CHARACTERISATION AND ENCAPSULATION OF *MORINGA oleifera* EXTRACTS

by

Toyosi Timilehin George

Thesis submitted in fulfilment of the requirements for the degree

Master of Science and Technology: Food Science and Technology

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

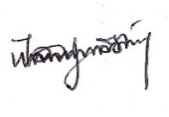
Supervisor: **Dr Anthony O. Obilana**
Co-supervisors: **Dr Ayodeji B. Oyenihi**
Mr Stefanus Rautenbach

Bellville Campus,

July 2021

DECLARATION

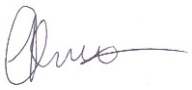
I, **Toyosi Timilehin George**, declare that the content of this thesis represent my own original work, and that the thesis has not previously been submitted for academic examination toward any qualification. Furthermore, it represents my own opinion and not necessarily those of the Cape Peninsula University of Technology.



20th July, 2021

…….. ……

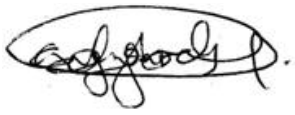
Toyosi Timilehin George Date



21st July 2021

……..... ……..

Dr Anthony O. Obilana (Supervisor) Date



21st July 2021

…… ……

Dr Ayodeji B. Oyenihi (Co-supervisor) Date

21 July 2021

…… ……

Mr Stefanus Rautenbach (Co-supervisor) Date

ABSTRACT

Moringa oleifera Lam. (Family: Moringaceae) has been well-documented for the high presence of bioactive and phytochemical compounds with health-promoting potential. However, these phytochemicals are susceptible to damage during processing and storage. The use of encapsulation to improve the functionality and stability of phytochemicals has been established as a viable way of protecting them. In this study, microcapsules containing *M. oleifera* extracts were developed using maltodextrin (MD) and Gum Arabic (GA) coating materials, individually and as a combination (MDGA). Bioactive compounds in *M. oleifera* leaf powder (MoLP) and seed powder (MoSP) were extracted using 60% ethanol (EtOH), acidified methanol (Ac. MeOH) and water (H_2O) and their phytochemical compositions characterized by spectrophotometry and liquid chromatography-mass spectrometry (LC-MS). The antioxidant capacities were evaluated using the oxygen radical antioxidant capacity (ORAC), ferric reducing antioxidant power (FRAP) and 2-diphenyl-1-picrylhydrazyl (DPPH) assays. Furthermore, to determine the optimum microcapsule preparation conditions for maximum encapsulation efficiency, the coating material, core/coating ratio, as well as ultrasonication time, were varied and their efficiencies determined by response surface methodology (RSM). The newly developed microcapsules from MD, GA and MDGA were analysed using scanning electron microscopy for morphological properties; thermogravimetric analysis (TGA) for thermal properties; X-ray diffraction for crystallinity patterns; and Fourier transform infrared (FTIR) spectroscopy for the structural composition of the microcapsules. Additionally, the physical and functional properties of the microcapsules were measured to determine the effect coating materials had on production.

The EtOH MoLP extract had the most phenolic (24.0 ± 0.4 mg GAE/g) and flavonoid (14.1 ± 0.2 mg QE/g) contents with the former showing a strong positive correlation with the antioxidant (ORAC) value. The LC-MS profiling of extracts also revealed the presence of many bioactive compounds such as neochlorogenic acid, 3-p-coumarylquinic acid, rutin, quercetin 3-galactoside, kaempferol O-rutinoside, malic acid, citric acid, etc. and these were more abundant in the EtOH MoLP, hence, was chosen for encapsulation. Based on RSM, the optimum preparation conditions were found to be 7.5: 2.5 for MD/GA coating material; 1: 8.5 for core/coating ratio; and the ultrasonication time of 13.3 minutes which gave an expected encapsulation efficiency (EE) of 89.9%. This was then validated, resulting in an EE value of 84.9%. Microcapsules of EtOH MoLP were thus developed based on these conditions using different carriers; the obtained EE differed significantly ($p < 0.05$) ranging from 72.9% (GA) to

85.7% (MDGA). The type of coating material used also significantly (p < 0.05) impacted the physical properties of the microcapsules. The bulk and tap density of the microcapsules ranged from 0.177 to 0.325 g/ml and 0.126 to 0.295 g/ml respectively while the moisture content ranged from 1.5 to 1.8%. All microcapsules had water solubility capacity between 86.4% for GA to 98.7% for MD. TGA indicated that encapsulation enhanced the thermal stability of the active compounds as the maximum thermal degradation temperature of the microcapsules was observed around 341 ˚C compared to the 320 ˚C obtained for the non-encapsulated extracts. Also, SEM analysis of MDGA microcapsule was spherical with dented surfaces whereas those of MD and GA showed amorphous, flake-like glassy appearances. The amorphous crystalline patterns of the microcapsules and MoLP extracts were also confirmed by X-ray diffraction. FTIR analysis after encapsulation, showed the presence of new spectra at 1177, 1382 and 1411cm^{-1} for MDGA, MD and GA microcapsules respectively were noted, indicating modifications in the structural patterns. Furthermore, storage stability tests performed over 28 days at 4, 25 and 40 ˚C showed that microcapsules were most stable at 4 ˚C although stability differed significantly (p < 0.05) with the coating material type and temperature. Using the simulated *in vitro* gastrointestinal model, MDGA microcapsule had the highest percentage polyphenol release profile, which was more pronounced in the gastric mucosa than in the intestine, despite the MD and GA microcapsules having higher TPC values. The antioxidant values (FRAP and DPPH) did not significantly (p > 0.05) differ among microcapsules made from the different coating materials.

Overall, the microencapsulation of *M. oleifera* leaf extract using MD and GA coating materials were successful and efficient with the microcapsules displaying enhanced stability properties. The microcapsules also masked the deep green coloration of the extracts but retained the phytochemical attributes and so may be effectively be used in the fortification of foods. The inclusion of *M. oleifera* leaf microcapsules in traditional South African foods is therefore encouraged due to the plethora of medicinal phytochemicals present therein that can be harnessed for their immense health benefits.

Keywords: *Moringa oleifera*, Microencapsulation, Response surface methodology, Plant extracts

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people and bodies that were crucial to the success of my research;

To God the creator of all, whose fatherhood I have enjoyed over the years and I keep enjoying daily.

To Dr. Anthony Obilana, Dr. Ayodeji Oyenihi and Mr Fannie Rautenbach my supervisors at the Cape Peninsula University of Technology. Dear sirs, I am forever indebted for your guidance, patience, diligence and tolerance in correcting my writings. Your encouragements, support and assistance during the period of my study proved pivotal to my success.

I would also like to appreciate Prof Victoria Jideani for facilitating the research methodology workshop in my first year, this changed my perception and view of research and writing, and for this I am grateful.

The effort of Mr. O. Wilson the senior laboratory technician at the Food Science and Technology Department Cape Peninsula University of Technology Bellville campus whose help with the procurement of chemicals and consumables proved pivotal is well appreciated.

I am grateful to Ms. Christa Van Schalkwyk, Ms Lamla Spilito and Mr. Thembilani Xolo for their support and assistance with lab works and schedules.

I am grateful to the Head of Department Food science and technology, CPUT Prof Jessy Van Wyk for providing a conducive atmosphere for students to thrive.

I am equally appreciative of the efforts and help of the staffs and students of the Oxidative stress research center CPUT during my laboratory experiments and I am grateful for the conducive work atmosphere provided.

I would also like to appreciate the following persons who stood by me and were sources of inspiration to me throughout my study; my mother Mrs O.O George, my siblings Deji, Tobi, Mayowa and Daniel, my family members; Mrs. Olaide Idjoko and Dr. Adewunmi Bodede

I am grateful to the following people; Dr. Samson Oyeyinka whose guidance has proved fruitful in my research journey, Mr. Daniel Lucky, Mr. Oladipo Tunde who was instrumental in putting me through the use of important materials and software during my program, Dr Babatunde Olawoye of the Technical University, Ibadan for help with Designing of experiment and Mr Ovia Kamathi for help after hours with laboratory works and schedules.

My gratitude will not be complete if I fail to mention the great input of my best friend and lover Tomisin Oyinlola Latinwo whose words of encouragement, moral support, love and care were of

immense of mportance during this journey. Thank you for pumping me daily with gallons full of inspiration. Babe, I am grateful and this platform presents me the opportunity to tell the whole world how much I love and cherish you.

I would like to appreciate the center for postgraduate studies Cape Peninsula University of Technology (CPUT) for putting together workshop sessions that were pivotal for my growth throughout this journey.

Finally, I am grateful to the South African National Research Foundation for providing me with the 2019 study bursary under the Thutuka grant and Center for postgraduate studies (CPGS), Cape Peninsula University of Technology (CPUT) for study bursary for the year 2020 and 2021.

Note: Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation and the CPUT CPGS.

DEDICATION

I dedicate this project to the memory of my late father Mr. Oluseye Kehinde George whose immense contribution to my academics cannot be quantified in words. Dear father, you wrote the story of your life in tears and these few lines will never be enough for me to write anf thank you for all you did. I am proud and honoured to have been fathered by you and I am here pursuing and doing what you and I have always believed is my forte, I promise never to stop until I reach the apogee of my career. I love you and will always do, rest on dear father!

Table of Contents

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

Acronym/Abbreviations	Definition/Explanation
AAE	Ascorbic acid equivalent
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
BBD	Box Beinkhen Design
DPPH	2,2-diphenyl-1-picrylhydrazyl
EE	Encapsulation efficiency
FRAP	Ferric reducing antioxidant power
FTIR	Fourier transform infrared spectroscopy
GA	Gum Arabic
GA microcapsules	MoLP extract microcapsules developed with gum Arabic coating
GAE	Gallic acid equivalent
MD	Maltodextrin
MD microcapsules	MoLP extract microcapsules developed with maltodextrin coating
MDGA microcapsules	MoLP extract microcapsules developed with maltodextrin gum arabic mixture coating
MoLP	*Moringa oleifera* leaf powder
MoSP	*Moringa oleifera* seed powder
ORAC	Oxygen radical absorbance capacity
QE	Quercetin equivalent
RSM	Response surface methodology
SEM	Scanning electron microscopy
SGP	Simulated gastric phase
SIP	Simulated intestinal phase
TFC	Total flavonoid content
TGA	Thermogravimetric analysis
TPC	Total phenolic/polyphenol content
TPTZ	2,4,6-tri[2-pyridyl]-s-triazine
WAI	Water absorption index
WSC	Water solubility capacity
XRD	X-ray diffraction

CHAPTER ONE
MOTIVATION AND BACKGROUND OF THE STUDY

1.1 Introduction

Biologically active compounds in plants are important ingredients in the preparation of functional foods so that adequate healthy compounds beyond basic nutrients present in them can be consumed and function as a prophylactic in the prevention of chronic diseases (Belšćak-Cvitanović *et al.*, 2011; Taneja and Singh, 2012). Public interest and demand in plants containing these bioactives continue to grow as preference tends towards exploring the deluge of naturally occurring, healthy compounds in plants in the production of nutraceuticals (Đorđević *et al.*, 2014). Some plants are rich in these active compounds otherwise known as phytochemicals with important health functions against many lifestyle diseases, and their presence in these plants has generated wide attention. One such plant that has been reported to be rich in these bioactives is *Moringa oleifera*.

Moringa oleifera Lam was originally domiciled in the sub-Himalayan province of India, Pakistan and Bangladesh (Anwar *et al.*, 2007; Oyeyinka and Oyeyinka, 2018; Padayachee and Baijnath, 2020). It is also well known in the tropical and subtropical regions of the world. It can thrive in areas with a prolonged drought period which makes it available all year round as a reliable and resourceful plant (Leone *et al.*, 2016; Raja *et al.*, 2016). The plant is highly nutritious, and enhances the nutritional, health status and food security of the consuming populace (Moyo *et al.*, 2016; Oyeyinka and Oyeyinka, 2018; Bolarinwa *et al.*, 2019). Despite its importance and functionality, Abioye, (2015) stated that the plant's vast potential is still largely untapped with current use in the food production industry lower than expected.

Past investigations on this plant have reported the presence of secondary metabolite compounds, mainly polyphenols, which are important to human health (Anwar *et al.*, 2007; Ma *et al.*, 2020; Padayachee and Baijnath, 2020). The presence of polyphenols has made the conventional use of the plant for the treatment of tumours, diabetes, hepatotoxic and fertility defects, popular than its use as functional inclusion compounds in food (Asare *et al.*, 2012; Omodanisi *et al.*, 2017; Oguntibeju *et al.*, 2019). The plant is also used in traditional medicine practices which have been claimed by many indigenous cultures and communities mostly based on folklore (Anwar *et al.*, 2007; Razis *et al.*, 2014). Airouyuwa and Kaewmanee, (2019) reported that the plant is rich in bioactive and antioxidant compounds such as flavonoids, phenols, amongst others and are beneficial to the human body. In recent years, attention has been given

to the nutritional, bioactive components and characteristic features of *M. oleifera* in many countries of the world where it is non-native, and because of its vast use and application, as it is believed to be an important ingredient in the production of functional foods. Therefore, it is safe to say that *M. oleifera* contains potential medicinal and nutraceutical compounds that can aid in the production of functional and healthy foods that do not only contain nutrients but protect and fight against diseases.

Biologically active compounds in plants are mostly unstable and susceptible to damage by the influence of certain environmental, processing, and storage conditions (Belšćak-Cvitanović *et al.*, 2011; Tomšik *et al.*, 2019). *M. oleifera* bioactives are prone to deterioration because of process conditions such as high temperature, as well as low solubility characteristics and bioavailability which may lessen their potency as active agents at their site of action (Airouyuwa and Kaewmanee, 2019). In addition to this, they are astringent in taste, somewhat bitter, and maybe unstable during storage. Hence, encapsulation will be a suitable procedure for maintaining and keeping the chemical integrity of the plant's extracts as well as masking their astringent taste and flavour (Tomšik *et* al., 2019).

Encapsulation is a technique used in the stabilization and preservation of bioactive compounds as it offers the necessary protection to these active compounds from external influences that may cause damage to their structure and function (Poshadri and Kuna, 2010; Tomšik *et al.*, 2019). The active or core agents are entrapped in carrier matrices in the form of micro or nanostructures (Drosou *et al.*, 2017). This procedure was proposed and reported as an approach that has great potential in the stabilization of biologically active compounds, controls and minimizes release as well as enhances their availability in biological systems (Mahfoudhi *et al.*, 2016). Since encapsulation helps to mask bitter and astringent tastes as well as off-flavour of bioactive compounds (Gharsallaoui *et al.*, 2007; Vinceković *et al.*, 2017), it is essential to entrap important biological components in a suitable carrier matrix that offers good coating, is safe and biodegradable and makes their absorption in human systems easy. This is the first study, to the best of my knowledge, that focuses on the encapsulation and characterization of *M. oleifera* extracts using maltodextrin, gum Arabic and their combination. The objective of this study was to demonstrate the encapsulation of bioactive compounds from *M. oleifera* using maltodextrin and gum Arabic and evaluate the different properties of the resulting microcapsules. This study also aimed to determine how different coating materials influence the physical, thermal, structural, and storage properties of the microcapsules as well as study their release profile using an *in vitro* simulated gastrointestinal model.

