Singh & Pandey (2011). These

are similar to results obtained by Wang et al. (2011) as to the rehydration ratio of pumpkin (11.09). However, the study's results indicate that cowpea and pumpkin are suitable for soup production due to their ability to absorb water into the cells as demonstrated by their rehydration ratio. According to Berk (2009), dehydrated foods should have the ability to regain their original moisture content, volume, shape and quality when rehydrated. This is important for soup production as it ensures that the ingredients can absorb water into their cells, resulting in a desirable texture and flavor in the final product therefore, cowpea and pumpkin possess this ability.

## **4.2. Effect of cowpea powder and pumpkin powder on the physical properties of cowpea-pumpkin composite soup**

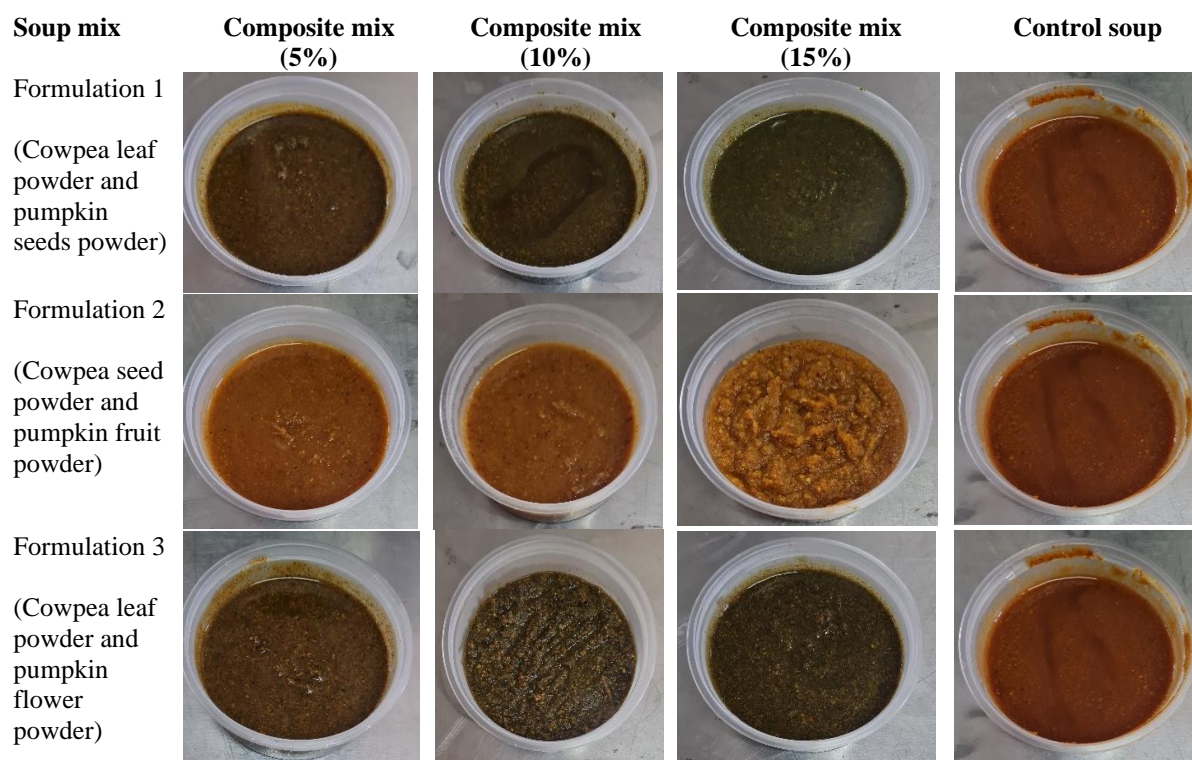
### **4.2.1. Appearance and colour of soup samples**

Using the control soup as a base, the composition of the soup mix formulations varied following the addition of bulleted components at 5%, 10% or 15% as follows:

- Formulation 1: increasing proportions of powdered cowpea leaf and pumpkin seed.
- Formulation 2: increasing proportions of powdered cowpea seed and pumpkin fruit.
- Formulation 3: increasing proportions of powdered cowpea leaf and pumpkin flower.

Photographs of cowpea-pumpkin composite soup formulations are shown in Figure 4.1. As indicated in column 4, the control soup mix samples had an appealing golden-brown colour. Regarding formulation 1, following the addition to 5% of the compositemix, the colour of the soup deepened slightly with slight gold and green shades. As the formulation increased to 10% and then 15%, the green colour in particular increased in intensity. From a colour perspective, the appearance of formulation 1 was similar to that of formulation 3. Cowpea leaf powder was common to these two soup preparations and so contained chlorophyll pigments (Li et al., 2018) that imparted a dark green colour to the soup and the intensity of the green colour in the various formulations increased together with the percentage increase in the formulations to 10% and then 15%. In terms of texture, formulation 1 appeared very different from formulation 3 which appeared to be relatively thicker and grainier. In addition to dark colour, formulation 3 had a coarse texture probably due to the presence of pumpkin flower powder. Cowpea leaf and pumpkin flower powder can also make soup appear thicker and grainier due to their high fiber content which adds bulk to the soup. The fibrous nature of these ingredients also contributes to the grainier texture as they do not dissolve completely.

In contrast, formulation 2 with 5% composite mix was an eye-catching, light golden-brown colour which was similar to the control soup. The difference between the appearance of formulation 2 soups and those of formulation 1 and 3 was the exclusion of vegetable leaf powders from the composite mix and this added consumer appeal to formulation 2. While all the formulation 2 soups retained the colour of the control soup, the 10% and 15% formulation 2 soup mixes increased in texture and graininess and appeared less appealing. In brief, a visual comparison of the three soup formulations according to their percentage composition indicated that formulation 2 soup colour and texture was most appealing at 5% and 10% addition of powdered cowpea seed and pumpkin fruit.



**Figure 4.1.** Photographs of samples of soup formulations

The colour of food largely affects its consumer appeal which ultimately has an influence on consumer expectations and purchase decisions. A colorimetric comparison of the control soup and formulation 2 soup samples showed that the intense golden-brown colour of these soups was associated with relatively high chroma values which ranged from 17.02 for the control soup and from 17.42 to 23.84 for the three formulation 2 soups containing increasing amounts of powdered cowpea seed and pumpkin fruit (Table 4.2).

The lightness intensity of the golden-brown colour increased with the addition of mixes from 5%, through 10% and on to 15% composite mixes as indicated by high L\* mean values (52.63 - 74.06) and chromaticity values (17.42 – 23.84) of formulation 2 compared to the control soup with L\* value (71.96) and chromaticity value of 17.02. This observation was also supported by high positive b\*-values (17.41-23.55) of formulation 2 when compared to the control soup (16.50). High positive b\*-values (responsible for yellowing) were probably due to the release of anthocyanins which are relatively high in the pumpkin fruit. The golden-brown colour of formulation 2 soup samples was probably imparted by carotenoid (mainly  $\beta$ -carotene, lutein, and zeaxanthin) which are predominantly present in pumpkin fruit and flours (Arifin et al., 2019). Carotenoids are abundantly occurring natural pigments responsible for the yellow, orange, and red colour of many fruits, vegetables, algae and bacteria (Atencio et al., 2022; Gordon & Bauernfeind, 1985). The intense golden-brown colour of formulation 2 soup samples may also be attributed to a combination of factors including browning and yellowing compounds, resulting from the Maillard reaction and the polymerization of anthocyanins with some phenolic compounds (Nistor et al., 2022).

**Table 4.2.** Colour of soup mixes

Soup mix	L*	a*	b*	C*
Control Mix	71.96 <sup>h</sup> ± 0.01	4.19 <sup>i</sup> ± 0.02	16.50 <sup>c</sup> ± 0.01	17.02 <sup>d</sup> ± 0.01
F1 (5%)	74.06 <sup>j</sup> ± 0.02	-3.20 <sup>f</sup> ± 0.02	19.83 <sup>g</sup> ± 0.01	20.09 <sup>g</sup> ± 0.01
(10%)	67.35 <sup>f</sup> ± 0.01	-0.43 <sup>b</sup> ± 0.02	15.13 <sup>a</sup> ± 0.01	15.14 <sup>a</sup> ± 0.01
(15%)	60.92 <sup>e</sup> ± 0.01	-0.63 <sup>d</sup> ± 0.02	16.48 <sup>c</sup> ± 0.02	16.49 <sup>b</sup> ± 0.02
F2 (5%)	72.49 <sup>i</sup> ± 0.01	3.72 <sup>g</sup> ± 0.01	23.55 <sup>h</sup> ± 0.01	23.84 <sup>h</sup> ± 0.01
(10%)	58.24 <sup>c</sup> ± 0.01	0.50 <sup>c</sup> ± 0.02	17.41 <sup>d</sup> ± 0.01	17.42 <sup>e</sup> ± 0.01
(15%)	55.29 <sup>b</sup> ± 0.00	0.96 <sup>e</sup> ± 0.02	17.51 <sup>e</sup> ± 0.02	17.54 <sup>f</sup> ± 0.02
F3 (5%)	71.37 <sup>g</sup> ± 0.02	3.81 <sup>h</sup> ± 0.05	26.86 <sup>i</sup> ± 0.01	27.13 <sup>i</sup> ± 0.01
(10%)	59.07 <sup>d</sup> ± 0.01	3.02 <sup>a</sup> ± 0.01	17.56 <sup>f</sup> ± 0.01	17.56 <sup>f</sup> ± 0.01
(15%)	52.63 <sup>a</sup> ± 0.01	3.20 <sup>f</sup> ± 0.01	16.29 <sup>b</sup> ± 0.02	16.60 <sup>c</sup> ± 0.02

Note: Mean ± Standard deviation. Mean values followed by different letters in a column are significantly different  $p < 0.05$  (LSD). L\* denotes lightness from black (0) to white (100), a\* redness (+) to greenness (-), b\* yellowness (+) to blueness (-) (Singo and Beswa, 2018). The chroma (C\*) coordinate indicates colour intensity (Hssaini et al., 2021).

The addition of more cowpea leaf resulted in a darker colour of the soup formulations 1 and 3 as shown by a decrease in L\*-values while a\*-values shifted from positive (redness) to negative (greenness) for formulation 1. In these formulations, the soup samples with a 5% of composite mix had the highest value of lightness (L\*) (74.06; 72.49 and 71.37, respectively). The intensity of the green colour of soup samples from formulations 1 and 3 could be attributed to the presence of cowpea leaves. Green plant leaves are known to contain high content of chlorophyll, a pigment responsible for the green colour. Increasing the concentration of

composite mix resulted in a further decrease in L\*-values (74.06-60.92 for formulation 1, 72.49 - 55.29 for formulation 2 and 71.37-52.63 formulation 3, respectively) and b\*-values (+ve), a\*-values (+ve) also decreased. The darker colour of the soup samples blended redness and yellowness. However, the overall colour difference values indicate a significant effect of cowpea leaf addition on the colour of formulations 1, 2 and 3.

#### **4.2.2. Rheological properties of soups**

Other physical parameters measured in this study were the rheological properties of cowpea-pumpkin composite soup. The rheological properties of liquid (flowing foods) play a significant role in process and quality control during food processing (Setiawan et al., 2021).

##### **4.2.2.1. Viscosity**

Viscosity is one of the important attributes of liquid foods like soups and has an effect on the mouthfeel and swallowability of food. While eating food that contains both liquid and solid phases, the liquid component tends to enter the hypopharynx before swallowing and this leads to high risk of aspiration (Yamada et al., 2017). According to Wendin (2010), patients who suffer from chewing and swallowing disorders e.g., dysphagia, may have difficulties ingesting normal food and liquids and need a texture-modified diet. Hence soups with higher viscosity observed in this study would be easier to swallow and could be recommended for children, the elderly and people with dysphagia and may enable them to maintain adequate nutrition. Therefore, when developing a liquid food, it is crucial to ensure that its viscosity suits the swallowing abilities/limitations of the target consumers (e.g., children, people with dysphagia etc.). Soups are perceived as being easier to swallow and could be recommended to people with swallowing problems e.g., young children. However, the porosity and wobbly consistence of low viscosity soups had higher risk of getting into airways during swallowing. The selected rheological properties of cowpea-pumpkin soup samples measured in this study are presented in Table 4.3.

Among the formulation1 variations of cowpea leaf powder and pumpkin seeds powder, the variation with 5% was more viscous as shown by the highest viscosity mean value of 88.91 cP with a shear stress of 21.2 Pa at a shear rate of 497.2 s<sup>-1</sup>. This was followed by the variations with the highest concentration of cowpea leaf powder and pumpkin seeds powder (15%) at a viscosity of 56.6 cP and shear stress of 15.1 Pa at a shear rate of 497.2 s<sup>-1</sup>. These values show that the addition of cowpea leaf powder and pumpkin seeds powder in formulation 1

significantly increased its viscosity with reference to the control soup (viscosity of 33.2 cP, shear stress of 13.9 Pa) at the same shear rate (497.2 s<sup>-1</sup>).

**Table 4.3.** Rheological properties for reconstituted cowpea-pumpkin soup mixes at 26°C

Soup Mixes	Viscosity (cP)	Shear stress (Pa)	Shear rate (s <sup>-1</sup> )
ClMix	33.21 <sup>c</sup> ± 0.04	13.92 <sup>c</sup> ± 0.19	497.23 <sup>a</sup> ± 2.05
Formulation 1 (5%)	88.91 <sup>i</sup> ± 1.10	21.21 <sup>f</sup> ± 0.30	497.23 <sup>a</sup> ± 0.85
(10%)	31.92 <sup>c</sup> ± 0.13	36.38 <sup>h</sup> ± 0.19	752.56 <sup>c</sup> ± 0.41
(15%)	56.64 <sup>e</sup> ± 0.45	15.14 <sup>d</sup> ± 0.55	497.23 <sup>a</sup> ± 0.95
Formulation 2 (5%)	21.09 <sup>a</sup> ± 0.40	7.80 <sup>a</sup> ± 0.10	497.20 <sup>a</sup> ± 0.30
(10%)	62.75 <sup>f</sup> ± 0.16	38.55 <sup>i</sup> ± 0.11	653.24 <sup>b</sup> ± 0.29
(15%)	48.78 <sup>d</sup> ± 0.85	18.96 <sup>c</sup> ± 0.18	497.11 <sup>a</sup> ± 2.70
Formulation 3 (5%)	24.13 <sup>b</sup> ± 0.55	8.65 <sup>b</sup> ± 0.05	497.20 <sup>a</sup> ± 0.50
(10%)	80.67 <sup>h</sup> ± 0.25	65.13 <sup>j</sup> ± 0.04	823.82 <sup>d</sup> ± 0.14
(15%)	71.50 <sup>g</sup> ± 0.70	22.37 <sup>e</sup> ± 0.56	497.21 <sup>a</sup> ± 1.39

Note: Mean ± Standard deviation. Mean values followed by different letters in a column are significantly different  $p < 0.05$  (LSD).

The increase in viscosity of formulation 1 was probably due to the presence of stabilizer agents (dietary fibres) in pumpkin seed which has the ability to bind water tending to increase the consistency of the products and so leading to the formulation of viscous gels and consequently promoting higher viscosities (Baba et al., 2018). It might also be due to molecular interactions and to inability of the samples to rehydrate and form crosslinks due to the high concentration of fibre which sequester water molecules (Mona et al., 2021).

The formulation 2 soup sample with 10% cowpea seed powder and pumpkin fruit powder was the thickest among the variations of formulation 2. This sample had a viscosity of 62.75 cP, shear stress of 38.5 Pa, at a shear rate of 653.2 s<sup>-1</sup>. In contrast, the 5% Formulation 2 variation exhibited the lowest viscosity (21.1 cP) and shear stress (7.8 Pa) among all the formulations in this study, including the control soup. The reduction in viscosity of formulation 2 (5%) could have been caused by the lower starch content in the lowest concentration of cowpea seed powder and pumpkin fruit powder (5%). According to Santamaria et al. (2021), viscosity is directly related to the amount of starch present in the food.

The incorporation of 10% cowpea leaf powder and pumpkin flower powder in formulation 3 resulted in soup samples with the highest viscosity of 80.7 cP, shear stress of 65.1 Pa, and the highest shear rate of 823.8 s<sup>-1</sup> among the variations of formulation 3. The variation with 5% of cowpea leaf powder and pumpkin flower powder was less viscous (24.1 cP) among formulation

3 variations (80.7 and 71.5 cP) and the control soup (33.2 cP). The lowest viscosity at 5% addition of the control mix was also observed in formulation 2.

The incorporation of cowpea into the formulation must have affected the viscosity. This might be due to the interaction of starches with protein which can influence gelatinization and retrogradation of starches (Adenike, 2012). The results agree with those reported by Idowu et al. (2017) with mean values ranging from 21.9 – 58.5 cP and 63.3- 107.3cP in cocoyam-cowpea blends. Similar results (65-145 cP) were also recorded by Adenike (2012) in 8% cowpea flour concentrations of complementary foods.

However, the variation in viscosity and shear appears not to be linear as the formulations increase from 5% through to 15%. i.e., viscosity does not consistently increase as the percentage composition of the soups increases. This could be an indication that the viscosity of pumpkin or cowpea powder at different concentrations had a great impact on the increase or decrease of the viscosity of the soup.

Among the formulation 1 variations (cowpea leaf powder and pumpkin seed powder), It was noted that as the viscosity increased there was a drop in shear stress and shear rate. This is because a higher viscosity means the soup's resistance to flow increased which resulted in a lower shear rate and lower shear stress. As the viscosity decreased, the shear stress and shear rate increased. This means the soup's resistance to flow allowed easier movement of soup leading to a higher shear rate and higher shear stress. The decrease in viscosity allowed faster movement. This is a characteristic on a non-Newtonian food. According to Van Canneyt & Verdonck (2014), foods whose viscosity decreases with increase in shear rate and vice versa are called non-Newtonian foods. In comparison, formulation 2 variations (cowpea seeds and pumpkin fruit) and formulation 3 variations (cowpea leaf powder and pumpkin flower) show an increase in shear stress and shear rate as the viscosity increased. This means an increase in shear rate and shear stress increased molecular alignment in the soup and the resistance of the soup to flow resulting in an increase in viscosity. This could be due to the addition of pumpkin seed powder in formulation 1 and pumpkin fruit and pumpkin flower in formulations 2 and 3. Formulation 2 and 3 could be examples of Newtonian food, foods with a constant viscosity are called Newtonian (Van Canneyt & Verdonck, 2014). Since no studies have been found related to this, it was not possible to compare this with other researchers.

It can be concluded that Formulation 1 (cowpea leaf and pumpkin seed powder at 5%) and Formulations 3 cowpea leaf powder and pumpkin flower at 10% and 15% had the desirable

viscosity. Thus, at higher viscosity the soup is thicker and is thought to be of superior quality (Fellows, 2009).

### 4.3. Effect of cowpea powder and pumpkin powder on the proximate composition of cowpea-pumpkin composite soup

Proximate assay an important criterion to assess the overall composition and nutritional status of any ingredient intended for food use (Verma et al., 2019).

The data on proximate composition of 10 soup formulations are summarized in Table 4.4.

**Table 4.4.** Proximate composition of soup mixes (g/100 g)

Soup mixes	Crude fat	Moisture	Crude fibre	Crude protein	Ash	Carbohydrates
ClMix	1.73 <sup>a</sup> ±0.15	7.78 <sup>a</sup> ±0.19	6.27 <sup>a</sup> ±0.49	13.33 <sup>a</sup> ±0.58	0.48 <sup>bc</sup> ±0.03	70.42 <sup>c</sup> ±0.82
F1 5%	2.73 <sup>a</sup> ±0.06	8.29 <sup>abc</sup> ±0.09	17.39 <sup>b</sup> ±6.25	27.00 <sup>bc</sup> ±2.00	0.52 <sup>cd</sup> ±0.01	44.06 <sup>a</sup> ±4.97
F1 10%	2.80 <sup>ab</sup> ±0.17	8.06 <sup>ab</sup> ±0.17	12.36 <sup>ab</sup> ±0.33	23.00 <sup>bc</sup> ±2.00	0.49 <sup>bcd</sup> ±0.06	53.30 <sup>ab</sup> ±1.55
F1 15%	3.10 <sup>b</sup> ±0.10	7.81 <sup>b</sup> ±0.29	14.25 <sup>ab</sup> ±1.69	24.33 <sup>bc</sup> ±2.52	0.48 <sup>bc</sup> ±0.01	50.03 <sup>ab</sup> ±3.56
F2 5%	2.30 <sup>ab</sup> ±0.20	8.68 <sup>bc</sup> ±0.25	9.11 <sup>ab</sup> ±1.75	26.67 <sup>bc</sup> ±1.53	0.55 <sup>cd</sup> ±0.04	52.70 <sup>ab</sup> ±3.13
F2 10%	2.13 <sup>ab</sup> ±0.84	8.84 <sup>bc</sup> ±0.13	13.29 <sup>ab</sup> ±7.08	22.33 <sup>b</sup> ±1.15	0.47 <sup>bc</sup> ±0.01	52.93 <sup>ab</sup> ±6.63
F2 15%	1.87 <sup>ab</sup> ±0.72	8.27 <sup>abc</sup> ±0.76	11.03 <sup>ab</sup> ±1.12	25.67 <sup>bc</sup> ±1.53	0.41 <sup>ab</sup> ±0.03	52.76 <sup>ab</sup> ±1.72
F3 5%	1.73 <sup>ab</sup> ±0.59	9.07 <sup>cd</sup> ±0.10	5.98 <sup>a</sup> ±0.57	24.67 <sup>bc</sup> ±0.58	0.55 <sup>cd</sup> ±0.04	58.00 <sup>b</sup> ±0.43
F3 10%	2.93 <sup>ab</sup> ±0.38	8.89 <sup>bc</sup> ±0.10	10.30 <sup>ab</sup> ±1.50	28.33 <sup>c</sup> ±3.21	0.35 <sup>a</sup> ±0.03	49.21 <sup>ab</sup> ±3.91
F3 15%	2.30 <sup>ab</sup> ±0.46	9.74 <sup>d</sup> ±0.12	11.28 <sup>ab</sup> ±0.64	26.33 <sup>bc</sup> ±2.52	0.57 <sup>d</sup> ±0.02	49.79 <sup>ab</sup> ±2.45

Mean ± Standard deviation. Mean values followed by different letters in a column are significantly different  $p < 0.05$  (LSD).

#### 4.3.1. Crude Fat content

Crude fat represents, besides the true fat (triglycerides), other materials extractable with ether such as phospholipids, sterols, essential oils and fat-soluble pigments (Ranganna, 1986). It includes all fats and oils that are edible which may be produced from plants and animals (FAO, 2020). Fat provides energy and aids in transport of fat-soluble vitamins, insulates and protects internal tissues and contributes to important cell processes (Pamela et al 2005; Jones et al., 1985).

Table 4.4 depicts the crude fat content of cowpea-pumpkin composite soup. A linear increase in crude fat content of composite soup was observed when more pumpkin powder was added. A significantly higher mean value of fat content was recorded in F1 15% samples (3.10 g/100 g) followed by F3 10% samples (2.93 g/100 g) then F1 10% samples (2.80 g/100 g). The possible reason for higher fat content in F1 samples is the addition of pumpkin seeds. Pumpkin



seeds have been reported as excellent sources of fat (38– 49 g/100 g) (Longato, 2017; Rezig et al., 2016; USDA, 2011). The fat content of Formulation 2 at all levels of composite mix was significantly less than F1 formulations (1.87-2.30 g/100 g). Addition of pumpkin fruit which has a low-fat content could be a possible reason. Pumpkin fruit is rich in different nutrients but low in fat (Poliszko et al., 2019). The results also indicate that addition of composite mix decreased the fat content of the soup in F2 formulations from 2.30 g/100 g to 1.87 g/100 g. The results show that the control sample and F3 with 5% composite mix had the lowest fat content, this was probably due to non-addition of either pumpkin or cowpea seeds which contain moderate amounts of fat. The low-fat content recorded in this study confirms the fact that Indigenous leafy vegetables are generally low in fats. Khalid & Elharadallou (2013) also reported similar results fat content (2.30 g/100 g) in cowpea. These results are also comparable to those reported by Madode et al. (2011) where fat content of 1.74 g/100 g was observed in cowpea puree. However, based on the results of this observation, low fat content in cowpea-pumpkin composite soup is desirable especially with a growing health-conscious population.