1.2 Statement of Research Problem

Despite the important nutraceutical, functional and food applications ascribed to *M. oleifera* bioactives, the plant is still underutilized as a commercial food ingredient and product in South Africa and other parts of Africa. The food industry has concentrated all its efforts at ensuring that healthy foods are available to consumers while little focus has been geared towards making sure that intact bioactive compounds in these foods or plant products are delivered efficiently and effectively. With consumers becoming aware of the need to have these natural compounds such as those found in *M. oleifera* incorporated into their foods, it is imperative to deliver these compounds in a way that they do not undergo degradation and deterioration under storage and processing conditions. Many of these compounds are heat-labile, and if not protected properly may result in unfavourable end products when the bioactive compounds react directly with foods. Exposure to oxygen may result in deterioration as well as their bitter taste may also impact the sensory properties of food when they are included as fortifying ingredients. The effectiveness of encapsulation in creating a protective barrier and ensuring the stability of these active compounds during food processing and production has been demonstrated in works of literature (Chranioti *et al*., 2015; Premi and Sharma, 2017; Dadi *et al*., 2020). However, there is a paucity of research and information on the encapsulation of *M. oleifera* active compounds by maltodextrin and gum Arabic carriers. This study, therefore, characterized and encapsulated *M. oleifera* extracts using these coatings and using optimum preparation parameters and drying procedures for further analysis.

1.3 Objectives of the study

1.3.1 Broad Objective

The specific objectives of this study are;

i. To extract bioactive compounds from *M. oleifeira* leaf and seed powder using different solvents and determine their phytochemical constituents and antioxidant capacities.
ii. To determine the optimum preparation conditions for the encapsulation of the best *M. oleifera* extract determined in (i) using response surface methodology.
iii. To determine selected physical, functional and structural properties of *M. oleifera* microcapsules prepared from different coating materials as well as their release properties and storage stability.

1.4 Research Hypotheses

In this study, it is hypothesized that;

- Encapsulation can protect and preserve the quality of *M. oleifera* extracts.
- The encapsulation efficiency of the resultant microcapsules will be high at optimum preparation conditions.

1.5 Delineation of the research

The delimitations of this research are that:

Extraction of the bioactive compounds was done using three solvents and the best solvent extracts will be considered for bulk extraction of phytochemicals.

- *Moringa oleifera* leaf and seed powder are the two raw materials to be used.
- Optimization for the best preparation condition for *M. oleifera* microcapsules.
- One method of drying; freeze-drying was used in this study.
- Maltodextrin and gum Arabic were used as coatings.

1.6 Significance of the Research

The vast presence of beneficial compounds present in *M. oleifera* extracts has made it a viable candidate for the development of functional ingredients used in foods. With the continuous growth in interest and awareness of consumers towards the consumption of healthy and functional foods, *M. oleifera* can form a viable inclusion and become an important functional ingredient in the enrichment of commonly consumed staple and indigenous foods. The significance of encapsulation of the important bioactives present in *M. oleifera* will ensure that problems associated with astringent taste, instability, deterioration during storage and processing will be overcome which will promote the use of the plant beyond conventional applications. The production of *M. oleifera* microcapsules using various coating materials will improve the use and value of the plant from its current underutilized and undervalued state in the food industry, and enhance their health benefits potentially reducing malnutrition and global disease burden in Africa and beyond. The anticipated rise in their use will also lead to corresponding demand which will increase the market value of *M. oleifera* for farmers. It is believed that this study will contribute to the existing body of knowledge on the importance of *M. oleifera*, and create new frontiers for research as South Africa continues to promote research on the use of underutilized crops in the country and Africa towards attaining food security and sustainability the next couple of years.

1.7 Outcomes and Results of this study

Encapsulation will prove to be an efficient procedure in preserving beneficial compounds present in *M. oleifera* extracts. In addition to this, the effect of different carrier materials on the different properties of *M. oleifera* microcapsules and optimum preparation conditions for maximum efficiency of encapsulation will be known. Research findings have the potential for a minimum of one research article to be published in a DHET accredited journal and one local or international conference to be attended for the presentation of research outputs. A Master of Food Science and technology degree is expected to be awarded from this study.

1.8 Thesis Overview

This thesis contains six chapters and was written in a journal article format where each research chapter looks like a manuscript. The thesis overview is shown in Figure 1.1 below.

Chapter one is the introduction which details the motivation, design and background of the study. The chapter also details the statement of the research problem, the objective of the study, the research hypothesis, delineation or limitation of the study, the significance of this study as well as outcomes expected from this research work. Chapter two is the literature review which elucidates in clear terms the background of the research and the major variables embedded in the topic. Chapter three is the first research chapter which shows the extraction studies.

Chapter four discusses the optimization process for the preparation of *M. oleifera* microcapsules using the Box Behnken design (BBD) of the response surface methodology (RSM) for the determination of optimum preparation conditions to attain maximum encapsulation efficiency. Chapter five is the last research chapter which focuses on the effect of carriers/coating materials on the properties of the microcapsules developed from maltodextrin, gum Arabic and the combined maltodextrin gum Arabic (MDGA) coating mixture obtained using optimization. The optimum preparation parameters obtained in chapter 4 was used in the development of the microcapsules with differences in coating material used. Chapter six is the concluding chapter where the summary of the research findings and conclusions made were stated. The recommendations for future studies were also proposed.

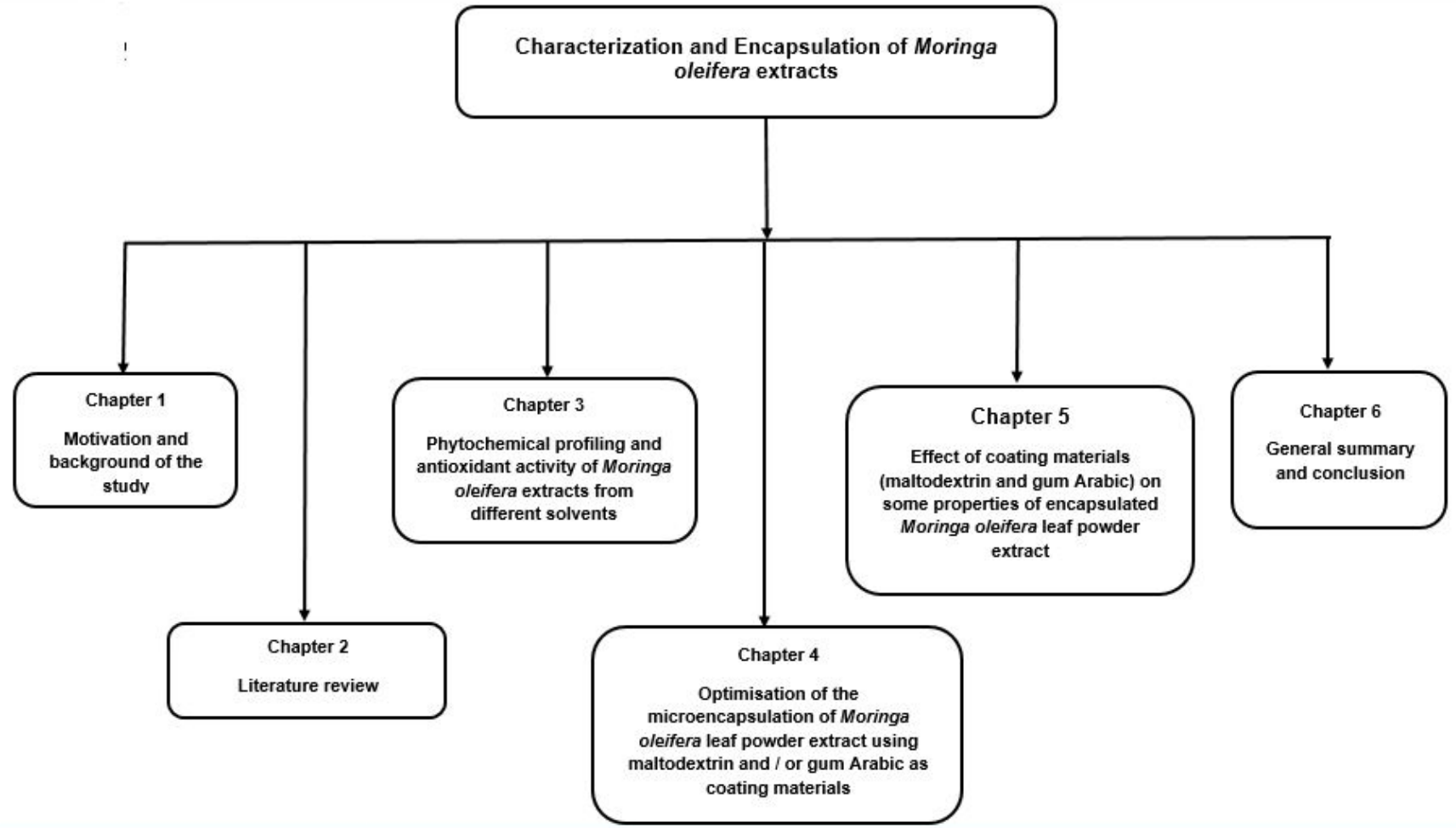


Figure 1.1: Thesis overview

1.9 REFERENCES

Abioye, V. (2015). Proximate composition and sensory properties of moringa fortified yellow maize-ogi. *African Journal of Food Science Research*, **3**, 155-159.

Anwar, F., Latif, S., Ashraf, M. & Hassan, A. (2007). *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *Phytotherapy Research*, **659**, 651–659.

Asare, G.A., Gyan, B., Bugyei, K., Adjei, S., Mahama, R., Addo, P., Otu-Nyarko, L., Wiredu, E.K. & Nyarko, A. (2012). Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. *Journal of Ethnopharmacology*, **139**, 265–272.

Belščak-Cvitanović, A., Stojanović, R., Manojlović, V., Komes, D., Cindrić, I.J., Nedović, V. & Bugarski, B. (2011). Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate-chitosan system enhanced with ascorbic acid by electrostatic extrusion. *Food Research International*, **44**, 1094–1101.

Bolarinwa, I.F., Aruna, T.E. & Raji, A.O. (2019). Nutritive value and acceptability of bread fortified with moringa seed powder. *Journal of the Saudi Society of Agricultural Sciences*, **18**, 195–200.

Drosou, C.G., Krokida, M.K. & Biliaderis, C.G. (2017). Encapsulation of bioactive compounds through electrospinning/electrospraying and spray drying: A comparative assessment of food-related applications. *Drying Technology*, **35**, 139–162.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, **40**, 1107–1121.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. & Bertoli, S. (2016). *Moringa oleifera* seeds and oil: Characteristics and uses for human health. *International Journal of Molecular Sciences*, **17**, 1–14.

Ma, Z.F., Ahmad, J., Zhang, H., Khan, I. & Muhammad, S. (2020). Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera*) as a potential functional food. *South African Journal of Botany*, **129**, 40–46.

Mahfoudhi, N., Ksouri, R. & Hamdi, S. (2016). Nanoemulsions as potential delivery systems for bioactive compounds in food systems: preparation, characterization, and applications in food industry. *Emulsions*, **93**, 365–403.

Moyo, B., Patrick, J.M., Arnold, H. & Voster, M. (2016). Nutritional characterization of Moringa

(*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, **10**, 12925–12933.

Oguntibeju, O.O., Aboua, G.Y. & Omodanisi, E.I. (2019). Effects of *Moringa oleifera* on oxidative stress, apoptotic and inflammatory biomarkers in streptozotocin-induced diabetic animal model. *South African Journal of Botany*, **129**, 354–365.

Omodanisi, E.I., Aboua, G.Y. & Oguntibeju, O.O. (2017). Therapeutic potentials and pharmacological properties of *Moringa oleifera* lam in the treatment of diabetes mellitus and related complications. *Tropical Journal of Pharmaceutical Research*, **16**, 1737–1746.

Osamede Airouyuwa, J. & Kaewmanee, T. (2019). Microencapsulation of *Moringa oleifera* leaf extracts with vegetable protein as wall materials. *Food Science and Technology International*, **25**, 533–543.

Oyeyinka, A.T. & Oyeyinka, S.A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, **17**, 127–136.

Padayachee, B. & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. *South African Journal of Botany*, **129**, 304–316.

Perumal, S. & Klaus, B. (2003). Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera Lam*.) Leaves. *Journal of Agricultural and Food Chemistry*, **51**, 2144–2155.

Poshadri, a & Kuna, A. (2010). Microencapsulation technology: A review. *The Journal of Research ANGRAU*, **38**, 86–102.

Raja, R.R., Sreenivasulu, M., Vaishnavi, S., Navyasri, D.., Samatha, G. & Geethalakshmi, S. (2016). *Moringa oleifera*-An Overview. *RA Journal Of Applied Research*, **2**, 620–624.

Razis, A.F.A., Ibrahim, M.D. & Kntayya, S.B. (2014). Health benefits of *Moringa oleifera*. *Asian Pacific Journal of Cancer Prevention*, **15**, 8571–8576.

Taneja, A. & Singh, H. (2012). Challenges for the Delivery of Long-Chain n-3 Fatty Acids in Functional Foods. *Annual Review of Food Science and Technology*, **3**, 105–123.

Tomšik, A., Šarić, L., Bertoni, S., Protti, M., Albertini, B., Mercolini, L. & Passerini, N. (2019a). Encapsulations of wild garlic (*Allium ursinum* L.) extract using spray congealing technology. *Food Research International*, **119**, 941–950.

Vinceković, M., Viskić, M., Jurić, S., Giacometti, J., Bursać Kovačević, D., Putnik, P., Donsì, F., Barba, F.J. & Režek Jambrak, A. (2017). Innovative technologies for encapsulation of

Mediterranean plants extracts. *Trends in Food Science and Technology*, **69**, 1-12.

CHAPTER TWO

LITERATURE REVIEW

This review of literature covers an overview of the *Moringa oleifera* plant, the extracts, various uses ascribed to the plant. This chapter also includes methods of encapsulation of biologically active compounds and their applications in the food industry and wall materials and hydrocolloids used in the process of encapsulation.

2.1 Origin and description of *Moringa oleifera*

Moringa (*Moringa oleifera*) is a tree with immense benefit to human health and nutrition (Figure 2.1). It is cultivated widely in several countries (Lin *et al.*, 2018), but was originally native to India, Pakistan, Bangladesh and other middle eastern countries (Anwar *et al.*, 2007; Oyeyinka and Oyeyinka, 2018; Padayachee and Baijnath, 2020). The crop has become naturalized in the tropics and sub-tropic regions of the world (Anwar *et al.,* 2007; Moyo, 2012; Moyo *et al.*, 2016), with increasing popularity in various African countries (Bolarinwa *et al.*, 2019). In South Africa, the plant is receiving much attention due to the many inherent benefits and importance to human health and is being cultivated in the Free State, Mpumalanga, Gauteng and KwaZulu-Natal provinces.

Moringa oleifera is known by different names in different regions of the world as summarized in Table 2.1. Many authors have also named the plant, these names includes; the horseradish and drumstick tree (Anwar *et al.*, 2007; Chukwuebuka, 2015; Gupta *et al.*, 2018), the ben-oil tree (Chukwuebuka, 2015; Gupta *et al.*, 2018), the mother's best friend (Chukwuebuka, 2015), the never die tree (Gupta *et al.*, 2018) and the miracle tree (Oyeyinka and Oyeyinka, 2018) because of its nutritional use, the presence of deluge of phytochemicals and its wide application as a remedy for the treatment of several ailments.

The plant is grown for its economic value (Özcan, 2018), it is drought-resistant and rarely afflicted by plant diseases and pests (Chukwuebuka, 2015; Raja *et al.*, 2016; Gupta *et al.*, 2018). Hence its economic benefits can be tapped in tropical, subtropical and sub-Saharan areas that experience low rainfall. *Moringa oleifera* plays an important role in food security since every part of the plant is beneficial and has been used to boost the nutritional composition of foods. For example, it is an important vegetable diet in many developing countries of the world (Chukwuebuka, 2015; Raja *et al.*, 2016; Oyeyinka and Oyeyinka, 2018). In addition, the

beneficial effect of many parts of this plant makes it an important plant for food enrichment, fortification,

Figure 2.1 – *Moringa oleifera* leaf and seed Adapted from Kou *et al.* (2018)

supplement development ingredient as well as an important ingredient for the formulation of functional foods. At least four notable parts of the *Moringa* plant are reported to be edible (Anwar *et al.*, 2007; Jideani and Diedericks, 2014; Ma *et al.*, 2019) these include; the pods, leaves, seeds and roots. Özcan, (2018), reported that the pods of the plant can be eaten as vegetables during various growth and developmental stages. The leaves of the plant have been used in the feeding of cattle (Raman *et al.*, 2018). The seeds are fried and consumed as food (Özcan, 2018) and contain a significant amount of oil reportedly fit for human consumption (Leone *et al.*, 2016). Therefore, it is safe to conclude that the leaf can be consumed as vegetables by man and animals and its seed as snacks to fully harness the many nutritional benefits of the plant.

Extracts from the leaves have been reported to improve and increase the nutritional, functional, organoleptic and sensory properties of some foods when incorporated in them, these food include bread, juices, milk, soup sauces and noodles (Mukunzi *et al.*, 2011). Other authors stated that their inclusion in the preparation of some foods affects the sensory properties of the food (Karim *et al.*, 2013; Arise *et al.*, 2015). Hence, their addition should be in a controlled and optimum quantity to get a desired and acceptable product. The miracle plant also contains several bioactive compounds that can be used in the development of novel functional products in the food industry (Saucedo-Pompa *et al.*, 2018). The presence of this bioactive in the plant makes it a viable antimicrobial and antioxidant ingredient in the development of functional foods

(Saucedo-Pompa *et al.*, 2018). Some of such antioxidants include; vitamins, flavonoids, carotenoids; a precursor to vitamin A production in the body, phenolic compounds, several vitamins and minerals (Ibrahim *et al.*, 2019). Hence should be exploited for use especially for human health.

Apart from its rich nutritional quality, various parts of the tree have been used in traditional and herbal medicine (Lin *et al.*, 2018). Awuku *et al.* (2012) reported that various parts of the plants are used in indigenous and traditional medicine /decoctions/concoctions for the treatment of ailments and diseases, because of the presence of several phytochemical compounds. It has also been validated by some authors for its prophylactic and therapeutic properties (Anwar *et al.*, 2007; Mukunzi *et al.*, 2011; Jideani and Diedericks, 2014; Omodanisi *et al.*, 2017a; Omodanisi *et al.*, 2017b; Oguntibeju *et al.*, 2019; Padayachee and Baijnath, 2019). For instance, in Malawi, the roots of the plant have been used for the treatment of fevers in infants (Sagona *et al.*, 2019). It has also been used in the treatment of diabetes (Oguntibeju *et al.*, 2019; Ma *et al.*, 2020), hyperglycemia-induced inflammation (Omodanisi *et al.*, 2017b) and cancer (Sagona *et al.*, 2019). The seed powder has an appreciable amount of a chemical compound known as selenium which has great antioxidant potential and helps lower the risk of breast and colorectal cancer (Sagona *et al.*, 2019). Medicinally the leaves have also been used in the treatment of hiccoughs (Chukwuebuka, 2015). Some studies have also shown the anti-microbial importance of the plant extracts (Govardhan Singh *et al.*, 2013; Sagona *et al.*, 2019). Research point to the vast potential embedded in various parts of the miracle plant that has been studied and areas that yet offer a wide array of untapped potential present in the plant but not currently studied.

Table 2.1 Common names of *M. oleifera* in different countries of the world

Country	Language	Common Name
Burkina Faso	Mooré	Arzan Tiiga
Brazil	Portuguese	Cedra
Côte D'Ivoire	Dioula	Arjanayiiri
Ethiopia	Giddigna	Haleko, Aleko
Germany	German	Behenbaum
Ghana	Dagari	Zangala
India	Hindi	Sahjan
	Gujarati	Saragvo
	Marathi	Shevga
	Malayalam	Muringakaya
Indonesia	Indonesian	Kelor
Malawi	Senna	Nsagoa
	Yao	Kalokola
Nepal	Nepali	Sawijan
Nicaragua	Nicaguaran	Marango
Nigeria	Hausa	Zogale
	Yoruba	Ewe igbale
South Africa	Xhosa	Imoringa
Sudan	Dinka	Anid
Thailand	Thai	ma rum
Zimbabwe	Tonga	Mupulanga

Adapted from Raja *et al.* (2016)

2.2 Nutritional composition and food uses of *Moringa oleifera*

Table 2.2 shows the proximate composition of *M. oleifera*. Research has reported varying values with differences purportedly ascribed to variation in the cultivar and planting location (Oyeyinka and Oyeyinka, 2018). Dry leaf samples have been reported to contain protein of 30.29%, with 19 amino acids (Moyo *et al.*, 2011). Protein value was corroborated by the study of Cuellar-Nuñez *et al.*, (2018) who reported a protein content of 30.60 – 35.50% in the dried leaves. These values are comparable and even higher than the protein content of many leguminous plants considered to have high protein levels. Özcan, (2018) also reported a value of 9.98% for the protein content of the seed, crude protein content of 25.16 % in dried Moringa flower was previously reported by Arise *et al.* (2015).

The plant also contains a carbohydrate content of 31. 00 – 43.40% and moisture content of 9.50 – 11.90% (Moyo *et al.*, 2011; Cuellar-Nuñez *et al.*, 2018). Previous studies have shown that different parts of the Miracle tree contain a wide array of nutrients and bioactive compounds. The leaves are rich in vitamins A, B vitamins which includes thiamine and niacin, vitamin C and E, nicotinic acid, pyridoxine, riboflavin, β-carotene, amino acids, minerals, proteins and several other phenolic compounds (Moyo *et al.*, 2011; Jideani and Diedericks, 2014; Chukwuebuka, 2015; Gopalakrishnan *et al.*, 2016). The vitamin C content of the leaves is reportedly higher than those found in fruits and plants conventionally known for high vitamin C content such as oranges (Anwar *et al.*, 2007; Chukwuebuka, 2015; Oyeyinka and Oyeyinka, 2018). The leaves are also rich in linoleic and oleic acids which are essential fatty acids (Moyo *et al.*, 2011; Jideani and Diedericks, 2014). The inclusion of the leaves in the human diet is capable of supplying these important nutritive components which cannot be synthesized by the body.

The leaf of the plant can improve the nutritional status among the consuming populace thereby boosting food security (Arise *et al.*, 2015; Oyeyinka and Oyeyinka, 2018). The leaves could also serve to combat malnutrition amongst children, pregnant women and nursing mothers in developing countries and regions of the world (Arise *et al.*, 2015; Moyo *et al.*, 2016). Because of the numerous nutritional benefits of the plant, it has been used as a nutritional supplement (Gupta *et al.*, 2018), food fortifier (Abioye, 2015; Arise *et al.*, 2015; Gopalakrishnan *et al.*, 2016; Oyeyinka and Oyeyinka, 2018; Bolarinwa *et al.*, 2019) as well as an enrichment plant for several foods deficient in essential and non-essential nutrients (Abioye, 2015). Thus making moringa an important crop among the vulnerable populace. Since nutrition plays a very important and pivotal role in the proper wellbeing of humans, the potential of the plant should be fully harnessed by food industries as a natural means of improving the nutritional qualities of

foods. Hence, it can be used to fortify and enrich foods low in nutrients and for the fortification and enrichment of popular children's food such as breakfast cereals.

Moringa oleifera can improve the yield of milk in nursing mothers and it has been termed "The Mother's Best Friend" (Chukwuebuka, 2015). Fresh leaves also help to boost milk production in nursing mothers and reduce anaemia, a condition that results due to a shortage of blood in the body (Anwar *et al.,* 2007; Padayachee and Baijnath, 2019). Padayachee and Baijnath, (2019), stated that the plant is now widely known for the significant health values it confers on consumers.

Sahay *et al.* (2017) reported in their review that when bread was fortified using 5% *M. oleifera* powder, an increase of 17% was noticed in the protein content of the resulting product. The protein content reported for the dry leaves and products fortified with it shows its importance and as such leaves can be included in the diets of individuals suffering from protein malnutrition otherwise known as kwashiorkor. Apart from the leaf powder, seed powder was also found to contain about 40% crude protein (Wang *et al.,* 2019). Bolarinwa *et al.* (2019) reported an increased protein content for bread fortified with the seed powder and this result was in tandem with Oyeyinka and Oyeyinka, (2018) where an increased protein content was reported for bread fortified with the leaf powder and increased protein content in *moringa* fortified *ogi*. About 100% increase was noticed in protein content when *Ogi* was fortified with 15% *moringa* leaf (Abioye, 2015). These studies have all shown that every edible part of the plant is rich in protein and may be a potential enrichment plant for protein deficient foods.

Carbohydrate content of 53.6% was reported for *M. oleifera* flower by Arise *et al.* (2015). The carbohydrate content of fresh leaves (12.5 %), dry leaves (41.2%), leaf powder (38.2 %), seeds (8.67%) and pods (3.7%) were also stated in the review of Gopalakrishnan *et al.* (2016) . Bolarinwa *et al.* (2019) fortified bread with different levels of moringa seed powder and reported a marginal decrease in carbohydrate content present in the bread as levels increased. The decrease in this study can be associated with the decrease in the wheat flour used as control and an increase in the seed powder.

The moringa leaf was reportedly high in dietary fibre content with a value of 18.75% (Cuellar-Nuñez *et al.*, 2018a) and 19.2% for the leaf powder (Gopalakrishnan *et al.*, 2016), however, Chukwuebuka, (2015) earlier recorded a crude fibre content of 35% for the leaf. Differences in crude fibre values may be ascribed to different planting locations, cultivar as well as variation in methods of determination. The dietary fibre content of the study conducted by Arise *et al.* (2014) on *M. oleifera* flower used in the fortification of weaning foods indicated an

increase in fibre content as fortification proportion increases, the result of the proximate analysis shows a fibre content of 7.5%. Additionally, Bolarinwa *et al.*, (2019) reported an increase in the fibre content of bread fortified with the seed powder. Other foods fortified with *M.oleifera* generally experienced increases in fibre contents. This further exemplifies that the leaf, seed and flower contain a significant quantity of fibre which is important for digestion as it causes distention and motility in the bowel of humans.

Moringa oleifera is rich in vitamins and minerals with a study by Moyo *et al.* (2016) stating the presence of several minerals (macro and micro-elements) in dried leaves. Generally, the fresh and dried leaves of *M. oleifera* were reported to be high in minerals (Sahay *et al.*, 2017), when compared with other plants. The presence of important mineral elements further reinforce that the leaf of the plant has great fortification and enrichment potential. The presence of a high amount of iron (Fe) in the plant leaves in comparison with other plants also attests to the earlier surmise (Moyo *et al.*, 2016). Bolarinwa *et al.* (2019), reported higher calcium (Ca) content than that present in potato-bread fortified with soy flour when the seed powder of *Moringa* was used in the fortification of wheat bread.

A study by Leone *et al.* (2016), showed B vitamins such as riboflavin, niacin, and thiamine to be present in the leaves of the plant. The presence of vitamin A and C has also been reported (Sahay *et al.*, 2017). The seed of the plant also contains fatty acids (essential and non-essential alike). Essential fatty acids are important dietary ingredient, they cannot be synthesized by the body but only supplied via the diet, with *M. oleifera* seeds potentially serving as an essential fatty acid source for consumers. Table 2.3 shows the fatty acids content present in *M. oleifera* seeds. The presence of all these important micronutrients has made it pertinent for the vast richness of this plant to be harnessed by researchers for consumer's benefit as vitamins and minerals are important micronutrients useful in the boosting of the immune system and preventing micronutrient malnutrition.

Table 2.2 - Proximate and Nutritional composition of *Moringa oleifera* leaf, flower and seed (%)

Nutrient	Dried leaves	Flower	Seed
Carbohydrate	30.99 – 43.20[a]	53.67[c]	10.59 – 18.00[d]
Protein	30.59 – 35.50[a]	25.16[c]	7.80 – 37.60[d]
Fat	5.76 – 7.77[a]	1.57[c]	17.58 – 58.80[d]
Fibre	18.06 – 21.05[a]	7.55[c]	3.20 – 20.00[d]
Ash	10.16 – 14.27[a]	6.01[c]	2.46 – 8.40[d]
Vitamin A	47.00[b]	NR	NR
Vitamin C	62.00[b]	NR	NR
Calcium	19.00[b]	NR	NR
Potassium	7.00[b]	NR	NR
Phosphorus	16.00[b]	NR	NR
Magnesium	41.00[b]	NR	NR

Adapted from (Cuellar-Nuñez *et al.*, 2018)a (Raja *et al.*, 2016)b (Arise *et al.*, 2015)c (Özcan, 2018)d

NR: Not reported

Table 2.3 - Fatty Acid composition of *Moringa oleifera* seed oil

Fatty acids	Content (%)
Arachidic	0.60 – 5.00
Behemic	2.52 – 7.24
Eicosenoic	1.50 – 9.64
Lauric	0.10 – 2.87
Lignoceric	1.30 – 10.98
*Linoleic	0.27–22.66
*Linolenic	0.20–32.53
Margaric	0.03–1.40
Oleic	67.9–85.00
Palmitic	5.73 – 17.06
Palmitoleic	1.32–3.70
Stearic	0.80–7.60

Names with a superscript (*) indicates essential fatty acid Adapted from Özcan, (2018)

2.3 Bioactive and Phytochemical composition of *Moringa oleifera*

For many years, man has used biologically active compounds through folklore medicine as prophylactic and therapeutic ingredients for healthy living. Biologically active compounds in plants have various health-benefiting importance and advantage. More recently, new research strides have detailed the use of phytochemical compounds in plants in the development of functional foods (Saucedo-Pompa *et al.*, 2018). Phytochemicals are important groups of bioactives, they are secondary metabolite compounds, non-nutritive in nature, present in plants (Anwar *et al.*, 2007; Jideani and Diedericks, 2014; Awika and Duodu, 2017; Padayachee and Baijnath, 2020), originally used as a defence mechanism against predators but has now found importance in human health and disease (Anwar *et al.*, 2007; Chukwuebuka, 2015; Leone *et al.*, 2016). Some phytochemicals do not have less dietary importance, but they have been identified for their important role in disease prevention and treatment (Fahey, 2005; Kurmukov, 2013). They are classified into five important groups based on their chemical structure and molecular properties as polyphenols, alkaloids, carotenoids, terpenoids and sulphur-containing phytochemical compounds (Ma *et al.*, 2020). While the presence of these important compounds may not be fully known, it may have informed their use in folklore medicine with promising responses obtained among traditional healers.