#### ***4.3.2. Moisture content***

The moisture content of cowpea-pumpkin composite soup was significantly high in F3 samples, a formulation of cowpea leaf powder and pumpkin leaf powder ranging from (8.89 – 9.74 g/100 g). It was observed that the control sample had the lowest moisture content (7.78 g/100 g) compared to samples F1 (7.81-8.29 g/100 g) and F2 (8.27-8.84 g/100 g). However, basing on these mean values there was no significant difference between all the formulated soup samples. Similar results were also observed by Pereira (2020) who also noted that high moisture content (8.46 – 9.55 g/100 g) in food present unfavourable conservation of products due to rapid decomposition. Moreira et al. (2020) also reported similar results on the effect of different cooking techniques on carotenoids and antioxidant of biofortified pumpkins. The results are also comparable to those obtained by Momanyi et al. (2020) who reported slightly higher moisture content ranging from 9.43 -9.45 g/100 g. The results obtained are significantly higher compared with the moisture content of pumpkin seed and rind (4.32-5.96 g/100 g) reported by Nyam et al. (2013), Habib et al. (2015) and those reported by Uduwerella et al. (2021) for pumpkin seed flour (6.33 g/100 g). High moisture content in food is an indication of their susceptibility to microbial attack and spoilage (Abubofuor et al., 2016) while lower moisture content ensures better shelf life and reduces the microbial spoilage of food (Ajayi et al., 2006).

### **4.3.3 Crude fibre content**

Dietary fibre is an indigestible carbohydrate found in structural components of plants (Sharma et al., 2012). It is the organic residue which remains after the food sample has been treated under standardized conditions with petroleum spirit, boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution and alcohol. The crude fibre consists largely of cellulose together with a little lignin (Verma et al., 2019). However, the health benefits of eating fibre rich diet are immense including prevention of constipation, regulation of blood sugar, protecting against heart diseases, reducing high levels of and prevention of certain form of cancers (Sharma et al., 2012). Fibres are not only desirable for their nutritional properties but also for their functional and technological properties and because of this, they could be used as food ingredients (Scieber et al., 2001).

The results for determination of crude fibre are presented in Table 4.4. Except for the control sample and F3 5% samples with low fibre content of 5.98 g/100 g and 6.27 g/100 g, the crude fibre of cowpea-pumpkin composite soup is significantly high. It was observed that F1 samples had the highest crude fibre content ranging from 12.36g/100g to 17.39 g/100 g (i.e., F1 5% = 17.39 g/100 g; F1 10% = 12.36 g/100 g, and F1 15% = 14.25 g/100 g). The high fibre content in F1 samples was probably due to the high crude fibre content found in pumpkin seeds that were incorporated in the formulation. However, the results are slightly lower than those reported by Nyam et al. (2013), who reported a high fibre content of 31.48 g/100 g in an investigation to determine the proximate composition pumpkin seed bread. Formulations F2, F3 10%, and F3 15% samples had slightly lower crude fibre content varying from 9.11 g/100 g to 13.29 g/100 g (F2 5% 9.11; F2 10% 13.29 g/100 g; F2 15% 11.03 g/100 g; F3 10% 10.30 g/100 g and F3 15% 11.20 g/100 g). The results indicate that there was not much difference between the mean values of these samples. The high fibre content in cowpea and pumpkin helps in regulating the digestive system aiding bowel health and weight management (Darkwa & Darkwa, 2013). Based on the results, it can be concluded that cowpea-pumpkin composite soup has a vital role to play in people's health.

### **4.3.4. Crude Protein content**

Proteins are large molecules made of amino acids. They are found in animal and plant foods and are the main structural constituents of the cells and tissues of the body (FAO, 2020) Substances, such as enzymes, hormones and antibodies are protein in nature and are necessary for the proper functioning of an organism. Lysine is the limiting amino acid in cereal proteins

whereas legumes contain adequate amount of lysine (Khattab & Arntfield, 2009). Proteins are necessary for growth and development of the body, for body maintenance and the repair and replacement of worn out or damaged tissues, to produce metabolic and digestive enzymes and they are essential constituents of certain hormones (FAO, 2020). According to Khalid et al. (2012), the continuous increase in population makes it difficult for people to afford buying animal products regularly. It is therefore recommended to enhance the protein quality of the diet through easily available and accessible plant protein sources to improve the nutritional status of the low-income group of population (Khattab & Arntfield, 2009). Crude protein measures the total nitrogen content of a product, and from that, estimates the amount of the protein within that product (Verma et al., 2012).

Results of the present study (Table 4.4) revealed that the highest crude protein content was observed in F3 10% (28.33 g/100 g) and the lowest was the control sample with 13.33 g/100 g. However, the results reveals that there was no significant difference in the mean values of all formulations at all levels of composite mix incorporation ranging from 22.33 g/100 g to 27.00 g/100 g (F1 23.00 g/100 g to 27.00 g/100 g; F2 22.33 g/100 g to 26.61 g/100 g; F3 24.67 g/100 g to 28.33 g/100 g). A possible reason for this is the addition of cowpea and pumpkin reported to be high in protein by various authors. Several authors reported that Indigenous vegetables are essential sources of proteins (Schreinemachers et al., 2018; Gido et al., 2017; Meldrum et al., 2018; Njume et al., 2014; Sharma et al., 2012; Khalid et al., 2012; Appiah et al., 2011; Kumari et al., 2021). Total crude protein was significantly low in the control sample where neither cowpea nor pumpkin was added. This is a clear indication that the addition of cowpea and pumpkin increases the protein content in food and can provide adequate nutrition vital to human survival. The obtained results agree well with those reported by Khalid & Elharadallou (2013) who reported 22.30 - 26.73 g/100 g protein content in cowpea flour, Sharma & Lakhawat (2017) reported 29.65 g/100 g protein content in pumpkin seeds. Nyam et al. (2013) reported protein content ranging from 20.21 to 23.89 g/100 g in pumpkin and Bunde-Tsegba et al. (2020), reported 24.80 g/100 g protein content in locust beans. Furthermore, protein content of 21.35 g/100 g was also reported by Momanyi et al. (2020) in cowpea and sorghum snack bars and lastly Naiker et al. (2019) reported 24.30 - 26.33 g/100 g protein content in cowpea flour. In contrast, the results are significantly higher than protein content of 9.6 g/100 g reported by See et al. (2007) in pumpkin flour. Current nutrition guidelines recommend 50 g – 56 g of protein daily (US FDA, 2022; NIH), the findings of this study reveal that cowpea and pumpkins are the vital sources for this. Consumption of cowpea-

pumpkin soup would be an important step towards relieving protein malnutrition in the poor countries of the world.

#### **4.3.5. Ash content**

Ash content of a food stuff is inorganic residue which remains after destruction of organic matter. It is also defined as a measure of the total amount of mineral content or inorganic material that remains after milling (Ejiofor & Kporna, 2019). It may not necessarily be exactly equivalent to the mineral matter as some changes may occur due to volatisation or some interaction between constituents (Ranganna, 1986). According to Adubofuor et al. (2016), food with high ash content is expected to have high concentration of various mineral elements which are expected to speed up metabolic processes, improve growth and development.

Even though the mean values for ash content were slightly different (Table 4.4), there was no significant difference in the ash content of all the samples. However, it was noted that the ash content in F1 progressively decreased with addition of cowpea and pumpkin from 0.52 to 0.48 g/100 g. A similar trend was also noted in F2 samples, wherein there was a reduction from 0.55 to 0.41 g/100 g. It is apparent that the incorporation of the composite mix consisting cowpea and pumpkin had a negative effect on the ash content as evidenced by similar observations in F2 where the mean values of ash decreased from 0.55 g/100 g to 0.41 g/100 g. A possible explanation for this decrease in ash content may be attributed to the inherent low ash content of cowpea and pumpkin. However, a different trend was reported in F3 samples as the highest ash content was observed in F3 15% (0.57 g/100 g), then F3 5% (0.55 g/100 g) then lastly the lowest of 0.35 g/100 g in F3 10%. The high ash content in F3 could be an indication that cowpea and pumpkin leaves combined produce high ash content as compared to other edible parts of these two indigenous vegetables. The results observed in this study are comparable to those obtained by Nyam et al. (2013) ranging from 0.41 to 0.68 g/100 g in pumpkin seeds and rind. Furthermore, the results were significantly lower from those reported by other researchers. Momanyi et al. (2020) reported ash content of 1.33 g/100 g and 2.26 g/100 g in sorghum and cowpea blend snack bar. Mean values of 2.71 – 3.64 g/100 g were reported by Naiker et al. (2019) in cowpea flour. Pereira (2020) reported ash content in pumpkin flour varying from 5.8 - 9.4 g/100 g and Saeleaw & Schleining (2011) also found ash content of 5.4 g/100 g in pumpkin flour.

#### **4.3.6. Carbohydrate content**

Carbohydrates have a wide range of physiological effects which are important for human health. They are the main source of energy and provide ideal fuel (glucose for body to function optimally (Verma et al., 2012). Carbohydrates provide energy to the cells particularly the brain cells that require glucose for metabolism. Energy is crucial in maintaining the metabolic rate of the body, growth, and synthesis of new tissues, muscular activity and physiological functions (Momanyi et al., 2020). Carbohydrates also have an impact on the mouthfeel of a product by increasing viscosity and providing textural cues perceived by the somatosensory system which makes them a vital formulation ingredient (Kokkinidou et al., 2018). Carbohydrates, especially starch have higher stability for granules swelling, high temperature resistant, higher shear stability and are used as viscosifiers and texturizers in soups (Egharevba, 2019).

Table 4.4 depicts the carbohydrate content of cowpea-pumpkin composite soup. Except for F1 5% samples with significantly low carbohydrate content (44.06 g/100 g), the carbohydrate content in all other formulated samples varied significantly with higher mean values ranging from 49.21 to 70.42 g/100 g. The control sample exhibited the highest carbohydrate mean value (70.42 g/100 g) in comparison to all experimental soup samples. The reason behind this noteworthy disparity may be attributed to the absence of cowpea and pumpkin in the control sample. Nevertheless, there was no significant variation between the mean values of F1 (44.06 - 50.03 g/100 g), F2 (52.70 - 52.93 g/100 g) and F3 (49.21 - 58.00 g/100 g). The probable explanation for the similarity in carbohydrate content is the dilution effect due to addition of cowpea and pumpkin in all the formulations which resulted in reduction of the carbohydrate content. These results were similar to the carbohydrate content (49.11 g/100 g) of pumpkin seeds reported by Nyam et al. (2013). Pereira (2020) also reported carbohydrate content varying from 54.2 to 61.8 g/100 g in pumpkin flour. Momanyi et al. (2020) observed slightly higher carbohydrate content of 61.1- 73.25 g/100 g in sorghum and cowpea. However, the values are lower compared to the results found by Saeleaw & Schleining (2011) who reported 79.57 g/100 g of total carbohydrate in pumpkin flour. From these findings, it can be concluded that cowpea- composite soup could make better sources of energy in people's diets. The decrease in carbohydrate content reported after addition of cowpea and pumpkin makes them low calorie and easy to digest raw materials.

#### 4.4. Effect of cowpea powder and pumpkin powder on consumer acceptability of cowpea-pumpkin composite soup

The data for consumer acceptance of soup samples is presented in Table 4.5. The results indicate that consumer acceptance of soup appearance for formulations F1 (6.61 – 7.27) and F2 (6.99 – 7.00) did not exhibit any significant deviation from that of the control sample, despite their mean values being slightly lower than the control samples (7.64). The similarity for consumer acceptance of F1 and F2 samples was probably due to the appealing colour resulting from the addition of pumpkin seeds in F1 and pumpkin fruit in F2 samples. This observation serves as a clear indication that colour plays a very crucial role in the acceptance of food. Similar results were also discovered by Arifin et al. (2019) in a study to evaluate the sensory acceptability of muffins made from pumpkin puree who muffins made from pumpkin puree noted that colour in is one of the most important attributes that directly affect the consumer preference of any product.

**Table 4.5.** Consumer acceptability of cowpea/pumpkin composite soup

Formulations	Sensory attributes				
	Appearance	Aroma	Taste	Mouthfeel/texture	Overall acceptability
Control	7.64 <sup>c</sup> ± 1.74	7.44 <sup>d</sup> ± 2.16	6.99 <sup>d</sup> ± 2.39	6.90 <sup>c</sup> ± 2.45	7.09 <sup>c</sup> ± 2.42
F1 (5%)	7.27 <sup>c</sup> ± 1.81	7.14 <sup>d</sup> ± 2.03	6.64 <sup>cd</sup> ± 2.46	6.36 <sup>bc</sup> ± 2.50	6.51 <sup>bc</sup> ± 2.53
F1 (10%)	6.61 <sup>bc</sup> ± 2.20	6.51 <sup>bcd</sup> ± 2.25	6.24 <sup>bcd</sup> ± 2.38	5.90 <sup>abc</sup> ± 2.58	6.16 <sup>abc</sup> ± 2.53
F1 (15%)	6.87 <sup>bc</sup> ± 2.04	6.70 <sup>cd</sup> ± 2.00	6.20 <sup>bcd</sup> ± 2.44	6.07 <sup>abc</sup> ± 2.49	6.17 <sup>abc</sup> ± 2.57
F2 (5%)	6.99 <sup>c</sup> ± 2.03	6.59 <sup>bcd</sup> ± 2.11	6.20 <sup>bcd</sup> ± 2.52	6.04 <sup>abc</sup> ± 2.61	6.29 <sup>bc</sup> ± 2.62
F2 (10%)	6.74 <sup>bc</sup> ± 2.13	6.53 <sup>bcd</sup> ± 2.28	6.10 <sup>bcd</sup> ± 2.53	5.87 <sup>abc</sup> ± 2.65	6.03 <sup>abc</sup> ± 2.56
F2 (15%)	7.00 <sup>c</sup> ± 2.02	6.70 <sup>cd</sup> ± 2.10	6.27 <sup>bcd</sup> ± 2.43	6.10 <sup>abc</sup> ± 2.56	6.44 <sup>bc</sup> ± 2.51
F3 (5%)	5.69 <sup>ab</sup> ± 2.93	5.54 <sup>abc</sup> ± 2.90	5.29 <sup>ab</sup> ± 3.07	5.20 <sup>ab</sup> ± 3.05	5.39 <sup>ab</sup> ± 3.13
F3 (10%)	5.34 <sup>b</sup> ± 2.95	5.39 <sup>ab</sup> ± 2.98	5.16 <sup>ab</sup> ± 3.21	4.96 <sup>ab</sup> ± 3.09	5.20 <sup>ab</sup> ± 3.24
F3 (15%)	4.99 <sup>a</sup> ± 3.20	5.04 <sup>a</sup> ± 3.12	4.64 <sup>a</sup> ± 3.28	4.64 <sup>a</sup> ± 3.29	4.76 <sup>a</sup> ± 3.30

Mean ± Standard deviation. Mean values followed by different letters in a column are significantly different  $p < 0.05$  (LSD).

Higher amount of pumpkin puree in muffin provided better appearance and a pleasant yellow /orange colour (Arifin et al., 2019). In support of this, the initial study reveals that F2 samples with 15% pumpkin fruit scored the highest acceptance on appearance. Pumpkin products have proved to be the best in appearance and addition of pumpkin flour into recipes produced desirable effects (Kiharason et al., 2017; Arifin et al., 2019; Islam et al., 2014; Hossein Ghaboose 2018; Borro R & Gemora, 2016; Islam et al., 2014; Hossein Ghaboose, 2018). The addition of pumpkin seeds in F1 formulation could have neutralized the colour of cowpea leaves which are less acceptable.

The appearance of formulation 3 (F3) at all levels of composite mix incorporation was significantly less acceptable (4.99-5.69) compared to that of the control (7.64). According to Matenge et al. (2012), the combination of different ingredients used in the preparation of the food samples might have masked the natural colour and texture of the dried cowpea leaves that could be less acceptable. This justifies the lowest acceptance scores for F3 samples (F3 5% (5.69); F3 10% (5.34); F3 15% (4.99) by the consumers. The initial study revealed that the dark green colour from cowpea leaves of F3 samples was not welcomed by many and this gave all F3 soup samples a lower rating. It also an indication that products made from cowpea powder produces a dark green colour which is unacceptable to consumers.

However, the high acceptability of the control sample could be due to the non-addition of indigenous vegetables (cowpea and pumpkin) and due to the common ingredients combined during formulation such as tomato powder, mushroom powder and onion powder familiar to consumers.

The findings of this study show that highest aroma scores were observed for the control (7.44) which was not significantly different from F1 and F2 samples with similar mean values ranging from 6.51-7.14. The addition of pumpkin seed powder in F1 samples contributed to the acceptable aroma. In a study by Biggins et al. (2013), cakes made with ground pumpkin seed meal were found to be more viscous with higher water activity, nice aroma and liked by consumers. The same results are also noted for F2 samples in this study, a combination of cowpea seeds and pumpkin fruit produced a good aroma. Moongngarm et al. (2014), found out that most panellists scored the cookies substituted with cowpea seed powder to be similarly acceptable as the control cookies for odour and overall liking. In addition, Hossein Ghaboose (2018) also revealed that cookies filling with 25% pumpkin puree was the most liked for their aroma compared to those without pumpkin puree.

The F3 samples received the lowest aroma scores, with the formulation containing 5% composite mix was scoring 5.54, while the formulation with 10% composite mix scored 5.39 and the formulation with 15% had a score of 5.04. The low mean scores were probably due to the strong odour of cowpea leaves which was probably deemed highly unacceptable by consumers.

The results show that consumer acceptance of soup taste for formulations F1, F2 and control samples were somehow similar as shown by their acceptance scores ranging from 6.10 to 6.99. The rich nutty taste of pumpkin seed powder used in formulating F1 samples could be the

reason for consumer acceptance. According to the findings reported by Jeong-Yeon et al. (2016), pumpkin seeds possess a distinct taste and flavour. The taste of F2 samples were found to be equally well-accepted as the F1 samples. This is likely attributable to the inclusion of cowpea seed and pumpkin fruit powders in the soup formulations. In a study by Borro & Gemora (2016), it was discovered that pumpkin fruit added to cake tasted savoury and proved to be very acceptable. It was also noted that pumpkin fruit is sweet (Dhiman et al., 2009) and can be used in many mixes because of its attractive flavour, colour and sweetness (Jesmin et al., 2016; Sathiya Mala et al., 2018; Arifin et al., 2019). In addition, the development of a bean like off - flavour of cowpea powder as protein supplement was noted (Campbel, 2016; Tortoe et al., 2014) which could be one of the reasons for acceptance of F2 samples.

The F3 formulations containing 5%, 10%, and 15% composite mix were observed to have the lowest acceptance scores for the taste, with mean values of 5.29, 5.16 and 4.64, respectively. This finding indicates that an increase in the concentration of pumpkin flower and cowpea leaf powder resulted in an unacceptable soup taste. A study by Borro & Gemora (2016) suggests that even though some people dislike the taste of pumpkin flowers, but with mild flavour, they can be easily mixed into casseroles, blended vegetable serving and other dishes.

The control soup was highly rated for mouthfeel/texture (6.90), it was not found to significantly varied from F1 and F2 formulations containing 5%, 10%, and 15% composite mix. The F1 formulations were scored 6.3, 5.90, and 6.07, respectively, while F2 formulations received 6.10, 6.04, and 5.87, respectively. It is noteworthy that food items made from pumpkin powder were more widely accepted in terms of their texture and acceptability as reported by Sathiya Mala (2018). The lowest scores were recorded for F3 samples with 5% 5.20; 10% 4.96 and 15% 4.64. From the present study, the mouthfeel/texture score of soup decreased with the increasing incorporation of cowpea leaf powder and pumpkin flower powders in the formulations of the soups. The comparative acceptance for texture could have been attributed to the combination of different ingredients used in the preparation of the food samples such as the tomato powder, onion powder, mushroom powder that might have masked the natural colour and texture of the dried cowpea leaves that could be less acceptable.

The results indicate that the overall acceptance of control, even though slightly high (7.09) than compared to the experimental formulations, didnot significantly differ from that of the F1 (ranging from 6.16 to 6.51) and F2 (ranging from 6.03 to 6.44). F3 samples exhibited the lowest overall acceptance mean values ranging from 4.76 to 5.39. The findings also showed that the



overall acceptance of soups decreased with the increased inclusion of cowpea leaf and pumpkin flower.

In general, overall acceptability of soups depends on the individual data for the sensory attributes like appearance, aroma, taste and mouthfeel. All soups coincided in the range of “like extremely” to “like very much” for soups made from pumpkin fruit powder and pumpkin seeds and that made from cowpea seeds powder and pumpkin fruit powder. Based on these findings, it can be concluded that pumpkin and cowpea produce likeable organoleptic qualities in formulated food products.

#### 4.5. Effect of cowpea powder and pumpkin powder on total phenolic content, antioxidant activity and flavonoid compounds of cowpea-pumpkin composite soup

##### 4.5.1. Total phenolic content, antioxidant activity

The gap in the current literature to which this study is addressed includes an assessment of the antioxidant potential of a combination of cowpea-pumpkin powders. The main aim of this experiment was to evaluate the effect of cowpea powder and pumpkin powder on antioxidant potential and the total phenolic content of cowpea-pumpkin composite soup. The results from the current study regarding the determination of Total Phenolic of soup samples are shown in Table 4.6.

**Table 4.6.** Total phenolic content and antioxidant activity of soup samples

Soup mix	TPC (mgGAE/100 g)	ABTS ( $\mu$ M TE/g sample)	DPPH ( $\mu$ M TE/g sample)
Control Mix	0.44 <sup>c</sup> $\pm$ 0.01	77.06 <sup>h</sup> $\pm$ 3.75	15.97 <sup>b</sup> $\pm$ 0.03
F1 (5%)	0.32 <sup>b</sup> $\pm$ 0.01	57.01 <sup>d</sup> $\pm$ 3.43	16.40 <sup>f</sup> $\pm$ 0.04
(10%)	0.30 <sup>b</sup> $\pm$ 0.01	41.12 <sup>b</sup> $\pm$ 1.71	16.24 <sup>cd</sup> $\pm$ 0.03
(15%)	0.25 <sup>a</sup> $\pm$ 0.01	32.94 <sup>a</sup> $\pm$ 1.03	16.33 <sup>ef</sup> $\pm$ 0.04
F2 (5%)	0.32 <sup>b</sup> $\pm$ 0.01	61.74 <sup>de</sup> $\pm$ 1.92	15.91 <sup>ab</sup> $\pm$ 0.01
(10%)	0.47 <sup>c</sup> $\pm$ 0.02	48.96 <sup>c</sup> $\pm$ 0.61	15.90 <sup>ab</sup> $\pm$ 0.02
(15%)	0.54 <sup>d</sup> $\pm$ 0.03	48.32 <sup>c</sup> $\pm$ 1.35	15.88 <sup>a</sup> $\pm$ 0.01
F3 (5%)	0.54 <sup>d</sup> $\pm$ 0.01	66.47 <sup>ef</sup> $\pm$ 1.78	16.22 <sup>c</sup> $\pm$ 0.02
(10%)	0.63 <sup>e</sup> $\pm$ 0.02	72.40 <sup>gh</sup> $\pm$ 0.71	16.31 <sup>de</sup> $\pm$ 0.07
(15%)	0.63 <sup>e</sup> $\pm$ 0.01	68.58 <sup>fg</sup> $\pm$ 0.49	16.29 <sup>cde</sup> $\pm$ 0.04

**Note:** Abbreviations include Total phenolic Content as TPC; 2,2'-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid antioxidant assay as ABTS; 2,2-diphenyl-1-picrylhydrazyl antioxidant assay as DPPH. Mean  $\pm$  Standard deviation. Mean values followed by different letters in a column are significantly different  $p < 0.05$  (LSD).

Total Phenolic Content (TPC) varied from 0.25 to 0.63 mg/GAE/100 g. Formulation 3 (cowpea leaf and pumpkin flower) had the highest TPC 0.54 – 0.63 mg/GAE/100 g followed by variations of formulation 2 (cowpea seeds and pumpkin fruit) with 0.32-0.54 mg/GAE/100 g, formulation 1 was the lowest with 0.25-0.32 mg/GAE/100g while the control sample had a TPC of 0.44 mg GAE/100 g. The TPC of formulation 1 decreased linearly as the concentration of the composite mix increased while an opposite trend was observed for formulations 2 and 3. Interestingly, it is assumed that the leaves and seeds could increase the TPC of the soup as they contain high amounts of phenolic compounds (Bai et al., 2018; Moreira-Araujo et al., 2018; Adjei-Fremah et al., 2015; Peiretti et al., 2017). However, the TPC of formulations 1 and 2 seem to have been affected by the presence of cowpea and pumpkin seeds which are known to be substantially high in dietary protein which co-exist with polyphenolic compounds (Rangel et al., 2004; Mansour et al., 1993; Da Silva et al., 2019). The interaction between protein and phenolic compounds probably resulted in formation of complexes which made these compounds less assayable (Czubiński & Dwiecki, 2016). TPC of formulation 3 increased with increased concentration of cowpea and pumpkin powders and this was probably due to the individual contribution of phenolic compounds naturally found in cowpea leaf and pumpkin flower (Santos et al., 2021; Cai et al, 2003; Peričin et al., 2009). Another potential factor could be the fact the absence of seeds in formulation 3 variants.

The concentration of composite mixes had a direct impact on the antioxidant activity of formulation 1 and formulation 2, resulting in a linear decrease. In the case of formulation 1, this decrease in antioxidant activity was evident in the ABTS radical scavenging activity, which decreased from 57.01 to 32.94  $\mu\text{M TE/g}$  sample, and the DPPH radical scavenging activity, which decreased from 16.40 to 16.24  $\mu\text{M TE/g}$  sample. This decrease corresponded with the decrease in TPC, which decreased from 0.32 to 0.25 mg GAE/100 g. On the other hand, formulation 2 exhibited opposing responses, as the antioxidant activity decreased from 61.74 to 48.32  $\mu\text{M TE/g}$  sample (ABTS) and 15.91 to 115.88  $\mu\text{M TE/g}$  sample (DPPH), while the TPC increased from 0.32 to 0.54 mg GAE/100 g.

The reasons for the antagonistic reactions between TPC and antioxidant activity in formulation 2 remain unclear, but it is possible that phenolic compounds were masked due to phenol-protein complexation. In contrast, formulation 3 showed a significant linear increase in TPC, ranging from 0.54 to 0.63 mg GAE/100 g, as well as an increase in antioxidant activity from 66.47 to 72.40  $\mu\text{M TE/g}$  sample (ABTS) and 16.22 to 16.31  $\mu\text{M TE/g}$  sample (DPPH). This observation supports the speculation that the presence of protein-rich ingredients had a negative effect on the extractability of phenolic compounds in formulations 1 and 2, as formulation 3, which did

not contain any protein-rich ingredients, exhibited higher TPC and antioxidant activity. The control had substantially higher ABTS radical scavenging activity (77.06  $\mu\text{M TE/g}$  sample) compared to the cowpea-pumpkin composite soup formulations (F1, F2, and F3), while the DPPH radical scavenging activity of the control was significantly lower than that of formulation 1 and formulation 3. The high TPC and antioxidant activity of formulation 3 can be attributed to the fact that the phenolic compounds of cowpea leaf powder and pumpkin flower powder did not interact with potential chelating constituents such as dietary proteins.

#### ***4.5.2. Some flavonoid compounds identified across soup formulations***

Flavonoids are important constituents of the human diet and are among the most potent antioxidants from plants. These compounds are present in cowpea, pumpkins and other indigenous vegetables like Amaranth leaves, moringa oleifera, purslane and sweet potato leaves (USDA, 2011). Studies suggest that dietary intake of flavonoid rich foods can have a protective effect against human diseases associated with oxidative stress (Sombie et al., 2018). The data on flavonoid compounds identified across the 9 soup formulations and a control are summarized in Table 4.7.

Results of the present study revealed that a total of 47 flavonoid compounds belonging to the flavones, chalcones, anthocyanins, flavanols, flavanones were detected in the formulation variations and a control. Among the variations, formulation 2 with cowpea seeds and pumpkin fruits had significantly higher number of flavonoid compounds (42). More of these compounds (19) were present in F2 5% samples. The higher number of these compounds could probably be due to the presence of cowpea seeds and pumpkin fruits which possess higher amounts of flavonoids. Several researchers have reported that cowpea seeds and pumpkin have been associated with higher total flavonoid content and are excellent sources of many natural poly-phenolic flavonoid compounds (Apeah Bah et al., 2017; Khalid & Elharadallou, 2012).

Among the variations of formulation 1, it appears that the number of compounds decreased from 12 to 1 along with an increase in cowpea leaf and pumpkin seed powder. A similar trend was also observed for the variations of formulation 2 where the number of compounds detected decreased from 19 to 9. However, the variations for formulation 3 with cowpea leaf and pumpkin flower followed an opposing trend where the number of compounds increased (6 to 15 compounds) with addition of cowpea leaf and pumpkin flower. This upward trend could be due to the addition of pumpkin flower. According to Gonzalez et al. (2022), volatile organic compounds are often associated with plants fragrances especially flowers.

About 15 flavonoid compounds were detected in the control sample (where no cowpea and pumpkin were added). About 24 flavonoid compounds (24) were detected in variations of formulation 1 with cowpea leaf and pumpkin seeds. Among these variations, only 1 compound *d-Proline, N-methoxycarbonyl, hexadecyl ester* was detected in F1 (15%). This is an indication that addition of cowpea leaf powder had an effect on the composition of flavonoid compound. Giada (2013) reported that excellent antioxidant activity is related to the presence of hydroxyl groups and the effectiveness of the flavonoids decreases with the substitution of hydroxyl groups for glycosides.

The findings of this study indicates that different sets of compounds were detected in each of the different formulation variations for example, none of the compounds detected in the control sample was detected in the F3 variations and none of the compounds detected in Formulation 3 variations was detected in the control sample. About 23 compounds which were not detected in F1 5% samples were also not detected in the F3 variations. These findings also reveal that 6 of the compounds detected in variations of F3 *n-Propyl acetate, 1H-Tetrazole-1,5-diamine, Propanenitrile, 3-hydroxy-, Methyl Alcohol, S-Isopropyl lactate* were not detected in any other formulation. Furthermore, the 14 compounds detected in F1 5% were also not detected in F2 10% and F2 15%. However, this difference of compounds may be due to the distinguishing feature of different powders and quantities in each formulation. Maguipa et al. (2022) also noted the individual contribution of each ingredient in each formulation on antioxidant activity. However, three compounds *2-Propynenitrile, 3-fluoro-, Methyl glyoxal, 4-(2-Acetoxyphenyl)-1-ethyl-3-methyl-5-(4-nitrophenyl) pyrazole* were dominant in all three formulations F1, F2 and F3 samples.

Since there are no studies for both cowpea and pumpkin that have been found to be related to these findings, it was not possible to compare this with other published research findings. However, Sombie et al. (2018) reported that cowpea seeds alone have a total flavonoid content ranging from 0.21 to 1.01 mgQE/100g. The USDA, (2011) also reports a flavonoid content of 1.63mgQE/100g in raw pumpkin fruit. Therefore, based on the results, it can be concluded that the consumption of cowpea and pumpkin could have a significant contribution towards a reduction of risks of several chronic diseases due to the presence of flavonoids compounds that are known to safeguard body cells from radical-induced oxidative damage.

**Table 4.7. Classification of some flavonoid compounds identified across soup formulations**

No	Name of Compound	Type of compound	Classification	Retention time (minutes)									
				Control	F1-5	F1-10	F1-15	F2-5	F2-10	F2-15	F3-5	F3-10	F3-15
1	2,4,6-Trimethoxyacetophenone	Flavonoid	Chalcone	12.19	-	-	-	12.18	-	-	-	-	-
2	2-Trifluoromethylbenzoic acid, tridec-2-ynyl ester	Not a flavonoid	Synthetic compound	9.10	-	15.282	-	-	9.09	-	-	-	-
3	2-Undecen-4-ol	Flavonoid	Flavones	16.18	6.13	-	-	16.22	16.15	-	-	-	-
4	7-Chloro-1,3,4,10-tetrahydro-10-hydroxy-1-[[2-[1-pyrrolidinyl]ethyl]imino]-3-[3-(trifluoromethyl)phenyl]-9(2H)-acridinone	Flavonoid	Flavones	23.27	9.92	-	-	-	-	6.98	-	-	-
5	9H-xanthen-9-ol, 9-(1,1-dimethylethyl)-	Not a flavonoid	Natural compound (Xanthone)	17.71	-	-	-	-	16.44	-	-	-	-
6	Butanenitrile	Not a flavonoid	Nitrile compound	8.50	-	8.36	-	6.72	-	-	-	-	-
7	Carbonic acid, decyl phenyl ester	Not a flavonoid	Ester compound	9.95	-	-	-	-	9.92	-	-	-	-
8	Carbonocyanidic acid, ethyl ester	Flavonoid	Anthocyanins	23.33	-	23.92	-	23.32	-	-	-	-	-
9	Cyclopentane, 1,2,3,4,5-pentamethoxy-, stereoisomer	Flavonoid	Flavone	14.98	-	-	-	14.98	-	-	-	-	-
10	d-Proline, N-methoxycarbonyl-, hexadecyl ester	Flavonoid	Anthocyanins	11.18	-	-	12.076	11.28	-	-	-	-	-
11	Glutaric acid, hept-2-yl 3-octyl ester	Not a flavonoid	Ester	4.98	-	-	-	-	4.01	-	-	-	-
12	N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine	Flavonoid	Methylated flavonoid	7.41	-	6.15	-	7.38	-	-	-	-	-
13	Naphthalene	Not a flavonoid	Polycyclic aromatic hydrocarbon	5.60	-	-	-	5.61	-	-	-	-	-
14	Pyrrole-2,5-dicarboxylic acid, 4-(2-diethylamino)ethyl-3-methyl-, 2-ethyl ester	Flavonoid	Anthocyanins	10.31	-	-	5.830	10.41	10.13	-	-	-	-
15	Tetradonium Bromide	Flavonoid	Flavonols	20.27	-	18.29	-	18.24	18.20	-	-	-	-
16	3-Butene-1,2-diol, 1-(2-furanyl)-	Not a flavonoid	Simple organic compound	-	-	13.97	-	-	2.92	-	-	-	-
17	Pyridine, 3-phenyl-	Not a flavonoid	Heterocyclic aromatic compound	-	-	26.98	-	-	26.98	-	-	-	-
18	Pyrrolidine-2-carboxamide, N-heptyl-N-methyl-	Not a flavonoid	Chemical compound	-	-	16.17	-	2.98	-	-	-	-	-
19	Trifluomeprazine	Not a flavonoid	Organic compound	-	-	20.94	-	-	20.20	-	-	-	-
20	Xanthene, 9,9-dimethyl-	Not a flavonoid	Chemical compound	-	-	12.18	-	-	12.18	-	-	-	-
21	Pregnan-20-one, 3-(acetyloxy)-5,6-epoxy-6-methyl-, cyclic 20-(1,2-ethanediyl acetal), (3 $\beta$ ,5 $\beta$ ,6 $\beta$ )-	Not a flavonoid	Steroid compound	-	-	8.10	-	6.10	-	-	-	-	-
22	Tetrahydropyran Z-10-dodecenoate	Flavonoid	Flavanones	-	7.64	10.23	-	8.19	7.60	7.12	-	-	-
23	4-(2-Acetoxyphenyl)-1-ethyl-3-methyl-5-(4-nitrophenyl)pyrazole	Flavonoid	Flavones	-	6.43	-	2.80	-	2.69	ND	2.57	-	-

24	2-(2,4,6-Trinitrophenyl)-3-oxobutanoic acid,ethyl ester	Not a flavonoid	Synthetic compound	-	2.92	-	-	-	-	ND	-	-	2.79
25	3-Methylbutyl N-heptafluorobutyryltryptophanate	Not a flavonoid	Synthetic compound	-	18.37	-	-	-	-	29.79	-	-	-
26	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid	Flavones	-	6.57	-	-	-	-	6.52	-	-	-
27	5-Hydroxymethylfurfural	Not a flavonoid	Organic compound	-	7.77	-	-	-	-	7.65	-	-	-
28	Glycolaldehyde dimer	Not a flavonoid	a oxoaldehyde compound	-	2.97	-	-	-	-	-	-	-	2.90
29	Methyl glyoxal	Not a flavonoid	a oxoaldehyde compound	-	7.93	-	-	-	-	2.93	2.63	2.70	-
30	N,N-dimethyl-4-tert-butylamphetamine	Not a flavonoid	Synthetic compound	-	29.62	-	-	5.12	-	-	-	-	-
31	tert-Butyldimethylsilylamine	Not a flavonoid	Organosilicon compound	-	11.35	-	-	-	-	-	-	-	27.33
32	2-Propynenitrile, 3-fluoro-	Not a flavonoid	Chemical compound	-	22.22	-	-	17.59	-	11.09	25.70	23.17	6.21
33	Acetic acid, hydrazide	Not a flavonoid	Simple organic compound	-	4.51	-	-	-	-	2.90	-	-	-
34	Thioacetic acid	Not a flavonoid	Simple organic compound	-	7.96	-	-	-	-	-	-	-	3.00
35	Chloromelfoquine	Not a flavonoid	Synthetic compound	-	-	-	-	-	23.32	9.87	-	-	-
36	Aminoacethydrazone	Not a flavonoid	Chemical compound	-	-	-	-	-	-	2.62	-	-	2.80
37	Ethanamine, N,N-difluoro-	Not a flavonoid	Chemical compound	-	-	-	-	-	-	2.91	-	2.60	-
38	Hydrazinecarboxamide	Not a flavonoid	Chemical compound	-	-	-	-	-	-	3.65	-	-	2.98
39	Methylsulphonamide, N-ethyl-N-tetradecyl-	Not a flavonoid	Organic sulphur containing compound	-	-	-	-	-	-	27.87	-	4.51	-
40	Ethanol, 2,2-dichloro-	Not a flavonoid	Chemical compound	-	-	-	-	-	-	11.30	-	2.67	-
41	2-Benzyl-3-methoxycyclopropanecarboxylic acid, methyl ester	Flavonoid	chalcones	-	-	-	-	5.67	-	-	-	ND	28.83
42	2-Chloroethanol	Not a flavonoid	Alcohol compound	-	-	-	-	-	-	-	-	2.74	2.73
43	(S)-Isopropyl lactate	Not a flavonoid	ester	-	-	-	-	-	-	-	-	2.71	2.69
44	Methyl Alcohol	Not a flavonoid	Simple alcohol compound	-	-	-	-	-	-	-	-	2.85	2.61
45	Propanenitrile, 3-hydroxy-	Not a flavonoid	Organic compound	-	-	-	-	-	-	-	2.89	2.59	2.66
46	1H-Tetrazole-1,5-diamine	Not a flavonoid	Organic compounds	-	-	-	-	-	-	-	2.93	-	2.94
47	n-Propyl acetate	Not a flavonoid	Ester	-	-	-	-	-	-	-	2.78	-	2.614

#### ***4.5.3. Compounds that were detected only in specific formulations***

Results of compounds that were detected only in specific formulations is presented in Table 4.8. This study allowed the identification of 177 compounds in three soups formulations. Among the 3 formulations (F1, F2 and F3), 16 to 90 compounds were detected in each formulation. Of the 3 formulations, the highest number of compounds, 50.8% were identified in variations of formulation F1 with cowpea leaf and pumpkin seeds. This formulation presents a large variety of volatile compounds with retention time varying from 3.08 min to 26.51 minutes. Among the variations of Formulation 1, F1 5% 2-Furancarboxylic acid retention was the predominant compound that was identified.

About 45% of the total compounds identified were from variations of formulation 2 with cowpea seeds and pumpkin fruit. This was the second highest number of compounds after formulation 1 variations and had high retention times also second highest in retention time 2.79 - 29.47 from formulation 3 variations.

The compound profile of F3 formulations with cowpea seeds and pumpkin fruit were quite similar to each other and show 2 predominant compounds 2-Propanol, 1- and Ethanol. Nevertheless, only 26% of compounds were detected in the variations of formulation 3. However, this formulation variation had the highest retention time 2.58 minutes to 31.24 minutes.

For the control sample, only 16 compounds 1H-Benzimidazole, 2-Diethylamino-N-naphthalen-1-yl-acetamide, 2-Propynal and others were identified with retention times of 3.33 minutes to 23.93 minutes.

However, the large variety of volatile compounds observed is due to the unique soup formulations of the present study. Furthermore, the lowest retention time 2.58 minutes was identified for Ethane, nitro- amongst the F3 variations and also the highest retention time 31.24 was also identified amongst the F3 variations for Carbon disulfide. The different retention time could be explained by the different chemical composition of each compound.

**Table 4.8. Compounds that were detected only in specific formulations and their retention times.**

Soup mix	flavonoids	Organic compounds	Synthetic compounds	Chemical compounds	inorganic compounds	Phenolic compounds	Hydrocarbon compound	Alcohols
Control	Atropic acid (6.79 min)	1H-Benzimidazole (9.37 min)	2-Diethylamino-N-naphthalen-1-yl-acetamide (4.58 min)	DL-Alanine, N-methyl-N-(but-3-yn-1-yloxy-carbonyl)-, tetradecyl ester (8.88 min)	-	-	-	-
	-	2-Propynal (4.64 min)	2-Pyrrolidinone, 5-(hydroxymethyl)- (23.93 min)	Heptyl ethylphosphonofluoridate (16.81 min)	-	-	-	-
	-	3-Piperidinol, 1-ethyl- (4.35 min)	-	-	-	-	-	-
	-	Acetone, 1-[4-(dimethylaminoethoxy) phenyl]- (20.95 min)	-	-	-	-	-	-
	-	Benzenehexanenitrile, $\beta,\beta$ -dimethyl- $\epsilon$ -oxo- (3.33 min)	-	-	-	-	-	-
	-	Benzoic acid, 3-methylbutyl-2 ester (7.74 min)	-	-	-	-	-	-
	-	Benzoic acid, 4-amino-, 4-acetoxy-2,2,6,6-tetramethyl-1-piperidinyl ester (24.54 min)	-	-	-	-	-	-
	-	Ethanesulfonyl chloride (5.176 min)	-	-	-	-	-	-
	-	Ethyl 2-hydroxybenzyl sulfone (9.01 min)	-	-	-	-	-	-
	-	N,N-Diethyl-2-aminoethanol, tert-butyl dimethylsilyl ether (7.11 min)	-	-	-	-	-	-
-	Phenylacetic acid, 2-diethylaminoethyl ester (3.60 min)	-	-	-	-	-	-	
F1(5%)	1,2,3-Propanetriol, 1-acetate (9.39 min)	2(3H)-Furanone, 5-hexyldihydro- (5.36 min)	1-Propanesulfonyl chloride (6.34 min)	1H-Imidazole, 1-methyl-5-nitro- (10.31 min)	-	4-Acetoxy-3-methoxystyrene (8.84 min)	5,7-Dodecadiene, (E,Z)- (19.76 min)	-
	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)- (16.82 min)	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- (12.68 min)	Dinocap (24.56 min)	1H-Indene, 1-hexadecyl-2,3-dihydro- (24.50 min)	-	-	-	-
	2-Butenoic acid, 3-methyl-, methyl ester (4.56 min)	2H-Tetrazole, 2-methyl- (9.95 min)	Ethyl 2-cyano-3-(4-methacryloyloxyphenyl)acrylate (18.11 min)	5-Ethoxy-3,4-dihydro-2H-pyrrole-2-carboxylic acid, ethyl ester (4.55 min)	-	-	-	-
	2-Diethylamino-N-[4'-(2-diethylamino-acetyl-amino)-3,3'-dimethoxy-biphenyl-4-yl]-acetamide (3.62 min)	4-Hexen-3-one, 5-methyl- (4.60 min)	Methyl 2-[methoxy(methyl)amino]-2-methylpropanoate (6.42 min)	Acetic acid, hydroxy-, propyl ester (3.31 min)	-	-	-	-



	2-Furancarboxylic acid (4.02 min)	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- (7.12 min)	Phosphoramidous difluoride (5.75 min)	Benzeneethanamine, 2-fluoro- $\beta$ ,3,4-trihydroxy-N-isopropyl- (20.19 min)	-	-	-	-
	2-Furancarboxylic acid, 3-methyl-, methyl ester (7.36 min)	9-Octadecenamide, (Z)- (21.79 min)	-	Carbonochloridic acid, ethyl ester (5.17 min)	-	-	-	-
	Orcinyl di-angelate (20.00 min)	Fumaric acid, ethyl 3,4,5-trichlorophenyl ester (13.18 min)	-	Chlorfenapyr (20.04 min)	-	-	-	-
	-	Hexadecanamide (18.19 min)	-	Difluoroisothiocyanatophosphine (10.02 min)	-	-	-	-
	-	1-Norvaline, N-ethoxycarbonyl-, hexadecyl ester (4.33 min)	-	Furan, 2-(dichloromethyl)-tetrahydro- (5.09 min)	-	-	-	-
	-	Methanone, (1-hydroxycyclohexyl) phenyl- (14.96 min)	-	Methylazoxymethanol acetate (3.08 min)	-	-	-	-
	-	n-Hexadecanoic acid (18.06 min)	-	Monoethanolamine (26.51 min)	-	-	-	-
	-	Oleic acid	-	N-Dodecylmethylamine (12.06 min)	-	-	-	-
	-	Phenethylamine, N-benzyl-p-chloro- (7.54 min)	-	N-[3,3'-Dimethoxy-4'-(2-piperidin-1-yl-acetylamino)-biphenyl-4-yl]-2-piperidin-1-yl-acetamide (21.37 min)	-	-	-	-
	-	Phytol (19.54 min)	-	Phosphonic acid, [1-[(1-methylethylidene)amino]propyl]-, bis(trimethylsilyl) ester (12.28 min)	-	-	-	-
	-	Pyrimidine, 2,4,5-triamino- (10.98 min)	-	$\beta$ -alanine, N-cyclohexyl-N-[2-(4-methyl-1-piperidinyl)acetyl]-, ethyl ester (5.62 min)	-	-	-	-
	-	Succinic anhydride (4.73 min)	-	$\beta$ -alanine, N-cyclohexyl-N-[2-(4-methyl-1-piperidinyl)acetyl]-, ethyl ester (5.62 min)	-	-	-	-
	-	Undecanoic acid, methyl ester (17.62 min)	-	DL-7-Azatriptophan (20.66 min)	-	-	-	-
	-	Butanoic acid, 2-cyano-3-methyl-, ethyl ester (5.95 min)	-	-	-	-	-	-
F1(10%)	Benzene, 1-methoxy-4-(methylthio)- (8.89 min)	4-Pyrimidinamine, 2,6-difluoro- (18.94 min)	1,3-Dioxolane, 2-(1,1-dimethylethyl)- (3.45 min)	Alanylalanine, N,N'-dimethyl-N-ethoxycarbonyl-, dodecyl ester (13.26 min)	-	-	Isobutane (2.64 min)	-
	Bis[2-(cinnamoyloxy)-1-naphthyl]methane (5.44 min)	Aminoacetic acid, 2-(2-fluoro-4,5-dimethoxybenzoyl)-, ethyl ester (24.64 min)	3,4-Methylenedioxy-N-ethylamphetamine (6.39 min)	Isophthalic acid, phenylethyl undecyl ester (6.59 min)	-	-	-	-
		Benzeneacetamide, N-methyl-N-[2-(dimethylamino)ethyl]-2-ethoxycarbonylamino- (19.96 min)	Benzedrex (20.26 min)	N-(2-Isopropoxy-phenyl)-4-phenoxy-benzamide (16.47 min)	-	-	-	-
		Butanenitrile, 4-bromo- (4.30 min)	$\alpha$ -[2-Piperidyl]-2,6-di-[p-trifluoromethylphenyl]-4-pyridinemethanol (23.33 min)	Oxamide, N-(3-methoxypropyl)-N'-cycloheptylidenamino- (17.06 min)	-	-	-	-