The most abundant class of phytochemical compounds present in moringa are polyphenols (Perumal and Klaus, 2003; Anwar *et al.*, 2007; Elwan *et al.*, 2018; Gupta *et al.*, 2018; Ma *et al.*, 2019). Polyphenols have been further classified as; phenolic acids, flavonoids, stilbenes, ligans and other classes (Oyenihi and Smith, 2019). In *M. oleifera*, the most studied are flavonoids and phenolic acids (Vongsak *et al.*, 2013; Al-Owaisi *et al.*, 2014). Phytochemical compounds are present in virtually every part of the tree with varying quantities reported (Anwar *et al.*, 2007; Prabakaran *et al.*, 2018; Padayachee and Baijnath, 2020). Although, the leaves are believed to have the best presentation of phytochemicals. Prabakaran *et al.* (2018) reported a varying polyphenol composition for different parts (leaves, seed, root, flower and bark extracts) of the plant (Table 2.4). The variation of phytochemicals in different parts of the plant is expected as the exposure of plant parts to nutrients and sunlight through photosynthesis varies. For example, the leaf is more likely to contain rich phytochemical compounds because they are exposed directly to photosynthesis. The presence of these compounds in *M. oleifera* also varies with cultivars, planting location, geographic and climatic conditions, extraction solvent and mechanisms among others. For instance, the total phenolic and flavonoid content of *M. oleifera* plants from Rwanda and China were reported and differs from each other (Mukunzi *et al.*,

2011). The authors reported a total phenolic (TPC) and flavonoid content (TFC) of 30.02 mg GAE/ g and 52.80 mg Rutin/g respectively for samples from Rwanda while a TPC and TFC of 24.65 mg GAE/g and 39.08 mg Rutin/g were reported for the sample obtained from China.

The presence of medicinal phytochemical compounds makes the plant a commonly used remedy for several non-communicable diseases. A high and potent antioxidant activity was reportedly present in aqueous and acetone extracts of the leaves (Nouman *et al.*, 2016; Oguntibeju *et al.*, 2019). The presence of flavonoids in *M. oleifera* has now become important in human nutrition as the human body cannot produce these compounds and as such can only be supplied via the diet (Lin *et al.*, 2018). Flavonoids have strong antioxidant activity and as such can trap free radicals in human systems (Makita *et al.*, 2016; Lin *et al.*, 2018; Kozłowska and Szostak-Węgierek, 2019).

The leaves were also reported to have an appreciable quantity of natural antioxidant compounds when it was compared with fruits such as strawberries which are commonly known for high antioxidant content (Oyeyinka and Oyeyinka, 2018). Other antioxidants obtained from the leaves of *M. oleifera* include zeatin, β – sitosterol amongst others (Padayachee and Baijnath, 2020). Carotenoids, mostly as β-carotene have also been reportedly present in the leaves of Moringa with antioxidant potentials in the scavenging of excess free radicals (Padayachee and Baijnath, 2020). Furthermore, the presence of vitamin C and vitamin E in the form of tocopherols have been reported (Padayachee and Baijnath, 2020).

Although the plant has been reported for the presence of many healthy compounds, their stability remains a challenge during the extended period of handling, storage, processing and inclusion. Additionally, most compounds present in the plant are astringent and bitter. Therefore, their incorporation in food may pose some challenges, which may have a telling effect on product quality. Additionally, phytochemicals have low solubility in aqueous phases, are prone to deterioration from environmental influences and have poor bioavailability in the human systems, these limitations may hinder their full potential in food systems (Mahfoudhi *et al.*, 2016). Therefore, the delivery, stability, protection and absorption of these beneficial compounds in food and the human body must be considered, for efficient delivery and optimum use.

Table 2.4 – Bioactive composition of some parts of different variants of *Moringa oleifera*

Moringa plant part	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/mg)
Leaf Extract	89 – 200	40 – 69
Root Extract	32 – 112	11 – 41
Seed Extract	20 – 55	10 – 33
Flower Extract	45 – 89	19 – 50
Bark Extract	22 – 69	11 – 30

Adapted from Prabakaran *et al.*, (2018)

2.3.1 Polyphenols

Polyphenols are diverse groups of non-nutritive secondary metabolite compounds present in plants that have been studied widely for their positive importance on human health (Cheynier, 2005; Bhullar and Rupasinghe, 2015; Jakobek, 2015; Oyenihi and Smith, 2019; Zhang *et al.*, 2019). They are further divided into phenolic acids, stilbenes, lignin, flavonoids and others (Fig 2.2) (Bhullar and Rupasinghe, 2015; León-González *et al.*, 2015; Oyenihi and Smith, 2019). Among these classes, flavonoids and phenolic acids are the most studied. They have been reported for their anti-carcinogenic (León-González *et al.*, 2015; Amoako and Awika, 2016; Oyenihi and Smith, 2019; Zhang *et al.*, 2019), anti-obesity (D'Archivio *et al.*, 2013), anti-oxidant (Annegowda *et al.*, 2013), anti-diabetic (Dhongade *et al.*, 2017; Ganesan and Xu, 2018; Ma *et al.*, 2019) properties as well as their protective and therapeutic role against degenerative and cardiovascular diseases (Dykes and Rooney, 2007; Amoako and Awika, 2016; Awika and Duodu, 2017; Ganesan and Xu, 2018; Zhang *et al.*, 2019). The health benefits of these compounds present in plants can be further exploited by encouraging the consumption of plant-based diets.

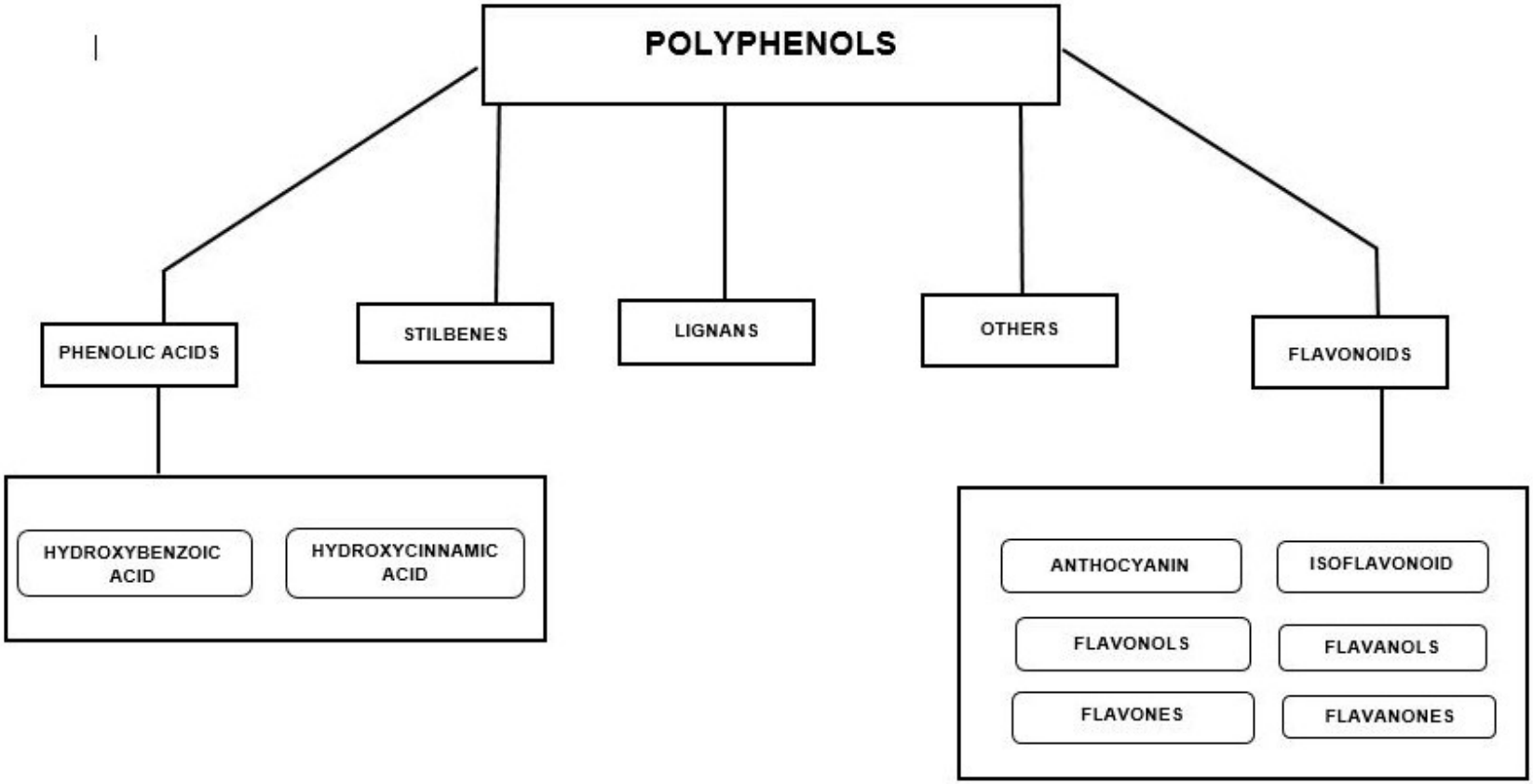
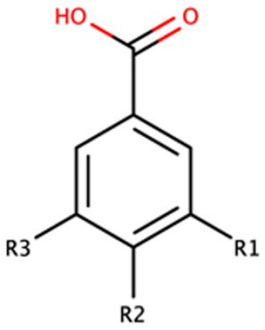


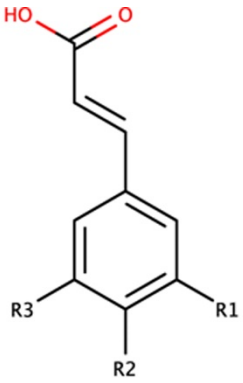
Fig 2.2: Polyphenols and their broad classifications, adapted from (Oyenihi and Smith, 2019).

2.3.1.1 Phenolic acids

Phenolic acids are polyphenol compounds with a single carboxylic acid group (Cheynier, 2005; Kurmukov, 2013). They are further divided into hydroxybenzoic and hydroxycinnamic acid (Fig. 2.3), based on the position of their carbon bond (Cheynier, 2005; Dykes and Rooney, 2007; Kurmukov, 2013; Bento-Silva *et al.*, 2019; Oyenihi and Smith, 2019). Example of phenolic acids includes; coumaric acid, caffeic acid, quinic acid protocatechuic acid, cinnamic acid, ferulic acid, ellagic acid (Leone *et al.*, 2016; Prabakaran *et al.*, 2018; de la Rosa *et al.*, 2019). These compounds have been reported for their importance against the damaging effect of oxidative stress in the human body (Kurmukov, 2013; Gupta *et al.*, 2018; Prabakaran *et al.*, 2018).

The presence of phenolic compounds may be the basis for the use of *M. oleifera* as therapeutics against disorders that arise from oxidative stress. The *in vitro* antioxidant activity and antioxidant potential of phenolic acids in foods has been reported in studies by Moyo *et al.*, 2012b; Jayawardana *et al.*, 2015 and Falowo *et al.*, 2018. Therefore, they have the potential to serve as a natural replacement for commonly used synthetically manufactured antioxidants in the food industry. They also contain antimicrobial activity (Bukar *et al.*, 2011; Moyo, 2012; Oluduro, 2012; Jayawardana *et al.*, 2015; Prabakaran *et al.*, 2018), which presents a cheap, readily available and non-synthetic source of antimicrobials for perishable foods. However, these benefits are marred by astringency and bitterness in taste and flavour (Cheynier, 2005), which may jeopardize their inclusion when developing functional foods. They can, therefore, be developed into microcapsules which could mask unpleasant taste and flavours, as well as important compounds from adverse environmental and processing conditions while providing stability and controlled release when used.





(a) (b)

Fig 2.3: (a) structure of hydroxybenzoic acid (b) structure of hydroxycinnamic acid (Bento-Silva *et al.*, 2019). R determines the specific phenolic acid compounds.

2.3.1.2 Flavonoids

Flavonoids are mostly present in fruits, vegetables and many other foods of plant origin. Over four thousand flavonoid compounds have been reported to be present in plants (Kurmukov, 2013), although Dykes and Rooney,(2007) stated that over 5000 of them have been identified in plants. They have two aromatic rings and are connected by three carbon links (Dykes and Rooney, 2007; Lin *et al.*, 2018; de la Rosa *et al.*, 2019; Kozłowska and Szostak-Węgierek, 2019) Fig. 2.4. They are generally made up of the diphenyl propane frame (C6 C3 C6) (Gulu, 2017; Kozłowska and Szostak-Węgierek, 2019). Examples of flavonoids are anthocyanins, flavonols, flavanols (catechin), flavones and flavanones (Dykes and Rooney, 2007; de la Rosa *et al.*, 2019; Kozłowska and Szostak-Węgierek, 2019). Flavonoids have dietary importance, and have been reported for their ability to prevent cardiovascular disorder, diabetes, cancer and other lifestyle diseases (Dykes and Rooney, 2007; Lin *et al.*, 2018). The presence and inclusion of these compounds in human diets may likely reduce the intake and use of synthetically manufactured drugs as a therapy for the aforementioned disorder, therefore the side effects associated with the usage of these drugs may be averted. Additionally, since they are found in many readily available plants, they are cost-friendly. Flavonoids have antioxidant functions (Kozłowska and Szostak-Węgierek, 2019) and as such can replace the synthetic antioxidants used in the food industry.

Moringa oleifera has been reported for the presence of flavonoid compounds (Leone *et al.*, 2016; Lin *et al.*, 2018). The most widely studied compounds in *M. oleifera* are flavonoids (Hamany Djande *et al.*, 2018). The flavonoid content (quantity) and composition in the plant differ with the planting location and the cultivar studied. For instance, Mukunzi *et al.* (2011) reported a total flavonoid content of 52.78 mg Rutin Eq/g for dry leaves of *M. oleifera* planted in Rwanda and 39.08 mg Rutin Eq/g China. The flavonoid profile composition of *M. oleifera* also differs with the planting location, for example, Makita *et al.* (2016) identified fourteen compounds in *M. oleifera* sourced from South Africa while ten compounds were reportedly present from samples sourced from Namibia. The major flavonoid components of the plant are apigenin, quercetin, isorhamnetin and kaempferol (Lin *et al.*, 2018b; Prabakaran *et al.*, 2018). The flavonoid component of *Moringa* is higher than turnip, cabbage, peas and carrots (Sultana and Anwar, 2008).