F1(15%)	5-Methoxy-N,N-Diisopropyltryptamine (6.92 min)	Ethoxycyclohexyldimethylsilane (6.13 min)	-	Pheniramine (27.53 min)	-	-	-	-
		Glutaric acid, hept-2-yl 3-hexyl ester (4.01 min)	-	-	-	-	-	-
		Thiourea, N,N'-dimethyl- (7.56 min)	-	-	-	-	-	-
		1,2,4,5-Tetrazine, 3,6-dipropyl- (2.74 min)	L-5-Propylthiomethylhydantoin (15.49 min)	1-Butanol, 2-methyl-, acetate (2.67 min)	-	1,4-Benzenediol, mono-tetradecyl ether (5.17 min)	Propene (3.85 min)	-
		1,4-Dimethyl-1,2,4-triazol-3-thione-5-one (7.16 min)	-	Alanylalanine, N,N'-dimethyl-N-ethoxycarbonyl-, hexadecyl ester (13.21 min)	-	-	-	-
		1-Butanone, 2-chloro-3-methyl-1-[4-(1-methylethyl)phenyl]- (4.34 min)	-	l-Isoleucine, N-methoxycarbonyl-, pentadecyl ester (8.57 min)	-	-	-	-
		2H-imidazole-2-thione, 1,3-dihydro-4-(2-methylpropyl)- (8.41 min)	-	l-Norvaline, n-propargyloxycarbonyl-, heptyl ester (8.86 min)	-	-	-	-
		3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- (5.19 min)	-	Octylamine, N,N-di(allyl)- (12.07 min)	-	-	-	-
		Ethanone, 1-[1-(4-amino-1,2,5-oxadiazol-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-2-morpholino- (3.03 min)	-	Spiroxamine (9.10 min)	-	-	-	-
		Pentanamide, 2-(dimethylamino)-N-[7-(hydroxyphenylmethyl)-3-(1-methylethyl)-5,8-dioxo-2-oxa-6,9-diazabicyclo[10.2.2]hexadeca-12,14,15-trien-4-yl]-3-methyl- (6.08 min)	-	Undecanoic acid isopropyl ester, 10-hydroxy-11-morpholin-4-yl- (3.17 min)	-	-	-	-
F2(5%)	2,3-Dihydrothieno[3,4-b][1,4]dioxine-5-carboxylic acid, cyclopropylamide (16.40 min)	Oxalic acid, allyl pentyl ester (3.12 min)	-	1-Piperidin-1-ylpropan-2-yl acetate (2.83 min)	-	-	-	
		-	-	Azepan-1-yl-acetic acid, acridin-9-ylmethylene-hydrazide (4.14 min)	-	-	-	
		-	-	Benadryl (20.21 min)	-	-	-	
		(3S,9aR)-3-Butyloctahydro-1H-pyrrolo[1,2-a]azepine (5.10 min)	-	3-t-Butyl-7a-dimethylaminomethyltetrahydropyrrolo[1,2-c]oxazol-1-one (25.02 min)	-	-	Benzene, 1,1',1"-[1-(bromomethyl)-2-methoxy-1-ethanyl-2-ylidene]tris- (16.52 min)	-
		-	1,2-Ethanediamine, N,N,N',N'-tetramethyl- (15.88 min)	9H-Pyrrolo[1,2-a]indol-9-one, 2-methyl- (27.25 min)	-	-	-	-
-	1,3-Oxathiolane, 2-[[2-(2-chloroethyl)thio]methyl]-2-methyl- 5.23 min)	Alanine, N-methyl-N-neopentyloxycarbonyl-, heptadecyl ester (18.96 min)	-	-	-	-		
-	1-[2-Pyridyl]-2,2-dimethyl-2-morpholino ethanol (6.40 min)	Anthrone (12.41 min)	-	-	-	-		

	-	3-Pentanamine (20.23 min)		Benzenamine, 4,4'-(1,2-ethanediy)bis- (8.68 min)	-	-	-	-
	-	Benzenesulfonamide, 5-bromo-2-fluoro-N-(2-pyridin-4-ylethyl)- (5.18 min)	-	Betahistine (20.16 min)	-	-	-	-
	-	Bromonitromethane (19.87 min)	-	1-Norvaline, n-propoxycarbonyl-, heptadecyl ester (7.65 min)	-	-	-	-
	-	Isoquinoline, 1,2,3,4-tetrahydro-1-[2-nitrobenzyl]-6,7-dimethoxy- (23.76 min)	-	Tioconazole (5.73 min)	-	-	-	-
	-	Pentanamide, 2-(dimethylamino)-4-methyl-N-[2-methyl-1-[[[3,3a,11,12,13,14,15,15a-octahydro-12,15-dioxo-13-(phenylmethyl)-5,8-ethenopyrrolo[3,2-b][1,5,8]oxadiazacyclotetradecin-1(2H)-yl]carbonyl]butyl] - (4.36 min)	-	-	-	-	-	-
	-	$\alpha$ -Bromo-2,4-difluorotoluene (16.36 min)	-	-	-	-	-	-
F2(10%)	-	4-Chlorobutanoic anhydride (3.61 min)	Benzoic acid, 4-(1-azepan-1-ylmethyl-2-oxo-1,2-dihydro-indol-3-ylideneamino)-, ethyl ester 7.91 min)	1,1,1,2,2,3,3-Heptafluoro-3-(1,2,2,2-tetrafluoroethoxy)propane (8.17 min)	-	-	Propane, 2,2-difluoro- (5.57 min)	-
	-	6-Hepten-2-one, 5,7,7-trichloro- (2.81 min)	Dipropargylaminomethyl-9-phenanthryl ketoxime (8.26 min)	1-Aminocyclopentanecarboxylic acid, N-methoxycarbonyl-, isohexyl ester (6.70 min)	-	-	-	-
	-	N,N-Dimethyl-3-butoxypropylamine (20.92 min)	Sotalol (3.10 min)	-	-	-	-	-
	-	Propanamide, N-(4-bromo-3-methylphenyl)- 3-(4-morpholyl)- (6.92 min)	-	-	-	-	-	-
F2(15%)	-	2,2'-Bithiazolidine (3.14 min)	-	1,2,5-Oxadiazole-3,4-dicarboxaldehyde, 4-(O-acetyloxime) 3-oxime, 2-oxide (7.82 min)	Hydroxylamine (3.42 min)	-	-	-
	-	3-Methyl-1,2-diazirine (8.00 min)	-	1,2-Ethanediamine, N,N,N',N'-tetraethyl- (5.44 min)	-	-	-	-
	-	3-Methyl-2-butenic acid, tridec-2-ynyl ester (6.26 min)	-	Carbohydrazide 2.94 min)	-	-	-	-
	-	Propanedioic acid, oxo-, bis(1-methylethyl) ester 2.99 min)	-	Hexafluoroisopropyl acrylate (11.21 min)	-	-	-	-
	-	1,3-Dioxolane, 2-pentadecyl- (29.47 min)	-	Oxalic acid, ethyl propyl ester (2.79 min)	-	-	-	-
	-	-	-	Propane, 1,1'-sulfonylbis- (3.03 min)	-	-	-	-
	-	-	-	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso-3.00 min)	-	-	-	-
	-	-	-	Silane 5.38 min)	-	-	-	-
	-	-	-	Thymine (5.61 min)	-	-	-	-

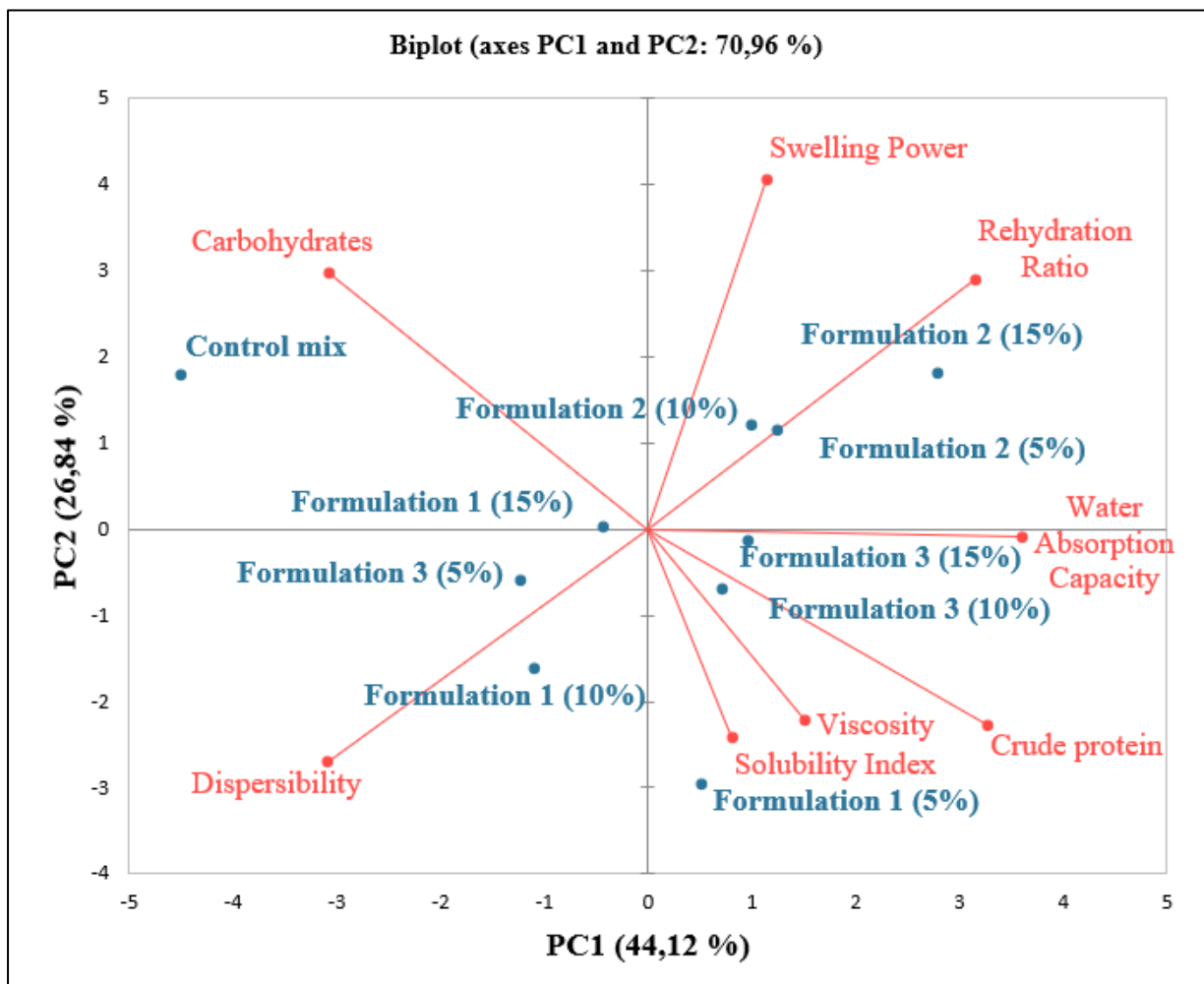
F3(5%)	2-Hexanol, 3-methyl- (2.66 min)	Methane, dipropoxy- (2.92 min)	-	1-Heptanamine, N,N-diheptyl- (25.34 min)	-	-	-	-
	4-Penten-2-one (2.76 min)	-	-	1-[3-(4-Bromophenyl)-2-thioureido]-1-deoxy-b-d-glucopyranose 2,3,4,6-tetraacetate (2.76 min)	-	-	-	-
	-	-	-	Argon (2.93 min)	-	-	-	-
	-	-	-	Carbon disulfide (31.24 min)	-	-	-	-
F3(10%)	-	Propanoic acid, 2-oxo- (2.98 min)	Glycine, N-acetyl-N-(trifluoroacetyl)-, methyl ester (3.00 min)	Hydrazine (2.80 min)	-	-	-	Ethanol (2.72 min)
	-	-	Pyrrole, 2-(2-naphthyl)-3,5-diphenyl- (29.40 min)	N8-(3-Chloro-4-fluorophenyl)-N2-(1-methylpiperidin-4-yl)pyrimido[5,4-d]pyrimidine-2,8-diamine (29.49 min)	-	-	-	Ethanol, 2,2,2-trifluoro- (2.63 min)
	-	-	-	-	-	-	-	Isopropyl Alcohol (2.66 min)
F3(15%)	-	1,1,1-Tribromopropanone (2.91 min)	-	2-Methylpropenoic acid, 2,2,3,4,4,4-hexafluorobutyl ester (30.06 min)	-	-	-	1-Octanol, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro- (2.85 min)
	-	Acetic acid, methyl ester (24.89 min)	-	2-Propanol, 1-hydrazino- (2.69 min)	-	-	-	1-Propanol (2.60 min)
	-	Ethanethioic acid, S-[(methylthio)methyl] ester (2.75 min)	-	2-Propanone, 1-hydroxy- (2.94 min)	-	-	-	2-Propanol, 1-amino-, (R)- (2.71 min)
	-	Ethane, nitro- (2.58 min)	-	-	-	-	-	-

## CHAPTER 5

### CORRELATION OF THE RESULTS USING PRINCIPAL COMPONENT ANALYSIS (PCA), CONTRIBUTIONS AND LIMITATIONS OF STUDY

#### 5.1. Correlation of the results using Principal Component Analysis (PCA)

In order to determine the relationships between soup formulations based on their functional properties, viscosity, carbohydrate and crude protein composition, the data was subjected to Principal Component Analysis (PCA). Principal Component 1 (PC1) accounted for 44.12% of the total variation, while Principal Component 2 (PC2) accounted for 26.84%. Together, these principal components explained 70.96% of the total variation, as illustrated in Figure 5.1.



**Figure 5.1.** Biplot of principal components PC1 and PC2 loadings

The vectors with acute angles indicate a positive correlation, while those with obtuse or right angles suggest no correlation. The soup formulations with similar characteristics were loaded closely together, while those with different attributes were placed further apart. Specifically, the formulation 1 containing 5% composite mix, Formulation 2 with 5% composite mix, Formulation 3 with 10% composite mix, and Formulation 3 with 15% composite mix were loaded on PC1. Their characterization was based on high viscosity, solubility index, crude protein, water absorption capacity, and rehydration ratio, as detailed in Tables 4.1, 4.3, and 4.4. However, viscosity and solubility index exhibited less significant contribution to PC1 loading compared to crude protein, water absorption capacity, and rehydration ratio.

The viscosity, solubility index, and water absorption capacity were closely related with formulation 1 containing 5% of composite mix and variations of formulations 3 containing 10 and 15% composite mix. The characterisation of formulation 1 (5%), formulation 3 (10%), and formulation 3 (15%) indicates that the rise in crude protein content has significantly enhanced the water absorption capacity and solubility of these formulations. As a result, the viscosity of these formulations has increased due to the interaction between proteins and water molecules.

Formulation 1 with 15% composite mix, and the soup from Formulation 2 with 10% composite mix were loaded on PC2. These formulations were characterized by a high carbohydrate content and high swelling power as shown in Table 4.4. Formulations with insignificant contribution were loaded on PC3, namely Formulation 1 containing 10% composite mix and Formulation 3 containing 5% composite mix and were associated with high dispersibility (Table 4.1) which had a negatively correlation with swelling power.

## **5.2. Contributions of study**

In the South African context, no studies have been conducted that shows the importance of combining different edible parts of indigenous vegetables in food formulations. In spite of several studies having been conducted in various parts of South Africa / globally with respect to indigenous leafy vegetables, this is the first study to investigate the physical, functional, nutritional value and antioxidant potential of soup made from a combination of cowpea and pumpkin. The holistic analysis of the present study added to the existing research by identifying the importance of combining different kinds of indigenous vegetables in food formulations. Additionally, combining the different edible parts (flowers, seeds, leaves and fruits) of cowpea and pumpkin can complement each other from a health perspective, if a part of one vegetable

is low in one nutrient it is adequately provided in the other vegetable. The confirmed result of this study also contributes to existing strategies to alleviate malnutrition using sustainable feeding strategies that require minimal funding and which provides a diverse diet using nutritionally dense indigenous leafy vegetables. Cowpea-pumpkin composite soup will also contribute as a sustainable strategy towards promoting food security and health in households. Producing cowpea-pumpkin soup provides full possibility for the consumption of indigenous leafy vegetables like cowpea and pumpkin.

Another unique aspect of this study became apparent when a preliminary meta-analysis of published literature indicated there were currently no reported research findings regarding the incorporation and analysis of cowpea and pumpkin in soup formulations. The findings contribute not only to the nutritional and health security but also safeguarding indigenous knowledge surrounding indigenous leafy vegetables like cowpea and pumpkin. The use of soup as opposed to just fortifying maize products is an improvement to food fortification.

The novel feature of this study of developing three soup formulations (Formulation 1 cowpea leaf powder plus pumpkin seed powder; Formulation 2 cowpea seed powder and pumpkin fruit; Formulation 3 cowpea leaf powder and pumpkin flower powder) adds to the existing literature on complementary foods. The combination of cowpea and pumpkin would provide complementary inputs to food products to improve their health and palatability. A combination of cowpea seed powder and pumpkin fruit exhibits golden-brown colour and an eye-catching appearance which is appealing to consumers. The low viscosity of cowpea-pumpkin is desirable in complementary foods due to increase in nutrient and energy densities without excessive increase in viscosity. The results of this study also add to the small amount of research that report the high solubility of foods which shows high digestibility of the food which may indicate excellent use for food.

The results of this study sought to close the gap between the availability of ILVs and exotic vegetables. It will do so by providing insight to policy makers that their policies are not in favour of ILVs. Therefore, they will review their policies existing policies on ILVs and raise awareness. This will not only increase their availability in the market but will also increase opportunities for the uses of ILVs. The availability of ILVs in the market will help consumers remove the negative critics that people hold against these leafy vegetables such as food for the old and poor, old-fashioned food and unhygienic since they grow in the wild.

Furthermore, development of cowpea-pumpkin soup can contribute immensely to improving the food baskets not only in South Africa but the globally. It will bring a renewed interest in the benefits of ILVs. Adoption, utilization, and consumption of cowpea-pumpkin soup will be a step forward towards combating the protein calorie malnutrition and iron deficiency in the parts of the world.

### **5.3. Limitations**

The COVID 19 pandemic and lockdown restrictions have significantly impacted the work environment. The necessity to prevent the spread of the virus and save lives has resulted in restricted access to laboratories, thereby necessitating sensory evaluations to be conducted outside of laboratory settings with limited attendance due to gathering restrictions. As such, the viewpoints of all those people who could not evaluate the samples is lacking. The questionnaires were formulated in English, which posed a challenge for some respondents who were illiterate. In certain instances, the questions had to be translated into Sepedi language to facilitate completion, which may have detracted from the interest of some participants.



## CHAPTER 6

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1. Conclusions

The first objective of this study was to assess the effect of cowpea powder and pumpkin powder on the functional properties of cowpea-pumpkin composite soup. The results of this study show that the water absorption capacity of cowpea and pumpkin is very high. The results of formulations 1, 2 and 3 were not significantly different from each other with mean values ranging from 185.7 – 263.3. However, increasing the cowpea seeds and pumpkin fruit into the mixture also increased the water absorption capacity of the sample. The findings from this study also indicate that cowpea and pumpkin have high swelling power and lower solubility index. The soup formulations also recorded higher rehydration ratio than the control sample. Dispersibility results recorded show that the control sample had the highest dispersibility as compared to the formulated mixes. Cowpea seeds and pumpkin fruit had the lowest dispersibility (22.7-47.0) compared to cowpea leaf and pumpkin flowers (49.3-56.7) which also had mean values lower than cowpea leaf powder and pumpkin seed (57.7-61.3).

Overall, the results reveal that cowpea and pumpkin have desirable functional properties which make them useful in many food systems where they could play functional roles. Furthermore, the water absorption of cowpea-pumpkin makes them useful not only for soups but for various products that require water retention for their textural integrity. The research will be valuable to food industries, researchers, food chemists and nutritionists and policy makers.

The second objective of this study was to assess the effect of cowpea powder and pumpkin powder on the physical properties of cowpea-pumpkin composite soup. The results show that the addition of cowpea leaf powder in F1 and F3 samples imparted a dark green colour to the soup which was not welcomed while pumpkin gave an attractive golden-brown colour to the soup in F2 samples. The intensity of the golden-brown colour Addition of pumpkin influences the colour of food because pumpkin contains a lot of B-carotene pigment which provides an intense orange yellow colour to food products.

Based on the results, Cowpea seeds and pumpkin fruit have very high viscosity which also increased with the addition of composite mix at different levels. Viscosity of cowpea leaf, pumpkin seeds and pumpkin flower was generally lower just like that of the control sample which had no cowpea or pumpkin addition.

The third objective of this study was to determine the effect of cowpea powder and pumpkin powder on the proximate composition of cowpea-pumpkin composite soup. The findings of this study showed that cowpea and pumpkin are rich in Proteins, carbohydrates, crude fibre, moisture and lower in ash and fat content. The fat content of all formulations ranged from 1.73 to 3.10 which confirms the fact that Indigenous vegetables are generally low in fat content which is desirable considering the growing health-conscious population. The results showed that cowpea and pumpkin are high in fibre content and the highest scores were reported for F1 samples cowpea and pumpkin seed. The results show that cowpea and pumpkin are high in protein and this is confirmed by the low mean value (13.3) reported for the control sample which had no cowpea or pumpkin as compared to the other formulations made from cowpea and pumpkin with mean values ranging from 22.33 to 28.33. The carbohydrate content of cowpea and pumpkin was significantly high in all formulations ranging from 44.6 to 58.0 even though they were lower than that of the control sample. The results indicate that the ash content in ILVs is generally very low as the mean values reported for all formulations were not significantly different ranging from 0.35 to 0.55. Low moisture content was reported for all formulations ranging from 7.81 -9.74 and this testifies that cowpea and pumpkin have a better shelf life and reduced microbial spoilage.

The results obtained in this study support the fact that cowpea and pumpkin have good physicochemical properties which could be exploited for nutrition and food formulation.

Furthermore, the present study has revealed that cowpea and pumpkin are valuable and excellent sources of protein which is cheaper than animal protein for rural and urban poor people. The study has also established that development of high nutritious products such as cowpea-pumpkin composite soup could significantly contribute towards alleviation of malnutrition and related health conditions for millions of people in the world. Moreover, soup production provides a full possibility for the consumption of Indigenous vegetables like cowpea and pumpkin. They have a high nutritive value are excellent sources of Protein and could significantly contribute towards alleviation of malnutrition and related health conditions.

The fourth objective of this study was to evaluate the effect of cowpea powder and pumpkin powder on consumer acceptability of cowpea-pumpkin composite soup. The results of the study revealed that the texture and dark green colour of dried cowpea leaves was not welcomed by many hence F3 samples (cowpea leaf and pumpkin flowers) had the lowest mean values in all attributes and overall acceptability. High acceptability was noted for control sample which

did not have any addition of cowpea or pumpkin. However, comparing the three formulations from cowpea and pumpkin, Formulation 1 (cowpea leaves and pumpkin seed) and Formulation 2 (pumpkin fruit and cowpea seeds) scored high mean values in terms of appearance, aroma, taste mouthfeel and overall acceptability.

The results reported in this study confirm that cowpea and pumpkin are very important vegetables which can be highly accepted in people's diets. It is also clearly observed that pumpkin powder could be successfully added to soup preparations to function as a natural nutrient source with remarkable health benefits and increasing consumer acceptance. The evaluation method used in this study has merit following from its relevance to panellists, the relaxed panellists contributed to improved analysis and accuracy of the results.

The last objective of this study was to evaluate the effect of cowpea powder and pumpkin powder on total phenolic content, antioxidant activity and total flavonoids of cowpea-pumpkin composite soup. Results indicated that formulations 2 and 3 possess the highest TCP levels ranging from 0.5 - 0.63 while formulation 1 had the lowest TCP content 0.25-0.32. Furthermore, it was also noted that Formulation 2 and 3 had also higher amounts of flavonoid compounds 28 to 42 while formulation 1 was the lowest with 24. The obtained results may however not be surprising because according to literature cowpea seeds and pumpkin flower and fruit are excellent sources of dietary phenolics the main cause for health promotion and antioxidant properties. Basing on these results it can be concluded that the consumption of cowpea and pumpkin is vital for a decline in several chronic diseases due to the presence of flavonoid compounds that safeguard body cells from radical-produced oxidative damage.

Overall, this study provides high-value information on the characteristics of pumpkin and cowpea powders for application in the food industry. The results reveal that Pumpkin and cowpea are amongst the important, nutritious and medicinal vegetables which can be consumed to cure different ailments. The study indicates that both cowpea and pumpkin are good sources of antioxidants which could be due to the presence of polyphenol compounds. Thus, they could be utilised as a source of supplement or further exploited for value addition as they are rich in nutrients and antioxidant components.

The results reported in this study confirm that cowpea and pumpkin are very important vegetables in people's diets because they have high protein and carbohydrate content which makes them suitable for addressing protein-energy malnutrition as well as in food product formulations. It can be concluded that pumpkin and cowpea can be successfully used as

functional and nutritionally valuable food ingredients. The use of products made from ILV such as a soup, as opposed to just fortifying starch-based staple products, improves fortification as a way to combat malnutrition. Thus, consumption of indigenous vegetables can play a pivotal role in mitigating the stress of malnutrition and help people meet their recommended daily nutrient requirement. There is evidence from this study conducted in South Africa that indigenous vegetables can contribute significantly to a nutritionally balanced diet by complementing less nutritious staple foods and thereby meeting the recommended daily nutrient intake of consumers of a staple diet. Development of cowpea-pumpkin soup provides a full possibility for the consumption of cowpea and pumpkin.

## **6.2. Recommendations**

Although this research has brought into light some significant results, the study suggests the following recommendations:

- Based on the results of this study it is recommended that cowpea-pumpkin could be highly valuable in terms of their high fibre content when producing new food products for people who need fibre in their diet.
- Further investigation is required to develop baby food products incorporating cowpea-pumpkin which are high in protein to help ease the problem of malnutrition among children.
- Limited comparability is another issue hence repeated experiments are required over several seasons, regions and sites to strengthen the validity of the results.
- The present study is a South African study and locally grown cowpea and pumpkin might show themselves to have a unique proximate composition and so unique benefits.
- Rivers particularly in Limpopo may be polluted with heavy metals from mining activities more studies are required to determine heavy metal content of ILVs and specifically cowpea and pumpkin.
- Further studies are required to determine other important attributes like the shelf life which were not considered in this study which also play a pivotal role in product development.
- Further nutritional profiling of cowpea-pumpkin soup is needed to determine the vitamin and amino acid content.

- To boost the utilisation of cowpea and pumpkin, recipes can be developed and introduced in popular eating places school feeding programmes and government institutions like prisons and hospitals.
- Based on the results of sensory evaluation, improving the appearance and taste of F3 soup is very important.
- There is need for in depth participatory studies on the indigenous knowledge systems about ILVs as well as on the current importance of cowpea-pumpkin in the household economy.
- More information is required on commercial processing of food products using various parts of cowpea and pumpkin plants.
- Research and policy intervention as well as extension workers need to educate people about the importance of Indigenous vegetables like cowpea and pumpkin and the edibility of parts like flowers and seeds and the nutritional benefits attached to them.
- There is need to promote the consumption of ILVs through awareness and campaigns.
- HIV positive people and other sick people should be encouraged to consume ILVs more so that their immune system can be strengthened.
- Cowpea-pumpkin seeds, fruits, leaves and flowers can be converted into extracts which become more concentrated source of nutrients and can be utilised in medicines and novel food products.
- Phytochemicals and proteins present in cowpea-pumpkin constituent parts can be extracted to develop safe and novel antioxidant and microbial drugs.
- After approval from scientific communities, cowpea-pumpkin based nutraceuticals and cosmetics can be commercially marketed for the well-being of humans.

## REFERENCES

- Abbas, K.A., Abdulkarim, S.M., Saleh, A.M. & Ebrahimian, M. (2010). Suitability of viscosity measurement methods for liquid food variety and applicability in food industry. A Review. *Journal of Food, Agriculture & Environment*, 8(3&4):100-107.
- Abebe, B. K., & Alemayehu, M. (2022). A review of the nutritional use of cowpea (*Vigna unguiculata* L. Walp) for human and animal diets. *Journal of Agriculture and Food Research*, 10, 100383. <https://doi.org/10.1016/j.jafr.2022.100383>
- Adebooye, O.C. & Opabode, J.T. (2004). Status of conservation of the indigenous leaf vegetables and fruits of Africa. *African Journal of Biotechnology*, 3(12): 700-705. Available at: <http://www.academicjournals.org/AJB>.
- Adegunwa, M.O., Bakare, H.A. & Akinola, O.F. (2012). Enrichment of noodles with soy flour and carrot powder. *Official Journal of Nigerian Institute of Food Science and Technology*, 30(1): 74-81.
- Adejuyitan, J.A., Otunola, E.T., Akande, E.A., Bolarinwa, I.F. & Oladokun (2009). Some physicochemical properties of flour obtained from fermentation of Tigernut (*Cyperus esculentus*). *Food Sci*, 3(2): 51- 055
- Adenike, O.M. (2012). Effect of germination, boiling and co-fermentation on the viscosity of maize/cowpea mixture as complementary infant food. *American Journal of Food Technology*, 7(1):1-12. <https://doi.10.3923/ajft.2012.1.12>
- Aderinola, T.A. (2018). Effect of pumpkin leaves on the chemical composition and antioxidant properties of smoothies. *Proceedings of the 4<sup>th</sup> regional food science and technology summit (Refrosts)* Akure, Ondo State Nigeria.
- Adewale, B., Oluwatonyin, M. & Aigbe, O.D. (2016). Contribution of cowpea (*Vigna unguiculata*) to food security in sub-Sahara Africa. *Global Journal of Plant Breeding and Genetics*, 3(2): 154-158.
- Adjei-Fremah, S., Jackai, L. E. N., & Worku, M. (2015). Analysis of phenolic content and antioxidant properties of selected cowpea varieties tested in bovine peripheral blood. *American Journal of Animal and Veterinary Sciences*, 10(4), 235–245. <https://doi.org/10.3844/ajavsp.2015.235.245>
- Adubofuor, J., Amoah, I. & Agyekum, P.B. (2016). Physicochemical properties of pumpkin fruit pulp and sensory evaluation of pumpkin-pineapple juice blends. *American Journal of Food Science and Technology*, 4(4):89-96. <http://pubs.sciepub.com/ajfst/4/4/1>.
- Ajayi, S. A., Berjak, P., Kioko, J., Dulloo, M. E., & Vodouhe, R. (2006). Responses of fluted pumpkin (*Telfairia occidentalis* Hook. f.; Cucurbitaceae) seeds to desiccation, chilling and hydrated storage. *South African Journal of Botany*, 72(4), 544–550. <https://doi.org/10.1016/j.sajb.2006.02.001>
- Aju, P.C., Labode, P., Uwalaka., R.E. & Iwuanyanwu, U.P. (2013). The marketing potentials of indigenous leafy vegetables in south-eastern Nigeria. *International Journal of Agri Science*, 3(9): 667-677.
- Ajuru, M. & Nmom, F. (2017). A review on the economic uses of species of Cucurbitaceae and their sustainability in Nigeria. *American Journal of Plant Biology*, 2(1): 17-24. <http://dx.doi.org/10.11648/j.ajpb.20170201.14>

- Akinboade, O.A. & Adeyefa, S.A. (2018). An analysis of variance of food security by its main determinants among the urban poor in the city of Tshwane, South Africa. *Social Indicators Research*, 137(1): 61-82. <http://doi:10.1007/s11205-017-1589-1>
- Akintade, A.O., Awolu, O.O. & Ifesan BO. (2019). Nutritional Evaluation of Fermented, Germinated and Roasted Pumpkin (*Cucurbita maxima*) Seed Flour. *Acta Universitatis Cibiniensis. Series E: Food Technology*, 23(2):179-186. <http://dx.doi.org/10.2478/auaft2019-00212>.
- Amaral, M.M., Herrin, W.E. & Gulere, G.B. (2018). Using the Uganda National Panel Survey to analyze the effect of staple food consumption on undernourishment in Ugandan children. *BMC Public Health*, 18(32): 1-12. <https://doi.org/10.1186/s12889-017-4576-1>
- Amin, M.Z., Islam, T., Uddin, M.R., Uddin, M.J., Rahman, M.M. & Satter, M.A. (2019). Comparative study on nutrient contents in the different parts of indigenous and hybrid varieties of pumpkin (*Cucurbita maxima* Linn.). *Heliyon*, 5:e02462 <https://doi.org/10.1016/j.heliyon.2019.e02462>
- Amin, T., Naik, H.R., Hussain, S.Z., Jabeen, A. & Thakur, M. (2018). In-Vitro antioxidant and antibacterial activities of pumpkin, quince, muskmelon and bottle gourd seeds. *Journal of Food Measurement and Characterization*, 12: 182-190. <https://doi.org/10.1007/s11694-017-9629-8>
- Antova, G.A., Stoilova, T.D. & Ivanova, M.M. (2014). Proximate and lipid composition of cowpea (*Vigna unguiculata* L.) cultivated in Bulgaria. *Journal of Food Composition and Analysis*, 33(2):146-152. <https://doi.org/10.1016/j.jfca.2013.12.005>
- AOAC. (1980). Association of Official Analytical Chemists. Official methods of analysis of AOAC international, Washington DC, USA.
- AOAC. (2010). Association of Official Analytical Chemists. Official methods of analysis of AOAC international, 18<sup>th</sup> Ed., Washington DC, USA.
- AOAC. (2016). Association of Official Analytical Chemists. Official methods of analysis of AOAC international, 13<sup>th</sup> Ed., Gaithersburg, MD, USA.
- Aoudi, F., Ayari, S., Ferhi, H., Roussos, S. & Hamdi, M. (2011). Gamma irradiation of air-dried olive leaves: Effective decontamination and impact on the antioxidative properties and on phenolic compounds. *Food chemistry*, 127(3), 1105-1113.
- Apea-Bah, F.B., Minnaar, A., Bester, M.J. & Duodu, K.G. (2016). Sorghum-cowpea composite porridge as a functional food, Part 11: Antioxidant properties as affected by simulated in vitro gastrointestinal digestion. *Food Chemistry*, 197: 307-315. <http://dx.doi.org/10.1016/j.foodchem.2015.10.121>.
- Apea-Bah, F.B., Serem, C.S., Bester, M.J. & Duodu, K.G. (2017). Phenolic composition and antioxidant properties of koose, a deep-fat fried cowpea cake. *Food Chemistry*, 237: 247-256. <http://dx.doi.org/10.1016/j.foodchem.2017.05.109>.
- Appiah, F., Asibuo, J.Y. & Kumah, P. (2011). Physicochemical and functional properties of bean flours of three cowpea (*Vigna unguiculata* L.Walp) varieties in Ghana. *African Journal of Food Science*, 5(2):100-104. <http://www.academicjournals.org/ajfs>
- Aremu, M.O., Olaofe, O. & Akintayo, T.E. (2006). Compositional evaluation of cowpea (*Vigna unguiculata*) and scarlet runner bean (*Phaseolus coccineus*) varieties grown in Nigeria. *Journal of Food, Agriculture and Environment*, 4(2): 39-43.

- Arifin, N., Siti Nur Izyan, M.A. & Huda-Faujan, N. (2019). Physical properties and consumer acceptability of basic muffin made from pumpkin puree as butter replacer. *Food Research*, 3(6): 840-845. <http://www.myfoodresearch.com>
- Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R. & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, 8(4): 96. <https://doi.org/10.3390/plants8040096>
- Asare, A.T., Agbemafle, R., Adukpo, G.E., Diabor, E. & Adamley, K. A. (2013). Assessment of functional properties and nutritional composition of some cowpea (*Vigna unguiculata* L.) genotypes in Ghana. *Journal of Agricultural and Biological Science*, 8(6): 465-469
- Asif, M., Raza Naqvi, S. A., Sherazi, T. A., Ahmad, M., Zahoor, A. F., Shahzad, S. A. & Mahmood, N. (2017). Antioxidant, antibacterial and antiproliferative activities of pumpkin (cucurbit) peel and puree extracts-an in vitro study. *Pakistan journal of pharmaceutical sciences*, 30(4):1327-1334.
- Atencio, S., Verkempinck, S.H.E., Reneike, K., Hendrickx, M. & Van Loey, Ann. (2022). Heat and light stability of pumpkin-based carotenoids in a photosensitive food. A carotenoid-coloured beverage. *Foods*, 11(3):485. <https://doi.org/10.3390/foods11030485>
- Aukkanit, N. & Prakrit, S.S.Z. (2016). Effect of dried pumpkin powder on physical, chemical and sensory properties of noodle. Proceedings of Academics World. 52<sup>nd</sup> International conference, Los Angeles, USA.
- Aurand, L. W., Woods, A. E., & Wells, M. R. (1987). Sampling and proximate analysis. In Food Composition and Analysis. Springer Netherlands. 19-34 [https://doi.org/10.1007/978-94-015-7398-6\\_2](https://doi.org/10.1007/978-94-015-7398-6_2)
- Awika J.M., Rooney, L.W., Wu, X., Prior, R.L. & Zevallos, L.C. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry*, 51: 6657-6662
- Awika, J.M. & Duodu, K.G. (2017). Bioactive polyphenols and peptides in cowpea (*Vigna unguiculata*) and their health promoting properties, A review. *Journal of Functional Foods*, 38(2017): 686-697. <http://dx.doi.org/10.1016/j.jff.2016.12.002>.
- Awuchi, C. G., Igwe, V. S. & Echeta, C. K. (2019). Functional properties of foods and flours. *International Journal of Advanced Academic Research*, 5: 139-160.
- Baba, W.N. (2018). Techno-functional properties of yoghurts fortified with walnut and flaxseed oil emulsions in guar gum. *LWT-Food Science and Technology*, 92:242-249.
- Badr, S.E.A., Shaaban, M., Elkholy, Y.M., Helal, M.H., Hamza, A.S., Masoud, M.S. & El Safty, M.M. (2011). Chemical composition and biological activity of ripe pumpkin fruits (*Cucurbita pepo* L.) cultivated in Egyptian habitats. *Natural Product Research*, 25(16):1524-1539. <https://doi.org/10.1080/14786410903312991>.
- Bai, Z., Huang, X., Meng, J., Kan, L., & Nie, S. (2018). Survey of Chinese cowpea cultivars for phenolic compounds and antioxidant activity. *Current Topics in Nutraceutical Research*, 17(4), 337–348. <https://doi.org/10.37290/ctnr2641-452x.17:337-348>
- Barakat, H. & Hassan, M.F.Y. (2017). Chemical, nutritional, rheological, and organoleptical characterizations of stirred pumpkin-yoghurt. *Food and Nutrition Sciences*, 8: 746-759. <https://doi.org/10.4236/fns.2017.87053>
- Barba, F. J., Cortés, C., & Esteve, M. J. (2012). Study of Antioxidant Capacity and Quality Parameters in An Orange Juice – Milk Beverage After High-Pressure Processing



- Treatment, *Food and Bioprocess Technology*, 5:2222–2232. <http://doi.org/10.1007/s11947-011-0570-2>
- Bardaa, S., Halima, N.B., Aloui, F., Mansour, R.B., Jabeur, H., Bouaziz, & Sahnoun, Z. (2016). Oil from pumpkin (*Cucurbita pepo* L.) Seed: Evaluation of its functional properties on wound healing in rats. *Lipids in Health and Disease*, 15: 73. <https://doi.org/10.1186/s12944-016-0237-0>
- Batista, J.E.R., Braga, L.P., Oliveira, R.C., Silva, E.P. & Damiani, C. (2018). Partial replacement of wheat flour by pumpkin seed flour in the production of cupcakes filled with carob. *Food Science and Technology*, 38(2): 250-254. <https://doi.org/10.1590/1678-457X.36116>
- Batool, M., Ranjha, M.M.A.N., Roobab, U., Manzoor, M.F., Farooq, U., Nadeem, H.R., Nadeem, M., Kanwai, R., AbdElgawad, H., Soad, K., Jaounni, A.I., Selim, S. & Ibrahim, S.A. (2022). Nutritional value, phytochemical potential and therapeutic benefits of pumpkin (*Cucurbita* sp). *Plants (Basel)*, 11(11):1394. <https://doi.org/10.3390/plants11111394>
- Berk, Z. (2009). Dehydration. In *Food process Engineering and Technology*. Third ed. 1-29. <https://doi.org/10.1016/B978-0-12-812018-7-7.00001-4>
- Berk, Z. (2018). Physical properties of food materials. In *Food Process Engineering and Technology*. Third ed. 1-29. Academic Press <https://doi.org/10.1016/B978-0-12-812018-7-7.00001-4>
- Biama, P.K., Fara, A.K., Mutungi, M., Osuga, I.N. & Kuruma, R.W. (2020). Nutritional and technological characteristics of new cowpea (*Vigna unguiculata*) lines and varieties grown in Eastern Kenya. *Food and Nutrition Sciences*, 11(5): 416-430. <https://doi.org/10.423/fns.2020.115030>.
- Biebinger, R. & Hurrell, R.F. (2008). Vitamin and mineral fortification of foods. Food fortification and supplementation technological, safety and regulatory aspects. 4th ed. Woodhead. Ottawa.
- Biggins, A., Qian Oo, Y. & Weaver, A. (2013). The effect of replacing cake flour with ground pumpkin seeds in cake. *Nutrition Research Project*, 453
- Birol, E., Meenakshi, J.V., Opainde, A., Perez, S. & Tomlins, K. (2015). Developing country consumers' acceptance of biofortified foods: a synthesis. *Food Security*, 7: 555-568. <http://doi.10.1007/s12571-015-0464-7>
- Boari, A.J., Cordovil, G.A. & Kauffmann, C.M. (2022). Bean common mosaic virus infecting manteiguinha cowpea (*Vigna unguiculata*): Seed transmission, evaluation of yield loss, and genetic resistance. *Tropical Plant Pathology*, 47: 450–455. <https://doi.org/10.1007/s40858-021-00490-w>
- Bochnak, J. & Świeca, M. (2020). Potentially bio-accessible phenolics, antioxidant capacities and the colour of carrot, pumpkin and apple powders—effect of drying temperature and sample structure. *International Journal of Food Science & Technology*, 55(1), 136-145.
- Borro, R. & Gemora, R. (2016). Sensory acceptability of squash (*Cucurbita maxima*) in baking cake. *Frontiers in Food and Nutrition*, 2(1): 1-13.
- Bunde-Tsegba, C.M., Abankwa, K. & Tersoo-Abiem, E.M. (2020). Physicochemical quality and consumer acceptability of condiment from pumpkin (*Cucurbita pepo*) seeds. *Journal of Current Research in Food Science*. 1(2):04-08 <http://www.foodresearchjournal.com>.