Flavonoids in *M. oleifera* has strong antioxidant activit as shown in mouse models exhibiting strong antioxidant activity of *M. oleifera* flavonoids because of their ability to scavenge free radicals and chelate metal ions (Makita *et al.*, 2016b; Jahan *et al.*, 2018; Lin *et al.*, 2018). The numerous dietary and associated health benefits of flavonoids in the plant is crucial and hence must be properly delivered in the best possible way for human consumers.

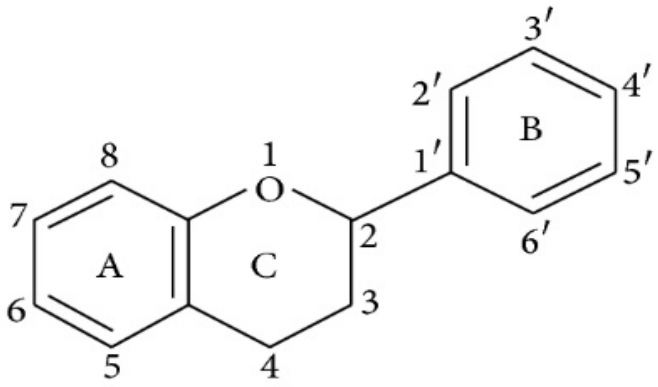


Fig. 2.4 – Structure of Flavonoid, adapted from Kozłowska and Szostak-Węgierek, (2019)

2.4 Extraction of phytochemicals and bioactives of *Moringa oleifera*

Generally, phytochemicals are extracted from plants to separate the active and core compounds from other components of the plant. This is done to separate the metabolite components of the plant from pulp, insoluble and residual components (Azwanida, 2015).

Table 2.5 – TPC (mg GAE/g) **and TFC (mg QE/g) of some parts of *M. oleifera* using different solvents**

Plant	Acetone		Ethanol		Methanol		Aqueous	
Part	TPC	TFC	TPC	TFC	TPC	TFC	TPC	TFC
Leaf	89	40	90	55	112	62	102	69
Flower	45	19	55	19	68	30	45	19
Seed	20	10	28	14	42	11	25	12

TPC: Total phenolic content TFC: Total flavonoid content, Adapted from Prabakaran *et al.* (2018)

Essential compounds in *M. oleifera* have been extracted using different methods and solvents (Prabakaran *et al.*, 2018), for example, acetone, ethanol, methanol and water were used for extracting some parts of the moringa plant (Prabakaran *et al.*, 2018; Oguntibeju *et al.*, 2019). The properties of some of these solvents vary and as such can affect extraction yield, phytochemical components of extracts and consequently the antioxidant activity of these extracted compounds. A study by Prabakaran *et al.* (2018) determined the phenolic and flavonoid content of leaf extracts of *M. oleifera* using acidified ethanol, acetone, ethanol, methanol and water varies significantly (Table 2.5), with variations likely associated with inherent quality attributes of the solvents such as polarity.

Various methods of extraction have also been used over the years, which include; percolation, maceration, decoction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical fluid extraction (Azwanida, 2015; Rodríguez-Pérez *et al.*, 2015; Zhong *et al.*, 2018; Dadi *et al.*, 2019c). The crude extracts from the plant using solvents such as ethanol and methanol had effective *in vivo* antioxidant activity when tested using mouse models (Padayachee and Baijnath, 2020). Ethanol extracts of the moringa seeds were reported to contain phytochemicals such as glycosides, alkaloids, etc., when profiled using thin-layer chromatography (Padayachee and Baijnath, 2020). The antioxidant potential of the methanol pod extract was reported to have potent free radical scavenging activity comparable to vitamin C (Mukeshbai, 2011). The leaf extract was also found effective as a natural anti-apoptotic and

anti-inflammatory therapy against liver and kidney diseases (Lin *et al.*, 2018; Oguntibeju *et al.*, 2019).

2.5 Encapsulation technology

Over the years, the use of biologically active compounds in enhancing the nutritional and functional property of foods has been limited (Mahfoudhi *et al.*, 2016), due to active compounds such as polyphenols becoming unstable in the presence of certain factors such as pH, oxygen heat, light etc. (Cheynier, 2005; Comunian and Favaro-Trindade, 2016; Ballesteros *et al.*, 2017b; Gómez *et al.*, 2018). Encapsulation has been found efficient in the delivery, stability and controlled release of active compounds. Encapsulation is a technique that results in the coating/entrapment of a material or mixture of materials in another material or system to offer adequate protection (Madene *et al.*, 2006; Gharsallaoui *et al.*, 2007; Poshadri and Kuna, 2010; Poornima and Sinthya, 2017). The active or core compounds are usually entrapped in another material known as coating, shell, carrier, or wall material (Madene *et al.*, 2006; Poshadri and Kuna, 2010a; Nedovic *et al.*, 2011; Saifullah *et al.*, 2019). These coating materials are usually polymers.

Generally, entrapped capsules have very small diameters that may be micro or nanometre-sized (Zuidam and Nedović, 2010; Drosou *et al.*, 2017; Poornima and Sinthya, 2017). Most encapsulation procedures are based on drying processes while others are based on chemical processes (Nedovic *et al.*, 2011; Ballesteros *et al.*, 2017). It is important to protect and preserve active agents present in many food products to maintain the functionality and stability of these compounds. Hence, this technology has given rise to the development and application of functional food products in the world food market (Schrooyen *et al.*, 2001; Silva *et al.*, 2015). In the pharmaceutical and food industries, encapsulation technology has been regularly applied due to its ability to offer sufficient protection to active agents from inactivation, increasing the stability of bioactive compounds (Rahmam *et al.*, 2016). Encapsulation can significantly improve the dispersibility of active compounds (Bao *et al.*, 2019), enhance their bioavailability, and improve the controlled release of bioactive compounds (Mahfoudhi *et al.*, 2016; Rahmam *et al.*, 2016). The technology also helps to shroud distasteful flavours, bitter taste and unpleasant aromas in foods resulting from some of these functional compounds (Nedovic *et al.*, 2011; Ballesteros *et al.*, 2017). Since different studies have shown the potential of this technology to help in the masking of unpleasant aromas and flavours, the chances of the acceptability of food products that contain these functional compounds is heightened.

Furthermore, to minimize and avoid losses during processing, bioactive, flavour and volatile materials in foods are entrapped in protective coatings called carriers. It was also reported that to limit the degradation or loss of aromas and flavours during processing and

storage, it becomes pertinent and needful to provide an efficient protective coating on volatile ingredients before being incorporated into foods and beverages (Madene *et al.*, 2006). Several encapsulation technologies have been put into application in the food and pharmaceutical industry, although only a few have found application in the food industry. It is pertinent to fully harness the potential of this technology for the delivery of these core materials. Despite the many encapsulation techniques that have been identified in literature, none has been considered widely and generally accepted for the protection of all bioactive compounds in foods. This is because each of these components has its own unique and peculiar characteristic feature ranging from polarity to molecular weight (de Vos *et al.*, 2010).

Nedovic *et al.* (2011) reported that the encapsulation of materials is mostly from liquid form to dried powders, therefore many of the techniques used for this process are usually based on drying. Examples of such encapsulation techniques include spray-drying, fluidized-bed coating, freeze-drying, coacervation etc (Ballesteros *et al.*, 2017). Spray drying is the most widely used encapsulation technology in the food and pharmaceutical industry because it is cheap and flexible (Ballesteros *et al.,* 2017), however, it has a major disadvantage in that it affects the retention of heat-labile and sensitive bioactive compounds (Ballesteros *et al.*, 2017). Cilek *et al.* (2012) however, posited that freeze-drying is suitable for drying heat-sensitive functional components because it keeps the initial properties of the compound.

In the encapsulation of bioactive components, the carrier material is as important as core materials. Benelli and Oliveira, (2019) posited that some materials including; gums, lipids, carbohydrates (majorly polysaccharides), proteins, alginate, cyclodextrins and other natural and synthetic polymeric materials have found wide use as encapsulating agents. The choice of which carrier material and technique to use is dependent on several factors, for instance, in choosing a carrier medium, one must put the following into consideration; the kind of protection the encapsulate offers to the resulting product of encapsulation, likely constraints for the wall/carrier material which may include the kind of encapsulation technique chosen, the concentration of the microcapsules formed, the kind of release and bioavailability desired, the stability of active agents as well as cost implications (Nedovic *et al.* 2011). Similarly, Devi *et al.* (2017) reported that the efficiency of encapsulation depends on the coating or carrier material used which subsequently determines its thermal and storage stability as well as the solubility property and release at the site of action. In all, it is important to protect and prevent functional and active components of food from degradation and inactivation as masking off-flavours present in these materials helps to increase the acceptability index among consumers as well

as offering them beneficial materials essential for wellness and good health. Since encapsulation gives an effective method of covering an active compound with protective wall coatings or material, hence, this technology offers a wide array of advantages (Nedovic *et al.* 2011), Table 2.6 presents different encapsulation method and procedures together with their associated advantages and disadvantage.

2.5.1 Spray drying

Spray drying was reported as one of the most widely used techniques of encapsulation in the food industry (Nedovic *et al.,* 2011). It is the most used method of encapsulating food ingredients and biologically active compounds present in foods (Drosou *et al.,* 2017). Loughrill *et al.* (2019), stated that spray drying has been used in the food industries for the encapsulation of oils, phenols, antioxidants and flavour compounds obtained from plants and other food commodities (Table 2.6). The use of spray drying in the pharmaceutical and biopharmaceutical processing is on the high side because it is seen as an effective drying and formulation technology (Ziaee *et al.*, 2019). The micro and nanocapsules fed into the dryer to give tiny droplets of particles of varying size.

Usually spray dryers are classified based on the pattern of flow during drying in the spray drying chamber which is an important factor defining the air-droplet interaction (Ziaee *et al.*, 2019). The four major components of a typical spray dryer are; the drying chamber (where drying of liquids takes place), the atomizer (also known as the nozzle, where the liquid substances are atomized), the aspirator, and collection cyclone (where the dehydrated particles are disembodied from the outlet gas stream) (Ziaee *et al.,* 2019). The coating material is prepared in a homogenizer, a prescribed amount of the coating material powder is dissolved in a known quantity of deionized water and then mixed accordingly. The active or core material is then mixed with the aqueous wall material mixture and the resultant substance which is usually an emulsion is then fed into the spray dryer (Chuyen *et al.*, 2019). The method described here was used in the encapsulation of carotenoid-rich oil extract from Gac peel (Chuyen *et al.*, 2019), other authors have used similar methods in preparing spray-dried microcapsules. When compared to lyophilisation or freeze-drying techniques, it offers an economic advantage as it is 4 – 7 times less than the cost of freeze-drying (Eratte *et al.*, 2018). However, in terms of efficiency, it is not as efficient as the latter (Ziaee *et al.*, 2019).

A disadvantage of this method is that it can lead to the deterioration of heat-labile compounds. It can also result in the collapse of the encapsulant carrying the active compounds

(Coronel-Aguilera and San Martín-González, 2015). Observations from the experiment of Chuyen *et al.* (2019) in the encapsulation of oil-rich carotenoid extracts from Gac peel using spray drying shows that low inlet temperature with high feed rate resulted in a final product that is inadequately dried and as such stuck to the spray drying chamber. This result was in tandem with Pellicer *et al.* (2019) who reported that the pump/flow rate of the feed and inlet temperature affected the drying time, with a high flow rate and low temperature requiring a longer drying time. Chuyen *et al.* (2019) reported that when a high inlet temperature with a low feed rate was used, a dried product was obtained, although this resulted in the degradation of the carotenoids present in the encapsulated microcapsules. This shows that the inactivation of the active agents may occur at a very high temperature because of the heat-labile nature of these materials.

A predictive model was then developed in the study of Chuyen *et al. (*2019) which gave an optimum spray drying condition that includes an inlet temperature of 160 ℃, a feed rate of 180 mL/h, emulsion composition of 25% total solid with the ratio of active agent to wall material being 3:10. This resulted in a product that has 80% carotenoid retention and 82% antioxidant capacity when compared to the preliminary experiment where degradation of active agents and inadequately dried emulsion was found to be a problem (Chuyen *et al.,* 2019). However, Choińska-Pulit *et al.* (2015) reported that air inlet temperature that exceeds 120 ℃ and outlet temperature above 60 ℃ was found to reduce the viability of probiotic bacteria. These values are lower than the values reported as the optimum temperature in the predictive model stated earlier although feed rate values were not reported for the latter which may account for discrepancies in values. Discrepancies may also result from the difference in the core materials being encapsulated as microorganisms tend to be more temperature-sensitive.

Table 2.6 Encapsulation technologies and their applications

Encapsulation technology	Previous application	Advantages	Disadvantages	References
Spray drying	Encapsulation of strawberry Encapsulation of carotenoid from gac-peel Retention of saffron bioactive components Microencapsulation of natural anthocyanins Encapsulation of eggplant peel extract	High encapsulation yield Excellent entrapment efficiency	Not suitable for heat-labile compounds	(Pellicer *et al.*, 2019) (Chuyen *et al.*, 2019) (Rajabi *et al.*, 2015) (Akhavan Mahdavi *et al.*, 2016) (Sarabandi *et al.*, 2019)
Freeze drying/lyophilization	Microencapsulation of phenolic compounds from sour cherry pomace Encapsulation of brown seaweed pigment Microencapsulation of grape skin phenolic extracts Encapsulation of non-dewaxed propolis Encapsulation of grape seed extracts	Effective for the drying of heat-sensitive substances.	Longer dehydration period Not cost-effective	(Cilek *et al.*, 2012) (Indrawati *et al.*, 2015) (Kuck and Noreña, 2016), (Šturm *et al.*, 2019) (Yadav *et al.*, 2019)

Complex coacervation	Microencapsulation of Palm oil using Chitosan/Pectin wall material Encapsulation of cinnamaldehyde using gelatin/pectin complex	High encapsulation efficiency and yield. Good particle size distribution. High retention index in the encapsulation of carotenoids. Improved thermal stability		(Jia *et al.,* 2016) (Rutz *et al.,* 2017) (Muhoza *et al.* 2019)
Spray chilling	Encapsulation in the pharmaceutical, flavour and food industries Encapsulation of lipid-coated microcapsules Encapsulation of minerals, vitamins and acidulants.	Inexpensive compared to freeze-drying High process efficiency Excellent entrapment index		(Nedovic *et al.*, 2011) (Arslan-Tontul and Erbas, 2017) (Poornima and Sinthya, 2017)
Fluidized bed coating	Encapsulation of Spray-dried β – carotene emulsion	Short encapsulation time. Controllable and makes automation easy. Formation of uniform layers around the core.		(Coronel-Aguilera and San Martín-González, 2015) (Poornima and Sinthya, 2017)
Electro spraying	Encapsulation of oils in the industry. e.g Peppermint oil	Efficient and viable		(Rahmam *et al.*, 2016)
Coacervation	Microencapsulation of Oxalic acid	Completely entrapment Excellent encapsulation efficiency – 99%	Imperfect micro-particles. Costly. Sensitive to pH and ionic strength	(Jia *et al.*, 2016) (Meng *et al.*, 2017)

Both studies indicated that encapsulation efficiency and retention of active agents is dependent on the operating condition which includes, temperature and the feed or pump rate of the spray dryer. The optimization, however, did not state the role of the active agent and the carrier material in maintaining stability and retention. Because of the variation in values reported for encapsulation efficiency in the studies stated above, further works on the best process conditions for encapsulation via spray drying should be embarked upon so that they can be adapted for use in the industry.