- Bvenura, C. & Afolayan, A.J. (2015). The role of wild vegetables in household food security in South Africa: A review: *Food Research International*. <http://doi.org/10.1016/j.foodres.2015.06.013>
- Bvenura, C. & Afolayan, A.J. (2016). Nutrient composition of some leafy wild vegetables in the Eastern Cape, South Africa. *South African Journal of Botany*, 103: 310. <http://dx.doi.org/10.1016/j.sajb.2016.02.027> .
- Bvenura, C. & Sivakumar, D. (2017). The role of wild fruits and vegetables in delivering a balanced and healthy diet. *Food Research International*, 99(1): 15-30. <https://doi.org/10.1016/j.foodres.2017.06.046>
- Cai, R., Ns, H., & Jalaluddin, M. (2003). High-Performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. *Journal of Agricultural and Food Chemistry*, 51(6), 1623–1627. <https://doi.org/10.1021/jf020867b>
- Campbell, L., Euston, S.R. & Ahmed, M.A. (2016). Effect of addition of thermally modified cowpea protein on sensory acceptability and textural properties of wheat bread and sponge cake. *Food Chemistry*, 194: 1230-1237. <http://dx.doi.org/10.1016/j.foodchem.2015.09.002>.
- Carvalho, C.C.R. & Caramujo, M.J. (2018). The various roles of fatty acids. *Journal of Synthetic Chemistry and Natural Product Chemistry*, 23(10): 2583. <http://doi.org/10.3390/molecules23102583>
- Chakona, G. & Shackleton, C.M. (2017). Voices of the hungry: A qualitative measure of household food access and food insecurity in South Africa. *Agriculture & Food Security*, 6(66): 1-17. <https://doi.org/10.1186/s40066-017-0149-x>
- Chen, H., Chen, H., Hu, L., Wang, L., Wang, S., Wang, M.L. & Cheng, X. (2017). Genetic diversity and a population structure analysis of accessions in the Chinese cowpea (*Vigna unguiculata* (L.) Walp) germplasm collection. *The Crop Journal*, 5(2017): 363-372. <http://dx.doi.org/10.1016/j.cj.2017.04.002>
- Chen, L. (2012). Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. *Bioresource Technology*, 111:208-214. <https://doi.org/10.1016/j.biortech.2012.02.033>
- Chikwendu, J.N., Igbatim, A.C. & Obizoba, I.C. (2014). Chemical composition of processed cowpea tender leaves and husks. *International Journal of Scientific and Research Publications*, 4(5).
- Chinaza, G.A., Ingwe, S.V. & Chinelo, K.E. (2019). The functional properties of foods and flours. *International Journal of Academic Research Sciences, Technology and Engineering*, 5(11).
- Chiou, A., Karathanos, V. T., Mylona, A., Salta, F. N., Preventi, F., & Andrikopoulos, N. K. (2007). Currants (*Vitis vinifera* L.) content of simple phenolics and antioxidant activity. *Food Chemistry*, 102(2), 516-522.
- Czubiński, J., & Dwiecki, K. (2016). A review of methods used for investigation of protein-phenolic compound interactions. *International Journal of Food Science & Technology*, 52(3), 573–585. <https://doi.org/10.1111/ijfs.13339>
- Da Silva, A. C., Da Costa Santos, D., Lopes Teixeira, D., Da Silva, P. B., Santos, R. C. D. & Siviero, A. (2019). Cowpea: a strategic legume species for food security and health. In *IntechOpen eBooks*. <https://doi.org/10.5772/intechopen.79006>

- Damoradan, S., Parkin, K.L. & Fennema, O.R. (2010). *Química de los alimentos de Fennema*. (3rd. ed) Zaragoza: Acribia.
- Darkwa, S. & Darkwa, A.A. (2013). The use of indigenous green leafy vegetables in the preparation of Ghanaian Dishes. *Journal of Food Processing and Technology*, 4(12):286.
- Darwish, A.M. (2020). Influence of inulin and pumpkin powder addition on sensory and rheological properties of low-fat probiotic yogurt. *Food and Public Health*, 10(4): 88-96. <http://doi:10.5923/j.fph.20201004.02>
- Dash, P. & Ghosh, C. (2017). Amino acid composition, antioxidant and functional properties of protein hydrolysates from *Cucurbitaceae* seeds. *Journal of Food Science Technology*, 54(13): 4162-4172. <http://doi.org/10.1007/s13197-017-2855-6>
- Deng, R., Mars, M., Ruud, G.M., Sman, V.D., Smeets, P.A.M., Janssen, A.E.M. (2020). The importance of swelling for in vitro gastric digestion of whey protein gels. *Food Chemistry*, 330. <https://doi.org/10.1016/j.foodchem.2020.127182>.
- Dhiman, A.K., Sharma, K.D. & Attri, S. (2009). Functional constituents and processing of pumpkin: A review. *J Food Science and Technology*, 46(5):411-417.
- Dhlamini, V.V. (2017). Adult women in Eluyengweni, Swaziland: Their food practices and knowledge of indigenous green leafy vegetables. Dissertation presented to the University of Pretoria. Available at: <http://hdl.handle.net/2263/60802>
- Dinu, M., Soare, R., Hoza, G. & Becherescu, A.D. (2016). Biochemical composition of some local pumpkin population. *Agriculture and Agricultural Science Procedia*, 10: 185 – 191.
- Doyle, N., Swain, D., Roberts, J.J. & Cozzolino, D. (2017). The use of qualitative analysis in food research and technology: Considerations and reflections from an applied point of view. *Food Analytical Methods*, 10: 964-969. <http://doi.org/10.1007/s12161-016-0654-8>.
- Drimie, S. & Pereira, L. (2016). Advances in food security and sustainability in South Africa. *Advances in Food Security and Sustainability*, 1: 1-32. <http://doi.org.10.1016/bs.af2s.2016.09.002>
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1-42. <http://doi.org/10.2307/3001478>.
- Dweba, T.P. & Mearns, M.A. (2011). Conserving indigenous knowledge as the key to the current and future use of traditional vegetables. *International Journal of Information*, 31 (6): 564-571.
- Dyshlyuk, L., Babich, O., Prosekov, A., Ivanova, S., Pavsky, V. & Yang, Y. (2017). *In vivo* study of medical and biological properties of functional bakery products with the addition of pumpkin flour. *Bioactive Carbohydrates and Dietary Fibre*, 12: 20-24. <http://dx.doi.org/10.1016/j.bcdf.2017.09.001>.
- Ebere, R.A., Imungi, J. K. & Kimani, V.N. (2017). Proximate composition, energy contents and blood sugar responses of stiff porridge and rice meals consumed in Kenya. *Food Science and Quality Management*, 63. ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online) <https://iiste.org>
- Edokpayi, J.N., Rogawski, E.T., Kahler, D.M., Courtney, L.H., Reynolds, C., Nyathi, E., Smith, J.A., Odiyo, J.O., Samie, A., Bessong, P. & Dillingham, R. (2018). Challenges to sustainable safe drinking water: A case study of water quality and use across seasons in rural communities in Limpopo Province, South Africa. *Water Basel*, 10 (2): 1-28. <http://doi:10.3390/w10020159>.

- Egharevba, H.O. (2019). Chemical properties of starch in the food industry. <http://doi.org/10.5772/intechopen.87777>.
- Eicher, L., Knop., Aszodi., S., French, L.E. & Wollenberg, A. (2019). A systematic review of factors influencing treatment adherence in chronic inflammatory skin disease strategies for optimizing treatment outcome. *Journal of the European Academy of Dermatology and Venereology*, 33: 2253-2263.
- Ejiofor, E.J. & Kporna, J.D. (2019). Nutrient and sensory evaluation of cowpea – acha flour blend in pudding production. *Food Science and Technology*, 4(1): 2574-2701. <https://doi.org/10.23880/fsnt-16000174>.
- Ekalu, A. & Habila, J.D. (2020). Flavonoids: isolation, characterization, and health benefits. *Beni-Suef University Journal of Basic and Applied Sciences*, 9, 45. <https://doi.org/10.1186/s43088-020-00065-9>
- Ekpa, O., Palacios-Rojas, N., Fogliano, G.K.V. & Linnemann, A.R. (2018). Sub-Saharan African maize-based technological perspectives to increase the nutrition security impacts of maize-breeding programmes. *Global Food Security*, 17: 48-56. <http://doi.org/10.1016/j.gfs.2018.03.007>.
- Elinge, C.M., Muhammad, A., Atiku, F.A., Itodo, A., Peni, I.J., Sanni, O.M. & Mbongo, A.N. (2012). Proximate, mineral and anti-nutrient composition of pumpkin (*Cucurbita pepo* L) seeds extract. *International Journal of Plant Research*, 2(5): 146-150. <http://journal.sapub.org/plant>
- Emeka-Ike, P.C. & Chukwuemeka Ike, C.C. (2020). Evaluation of physicochemical properties and microbiological safety of pumpkin (*Cucurbita pepo*) seed composite flours. *GSC Biological and Pharmaceutical Sciences*, 12(03): 061-072 <https://doi.org/10.30574/gscbps.2020.12.3.0223>
- Evans, J.R. & Lawrenson, J.G. (2017). Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration. *Cochrane Database Syst. Rev.* CD000253.
- Faber, M., Olefofse, A. & Wenhold, P. 2010. African leafy vegetables consumed by households in the Limpopo Province and Kwazulu-Natal provinces in South Africa. *South African Journal of Clinical Nutrition* 23(1):30-38.
- Fang, Y., Selomulya, C. & Chen, X.D. (2008). Measurement of food powder reconstitution properties. *Drying Technology*, 26:3-14. <https://doi.org/10.1080/07373930701780928>
- FAO. (2012). Food and Agriculture Organisation of the United Nations. The state of food insecurity in the world. Food and Agriculture Organisation, Rome, Italy. <http://www.fao.org/docrep/016/i3027e/3027e.pdf>.
- FAO. (2020). Food and Agriculture Organisation of the United Nations. The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets. Rome, FAO. <https://doi.org/10.4060/ca9692en>
- Fellows, P.J. (2009). Rehydration. In *Food Processing Technology*. Third Edition Food and Agriculture Organisation of the United Nations (FAO). (2010). Composition of selected foods from West Africa. Food and Agriculture Organisation, Rome, Italy.
- Filbrandt, K.R. (2012). Effect of Pumpkin seed oil cake on the textural and sensory properties of white wheat bread. *American Psychological Association*, 6: 1-72.
- Filho, A., Elenilson, G., Silva, L.M.A., Teofilo, E.M., Larsen, F.H. & De Brito, E.S. (2017). Gene type evaluation of cowpea seeds (*Vigna unguiculata*) using HqNMR combined with

- exploratory tools and solid-state NMR. *Food Research International*, 91: 140-147. <http://dx.doi.org/10.1016/j.foodres.2016.12.007>.
- Gani, M., Jabeen, A., Majeed, D., Mir, S.A. & Dar, B.N. (2018). Proximate composition mineral analysis and antioxidant capacity of indigenous fruits and vegetables from temperate region of Indian Himalayas. *Journal of Food Measurement and Characterization*, 12: 1011-1019. <http://dx.doi.org/10.1007/511694-017-9716-x>
- Ghosh-Jerath, S., Singh, A., Magsumbol, M.S., Kamboj, P. & Goldberg, G. (2016). Exploring the potential of indigenous foods to address hidden hunger: Nutritive value of indigenous foods of Santhal tribal community of Jharkhand, India. *Journal of Hunger & Environmental Nutrition*, 11(4): 548-568. <http://doi.10.1080/19320248.2016.1157545>
- Giada, M.L.R. (2013). Food phenolic compounds: Main classes, sources and their antioxidant power. Oxidative stress and chronic degenerative diseases. A role for antioxidants. InTech. <https://doi.10.5772151687>
- Gido, E.O., Ayuya, O.I., Owuor, G. & Bokelmann, W. (2017). Consumption intensity of leafy African indigenous vegetables: Towards enhancing nutritional security in rural and urban dwellers in Kenya. *Agricultural and Food Economics*, 5: 14. <https://doi.org/10.1186/s40100-017-0082-0>.
- Giese, J. (2003). Colour measurement. In Foods Labotary. Food Technology Magazine. IFT News and publications. *Food Technology*, 57(12) <https://www.ift.org/news&publications>
- Glew, R.H., Glew, R.S., Chuang, L.T., Huang, Y.S., Millson, M., Constans, D. & Vanderjagt, D.J. (2006). Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbitasp*) and *Cyperas esculentus* nuts in the Republic of Niger. *Plant Foods for Human Nutrition*, 61(2): 49-54.
- Godecke, T., Stein. & Qaim, M. (2018). The global burden of chronic and hidden hunger: Trends and determinants. *Global Food Security*, 17: 21-29. <https://doi.org/10.1016/j.gfs.2018.03.004>
- Goncalves, A., Goufo, P., Barros, A., Dominguez, P.R., Trindade, H., Rosa, E.A.S., Ferreira, L. & Rodrigues, M. (2016). Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the Science of Food and Agriculture*, 96: 2941-2951.
- Gonzalez, A., Benfodda, Z., Benimelis, D., Fontaine, J.X., Molinie, R. & Meffre, P. (2022). Extraction and identification of volatile organic compounds in scentless flowers of 14 tillandsia species using HS-SPME/GC-MS. *Metabolites*, 12:628. <https://doi.org/10.3390/metabol12070628>
- Gordon, H.T., Johnson, L.E. & Bauernfeind, J.C. (1985). The use of beta carotene in bakery products. *Cereal Food World*, 30:274-276.
- Gramza-Michałowska, A., Sidor, A. & Kulczyński, B. (2017) Berries as a potential anti influenza factor: A review. *Journal of Functional Foods*, 37(17): 116–137.
- Granato, D., Masson, M.M. & Ribeiro, J.C.B. (2012). Sensory acceptability and physical stability of a prebiotic soy-based dessert developed with passion fruit juice. *Ciência e Tecnologia de Alimentos Caminas*, 32(1): 119-125. doi: <http://dx.doi.org/10.1590/S0101-20612012005000004>
- Gupta, S., Srivastava, A. & Lal, E.P. (2017). Indigenous leafy vegetables for food and nutritional security in two districts of Jharkhand, India. *Journal of Pharmacognosy and Phytochemistry*, 6(6): 901-909.

- Habib, A., Biswas, S., Abdul, H.S., Manirujjaman, M., Uddin, M., Islam, M., Hasan, M., Rahman, M., Asaduzzaman, M., Sohanur, R.M., Khatun, M., Islam, M.A. & Rahman, M. (2015). Nutritional and lipid composition analysis of pumpkin seed (*Cucurbita maxima* Linn). *Journal of Nutrition and Food Sciences*, 5(4): 374. <http://dx.doi.org/10.4172/2155-9600.1000374>
- Hama-Ba, F., Quattara F., Savadogo, A., Simpo, M. & Diwara. B. (2018). Study of the nutritional quality and acceptability of millet biscuits (*Pennisetum glaucum* L) supplemented with cowpea (*Vigna unguiculata* L) and bambara groundnut (*Vigna subterranea* L). *Journal of Agriculture, Science and Food Research*, 9(1): 1-4.
- Hamid, S., Muzaffar, S., Ahmed, W., Masoodi, F.A. & Bhat, M.M. (2016). Physical and cooking characteristics of two cowpea cultivars grown in temperate Indian climate. *Journal of the Saudi Society of Agricultural Sciences*, 15: 127-134. <http://dx.doi.org/10.1016/j.jssas.2014.08.002>.
- Hardisson, A. (2001). Mineral composition of the banana (*Musa acuminata*) from the island of Tenerife. *Food Chemistry*, 73: 153-161.
- Harouna, D.V., Venkataramana, P.B., Ndakidemi, P.A. & Matemu, A.O. (2018). Under-exploited wild vigna species potentials in human and animal nutrition: A review. *Global Food Security*, 18: 1-11. <http://dx.doi.org/10.1016/j.gfs.2018.06.002>
- Hart, T. & Vorster, I. (2006). Indigenous knowledge on the South African landscape: Potentials for agricultural development. (No 1). *Human Sciences Research Council*.
- Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M. & Allgeier, C. (2020). Plant Proteins: Assessing Their Nutritional Quality and Effects on Health and Physical Function. *Nutrients*, 12(12), 3704. <https://doi.org/10.3390/nu12123704>
- Hossein Ghaboose, S.H., Ardabili, S. M.S. & Kashaninejad, M. (2018). Physico-chemical, textural and sensory evaluation of sponge cake supplemented with pumpkin flour. *International Food Research Journal*, 25(2):854-860.
- Hssaini, L., Hernandez, F., Viuda, M.M., Charafi, J., Razouk, R., Houmanat, K., Ouaabou, R., Ennahli, S., Elothmani, D., Hmid, I., Fauconnier, M.L. & Hanine, H. (2021). Survey of phenolic acids, flavonoids and In Vitro antioxidant potency between fig peels and pulps. Chemical and Chemometric Approach. *Molecules*, 26(9):2574. <https://doi.10.3390/molecules26092574.PMID>
- Hussain, A., Kausar, T., Din, A., Murtaza, A., Jami, M.A., Noreen, S. & Iqbal, M.A. (2021) Antioxidant and Antimicrobial Properties of Pumpkin (*Cucurbita maxima*) Peel, Flesh and Seeds Powders. *Journal of Biology, Agriculture and Healthcare*.11(6):42-5. <http://doi.10.7176/JBAH/11-6-05>
- Idowu, M.A., Adeola, A.A., Olaniyan, D.J., Oke, E.K. & Omoniyi, S.A. (2017). Quality evaluation of cocoyam-cowpea flour blends and sensory attributes of their cooked paste (Amala). *Annals: Food Science and Technology*, 18(2): 183-191. <http://www.afst.valahia.ro>
- Idris, S. (2011). Compositional studies of *Telfairia occidentalis* leaves. *American Journal of Chemistry*, 1(2): 56-59. <https://dx.doi.org.10.5923/j.chemistry.20110102.12>, 7(5), 402-407.
- IFT (Institute of food Technologists). (2007). Sensory Evaluation Methods. *The Society for the Food Technologists*, Chicago, IL.

- Ilesanmi, J.O.Y. & Gungula, D.T. (2016). Amino acid composition of cowpea grains preserved with mixtures of neem (*Azadirachta India*) and moringa (*Moringa oleifera*) seed oils. *American Journal of Food and Nutrition*, 4(6): 150-156. <http://pubs.sciepub.com/ajfn/4/6/2>
- Islam, M., Jothi, J.S., Habib, M.R. & Iqbal, A. (2014). Evaluation of nutritional and sensory quality characteristics of pumpkin pies. *International Journal of Emerging Trends in Science and Technology*, 1(7): 1091-1097.
- Iwe M.O., Onyeukwu, U. & Agiriga, A.N. (2016). Proximate, functional & pasting properties of FARO 44 rice, African yam bean and brown cowpea seeds composite flour. *Cogent Food & Agriculture* (20) 2: 114-240. <http://dx.doi.org/10.1080/23311932.2016.1142409>
- Jayathilake, C., Rizliya, V., Afka, D., Ruksheela, B., Barana, C.J., Srinivas, N. & Ruvini, L. (2018). Cowpea: an overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture*, 98(13): 4793-4806. <https://doi.org/10.1002/jsfa.9074>
- Jena, A.K., Deuri, R., Sharma, P. & Singh, S.P. (2018). Underutilized vegetable crops and their importance. *Journal of Pharmacognocny and Phytochemistry*, 7(5), 402-407.
- Jeong, D. & Chung, H.J. (2018). Physical, textural and sensory characteristics of legume-based gluten free muffin enriched with waxy rice flour. *Journal of Food Science Biotechnology*, 28: 87-97. <https://doi.org/10.1007/s10068-018-0444-8>.
- Jeong-Yeon, K., Ko, M., Shinkim, D. & Lim, S.B. (2016). Enhanced production of phenolic compounds from pumpkin leaves by subcritical water hydrolysis. *Preventive Nutrition and Food Science*, 21(2): 132-137. <http://dx.doi.org/10.3746/pnf.2016.21.2.132>
- Jesmin, A.M., Ruhul, A.M. & Chandra, M.S. (2016). Effect of pumpkin powder on physico-chemical properties of cake. *International Research Journal of Biological Sciences*, 5(4),1-5.
- Jin, T., Zhou, J., Chen, J., Zhu, L., Zhao, Y. & Huang, Y. (2013). The genetic architecture of zinc and iron content in maize grains as revealed by QTL mapping and meta-analysis. *Breeding Science*, 63(3): 317-324.
- Johnson, L.A. (2000). Corn: The major cereals of the Americas. In: *Handbook of Cereal Science and Technology*. K. Kulp and J.G. Ponte, (eds). 2nd Ed. Dekker Inc, New York
- Jones, M.M., Johnson, D.O., Netlerville, J.T., Wood, J.I. & Joesten, M.D. (1985) *Chemistry and Society*. 5th ed., Saunders College Publishers U.S.A, 521-577.
- Joshi, N., Bains, K., & Kaur, H. (2020). Evaluation of Antioxidant Activity of Developed Instant Soup Mixes using Vegetable Leaf Powders from Unconventional Greens. *International Journal of Current Microbiology and Applied Sciences*, 9(1), 711–721. <https://doi.org/10.20546/ijemas.2020.901.077>
- Jothi, J.S., Hashem, S., Rana, M.R., Rahman, M.R.T. & Shams-Ud-Din. (2014). Effect of gluten-free composite flour on physico-chemical and sensory properties of cracker biscuits. *Journal of Scientific Research*, 6(3): 521-530. <http://dx.doi.org.10.3329/jsr.v6i3.16531>
- Kalsoom, K., Muhammad, H., Muhammad, A.K., Park, Yong., Kim, I., K., Shin, D.H. & Labal, A. (2021). Physicochemical properties and antioxidant potential of *Tateishikazii* vegetable soup. *Journal of Food Quality*, 1-10. <https://doi.org/10.1155/2021/8194219>.

- Kerksick, C. M., Jagim, A., Hagele, A., & Jäger, R. (2021). Plant Proteins and Exercise: What Role Can Plant Proteins Have in Promoting Adaptations to Exercise? *Nutrients*, 13(6), 1962. <https://doi.org/10.3390/nu13061962>
- Kevart, D. (2017). The top 52 healthiest vegetables on the planet. *Fitness World*. Available at: <http://www.wiki-fitness.com/vegetables-health-benefits-nutrition-facts> (accessed on 02 September 2023).
- Khalid, I. I. & Elharadallou, S.B. (2013). Functional properties of cowpea (*Vigna unguiculata* L. Walp), and lupin (*Lupinus Termis*) flour and protein isolates. *Journal of Nutrition and Food Sciences*, 3:6 <http://dx.doi.org/10.4172/2155-9600.1000234>
- Khalid, I.I., Elhardallou, S.B. & Elkhalifa, E.A. (2012) Composition and functional properties of cowpea (*Vigna unguiculata* L. Walp) flour and protein isolates. *American Journal of Food Technology*, 7(3): 113-122. <http://doi:10.3923/ajft.2012.113.122>
- Khattab, R.Y. & Arntfield, S.D. (2009). Nutritional quality of legume seeds as affected by some physical treatments: Part 2. Antinutritional factors. *LWT-Food Science and Technology*. 42: 1113-1118.
- Khuzwayo, W. (2014). Millions lack despite local veggies. Business report. Independent Newspapers. <http://www.iol.co.za/business/news/millions-lack-despite-local-veggies-1.66> (accessed on 16 March 2014).
- Kidane, B., Van de Maesen, L.T.G., Asfaw, Z., Sosef, M.S.M. & Van Anandel. (2015). Wild and semi-wild leafy vegetables used by the Maale and Ari ethnic communities in Southern Ethiopia. *Genet Resour Crop Evol*. 62:221-234. <http://dx.doi.org/10.1007/s/0722-014-0147-9>
- Kiharason, J.W., Isutsa, D.K. & Ngoda, P.N. (2017). Evaluation of sensory acceptability of bakery products prepared from blended wheat and pumpkin composite flour. *International Journal of Science and Nature*, 8(1): 1-7
- Kim, M. Y., Kim, E. J., Kim, Y. N., Choi, C. & Lee, B. H. (2012). Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts. *Nutrition Research and Practice*, 6(1):21-27. <http://doi.org/10.4162/nrp.2012.6.1.21>
- Kirigia, D., Winkelmann, T., Kasili, R. & Mibus, H. (2018). Development stage, storage, temperature and storage duration influence phytonutrient content in cowpea (*Vigna unguiculata* L. Walp.). *Heliyon*, 4(6): 1-24. <http://dx.doi.org/10.1016/j.heliyon.2018.e00656>
- Koes, R.E., Quattrocchio, F. & Mol, J.N.M. (1994). The flavonoid biosynthetic pathway in plants: Function and evolution. *BioEssays*, 16(2): 123-132. <http://doi.org/10.1002/bies.950160209>
- Kokkinidou, S., Peterson, D., Bloch, T. & Branston, A. (2018). The important role of carbohydrates in the flavor, function and formulation of oral, nutritional supplements. *Nutrients*. 10(6):742. <http://doi.org/100.3390/nu10060742>.
- Konsam, S., Thongam, B. & Handique, A.K. (2016). Assessment of wild leafy vegetables traditionally consumed by the ethnic communities of Manipur, northeast India. *Journal of Ethnobiology and Ethnomedicine*, 12(9): 1-15. <http://dx.doi.org/10.1186/s13002-016-0080-4>
- Krokida, M.K. & Marinos-Kouris, D. (2003). Rehydration kinetics of dehydrated products. *Journal of Food Engineering*, 57(1): 1-7.



- Krotz, L., Ciceri, E. & Giazzi, G. (2008). Protein determination in cereals and seeds. Available at: <https://www.foodqualityandsafety.com/article/protein-determination-in-cereals-and-seeds/> (accessed on 13 July 2023)
- Kulaitiene, J., Jariene, E., Dailcenko, H., Cerniauskiene, J., Wawrzyniak, A., Hamulka, J. & Juknevičienė, E. (2014). Chemical composition of pumpkin (*Cucurbita maxima* D.) flesh flours used for food. *Journal of Food, Agriculture & Environment*, 12(3&4): 61-64. Available at: <https://hdl.handle.net/20.500.12259/87746> (accessed on 05 September 2023)
- Kulczynski, B., Gramza-Michalowska, A. & Krolczyk, J.B. (2020). Optimization of extraction conditions for the antioxidant potential of different pumpkin varieties (*Cucurbita maxima*). *Sustainability*, 12(1305): 1-30. <http://doi:10.3390/su12041305>
- Kulkarni, K.D., Kulkarni, D.N. & Ingle, U.M. (1991). Sorghum malt-based weaning formulations preparation, functional properties and nutritive value. *Food and Nutrition Bulletin*, 13(4): 322-327.
- Kumari, N., Sindhu, S.C., Rani, V., Kumari, A. & Kumari, V. (2021). Effect of fermentation on functional properties of pumpkin seed flour. *The Pharma Innovation Journal*, 10(5): 1031-1033 <http://www.thepharmajournal.com>.
- Li, X. & Siddique, K.H.M. (2018) Future Smart Food- Rediscovering hidden treasures of neglected and underutilised species for zero hunger in Asia. FAO. Bangkok. 242.
- Li, X.J., Li, Z.G., Wang, X., Han, J.Y., Zhang, B., Fu., Y.J. & Zhao, C.J. (2016). Application of cavitation system to accelerate aqueous enzymatic extraction of seed oil from *Cucurbita pepo* L. and evaluation of hypoglycemic effect. *Food Chemistry*, 212: 403-410. <http://dx.doi.org/10.1016/j.foodchem.2016.05.185>.
- Lim, S. (2018). Eating a balanced diet: A healthy life through a balanced diet in the age of longevity. *Journal of Obesity & Metabolic Syndrome*. 27: 39-45. <https://doi.org/10.7570/jomes.2018.27.1.39>
- Liu, F., Xu, Q., Dai, R. & Ni, Y. (2015) Effects of natural antioxidants on colour stability, lipid oxidation and metmyoglobin reducing activity in raw beef patties. *Acta Sci. Pol. Technol. Aliment*, 14(25):37–44.
- Londonkar, R.L. & Awanti, B.S. (2014). Pharmacognostic and phytochemical screening of cowpea seeds (*Vigna unguiculata*). *International Journal of Phytomedicine*, 6: 510-514.
- Longato, F., Gonzalez, R.L., Peiretti, P.G., Meineri, G., Alvarez, J.A.P., Martos, M.V. & Lopez, J. F. (2017). The effect of natural ingredients (amaranth and pumpkin seeds) on the quality properties of chicken burgers. *Food Bioprocess Technology*, 10: 2060-2068. <http://dx.doi.org/10.1007/511947-017-1978-0>
- Madode, Y.E., Houssou, P.A., Linnemann, A.R., Hounhouigan, D.J., Nout, M.J.R & Van Boekel, M.A.J.S. (2011). Preparation, consumption and nutritional composition of West African cowpea dishes. *Ecology of Food and Nutrition*, 50(2): 115-136.
- Maguipa, T.C.L., Mbougoung, P.D. & Womeni, H.M. (2022). Evaluation of the sensory attributes of pepper soup beef hides and determination of the preservative potential of the spices used for its preparation. *Journal of Agriculture and Food Research*, 8(2022): 100293. <http://doi.org/10.1016/j.jafr.2022.100293>.
- Mala, K. S. & Kurian, A. E. (2016). Nutritional composition and antioxidant activity of pumpkin wastes. *International Journal of Pharmaceutical, Chemical & Biological Sciences*, 6(3), 336-344. <http://www.ijpcbs.com>

- Mamiro, P.S., Mbwaga, A.M., Mamiro, D.P., Mwanri, A.W. & Kinabo, J.L. (2011). Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. *African Journal of Food, Agriculture, Nutrition and Development*, 11(1): 4490-4506.
- Manjula, K. & Suneetha, C. (2014). Formulation and development of functional confectionery by incorporating pumpkin juice. *International Journal of Food, Agriculture and Veterinary Sciences*, 4(1): 47-52.
- Mansour, E. H., Dworschák, E., Lugasi, A., Barna, É., & Gergely, A. (1993). Nutritive value of pumpkin (*Cucurbita Pepo* Kakai 35) seed products. *Journal of the Science of Food and Agriculture*, 61(1), 73–78. <https://doi.org/10.1002/jsfa.2740610112>
- Maseko, I., Mabhaudhi, T., Tesfay, S., Tesfamicael, A., Fezzehazion, M. & Du Plooy, C.P. (2017). African leafy vegetables: A review of status, production and utilization in South Africa. *Sustainability*, 10: 16.
- Matenge, S.T.P., Van der Merwe, D., De beer, H., Bosman, M.J.C. & Kruger, A. (2012). Consumers' beliefs on indigenous and traditional foods and acceptance of products made with cow pea leaves. *African Journal of Agricultural Research*, 7:2243-2254.
- Mavengahama, S. (2013). The contribution of vegetables to food security and nutrition within selected sites in South Africa. Dissertation presented to Stellenbosch University. [http://cirettransdisciplinarity.org/biblio/biblio\\_pdf/Sydney\\_Mavengahama.pdf](http://cirettransdisciplinarity.org/biblio/biblio_pdf/Sydney_Mavengahama.pdf).
- Mbhenyane, X. G. (2017). Indigenous foods and their contribution to nutrient requirements. *South African Journal of Clinical Nutrition*, 30(4): 5-7.
- Meilgaard, M., Civile, G.V. & Carr, B.T. (2007). Sensory evaluation techniques. 4th ed. Boca Raton, FL: CRC Press.
- Meldrum, G.; Padulosi, S.; Lochetti, G.; Robitaille, R.; Diulgheroff, S. (2018). Issues and Prospects for the Sustainable Use and Conservation of Cultivated Vegetable Diversity for More Nutrition-Sensitive Agriculture. *Agriculture*, 8,112.
- Menkir, A., Liu, W., White, W.S., Maziya-Dixon, B. & Rocheford, T. (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. *Food Chemistry*, 109: 521-529.
- Menssen, M., Linde, M., Omondi, E.O., Onyango, M.A., Dinssa, F.F. & Winkelmann, T. (2017). Genetic and morphological diversity of cowpea (*Vigna unguiculata* (L) Walp.) entries from East Africa. *Scientia Horticulturae*, 226: 268-276. <http://dx.doi.org/10.1016/j.scienta.2017.08.003>
- Modawi, S.Y.H. (2006). Nutritional Evaluation and Functional Properties of Flour of Pumpkin seeds. Sudan University of Science & Technology
- Mohanta, R.K. (2016). Correct Citation of AOAC (Official Methods of Analysis of AOAC International). <https://doi:10.13140/RG.2.2.20883.89125>
- Mokganya, M.G. & Tshikhawe, M.P. (2018). Medicinal uses of selected wild edible vegetables consumed by Vhavenda of the Vhembe District Municipality, South Africa. *South African Journal of Botany*, 122: 184-188. <http://doi.org/10.1016/j.sajb.2018.09.029>.
- Momanyi, D., Owino, W. & Makokha, A. (2020). Formulation, nutritional and sensory evaluation of baobab based ready-to-eat sorghum and cowpea blend snack bars. *Scientific African*, 7: 2468-2276. <https://doi.org/10.1016/j.sciaf.2019.e00215>
- Mona, M.H.M., Mohammed, A.E., Heba, I.G., Mohammad, Y.A., Nora, H.M.E., Eman, M.H. & Galila, A.H.A. (2021). Pea peels as a value-added food ingredient for snack

- crackers and dry soup. (2021). *Scientific Reports*, 11:22747. <https://doi.org/10.1038/s41598-021-02202-5>
- Monica, S.J., John, S., Saraswathi, K., Madhanagopal, R. & Arumugam, P. (2020). Evaluation of antioxidant and antidiabetic potential of crude protein extract of pumpkin seeds (*Cucurbita maxima* L.). *Asian Journal of Biological and Life Sciences*, 9(2):152-157
- Montesano, D., Blasi, F., Simonetti, M., Santini, A. & Cossignani, L. (2018). Chemical and nutritional characterization of seed oil from *Cucurbita maxima* L. (var. Berrettina) pumpkin. *Foods*, 7(3): 30. <http://dx.doi.org/10.3390/foods7030030>
- Moongngarm, A., Sasanam, S., Pinsiri, W., Inthasoi, P., Janto, S. & Pengchai, J. (2014). Functional properties of protein concentrate from black cowpea and its application. *American Journal of Applied Sciences*, 11(10):1811-1818. <http://doi:10.3844/ajassp.2014.1811.1818>
- Moreira, L.A.S., Carvalho, L.M.J., Cardoso, F.S.S.N., Ortiz, G.M.D., Finco, F.D.B.A. & Carvalho, J.L.V. (2020). Different cooking styles enhance antioxidant properties and carotenoids of biofortified pumpkin (*Curcubita moscahata* Duch) genotypes. *Food Science and Technology*, 40(2):302-306. <http://dx.doi.org/10.1590/fst.39818>
- Moreira-Araújo, R. S. D. R., Sampaio, G. R., Soares, R. a. M., Da Silva, C. P., Da Mota Araújo, M. A., & Arêas, J. a. G. (2018). Identification and quantification of phenolic compounds and antioxidant activity in cowpeas of BRS Xiquexique cultivar. *Revista Caatinga*, 31(1), 209–216. <https://doi.org/10.1590/1983-21252018v31n124rc>
- Moreno, R.B. (2015). Sensory acceptability of squash (*Cucurbita maxima*) in making ice-cream. *Asia Pacific Journal of Multidisciplinary Research*, 3(1): 18-24.
- Moutaleb, O.H., Amadou, I., Amza, T. & Zhang, M. (2017). Physico-functional and sensory properties of cowpea flour-based recipes (akara) and enriched with sweet potato. *Journal of Nutritional Health & Food Engineering*. 7(4):325-330.
- Mtolo, M., Gerrano, A. & Mellem, J. (2017). Effect of simulated gastrointestinal digestion on the phenolic compound content and in vitro antioxidant capacity of processed cowpea (*V. unguiculata*) cultivars. *CyTA – Journal of Food*, 15(3): 391–399.
- Muhanji, G., Roothaert, R.L., Webó, C. & Mwangi, S. (2011). African indigenous vegetables enterprises and market access for small-scale farmers in East Africa. *International Journal of Agricultural Sustainability*, 9(1): 194-202.
- Mungofa, N., Malongane, F. & Tabit, F.T. (2018). An exploration of the consumption, cultivation and trading of indigenous leafy vegetables in rural communities in the greater Tubatse Local Municipality, Limpopo Province, South Africa. *Journal of Consumer Sciences*, 3: 53-67.
- Naicker, N., Mathee, A. & Teare, J. (2015). Food insecurity in households in informal settlements in urban South Africa. *South African Medical Journal*, 105(4): 268-270. <https://dx.doi.org/10.7196/SAMJ.8927>.
- Naiker, T.S., Gerrano, A. & Mellem, J. (2019). Physicochemical properties of flour produced from different cowpea (*Vigna unguiculata*) cultivars of Southern African origin. *Journal of Food Science and Technology*, 56(3):1541-1550. <https://doi.org/10.1007/s13197-019-03649-1>
- National Institute of Health (NIH). Nutrient Recommendations and Databases. Available Online: <https://ods.od.nih.gov/HealthInformation/nutrientrecommendations.aspx#dv> (accessed on 27 May 2023).

- Neugart, S., Baldermann, S., Ngwene, B., Wesonga, J. & Schreiner, M. (2017). Indigenous leafy vegetables of Eastern Africa - A source of extraordinary secondary plant metabolites. *Food Research International*, 100: 411-422. <http://dx.doi.org/10.1016/j.foodres.2017.02.014>
- Ngozi, O.P., Chukwuemeka, O. & Joy, A. (2014). Proximate and organoleptic assessment of indigenous dishes based on pumpkin leaves, pulp and seeds (*Cucurbita pepo*). *Advances in Biochemistry*, 2(5): 65-70. <http://www.sciencepublishinggroup.com/j/ab>
- Nielsen, S. S. (2006). Proximate assays in food analysis. In *Encyclopedia of Analytical Chemistry*. (eds R.A. Meyers and R.J. McGorin) <https://doi.org/10.1002/9780470027318.a1024>
- Nistor, M., Pop, R., Daescu, A., Pintea, A., Socaciu, C. & Rugina, D. (2022). Anthocyanins as key phytochemicals acting for the prevention of metabolic diseases: An overview. *Molecules*, 27(13):4254. <https://doi.org/10.3390/molecules27134254>
- Njume, C., Goduka, N.I. & George, G. (2014). Indigenous leafy vegetables (imifino, morogo, muhuro) in South Africa: A rich and unexplored source of nutrients and antioxidants. *African Journal of Biotechnology*, 13(19):1933-1942. <https://doi.10.5897/AJB2013.13320>
- Noomhorm, A. (2007). Overview of dehydration method on quality of fruit and vegetables. *SWU Sci.J.* 23:9-22.
- Nuss, E.T. & Tanumihardjo, S.A. (2011). Quality protein maize for Africa: Closing the protein inadequacy gap in vulnerable populations. *Advances in Nutrition*, 2: 217-224.
- Nyadanu, D. & Lowor, S.T. (2015). Promoting competitiveness of neglected and underutilised crop species: comparative analysis of nutritional composition of indigenous and exotic leafy and fruit vegetables in Ghana. *Genetic Resources and Crop Evolution*, 62: 131-140. <https://doi.org/10.1007/s10722-014-0162-x>
- Nyam, K.L., Lau, M. & Tan, C.P. (2013). Fibre from Pumpkin (*Cucurbita pepo* L.) Seeds and Rinds: Physico-chemical Properties, Antioxidant Capacity and Application as Bakery Product Ingredients. *Malaysian Journal of Nutrition*, 19(1):99-109.
- Ogrodowska, D., Tanska, M. & Brandt, W. (2017). The influence of drying process conditions on the physical properties, bioactive compounds and stability of encapsulated pumpkin seed oil. *Food Bioprocess Technology*, 10: 1265-1280. <http://dx.doi.org/10.1007/511947-017-1898-z>.
- Ogunwole, O.A. (2021). Production of biodiesel from watermelon (*Citrullus lanatus*) seed oil. *Leonardo Journal of Sciences*, 27: 63-74. <http://IJs.academicdirect.org/A27/063-074.pdf>
- Okonya, J.S. & Maas., B.L. (2014). Protein and iron composition of cowpea leaves. An evaluation of six cowpea varieties grown in Eastern Africa. *African Journal of Food, Agriculture, Nutrition and Development*, 14(5): 2129-2140.
- Oliveira, S.M., Brandao, T.R.S. & Silva, C.L.M. (2016). Influence of drying processes and pretreatments on nutritional bioactive characteristics of dried vegetables: A review. *Food Engineering Review*, 8: 134-163. <http://dx.doi.org/10.1007/s12393-015-912-0>.
- Olubi, O., Minnaar, J.V.F. & Jideani, V.A. (2021). Physicochemical, mineral and sensory characteristics of instant *Citrullus lanatus mucospermus* (Egusi) soup. *Foods*, 10(8): 1817. <http://doi.org/10.3390/foods10081817>.

- Ong, H.G. & Kim, Y.D. (2017). The role of wild edible plants in household food security among transitioning hunter-gatherers: evidence from the Philippines. *Food Security*, 9: 11-24. <http://doi.org/10.1007/s12571-016-1630-6>
- Oppong, D., Arthur, E., Kwadwo, S. O., Badu, E. & Patrick, S. (2015). Proximate composition & some functional properties of soft wheat flour. *International Journal of Innovative Research in Science, Engineering and Technology*, 4(2): 753-758. <https://doi.org/10.15680/IJIRSET.2015.0402097>
- Oroian, M. & Escriche, I. (2015) Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International*, 74 (2):10–36.
- Oulai, P., Lessoy, Z., Megnanou, R.M., Doue, R. & Niamke, S. (2014). Proximate composition and nutritive value of leafy vegetables consumed in Northern Cote D’ivoire. *European Scientific Journal*, 10(6): 1857-7881.
- Pala, R., Beyaz, F., Tuzcu, M., Er, B., Sahin, N., Cinar, V. & Sahin, K. (2018). The effects of coenzyme Q10 on oxidative stress and heat shock proteins in rats subjected to acute and chronic exercise. *J. Exerc. Nutr. Biochem.* 22(6):14–20.
- Pamela, C.C., Richard, A.H. & Denise, R.F. (2005). Lippincotts Illustrated Reviews Biochemistry 3<sup>rd</sup>edi, Lippincott Williams and Wilkins, Philadelphia, 335-388.
- Peiretti, P. G., Meineri, G., Gai, F., Longato, E., & Amarowicz, R. (2017). Antioxidative activities and phenolic compounds of pumpkin (*Cucurbita pepo*) seeds and amaranth (*Amaranthus caudatus*) grain extracts. *Natural Product Research*, 31(18), 2178–2182. <https://doi.org/10.1080/14786419.2017.1278597>
- Pereira, A.M., Krumreich, F.D., Ramos, A.H., Krolow, A.C.R., Santos, R.B. & Gularte, M.A. (2020). Physicochemical characterization, carotenoid content and protein digestibility of pumpkin access flours for food application. *Food Science and Technology (Campinas)*, 40(4). <https://doi.org/10.1590/fst.38819>
- Peričin, D., Krimer, V., Trivić, S., & Radulović, L. (2009). The distribution of phenolic acids in pumpkin’s hull-less seed, skin, oil cake meal, dehulled kernel and hull. *Food Chemistry*, 113(2), 450–456. <https://doi.org/10.1016/j.foodchem.2008.07.079>
- Poliszko, N., Kowalczewski, P.L., Rybicka, I., Kubiak, P. & Poliszko, S. (2019). The effect of pumpkin flour on quality and acoustic properties of extruded corn snacks. *Journal of Consumer Protection and Food Safety*. 14:121-129. <https://doi.org/10.1007/s00003-019-01216-6>
- Pongjanta, J., Naulbunrang, A., Kawngdang, S., Manon, T. & Thepjaikat, T. (2006). Utilisation of pumpkin powder in bakery products. *Journal of Science & Technology*, 28(1): 71-79.
- Pradeepkumar, T., Indira, V. & Sankar, M. (2015). Nutritional evaluation of wild leafy vegetables consumed by tribals in the Wayanad District of Kerala. *Proceedings of the National Academy of Sciences of India, Sec.B, Biological Science*. 85(1): 93-99. <http://www.10.1007/s40011-013-0271-x>
- Rabrenovic, B.B., Dimic, E.B., Novakovic, M.M., Tessevic, V.V. & Basic, Z.N. (2014). The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L) seeds. *LWT-Food Science and Technology* 55:521-527.
- Rai, K.N, Govindaraj, M. & Rao, A.S. (2012). Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance and Safety of Crops & Food*, 4(3): 119-125.

- Rajurkar, N.S. & Hande, S.M. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian J Pharm Sci*, 73(2):146-151. <http://www.10.4103/0250-474x91574>
- Ranganna. S. (1986). Handbook of Analysis and Quality Control for Fruit and Vegetable Products, 2 ed, Tata McGraw Hill Publication Company, New Delhi. ISBN 9780074518519.
- Rangel, A., Saraiva, K. P., Schwengber, P., Narciso, M. S., Domont, G. B., Ferreira, S. T., & Pedrosa, C. (2004). Biological evaluation of a protein isolates from cowpea (*Vigna unguiculata*) seeds. *Food Chemistry*, 87(4), 491–499. <https://doi.org/10.1016/j.foodchem.2003.12.023>
- Ranum, P.; Peña-Rosas, J. P. & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, 1312(1):105–112. <http://doi: 10.1111/nyas.12396>.
- Rezig, L., Riaublanc, A., Chouaibi, M., Gueguen, J. & Hamdi, S. (2016). Functional properties of protein fractions obtained from pumpkin (*Cucurbita maxima*) seed. *International Journal of Food Properties*, 19(1): 172-186. <http://doi:10.1080/10942912.2015.1020433>
- Roznowska, I.P. (2017). Physicochemical properties of starches isolated from pumpkin compared with potato and corn starches. *International Journal of Biological Macromolecules*, 101: 536-542. <http://dx.doi.org/10.1016/j.ijbiomac.2017.03.092>.
- Różyło, R., Gawlik-Dziki U., Dziki D., Jakubczyk A., Karaś M. & Różyło, K. (2014). Wheat bread with pumpkin (*Cucurbita maxima* L.) pulp as a functional food product. *Food Technology and Biotechnology*, 52: 430–438. <http://dx.doi.10.17113/ftb.52.04.14.3587>.
- Rueda, A., Seiquer, I., Olalla, M., Gimenez, R., Lara, L. & Cabrera-Vique, C. (2014). Characterization of fatty acid profile of argan oil and other edible vegetable oils by gas chromatography and discriminant analysis. *Journal of Chemistry*, 2014: 843-908. Available at <https://dx.doi.org/10.1155/2014/843908>.
- Saarvedra, M. J., Aires, A., Dias, C., Almeida, J.A., De Vasconcelos, M. C. B. M., Santos, P. & Rosa, E.A. (2015). Evaluation of the potential of squash pumpkin by-products (seeds and shell) as sources of antioxidant and bioactive compounds. *Journal of Food Science Technology*, 52(2): 1008–1015. <https://doi: 10.1007/s13197-013-1089-5>
- Saeleaw, M. & Schleining, G. (2011). Composition, physico-chemical and morphological characterization of pumpkin flour. In *Proceedings of the 11<sup>th</sup> International Congress on Engineering and Food “Food Process Engineering in a Changing World” (ICEF11)*. Athens: National Technical University.
- Santamaria, M., Garzon, R., Moreira, R. & Rossel, C.M. (2021). Estimation of viscosity and hydrolysis kinetics of corn starch gels based on microstructural features using a simplified model. *Carbohydrate Polymers*, 273. <https://doi.org/10.1016/j.carbpol.2021.118549>.
- Santos, A., Ceccon, G., Rodrigues, E. V. & Teodoro, P.E. (2016). Adaptability and stability of cowpea genotypes to Brazilian Midwest. *African Journal of Agriculture and Research*, 10: 3901-3908. <http://dx.doi.org/10.5897/AJAR2015.10165>
- Santos, R. A., Filho, A. S., Filho, A. J. C., Guilhon, G. M. S. P., & Santos, L. S. (2021). Analysis of phenolic compounds from cowpea (*Vigna unguiculata*) by HPLC-DAD-MS/MS. *Brazilian Journal of Food Technology*, 24. <https://doi.org/10.1590/1981-6723.07720>

- Sapirstein, H., Wu, Y., Filiz, K. & Graf, R. (2018). A study of factors influencing the water absorption capacity of Canadian hard red winter wheat flour. *Journal of Cereal Science*, 81:52-59. <https://doi.org/10.1016/j.jcs.2018.01.012>.
- Sathiya Mala, K., Aathira, P., Srinivasulu, E.K. & Sulochanamma, G. (2018). Effect of pumpkin powder incorporation on the physio-chemical. Sensory and nutritional characteristics of wheat flour muffins. *International Food Research Journal*, 25(3):1081-1087. <http://www.ifrj.upm.edu.my>.
- Schonfeldt, H. C., Gibson, N. & Vermeulen, H. (2010). The possible impact of inflation on nutritionally vulnerable households in a developing country using South Africa as a case study. *Nutrition Bulletin*, 35(3): 254-267 <https://doi.org/10.1111/j.1467-3010.2010.01837.x>
- Schonfeldt, H.C. & Pretorius., B. (2011). The nutrient content of five traditional South African dark green leafy vegetables – A preliminary study. *Journal of Food Consumption and Analysis*, 24(8): 1141-1146.
- Schreinemachers, P., Simmons, E.B. & Wopereis, M.C.S. (2018). Tapping the economic and nutritional power of vegetables. *Global Food Security*, 16: 36-45. <http://dx.doi.org/10.1016/j.gfs.2017.09.005>
- Scieber, A., Stintzing, F.C. & Carle, R. (2001). By-products of plant food processing as a source of functional compounds-recent developments. *Trends Food Science Technology*, 12:401-405.
- See, E.F., Nadiyah, W.A. & Aziah, A.A. (2007). Physico-chemical and sensory evaluation of breads supplemented with pumpkin flour. *ASEAN Food Journal*, 42(2):123-130
- Senekal, M., Nel, J.H., Malczyk, S., Drummond, L., Harbron, J. & Steyn, N.P. (2019). Provincial dietary intakes study (PDIS) prevalence and sociodemographic determinants of the double burden of malnutrition in a representative sample of 1 to under 10-year-old children from two urbanised and economically active provinces in South Africa. *International Journal of Environmental Research and Public Health*, 16(18): 3334. <http://doi.10.3390/ijerph16183334>
- Setiawan, B., Aulia, S.S., Sinaga, T. & Sulaeman, A. (2021). Nutritional content and characteristics of pumpkin cream soup with tempeh addition as supplementary food for elderly. *International Journal of Food Science*, 2021:1-8. <https://doi.org/10.1155/2021/6976357>
- Severini, C., Baiano, A., Pilli, T.D., Carbone, B.F. & Derossi, A. (2005). Combined treatments of blanching and dehydration: study on potato cubes. *Journal of Food Engineering*, 68(3): 289–296.
- Seymen, M., Uslu, N., Turkmen, O., Juhalm, F.A. & Ozcan, M.M. (2016). Chemical composition and mineral contents of some hull-less pumpkin seed and oils. *Journal of the American Oil Chemist's Society*, 93: 1095-1099. <http://dx.doi.org/10.1007/s11746-016-2850-5>.
- Shaheen, S., Ahmad, M. & Haroon, N. (2017). Food security: A global problem. In: *Edible Wild Plants: An Alternative Approach to Food Security*, Springer, Cham. 41-57. <https://doi.org/10.1007/978-3-319-63037-3-2>
- Shalaby, E.A. & Shanab, S. M. M. S. (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Indian Journal of Geo-Marine Sciences*, 42(5):556-564.