Dadi and Emire, (2019) reported an optimum inlet temperature of spray drying of 140 ℃ in the encapsulation of *M. stenopetala* extracts with maltodextrin and high methoxyl pectin (MD-HMP) complex. Other factors that contributed to the reported optimum condition include; core coating ratio and MD-HMP complex ratio. In addition, Dadi *et al.* (2019) stated that spray drying conditions harmed physical properties of microcapsule powders such as flowability. This report validated earlier reports by Akhavan Mahdavi *et al.* (2016) where spray drying and encapsulation conditions affected the physical properties of the powder, thereby resulting in poor flowability of powder from all samples.

Several studies have been done using spray drying and although reports of its wide use in the pharmaceutical and food industry are high, and its economic importance reported, its efficiency in the encapsulation of active agents that are sensitive to high temperature is still a cause for concern and future studies on this must focus to address this unevenness. Other studies where spray drying was used includes but not limited to the following; retention of saffron bioactive components (Rajabi *et al.*, 2015), microencapsulation optimization of natural anthocyanins (Akhavan Mahdavi *et al.*, 2016), encapsulation of eggplant peel extract as a natural antioxidant and color source (Sarabandi *et al.*, 2019) amongst others.

2.5.2 Spray chilling

Spray chilling is a multifunctional encapsulation technology for capturing active compounds under processing conditions that are not severe, resulting in increased stability of active compounds (Yin and Cadwallader, 2019). It is a technique of encapsulation whose use has found a wide range of applications in encapsulating drug molecules with minute details and in the production of nutraceuticals (Tomšik *et al.*, 2019). It has also found wide application in the flavour industry (Yin and Cadwallader, 2019), and currently gaining ground in the food industry (Tomšik *et al.,* 2019). In spray chilling the active agent is fused with the coating material and then atomised using chilled air, hence it differs from spray drying where heated air is used (Poshadri and Kuna, 2010).

Similarly, Poornima and Sinthya, (2017) opined that the active material and coating mixtures are atomised using chilled air, causing the wall to form a solid material around the active ingredient. In this technique of encapsulation, no heat is required because there is no water to be evaporated (Poornima and Sinthya, 2017). Nedovic *et al.* (2011) reported that spray chilling is widely used in the production of lipid-coated active agents. Similarly, Poshadri and Kuna, (2010), reported the use of hydrogenated or fractionated vegetable oil as outer material for spray chilling and more recently the use of hydrogenated palm oil as wall material was reported by (Arslan-Tontul and Erbas, 2017).

In terms of cost, it is the cheapest of all encapsulation technologies and has been used for the encapsulation of several organic and inorganic substances, including; vitamin, minerals, flavours and enzymes (Poshadri and Kuna, 2010). Arslan-Tontul and Erbas, (2017) reported similar efficiencies to freeze-dry, which is more expensive, during the encapsulation of probiotics, thus, it can be used as an alternative to freeze-drying based on cost. Carvalho *et al.* (2019) observed effectiveness and a high encapsulation efficiency value in their study on the characterization of microencapsulated ascorbic acid using spray chilling. Hence, it could be concluded that spray chilling is an effective method of encapsulation with high yield efficiency, low production cost as well as increased stability of core materials and as such could be considered for use commercially.

2.5.3 Freeze drying

Freeze drying is based on the principle of sublimation of water where there is no liquid-vapour phase during the processing (Cassanelli *et al.,* 2018). It is a popular process used in the encapsulation of heat-labile bioactive compounds (Pellicer *et al.,* 2019). This is because most biologically active compounds are heat-sensitive and may experience deterioration and inactivation during encapsulation using other methods that utilise heat. The powdered products obtained from freeze-drying usually have the following properties; low bulk density, high porosity good aroma and taste retention (Pellicer *et al.,* 2019). Although various studies have highlighted the benefit of this encapsulating procedure, however, a huge amount of energy consumed over a long period of processing time puts freeze-drying at a competitive disadvantage when compared with other techniques (Nedovic *et al.,* 2011).

According to Antonio *et al.* (2019), in a recent study on the stability of flavour compounds in strawberry, it was discovered that freeze-drying when compared with two other methods (spray and fluidized-bed coating) in the microencapsulation of the strawberry flavour, was best.

Also, in the encapsulation of phenolic compounds present in spent coffee ground extracts by freeze and spray drying, freeze-drying with 100% maltodextrin as coating material resulted in microcapsules with improved physico-chemical properties (Ballesteros *et al.,* 2017). The quantity of active compounds retained after the encapsulation procedure indicated effectiveness in the encapsulation of phenolic and flavonoid compounds (Ballesteros *et al.,* 2017). The results of this study were in agreement with the result of Ramírez *et al.,* (2015) in the modelling and stability of polyphenols in dried fruits where varying proportions of wall materials were used. The highest content of the phenolic compounds reported in this study was obtained using freeze-drying and a wall material containing maltodextrin of 100%. Hence the encapsulation efficiency and yield of this technique are largely dependent on the drying technique and wall material used. If this technique is to be used on a large scale, its economic implications must be put into consideration. Furthermore in a study conducted by Pellicer *et al.* (2019) on the stability of encapsulated strawberry comparing freeze-drying, spray-drying and fluidized bed coating, the authors reported in this study that the freeze-dried microcapsules had the highest drying yield of 87-88% significantly higher than spray dried (68%) and fluidized bed coating microcapsules (38%) which show that freeze-drying has an advantage over spray drying and fluidized bed coating in terms of drying efficiency and yield underlines the effectiveness of this technology in producing stable microcapsules. The result reported for drying yield was further reinforced by results from the study of Darvishi *et al.* (2012) who reported a similar value (77%) though a little lesser when *Yarrow lipolytica* lipase was freeze-dried. The results of these works reported pointed solely to one thing which is the effectiveness and high yield of this technology. Other studies on freeze-drying include microencapsulation of phenolic compounds from sour cherry pomace (Cilek *et al.*, 2012), encapsulation of brown seaweed pigment (Indrawati *et al.*, 2015), microencapsulation of grape skin phenolic extracts (Kuck and Noreña, 2016), limonene encapsulation in freeze-dried gellan systems (Evageliou and Saliari, 2017), encapsulation of non-dewaxed propolis (Šturm *et al.*, 2019) and encapsulation of grape seed extracts (Yadav *et al.*, 2019).

2.5.4 Electro-spraying

The potential of electro-spraying in the encapsulation of compounds in the food industry is still underexploited, although it has been used in the production of some microcapsules of proteins (Xu *et al.*, 2006). This process is otherwise known as electrostatic extrusion (Xu *et al.*, 2006).

Electro-spraying as a method of encapsulation makes use of very high voltage electrically charged jets from polymer solutions which become visco-electric when dried (Drosou *et al.*, 2017). This occurs by evaporating the solvent, which in turn produces polymeric structures of different sizes and properties (Drosou *et al.*, 2017).

Electro-spraying has been reported as an alternative method of encapsulating core compounds which is viable and effective because it does not make use of high temperature like spray drying (Rahmam *et al.,* 2016). Additionally, the production of well-dispersed droplets for high encapsulation yield was reported (Rahmam *et al.*, 2016) and hence can be considered for use on a large scale because of its efficiency and viability. This technology has been recommended and proposed for wider use by Koo *et al.*, (2014) in the encapsulation of oils and essential oils in the food industry. These authors also reported that the use of this technique makes the production of microcapsules of peppermint oil easy because the oil was properly entrapped without leaking. The apparatus used for the process of electro-spraying has a capillary which pumps the in-feed emulsion at a constant rate, an electricity source with voltage measurement of 1-30 kV and a collector that is attached to the counter electrode (Jia *et al.*, 2016).

2.5.5 Coacervation

Coacervation is a method of encapsulation that separates the liquid phase of a material used for coating in a polymeric solution (Poornima and Sinthya, 2017), it is a liquid-liquid phase transition procedure (Fang *et al.*, 2018). This encapsulation procedure has been around for a long time and has been used for the manufacture of several products in the food industry (Park and Yeo, 2007). In this process, a concentrated phase is formed, which results from the phase separation of macromolecules (Fang *et al.*, 2018). It is dependent on various factors which include; temperature, concentration, or the combination of both factors resulting in the separation of the components from the solvent thereby resulting in the formation of a condensed phase (Fang *et al.*, 2018). In using this technique, a change in pH results in the formation of a shell by a complex polymer, it makes use of fluid-fluid phase separation of an aqueous polymeric solution (Nazzaro *et al.*, 2012). Hence can be termed a chemical method of encapsulation.

Coacervation as a method of encapsulation can be fully explored through the use of electrostatic interactions, although water-repelling interactions are involved (de Vos *et al.*,

2010). The advantage of this technique is that the core material is completely entrapped by the carrier matrix which offers sufficient protection and barrier against external effects that may result in deterioration (Poshadri and Kuna, 2010). This method of encapsulation is said to be a promising with its potential yet to be fully harnessed in the food industry even though it has an encapsulation efficiency of 99% (Jia *et al.*, 2016). Although this method of encapsulation was reported to be expensive and sensitive to pH and ionic strength (Jia *et al.,* 2016), a major drawback and disadvantage to its use on a commercial scale. Several studies have used coacervation as a technology for the entrapment of biologically active compounds.

2.5.6 Complex coacervation

Complex coacervation is a physico-chemical method of encapsulation. In this technique, electrostatic attraction occurs between two oppositely charged molecules (Rutz *et al.*, 2017; Fang *et al.*, 2018) which results in the production of complex polymers that are positively charged (Tavares and Noreña, 2019). It has found wide use in the encapsulation of lipid compounds such as essential oils, vegetable oils and palm oil (Rutz *et al.*, 2017) and encapsulation of garlic extracts (Tavares and Noreña, 2019) amongst others. It was also reported that there is an increased interest in the study of complex coacervation technique of encapsulation using polysaccharides and proteins (Devi *et al.*, 2017), this is due to the efficiency of the method.

In complex coacervation, the coating materials are combined for better output in terms of encapsulation efficiency and yield. The technique was found to be an effective method of entrapping core materials present in palm oil using chitosan/pectin and chitosan/xanthan as carriers (Rutz *et al.*, 2017). The encapsulation efficiency and yield were high with a high retention index for the carotenoid content present in the palm oil. When used in the encapsulation of cinnamaldehyde using gelatin/pectin complex, it shows high coacervate yield and good particle size distribution (Muhoza *et al.*, 2019). Additionally, thermal gravimetric analysis of the resultant microcapsules show an increased degradation temperature (Muhoza *et al.*, 2019). This technique was reported to be excellent for curcumin entrapment, improved light and thermal stabilities of encapsulates when ovalbumin/κ-carrageenan were used as complex carriers (Xie *et al.*, 2019). Additionally, complexing protein with polysaccharides as wall materials has been reported to be of immense benefits to protein encapsulants as it reduces the effect of enzymatic degradation on the protein wall material as well as improve the mechanical and release characteristics of the formed capsules (Jia *et al.*, 2016).

Complex coacervation has also been used in the encapsulation of garlic extracts using whey protein isolate (WPI) and chitosan (Ch) as coating material, the optimal coacervation ratio of Ch to WPI was recorded at 0.2:1 with resultant microparticles highly hygroscopic indicating that they needed to be adequately packaged to prevent the absorption of moisture (Tavares and Noreña, 2019). Additionally, the solubility attributes of the microparticles were reportedly high and this has been attributed to the use of WPI which has a good solubility characteristic that makes their use in the production and development of food products important. Overall, the results obtained from FTIR shows that there was an interaction between extracts and wall material. Scanning electron microscopy (SEM) images also show that there were no cracks noticed in the microparticles indicating the good protection capacity the coacervates had on the extracts (Tavares and Noreña, 2019). Hence, the method of encapsulation can be considered for use in the encapsulation of other plant extracts.

2.5.7 Fluidized bed coating

Fluidized bed film coating is a method used in coating membranes as well as coating of bioactive compounds to give an output that is microporous in structure, and has found wide use in the chemical, biochemical and pharmaceutical industries (Capece and Dave, 2011). This process has been critically checked, touted, and found effective for its suitability in the encapsulation of bioactive compounds, although it is believed to be a viable alternative to the spray drying method of encapsulation. It involves a multi-phase procedure which is done simultaneously and with mass transfer and fluid dynamics (Benelli and Oliveira, 2019). The method is used in large batches for the coating of materials and it was discovered that encapsulation of particles occurs in a short space of time (Capece and Dave, 2011). It is controllable, thus making automation easy (Capece and Dave, 2011). Although a high heat and mass transport exerted may result in the inactivation of heat-labile active compounds.

During this procedure, an emulsion containing the wall and core material is spray-dried to produce a coated microcapsules powder in a fluidized bed (Coronel-Aguilera and San Martín-González, 2015). Some factors are considered because they affect the agglomeration (the formation of mass heaps) and formation of film particles which influences the efficiency of the coating, such parameters include; the solid circulating rate, pressure of the atomizing nozzles, coating temperature, humidity and the feed rate (Coronel-Aguilera and San Martín-González, 2015).

This coating technology results in the production of a uniform layer around the particles to be coated, this is because the coating agent is deposited on the surface of these particles which in turn results in increased protection of active ingredients as well as providing an adequate barrier between the active materials and the surroundings (Coronel-Aguilera and San Martín-González, 2015). This technique has found wide applications in industries particularly for the coating of solid particles such as pellets, granules and powders (Pellicer *et al.*, 2019).

2.6 Wall/Coating material for encapsulation

Food biopolymers in recent times have been used in entrapping active compounds during encapsulation in the food and drug industry (Labuschagne, 2018). The selection of suitable wall material is essential to protect the core material from deterioration (Poshadri and Kuna, 2010). Wall materials used in encapsulation primarily serve the purpose of protecting bioactive, enzymes, probiotics and flavour compounds in the food industry (Pellicer *et al.*, 2019). These materials carry out this protective role by providing a barrier between the active materials and their surroundings (Pellicer *et al.*, 2019). Some of the most widely used encapsulating agents include; maltodextrins, gum Arabic, modified starches, alginates, gelatin, pectin, carrageenan, carboxyl methylcellulose, xanthan and cyclodextrins (Pellicer *et al.*, 2019).

Wall materials used for encapsulation must be of food-grade and must not react unfavourably with the active/core material (Labuschagne, 2018). Additionally, materials to be considered as wall materials should have no odour, be water retaining and tasteless (Khanvilkar *et al.*, 2016). The wall materials that have been used in encapsulation include carbohydrates (majorly polysaccharides), protein and gums (Labuschagne, 2018). Proteins such as pea protein isolates and soy protein isolates have been used in encapsulation and their effects on the resulting micro-particles have been investigated (Osamede and Kaewmanee, 2019). Results from the study showed the effectiveness of vegetable proteins in encapsulating bioactive compounds and a comparative study involving the two wall materials indicated that soy protein isolate was better in terms of the physico-chemicak properties (Osamede and Kaewmanee, 2019). Some wall materials are soluble and while others are insoluble in water. Soluble wall materials can dissolve, react/interact with water to produce a viscous mixture (Taheri and Jafari, 2019). They are also known as hydrocolloids which have been described as group chain polymers that have the property of forming solutions or gels when dispersed in water (Milani and Maleki, 2012).

2.6.1 Hydrocolloids

'Hydrocolloid' as a term finds its root from the Greek lexicons, hydro meaning 'water' and Kolla referring to 'glue', they are ingredients used in the food and pharmaceutical industry that forms colloidal solutions in an aqueous phase (Yousefi and Jafari, 2019), which may be a result of the hydrophilic (water-loving) parts of their structure (Yousefi and Jafari, 2019). They have also been defined as a diverse group of long-chain polymers that are readily dispersive, fully or partially soluble, and prone to swell in water, they are among the most commonly used ingredients in the food industry and serve diverse purposes ranging from thickening, gelling, clarifying, clouding, whipping and flocculating agents, emulsifiers, stabilizers, fat replacers amongst others to encapsulating materials used in the encapsulation of flavour compounds (Li and Nie, 2019).

Li and Nie, (2019), posited that this group of polymers changes the physical characteristics and properties of solutions to form gels, thickeners, emulsifiers, coating materials, and stabilizers. A major reason for their use in the food industry lies in their capacity to alter the rheological characteristics of food systems (Milani and Maleki, 2012). They have been in use and widely studied for many years due to their functional and nutritional properties (Li and Nie, 2019). Their wide use in the food industry is due to their ability to bind with water and then alter the properties of ingredients present in foods (Li and Nie, 2019). Benelli and Oliveira, (2019), reported that hydrocolloids include; gums, lipids, carbohydrates (majorly polysaccharides), proteins, alginate and other natural and synthetic polymeric materials, although Burnside, (2014) reported gum Arabic and sodium alginate as the most studied of them all.

Their use as encapsulating agents may alter and disrupt the physical and sensory characteristics of the food systems they are being incorporated into hence, this must be taken into consideration during their selection (Yousefi and Jafari, 2019). Gao *et al.* (2017), reported that hydrocolloids can significantly affect the structure and functional properties of foods. This is because of their reaction with other components present in complex food matrices, they also reported that these compounds play a very important role in the final outlook of food structure and their stability which lend further credence to the very fact that they must be carefully selected considering factors that enable stability during and after the process.

Additionally, when selecting these compounds, their capacity to disperse the core/active material and give enough protection and stability to the resulting product is essential (Nizori *et al.*, 2018). Hence, the kind of active material to be encapsulated and the

encapsulation technology employed determine the selection of hydrocolloids and their reaction in food systems. A hydrocolloid used as a coating/wall material in encapsulation should possess good flow properties, additionally, ease of handling during the process of encapsulation should be of paramount interest (Nizori *et al.*, 2018). Furthermore, the kind of wall material selected for use in the encapsulation of active compounds was reported to have a direct effect on the stability of emulsion and the resultant micro-particles after drying and during the storage stage (Carneiro *et al.*, 2013). Several properties of the in-feed emulsion such as oxygen stability, viscosity, density etc. are largely influenced by the coating material selected for use (Carneiro *et al.*, 2013).

Certain wall materials have also been combined in encapsulation, for example, Devi *et al.* (2017) reported in their review the use of complex coacervates of polysaccharide-protein which was found to be among the widely used pair of biopolymers with their use gaining ground in the last decades in encapsulating various active ingredients. Additionally, the use of biopolymers that are natural as wall material in the entrapment of active agents present in plants is essential and widely studied because of their safety and ease of degradation in the gastrointestinal system (Xie *et al.*, 2019). Ovalbumin/κ-carrageenan complex has shown excellent potential as wall material in the nano-encapsulation of curcumin, a polyphenolic compound present in turmeric. It was observed that the complex offered a good protective coating, increased the antioxidant activities as well as thermal and light stability of the core material, although it was reported to have a low release rate in simulated gastric fluids (Xie *et al.*, 2019).

Gelatin and pectin complex coacervates were also used as carriers for cinnamaldehyde in a study conducted by Muhoza *et al.* (2019) where the encapsulation efficiency ranged from 85.2 to 89.2% and a high retention rate observed. Thermogravimetric analysis show that the complex coacervates greatly increased the inactivation temperature of cinnamaldehyde from between 180-220℃ to 400℃. This highlights the ability of the complex wall material formed to increase the thermal stability of the active agents they serve to protect. Chitosan/xanthan and chitosan/pectin were used in the encapsulation of palm oil using lyophilization by Rutz *et al.* (2017) which resulted in higher carotenoid retention, higher encapsulation efficiency and yield and its release in gastric fluids and water were found to be satisfactory. All these authors have reported good retention of core materials, high encapsulation efficiency and yield for active agents encapsulated with complex wall materials.

Gums are important hydrocolloid groups that are hydrophilic. When they are dispersed in this substance, they form a colloidal solution (Taheri and Jafari, 2019), gums including locust bean gum, carrageenan, xanthan, and gum Arabic have been used in the food industry, others include; Ghatti, Karaya, and Tragacanth (Taheri and Jafari, 2019). The efficiency of gum Arabic as a wall material for carrying active compounds is a result of its excellent solubility and poor viscous property in aqueous solutions, particularly when compared with other wall materials (Tirgar et al., 2015) such as soy and pea proteins, maltodextrin etc. Alginates and pectin improved the physical and chemical stabilities of anthocyanin (Gao et al., 2017). Cyclodextrin is the resultant effect of enzymatic modification of starch (Labuschagne, 2018), with the most commonly used and widely studied cyclodextrin being β – cyclodextrin (dos Santos et al., 2017).

Maltodextrin is also a modified starch that has found application in the food industry for coating compounds (Pellicer et al., 2019). It was reported for its ability to increase the stability of microcapsules, while gum Arabic was reported as a suitable coating material due to its low viscosity and good emulsifying capacity in the encapsulation of phenolic compounds (Cilek *et al.*, 2012). Tirgar *et al.* (2015) also reported that maltodextrin dissolves excellently in water, possesses low viscous property, exhibits poor retention property for volatile compounds and when used in the encapsulation of active compounds in foods lessens the level of undesirable reactions. Soy and pea protein isolates (SPI and PPI) have been used in the microencapsulation of Moringa and it was reported that the choice of wall material has a significant role to play on several parameters that were analyzed for. Soy protein isolate was reported to effectively mask the green colouration of Moringa than PPI (Airouyuwa and Kaewmanee, 2019). Microparticles that have high solubility were observed in a study on the encapsulation of garlic extract using whey protein concentrates /Chitosan (WPC/Ch) complex as wall materials, this was so because of the presence of whey protein concentrate in the wall material complex, which was reportedly soluble in water (Tavares *et al.*, 2019)

It is essential to critically and carefully select suitable wall materials during encapsulation for good encapsulation efficiency, high yield and better retention of active agents as well as stability and maximum protection. The summary of some major wall materials used for encapsulation is detailed in Table 2.7.

2.6.1.1 Maltodextrin

Maltodextrin (MD) is a carbohydrate compound obtained from partial hydrolytic modification of starches using acids or enzymes under a controlled condition (Castro *et al.*, 2016). They are classified based on their dextrose equivalent values (DE) (Pycia *et al.*, 2016). Dextrose equivalents are values that indicate the quantity of reducing sugars that are present in starch polymers (Barthold *et al.*, 2019). Because of some of their characteristic features of being odourless, tasteless and colourless, they have been widely used in the food industry as wall materials for the encapsulation of biologically active compounds (Castro *et al.*, 2016). Important properties of this coating material that make it suitable for selection includes their safety and optimum solubility, their biological compatibility was also found to be a factor that informs their choice and use as coating materials for biologically active compounds (Sarabandi *et al.*, 2019). Additionally, their low cost puts them ahead of other hydrocolloids (Marques *et al.*, 2014). In addition to their use as wall materials in the process of encapsulation, they have been used as texturizers, thickening agents and fat replacers (Pycia *et al.*, 2016). Maltodextrin has been used in different studies for the encapsulation of plant bioactives. They include encapsulation of natural anthocyanin compounds (Akhavan Mahdavi *et al.*, 2016), encapsulation of non–dewaxed propolis (Šturm *et al.*, 2019), saffron petal's anthocyanin (Mahdavee Khazaei *et al.*, 2014), brown seaweed pigment (Indrawati *et al.*, 2015), flaxseed oil (Carneiro *et al.*, 2013), antioxidant from spent ground coffee (Ballesteros *et al.*, 2017), phenolic compounds derived from cherry pulp (Cilek *et al.*, 2012) and rosemary essential oils (Turasan *et al.*, 2015) to mention a few.

When maltodextrin was used alongside gum Arabic in the encapsulation of anthocyanin in saffron petal, the water activity and moisture content of the resulting powder using 100% maltodextrin (M20) was higher than that of gum Arabic. Besides the higher water activity and moisture level, the ability of the encapsulated powder to absorb and exude water known as hygroscopicity was higher in micro-particles encapsulated with MD (Mahdavee Khazaei *et al.*, 2014). This result was in tandem with results of the study conducted on the encapsulation of eggplant peel extract by Sarabandi *et al.* (2019) where micro-particles from MD have higher water absorption tendencies than those from gum Arabic. This property can affect the shelf life of products as there is a correlation between high hygroscopic index, water activity, moisture content and the shelf life of products. The more the tendency of a food product to absorb water from the surrounding or exude water to the surrounding the more it becomes susceptible to spoilage and instability. Additionally, MD also has good entrapment efficiency, good

morphological characteristics. For example, Ballesteros *et al.* (2017) reported that MD exhibited spherical shape than gum Arabic and has particle sizes with a diameter of 30μm, the rounded shape obtained in the above study is similar to the shape reported in the study of Mahdavee Khazaei *et al.* (2014) in the encapsulation of saffron. The results reported for particles of microcapsules encapsulated using 100% M20 maltodextrin was evenly shaped with rounded corners. Spherical shapes of microcapsules help the coating to hold the component effectively consequently resulting in good encapsulation efficiency (Ballesteros *et al.*, 2017).

Table 2.7 Summary of some major wall materials used for Encapsulation

Wall Material	Source	Encapsulated compounds	Encapsulation Method	Observations	References
Whey protein isolate	Dairy milk waste	Grape seed polyphenols	Freeze drying	Encapsulation efficiency increased with addition of other wall materials.	(Yadav *et al.*, 2019)
		Sour cherry pomace	Freeze drying	Encapsulation efficiency is influenced by wall material and its formulation with other carriers.	(Cilek *et al.*, 2012)
		Garlic Extract	Complex coacervation	Microcapsules show good water solubility property.	(Tavares *et al.*, 2019)
		Bifidobacterium animalis	Spray drying	Exhibited good protective property when used with dextran conjugates.	(Loyeau *et al.*, 2018)
		Bilberry anthocyanin	Extrusion method	Good particle sizes obtained from whey protein gels.	(Betz and Kulozik, 2012)
Maltodextrin	Starch	*Moringa stenopelata* extracts	Spray drying	Lower digestibility when used alone but improved when added with HMP.	(Dadi *et al.*, 2019a)
		Eggplant extract	Spray drying	Improved final product and overall acceptability of the product.	(Sarabandi *et al.*, 2019)
		Grape polyphenols	Spray drying	Improved encapsulation efficiency and retention when combined with other carriers.	(Tolun *et al.*, 2016)
		Spent coffee ground	Spray and freeze drying	Good retention property of phenolic and flavonoid compounds.	(Ballesteros *et al.*, 2017)
		Saffron anthocyanin	Spray drying	Good stabilizing property of microcapsules.	(Mahdavee Khazaei *et al.*, 2014)

Gum Arabic	Tree exudate	Grape Polyphenols	Spray drying	Its use with maltodextrin resulted in good functional outlook of microcapsules and increased stability on storage.	(Tolun *et al.*, 2016)
		Grape seed polyphenols	Freeze drying	Blending with maltodextrin and whey protein concentrate increases release property and encapsulation efficiency.	(Yadav *et al.*, 2019)
		Sweet orange	Complex coacervation	Good flavour retention property.	(Jun-xia *et al.*, 2011)
		Spent coffee ground	Spray and freeze drying	Detrimental effect on the retention of coffee phytochemicals.	(Ballesteros *et al.*, 2017)
		Eggplant extracts	Spray drying	Noticeable influence on functional and physico-chemical properties of the microcapsules.	(Sarabandi *et al.*, 2019)

Additionally, values reported from this study indicated that the kind of material used as wall material affects the encapsulation efficiency and as such 100% MD used in the encapsulation of phenolic components present in spent coffee resulted in an excellent encapsulation efficiency of the particles (Ballesteros *et al.,* 2017). A 73% efficiency reported when MD was used in a study by Akhavan Mahdavi *et al.* (2016) indicated a high encapsulation efficiency when MD was combined with gum Arabic indicating that its efficiency as a wall material can be enhanced when it is used in combination with other wall materials. The antioxidant activity (A_a) and total phenolic content (TPC) has to do with surface phenolic content of the microcapsules. The better the entrapment potential of the core, the more the activity of the antioxidant when it is released. Sarabandi *et al.* (2019), reported the highest TPC and A_a for their study when 100% MD was used as the wall material, replacement of the carrier material with GA resulted in a significant decrease in TPC and consequently resulting in a decrease in A_a. This result is similar to the results reported by Ballesteros *et al.* (2017) for TPC and A_a when 100% MD was used with freeze drying. Hence, underlying the importance the choice of a suitable wall material for encapsulation plays in the process cannot be underestimated and reports from the foregoing has reinforced the efficiency and usefulness of MD as a suitable material for coating.

2.6.1.2 Gum Arabic

Gum Arabic (GA) is a natural sticky and gummy discharge from the stem of acacia tree, they are edible and contain non-viscous fibres (Ali *et al.*, 2009). Islam *et al.* (1997) in their review described it as the oldest and most popular and used exudate from trees that discharge gum. They are derived from *Acacia arabica* and *Acacia senegal* tree (Ahmad *et al.*, 2019). Structurally, it is said to contain six different moieties of carbohydrates which include; arabinopyranose, rhamnopyranose, arabinofuranose, galactopyranose, glucuropyranosyluronic acid and 4 – 0 methylglucuropyranosyl uronic acid (Islam *et al.*, 1997). The availability and cost of this wall material has been a cause for concern.