- Sharif, M.K., Butt, M.S., Sharif, H.R. & Nasir, M. (2017). Sensory evaluation and consumer acceptability. In: *Handbook of Food Science and Technology*, John Wiley and Sons. 10, 362-386.
- Sharma, G. & Lakhawat, S. (2017). Development, Quality Evaluation and Acceptability of Pumpkin Seed Flour Incorporated in Gravy. *J Nut Food Sci*, 7(613):1-4 <http://doi:10.4172/2155-9600.1000613>
- Sharma, K.D., Karki, S., Thakur, N.S. & Surekha, A. (2012). Chemical composition, functional properties and processing of carrot-a review. *Journal of Food Science and Technology*, 49(1): 22-32. <http://doi.10.1007/s13197-011-0310-7>
- Sharma, P., Kaur, G., Kehinde, B. A., Chhikara, N., Panghal, A., & Kaur, H. (2020). Pharmacological and biomedical uses of extracts of pumpkin and its relatives and applications in the food industry: a review. *International Journal of Vegetable Science*, 26(1):79-95.
- Shephard, G.S., Leggott, N.L., Stockenström, S., Somdyala, N.I. & Marasas, W.F. (2002). Preparation of South African maize porridge: effect on fumonisin mycotoxin levels. *South African Journal of Science*, 98: 393-396.
- Shevkani, K., Kaur, A., Kuma, S. & Singh, N. (2015). Cowpea protein isolates: Functional properties and application in gluten free rice muffins. *Journal of Food Science and Technology*, 63:927-933. <http://dx.doi.org/10.1016/j.lwt.2015.04.58>
- Singh, N.J. & Pandey, R.K. (2011). Rehydration characteristics and structural changes of sweet potato cubes after dehydration. *American Journal of Food Technology*, 6(8):709-716. <http://doi:10.3923/ajft.2011.709.716>
- Singh, S., Singh, D., Singh, L.B., Chand, S. & Roy, D. (2013). Indigenous vegetables for food and nutritional security in Andaman and Nicobar Islands, India. *International Journal of Agriculture and Food Science Technology*, 4(5): 503-512. <http://www.ripublication.com/ijafst.htm>
- Skandrani, I., Limem, I., Neffati, A., Boubaker, J., Sghaier, M. B., Bhourri, W. & Chekir-Ghedira, L. (2010). Assessment of phenolic content, free-radical-scavenging capacity genotoxic and anti-genotoxic effect of aqueous extract prepared from *Moricandia arvensis* leaves. *Food and chemical toxicology*, 48(2), 710-715.
- Sombie, P.A.E.D., Compaore, M., Coulibaly, A.Y., Ouedraogo, J.T., Tignegre, J.D. & Kiendrebeogo, M. (2018). Antioxidant and Phytochemicals Studies of 31 Cowpeas (*Vigna unguiculata* (L. Walp.)) Genotypes from Burkina Faso. *Foods*. 7(143)1-9 <https://doi:10.3390/foods7090143>
- Song, J., Wang, X., Li, D., Meng, L. & Liu, C. (2017). Degradation of carotenoids in pumpkin (*Cucurbita maxima* L.) slices as influenced by microwave vacuum drying, *International Journal of Food Properties*, 20(7): 1479-1487. <http://dox.doi.10.1080/10942912.2016.1212875>
- Sopan, B.A., Vasantrao, D.N. & Ajit, S.B. (2014). Total phenolic content and antioxidant potential of *Cucurbita maxima* (Pumpkin) powder. *International Journal of Pharmaceutical Sciences and Research*, 5(5): 1903-1907. <https://dx.doi.org/10.13040/IJPSR.0975-8232>
- Statistics South Africa (STATS SA), (2022). Inflation softens. Available at: <https://www.statssa.gov.za> (accessed on 17 July 2023).



- Stone, H. & Sidel, J.L. (1993). *Sensory Evaluation Practices*, 2nd ed. Academic Press Inc., San Diego, CA
- Stone, H. & Sidel, J.L. (2004). *Sensory Evaluation Practices*. 3<sup>rd</sup> ed. California: Elsevier Academic Press, pp. 247-277.
- Sulaiman, C. T., & Balachandran, I. (2012). Total phenolics and total flavonoids in selected Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences*, 74(3): 258–260. <https://doi.org/10.4103/0250-474X.106069>
- Surech, C. & Samsher, S. (2013). Assessment of functional properties of different flours. *African Journal of Agricultural Research*, 8(38); 4849-4852. <http://doi:10.5897/AJAR2013.6905>.
- Świątkiewicz, M. & Bojanowski, J. (2004). Efficiency of ensilaged high-lysine maize grain in pig fattening. *Journal of Animal Feed Science*, 13(2): 83-86.
- Timko, M. P., Ehlers, J. D. & Roberts, P. A. (2007). Cowpea. In pulses, sugar and tuber crops, Berlin Heidelberg, *Springer Berlin Heidelberg* (pp. 49–67). [https://doi.org/10.1007/978-3-540-34516-9\\_3](https://doi.org/10.1007/978-3-540-34516-9_3)
- Tortoe, C., Akonor, P.T., Nketia, M.O., Amengor, M.G., Hagan, L. & Padi, A. (2014). Assessing the sensory characteristics and consumer preferences of yam-cowpea-soybean porridge in the Accra Metropolitan Area. *International Journal of Nutrition and Food Sciences*, 3(2): 127-132.
- Uarrota, V.G., Bairros, A.F.M., Gindri, D., Leolato, L.S., Andrade, G.C., Nerling, D., Stefen, D., Arijama, M., Razao, E. H. & Rocha, M. (2019). From neglected and underutilised crops to powerful sources of Vitamin A. Three case studies of Mozambican cultivated *Tacca leontopetaloides*, cowpea and cassava. <https://doi.org/10.5772/intechopen.84829>
- Uduwerella, H.M.I.A., Arampath, P.C. & Mudannayake, D.C. (2021). Physicochemical and Functional Properties of pumpkin seed flour of *Curcubita maxima* and *Cucurbita moschata* species. *Tropical Research*, 32(2):201-211. <http://doi.org/10.4038/tar.v32i2.8467>
- UNICEF. (2020). What you need to know about malnutrition & feeding children. Available at: <https://www.unicef.org/southafrica/stories/what-you-need-know-about-malnutrition-feeding-children> (accessed on 19 August 2023).
- United States Department of Agriculture (USDA). (2011). National nutrient database for standard reference. Plants Profile. *Senna occidentalis* (L). *The plant database*. Available at: <http://plants.usda.gov/java/profile?symbol=SEOC2> (accessed on 9 June 2023).
- United States Department of Agriculture (USDA). (2019). Agricultural Research Service. *Food Data Central*. Available at: <https://fdc.nal.usda.gov/fdc-app.html/food-details/170188/nutrients> (accessed on 16 July 2023).
- United States Food and Drug Administration (US FDA) (2020). Daily value on the new nutrition and supplement facts labels. The new nutrition facts label. Available at: <https://www.FDA.gov/> (accessed on 02 May 2023).
- USDA. (2021). United States Department of Health and Human Services and U.S. Department of Agriculture. 2015 –2020 *Dietary Guidelines for Americans*. 8th Edition. Available at: <https://health.gov/our-work/nutrition-physical-activity/dietary-guidelines/previous-dietary-guidelines/2015> (accessed on 10 March 2023).

- Uusiku, N.P., Oelofse, A. & Duodu, K.G. (2010). Nutritional value of leaf vegetables for Sub-Saharan Africa and their potential contribution to human health. A review. *Journal of Food Composition and Analysis*, 23: 499-509.
- Van Canneyt, P. & Verdonck, P. (2014). Physical medicine and rehabilitation. In *Comprehensive Biomedical Physics*.
- Van Rensburg, W.S., Averbek, W., Slabbert, R., Faber, M., Van Jaarsveld, P., Van Heerden, I., Wenhold, F. & Oelofse, A. (2007). African Leafy Vegetables in South Africa. *Water SA*, 33(3): 317-326.
- Verma, A., Kushwaha, A., Kumar, A. & Baghel, S.S. (2019). Physico-chemical properties and nutritional composition of improved varieties of grain cowpea grown in Pantnagar. International Conference on Food Security through Agriculture & Allied Sciences. *Journal of Pharmacognosy and Phytochemistry*, 5: 334-338
- Verma, M., Sharma, A., Naidu, S., Bhadra, A.K., Kukreti, R. & Taneja, V. (2012). Curcumin prevents formation of polyglutamine aggregates by inhibiting Vps36, a component of the ESCRT-II complex. *PLoS ONE*. 7(8):e42923 <https://doi.org/10.1371/journal.pone.0042923>
- Vilakati, N., Taylor, J.R.N., MacIntyre, U. & Kruger, J. (2016). Effects of processing and addition of a cowpea leaf relish on the iron and zinc nutritive value of a ready-to-eat sorghum-cowpea porridge aimed at young children. *Food Science and Technology*, 73: 467-472. <http://dx.doi.org/10.1016/j.lwt.2016.06.022>
- Vorster, I.H.J., Van Willem, R.J. & Sonja, L. (2007). The importance of traditional leafy vegetables in South Africa. *African Journal of Food, Agriculture, Nutrition and Development*, 7(4): 1-13.
- Vu, D.T. & Nguyen, A.T. (2017) The neglected and underutilized species in the Northern mountainous provinces of Vietnam. *Genetic Resources and Crop Evolution*, 64, 1115-1124. <https://doi.org/10.1007/s10722-017-0517-1>
- Wafula, E.N., Franz, C.M.A.P., Rohn, S., Huch, M., Mathara, J.M. & Trierweiler, B. (2016). Fermentation of African indigenous leafy vegetables to lower post-harvest losses, maintain quality and increase product safety. *African Journal of Horticultural Science*, 9: 1-13.
- Wamalwa, E.N., Muoma, J. & Wekesa, C. (2016). Genetic diversity of Cowpea (*Vigna unguiculata* (L.) Walp). Accession in Kenya Gene Bank based on simple sequence repeat markers. *International Journal of Genomics*, 1-15. [http://doi: 10.1155/2016/8956412](http://doi:10.1155/2016/8956412)
- Wang, S.M., Yu, D.J. & Song, K.B. (2011). Physicochemical property of pumpkin slices dehydrated with red algae extract. *J Korean Soc. Appl. Biol. Chem*, 54(6):921-925. <http://dx.doi.org/10.389/jksabc.2011.139>.
- Ware, M. (2018). What are the health benefits of pumpkins? *Medical New Today*. <https://www.medicalnewtoday.com/articles/279610.php> (accessed on 5 January 2018)
- Waterman, P.G. & Mole, S. (1994). In: Analysis of Phenolic Plant Metabolites. London: Blackwell Scientific Publications. 1-20. <https://www.semanticscholar.org/paper/Analysis-of-Phenolic-Plant-Metabolites-Waterman-Mole/72b6339e6af0c9eb98fb5edcc7ad3dd4bff1d722>
- Weinmann, H. (1947). Determination of total available carbohydrates in plants. *Plant Physiology*, 22(3): 279-290. <https://doi.org/10.1104/pp.22.3.279>

- Wendin, K., Ekman, S. Bulow, M., Ekberg, O, Johansson, D., Rothenberg, E. & Stadin, M. (2010). Objective and quantitative definitions of modified food textures based on sensory and rheological methodology. *Food and Nutrition Research*, 54:5134. <https://doi.10.1016/s978-1-78242-334-8-00007-9>
- Wilhem, L.R., Luther, R., Dwayne, A.S. & Brusewitz, G.H. (2004). Physical properties of food materials. In *Food and Process Engineering Technology*, Chapter 2, 23-52. St Joseph, Michigan.
- Wootta, S. & Beckjr, G.R. (2013) Dental and skeletal applications of silica-based nano materials. In *Nanobiomaterials in Clinical Dentistry*.
- World Health Organisation (WHO). (2015). Promoting fruit and vegetable consumption around the world. World Health Organisation. Geneva, Switzerland. Available at: <http://www.who.int/dietphysicalactivity/fruit/en/index2.html> (accessed on 04 April 2023).
- World Health Organisation (WHO). (2020). Malnutrition. World Health Organisation. Geneva. Available at: <https://www.who.int/news-room/questions-and-answers/item/malnutrition> (accessed on 11 January 2023).
- World Health Organisation (WHO). (2020). The state of food security and nutrition in the world 2020. Transforming food systems for affordable healthy diets. Rome, FAO. <https://doi.org/10.4060/ca9692en>
- Yadav, N., Kau, D., Malaviya, R., Singh, M., Fatima, M. & Singh, L. (2018). Thermal and non-thermal processing on antioxidant potential of cowpea seeds. *International Journal of Food Properties*, 21(1): 21-35. <https://doi.org/10.1080/10942912.2018.1431659>
- Yamada, T., Matsuo, K., Izawa, M., Yamada, S., Masuda, Y., & Ogasawara, T. (2017). Effects of age and viscosity on food transport and breathing-swallowing coordination during eating of two-phase food in nursing home residents. *Geriatrics & Gerontology International*, 17(11): 2171–2177. <https://doi.org/10.1111/ggi.13056>
- Yang, J., & Lee, J. (2019). Application of sensory descriptive analysis and consumer studies to investigate traditional and authentic foods: A Review. *Foods*, 8(2), 54. <https://doi.org/10.3390/foods8020054>
- Yesilgurt, V., Halfon, B., Ozturk, M. & Topcu, G. (2008). Antioxidant potential and phenolic constituents of *Salvia cedronella*. *Food Chemistry*. 108(1): 31-39
- Zayas, J.F. (1997). Solubility of Proteins. In *Functionality of proteins in food*. Springer, Berlin, Heidelberg. <https://doi.org/10.1007/978-3-642-59116-7-2>
- Zhang, L., Chai, Z., Zhang, Y. & Wang, Y. (2016). Ethnobotanical study of traditional edible plants used by the Naxi people during droughts. *Journal of Ethnobiology and Ethnomedicine* 12: 39. <http://dx.doi.org/10.1186/s13002-016-0113-z>
- Zhao, Y., Du, S., Wang, H. & Cai, M. (2014). In vitro antioxidant activity of extracts from common legumes. *Food Chem* (152):462–466
- Zhou, C.L., Liu, W., Zhao, J., Yuan, C., Song, Y., Chen, D., Ni, Y.Y. & Li, Q.H. (2013). The effect of high hydrostatic pressure on the microbiological quality and physical-chemical characteristics of pumpkin (*Cucurbita maxima* Duch.) during refrigerated storage. *Innovative Food Science and Emerging Technology*, 21:24-34. <https://doi.org/10.1016/j.ifset.2013.11.002>

- Zhu, Y., Hsu, W. H., & Hollis, J. H. (2013). The impact of food viscosity on eating rate, subjective appetite, glycemicroresponse and gastric emptying rate. *PloS One*, 8(6), e67482. <https://doi.org/10.1371/journal.pone.0067482>
- Zia-UI-Haq, M., Ahmad, S., Amarowicz, R. & De Feo, V. (2013). Antioxidant activity of the extracts of some cowpea (*Vigna unguiculata* (L) Walp.) cultivars commonly consumed in Pakistan. *Molecules*, 18(2): 2005-2017. <https://doi.org/10.3390/molecules18022005>
- Zlabur, J. Š., Dobričević, N., Pliestic, S., Galic, A., Bilic, D. P., & Voca, S. (2017). Antioxidant potential of fruit juice with added chokeberry powder (*Aronia melanocarpa*). *Molecules*. 22(12):2158 <http://doi.org/10.3390/molecules22122158>

## APPENDICES

### Appendix 1: Ethical clearance



#### CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 26/04/2019

Dear Ms Mungofa

NHREC Registration # : REC-170616-051  
REC Reference # : 2019/CAES/076  
Name : Ms N Mungofa  
Student # : 44581998

**Decision: Ethics Approval from  
01/05/2019 to 30/04/2020**

**Researcher(s):** Ms N Mungofa  
[44581998@mylife.unisa.ac.za](mailto:44581998@mylife.unisa.ac.za)

**Supervisor (s):** Dr D Beswa  
[beswad@unisa.ac.za](mailto:beswad@unisa.ac.za); 011-471-2274

Dr JJ Sibanyoni  
[sibanjj@unisa.ac.za](mailto:sibanjj@unisa.ac.za); 011/471-2796

#### **Working title of research:**

The potential of *Vigna unguiculata* and *Cucurbita maxima* as complementary food ingredients

**Qualification:** PhD Consumer Science

Thank you for the application for research ethics clearance by the CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for a one-year period, **subject to the clarification of the sensory testing venue**. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

**Due date for progress report: 30 April 2020**

*Please note the points below for further action:*

1. The committee advises against using a boardroom for the sensory testing. The researchers cannot control all the variables in such an open environment and this may lead to unreliable results. For instance, odour cannot be tested properly in such an open environment, and the lighting could affect colour and visual impressions. Furthermore, tasters should have the option of spitting out the substances they are



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tasting. How will this be accommodated in the boardroom? The tasters may feel self-conscious to do so where others can see or hear them do it. The Unisa sensory laboratory has seven booths set up for this purpose, and the researchers are advised to make use of this facility to ensure that the data gathered is reliable.

2. The researcher indicates that there will a control soup; what will this soup be? Will it be the same base soup without the added complementary ingredients? If this is the case, what will happen if the tasters dislike the base soup? Will this not skew the results as they will most likely not like the soup with the complementary ingredients either?

*The low risk application was reviewed by the CAES Health Research Ethics Committee on 25 April 2019 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:

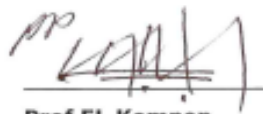
1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. 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.
5. 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.
6. 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.

7. 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 2019/CAES/076 should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,



**Prof EL Kempen**

**Chair of CAES Health REC**

E-mail: kempeel@unisa.ac.za

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**Prof MJ Linington**

**Executive Dean : CAES**

E-mail: lininmj@unisa.ac.za

Tel: (011) 471-3806

## Appendix 2: Permission Letter



Department of Life and Consumer Sciences  
University of South Africa  
Private Bag X6  
Florida, 1710

3 September 2021

Mr NW Phala  
Municipal Manager  
Greater Tubatse Municipality

Dear Madam

### **PERMISSION TO CONDUCT A RESEACH IN THE GREATER TUBATSE LOCAL MUNICIPALITY**

My name is Nyarai Mungofa and I am a PhD student in the Department of Life and Consumer Science at the University of South Africa. The title of my research is “The Potential of using vigna unguiculata and cucurbita maxima as complementary food ingredients”.

I hereby seek your consent to conduct a research in Greater Tubatse Local Municipality. The research study involves consumer acceptability of prepared soup formulations from cowpea and pumpkin. For further reference, I have provided you with a copy of my research proposal which includes a consent form and questionnaire to be used in the research process as well as a copy of the approval letter from the Ethics Committee of UNISA.

For more details on this project, you can contact my supervisor Dr D Beswa via Tel: 011 559 6000 and email: [beswad@uj.ac.za](mailto:beswad@uj.ac.za).

Your consideration in this regard will be greatly appreciated.

Yours faithfully

.....

Nyarai Mungofa (Researcher)



### Appendix 3: CONSENT FORM

**Title of research project:** The potential of *Vigna unguiculata* and *Cucurbita maxima* as complementary food ingredients

Dear Mr/Mrs/Miss/Ms \_\_\_\_\_

Date...../...../20.....

#### NATURE AND PURPOSE OF THE STUDY

Thank you for agreeing to participate in this important research study aimed at investigating the potential of using cowpea (*Vigna unguiculata*) and pumpkin (*Cucurbita maxima*) as complementary food ingredients.

The findings of this study will encourage utilization of locally available crops as strategies towards improving livelihood of consumers in low-income populations.

#### RESEARCH PROCESS

Three soup formulations and a control will be developed. Experiments will be done to determine the physical properties, functional properties, nutritional composition, total phenolic content and antioxidant potential of cowpea and pumpkin. A panel of untrained consumers of soup will then be recruited to rate the hot soup samples in terms of visual appearance, odour, texture, taste and overall acceptability using a 9-point Hedonic scale. Data will then be analysed.

#### 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 thesis 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

The findings of this investigation will unpack the potential of cowpea and pumpkin in addressing malnutrition and promoting food security in rural households. The findings will also raise awareness and understanding of the nutritional value and medicinal benefits of cowpea and pumpkin and their significant role in reducing micronutrient deficiency thereby changing people's attitude towards these vegetables. Appropriate recommendations will be made to the relevant authorities for possible intervention.

## INFORMATION (contact information of your supervisor)

If there is any question concerning this study contact Dr D Beswa, 011 471 2274, Department of Life and Consumer Sciences, UNISA.

## 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 of the university against any liability that I may incur during the course of the project.

I further undertake to make no claim against the university in respect of damages to my person or reputation that may be incurred as a result 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. ....

Will you need feedback on the outcome of this study? If so, please provide your email address:

.....  
.....  
.....

## Appendix 4: Questionnaire

### Consumer acceptability test: Soup

Gender:.....

Date:.....

Age group:.....

Sample number:.....

Panelist number:.....

Please indicate with a X how much you like or dislike the sample. Open container and smell sample before tasting.

#### Appearance

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

#### Aroma

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

#### Taste

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

#### Mouthfeel/texture

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

#### Overall acceptability

9	8	7	6	5	4	3	2	1
Like extremely	Like very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely



FETAKGOMO TUBATSE  
LOCAL MUNICIPALITY

Enquiries: K Shongwe (013)231-1090

05 August 2021

University of South Africa  
Department of Life and Consumer Sciences  
Private Bag X6  
FLORIDA  
1710

Attention: Ms N Mungofa

Dear Sir/Madam

**RE: REQUEST FOR PERMISSION TO CONDUCT AN ACADEMIC STUDY AT FETAKGOMO TUBATSE LOCAL MUNICIPALITY: "THE POTENTIAL OF USING VIGNA UNGUICULATA AND CUCURBITA MAXIMA AS COMPLEMENTARY FOOD INGREDIENTS"**

The above matter bears reference

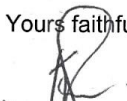
The Municipality hereby acknowledges the letter dated 03 September 2021, for the request to conduct a research study for the purposes of education fulfillment for Ms. N Mungofa.

The Municipality grants Ms. N Mungofa the permission to conduct her research study within Fetakgomo Tubatse Local Municipality. The municipal contact person to assist is Director: Local Economic Development and Tourism, Ms. Katleho Shongwe.

The Municipality requests a copy of the research paper upon completion as the research topic is within the jurisdiction area of Fetakgomo Tubatse Local Municipality.

Hoping that the information contained in this letter is sufficient.

Yours faithfully

  
\_\_\_\_\_  
NWAPHALA  
Municipal Manager

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