This polysaccharide compound cannot be digested by humans and can only be fermented in the large intestine by beneficial microbes to produce short-chain fatty acids (Ali *et al.*, 2009; Khalid *et al.*, 2014) and as such been classified as non-digestible carbohydrates otherwise known as dietary fibre (Khalid *et al.*, 2014). It has been used in the food industry as additives such as thickeners, stabilizers and emulsifiers (Ali *et al.*, 2009). In addition to its use as additives in the food industry, it has also been used as coating/wall material for the entrapment of core and active materials in plant extracts. Examples include their use as wall material alongside Maltodextrin (MD) and other wall materials in the encapsulations of bioactive components in saffron plant (Rajabi *et al.*, 2015), sweet orange oil (Jun-xia *et al.*,

2011), natural anthocyanin (Akhavan Mahdavi *et al.*, 2016), grape polyphenols (Tolun *et al.*, 2016), jussara pulp (Santana *et al.*, 2016), phenolic compounds in spent coffee (Ballesteros *et al.*, 2017b), walnut, peanut and pecan oils (Luna-Guevara *et al.*, 2017).

2.6.1.3 Whey protein concentrates and isolates

The by-products from cheese and rennet casein production in the dairy industry is known as whey protein and have been grouped based on their protein content into two, namely whey protein concentrates (WPC having 30 – 85% protein content) and whey protein isolate (WPI having a protein content > 90%) (Jiang *et al.* 2018). Whey protein concentrates constituents is given in the following proportion 7.2% immunoglobulins, 8.6% bovine serum albumin, 17.5% α-lactalbumin and 67.3% β-lactoglobulin, while WPI contains 6.9% immunoglobulins, 8.6% bovine serum albumin, 14.3% α-lactalbumin and 70.2% β- lactoglobulin (Teixeira *et al.* 2019). This protein has varying properties depending on the initial product, for example, a by-product in the production of cheese made from acidic coagulation of milk is known as acid whey (pH < 5.1) while the by-product obtained from cheese that is coagulated from rennet is known as sweet whey (pH > 5.6) (Teixeira *et al.* 2019). The low pH of acid whey makes it yield a more concentrated content of ash and calcium (Teixeira *et al.* 2019). Their use in the food industry includes the production of baby foods, baked products, emulsifiers, thickeners, in the production of fat spreads and in encapsulation where they serve as carriers for core materials (Jiang *et al.* 2018). In addition, they have good functional properties which can be further enhanced via Maillard reaction, a non-enzymatic browning reaction which has been found to enhance the functional properties of protein (Loyeau *et al.*, 2018). Its use as wall material in encapsulation includes; encapsulation of mulberry juice polyphenols (Khalifa *et al.*, 2019), *Bifidobacterium animalis* a lactic acid bacteria (Loyeau *et al.*, 2018) and garlic extracts (Tavares & Noreña, 2019). Its use as coating material in all of these studies was with other materials as seen in (Table 2.7).

Some properties of whey protein such as good protective effects, high product yield and easy flow have enhanced its use as wall material during encapsulation (Khalifa *et al.*, 2019). In the study of Khalifa *et al.* (2019), it was reported that the stability of gum Arabic as wall material was enhanced because of the presence of protein which was reportedly present in whey protein. In addition to this, Airouyuwa & Kaewmanee (2019) reported that protein enhances the release profile of polyphenols. This makes the use of protein as wall material/carrier an important inclusion in encapsulation to enhance the bioavailability of important compounds in the site of action.

2.7 Encapsulation of bioactive compounds

Bioactive compounds have been in use in different manufacturing and consumer-good producing firms such as pharmaceutical, food and chemical industries, this signifies that the most appropriate and standard method to extract these active components from plant materials are put into use (Azmir *et al.*, 2013). Most bioactive compounds are lipid/oil-loving compounds and because of this, they have poor solubility in water and subsequently, difficulty in incorporating them in food systems as well as poor assimilation in the digestive system (Donsì *et al.*, 2011).

One major disadvantage of bioactive compounds is that they undergo degradation easily under harsh and unfavourable environmental conditions such as heat, pH, presence of oxygen etc. Hence, the need to create a protective barrier around these compounds to prevent them from undergoing inactivation (de Vos *et al.*, 2010). The carrier and the core materials are homogenized prior to drying under defined preparation and processing conditions.

2.8 Optimization and design of experiment for encapsulation

The conventional way of determining the effects of various factors in an encapsulation experiment is the use of a phenomenon known as one-factor-at-a-time (OFAT). However, this method is not efficient in determining interactions between factors or variables. Apart from this limitation, using OFAT may lead to time and resources consumption, which may in turn result in conducting a large number of experiments (Paulo and Santos, 2017). A more efficient way of determining interaction among variables was developed and has been used to accurately model and optimize the conditions of an encapsulation experiment. Generally, in selecting viable and workable encapsulation factors, the functionality of the final product must be put into consideration. This is mostly determined by performing a preliminary screening test. From various encapsulation study, the most important factors that affect the functionality of the final product have been identified as core coating ratio, wall material ratio (in the case where more than one wall material is used), ultrasonication or mixing time, inlet drying temperature and flow rate (in the case of spray drying experiment) (Chuyen *et al.*, 2019; Dadi *et al.*, 2019b). For optimization, multivariate analysis has been used, with response surface methodology (RSM) the most used in the food industry and food encapsulation research (Paulo and Santos, 2017).

2.8.1 Response surface methodology

Response surface methodology (RSM) is a collection of mathematical and statistical techniques used to develop and analyse models in order to derive optimum conditions (best conditions) for experiments (Paulo and Santos, 2017; Oladipo and Betiku, 2019; Abdulgader *et al.*, 2020). In RSM, interactions between factors and responses are measured. Generally, factors are referred to as independent variables, because responses are dependent on them while responses dependent variables because the values obtained for responses are usually measured from the design (Paulo and Santos, 2017). Response surface methodology is divided into Box-Behnken Design (BBD), central composite design (CCD) and D-optimal design. The most commonly used in encapsulation experiment is BBD because of the ease associated with the design. Some studies have used BBD in designing an encapsulation experimental conditions, they include; encapsulation of *Moringa stenopetala* (Dadi *et al.*, 2019a,b), coffee oil flavour (Getachew and Chun, 2016), polyphenols in spray and freeze-dried fruits (Ramírez *et al.*, 2015) and encapsulation of carotenoid-rich oil from gac peel extract (Chuyen *et al.*, 2019) to mention a few.

The equation below represents the relationship between the independent variables and responses in an encapsulation study.

$$\mathrm{Y} = \mathrm{f}\,(\mathrm{xi} \ldots \mathrm{xn}) + £ \qquad \text{Equation (1)}$$

Where *Y* is the measured response *f* is the unknown function, *xi* to *xn* are the independent variables while £ is the error. Errors in RSM are generally measurement errors from statistical calculations.

In RSM, mathematical expressions can either be linear or polynomial equations, the simplest mathematical expression that can be used in RSM is a linear function (Paulo and Santos, 2017). It is given below as;

$$\mathrm{y} = \beta\mathrm{o} \sum_{\mathrm{i}=1}^{\mathrm{k}} \beta\mathrm{i}\mathrm{X}\mathrm{i} + £ \qquad \text{Equation (2)}$$

Where y is the response measured for the linear equation, βo is a constant, *xi* represents independent variables, *βi* represents coefficients of the independent variables, £ statistical and measurement error, and k stands for the number of variables. In this kind of model, a curvature is not obtained hence no interactions between variables are noticed (Paulo and Santos, 2017). To effectively study interactions between variables, a quadratic or second-order model must be used. Results obtained from laboratory experiments are fitted into the

experimental designs to compare experimental values with predicted values and models are then validated.

2.9 Conclusion

The importance and richness of extracts of *M. oleifera* have made the plant a potential candidate in the development of functional foods and nutraceuticals. The encapsulation of this compound will preserve their functionality and increase desirability when they are included in foods as fortificants and food enrichers. Optimum preparation conditions, as well as wall material for the encapsulation of these extracts, affect the efficiency of this process as well as selected properties of the microcapsules. Therefore, process parameters must be carefully selected.

2.10 References

Abdulgader, M., Yu, Q.J., Zinatizadeh, A.A., Williams, P. & Rahimi, Z. (2020). Application of response surface methodology (RSM) for process analysis and optimization of milk processing wastewater treatment using multistage flexible fiber biofilm reactor. *Journal of Environmental Chemical Engineering*, **8**, 103797.

Abioye, V.. (2015). Proximate composition and sensory properties of moringa fortified yellow maize-ogi. *African Journal of food science Research*, **3**, 155–159.

Ahmad, S., Ahmad, M., Manzoor, K., Purwar, R. & Ikram, S. (2019). A review on latest innovations in natural gums based hydrogels: Preparations & applications. *International Journal of Biological Macromolecules*, **136**, 870–890.

Akhavan Mahdavi, S., Jafari, S.M., Assadpoor, E. & Dehnad, D. (2016). Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. *International Journal of Biological Macromolecules*, **85**, 379–385.

Al-Owaisi, M., Al-Hadiwi, N. & Khan, S.A. (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine*, **4**, 964–970.

Alhakmani, F., Kumar, S. & Khan, S.A. (2013). Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of *Moringa oleifera*. *Asian Pacific Journal of Tropical Biomedicine*, **3**, 623–627.

Ali, B.H., Ziada, A. & Blunden, G. (2009). Biological effects of gum arabic: A review of some recent research. *Food and Chemical Toxicology*, **47**, 1–8.

Amoako, D. & Awika, J.M. (2016). Polyphenol interaction with food carbohydrates and consequences on availability of dietary glucose. *Current Opinion in Food Science*, **8**, 14–18.

Annegowda, H. V., Bhat, R., Tze, L.M., Karim, A.A. & Mansor, S.M. (2013). The free radical scavenging and antioxidant activities of pod and seed extract of *Clitoria fairchildiana* (Howard)- An underutilized legume. *Journal of Food Science and Technology*, **50**, 535–541.

Anwar, F., Latif, S., Ashraf, M. & Hassan, A. (2007). *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *Phytotherapy Research*, **659**, 651–659.

Arise, A.K., Arise, R.O., Sanusi, M.O., Esan, O.T. & Oyeyinka, S.A. (2015). Effect of *Moringa oleifera* flower fortification on the nutritional quality and sensory properties of weaning food. *Croatian Journal of Food Science and Technology*, **6**, 18–27.

Arslan-Tontul, S. & Erbas, M. (2017). Single and double layered microencapsulation of probiotics by spray drying and spray chilling. *Lwt*, **81**, 160–169.

Asare, G.A., Gyan, B., Bugyei, K., Adjei, S., Mahama, R., Addo, P., Otu-Nyarko, L., Wiredu, E.K. & Nyarko, A. (2012). Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. *Journal of Ethnopharmacology*, **139**, 265–272.

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**, 686–697.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N. & Omar, A.K.M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, **117**, 426–436.

Azwanida, N.N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, **04**, 3–8.

Ballesteros, L.F., Ramirez, M.J., Orrego, C.E., Teixeira, J.A. & Mussatto, S.I. (2017). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. *Food Chemistry*, **237**, 623–631.

Bao, C., Jiang, P., Chai, J., Jiang, Y., Li, D., Bao, W., Liu, B., Liu, B., Norde, W. & Li, Y. (2019). The delivery of sensitive food bioactive ingredients: Absorption mechanisms, influencing factors, encapsulation techniques and evaluation models. *Food Research International*, **120**, 130–140.

Barthold, S., Hittinger, M., Primavessy, D., Zapp, A., Groß, H. & Schneider, M. (2019). Preparation of maltodextrin nanoparticles and encapsulation of bovine serum albumin – Influence of formulation parameters. *European Journal of Pharmaceutics and Biopharmaceutics*, **142**, 405–410.

Benelli, L. & Oliveira, W.P. (2019). Fluidized bed coating of inert cores with a lipid-based system loaded with a polyphenol-rich *Rosmarinus officinalis* extract. *Food and Bioproducts Processing*, **114**, 216–226.

Bento-Silva, A., Koistinen, V.M., Mena, P., Bronze, M.R., Hanhineva, K., Sahlstrøm, S., Kitrytė, V., Moco, S. & Aura, A.M. (2019). Factors affecting intake, metabolism and health benefits of phenolic acids: do we understand individual variability? *European Journal of Nutrition*, **59**, 1275–1293.

Betz, M. & Kulozik, U. (2012). Microencapsulation of bioactive bilberry anthocyanins by means of whey protein gels. *Procedia Food Science*, **1**, 2047–2056.

Bhullar, K.S. & Rupasinghe, H.P.V. (2015). Antioxidant and cytoprotective properties of partridgeberry polyphenols. *Food Chemistry*, **168**, 595–605.

Bichi, M.H., Agunwamba, J.C., Muyibi, S.A. & Abdulkarim, M.I. (2012). Effect of Extraction

Method on the Antimicrobial Activity of *Moringa oleifera* Seeds Extract. *Journal of American science*, **6**, 134–140.

Bolarinwa, I.F., Aruna, T.E. & Raji, A.O. (2019). Nutritive value and acceptability of bread fortified with moringa seed powder. *Journal of the Saudi Society of Agricultural Sciences*, **18**, 195–200.

Bukar, A., Uba, A. & Oyeyi, T. (2011). Antimicrobial profile of *Moringa oleifera* lam. Extracts against some food – borne microorganisms. *Bayero Journal of Pure and Applied Sciences*, **3**, 43–48.

Burnside, E. (2014). Hydrocolloids and gums as encapsulating agents. In: *Microencapsulation in the Food Industry*. Pp. 241–252. Elsevier Inc.

Capece, M. & Dave, R. (2011). Application of fluidized bed film coating for membrane encapsulation of catalysts. *Powder Technology*, **211**, 199–206.

Carneiro, H.C.F., Tonon, R. V., Grosso, C.R.F. & Hubinger, M.D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering*, **115**, 443–451.

Carvalho, J.D. dos S., Oriani, V.B., Oliveira, G.M. de & Hubinger, M.D. (2019). Characterization of ascorbic acid microencapsulated by the spray chilling technique using palm oil and fully hydrogenated palm oil. *LWT - Food Science and Technology*, **101**, 306–314.

Cassanelli, M., Norton, I. & Mills, T. (2018). Role of gellan gum microstructure in freeze drying and rehydration mechanisms. *Food Hydrocolloids*, **75**, 51–61.

Castro, N., Durrieu, V., Raynaud, C. & Rouilly, A. (2016). Influence of DE-value on the physicochemical properties of maltodextrin for melt extrusion processes. *Carbohydrate Polymers*, **144**, 464–473.

Cheynier, V. (2005). Polyphenols in foods are more complex than often thought. *The American journal of clinical nutrition*, **81**, 223–229.

Choińska-Pulit, A., Mituła, P., Śliwka, P., Łaba, W. & Skaradzińska, A. (2015). Bacteriophage encapsulation: trends and potential applications. *Trends in Food Science and Technology*, **45**, 212–221

Chukwuebuka, E. (2015). *Moringa oleifera* “The Mother’s Best Friend.” *International Journal of Nutrition and Food Sciences*, **4**, 624.

Chuyen, H. V, Roach, P.D., Golding, J.B., Parks, S.E. & Nguyen, M.H. (2019). Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technology*, **344**, 373–379.

Cilek, B., Luca, A., Hasirci, V., Sahin, S. & Sumnu, G. (2012). Microencapsulation of phenolic compounds extracted from sour cherry pomace: Effect of formulation, ultrasonication time and core to coating ratio. *European Food Research and Technology*, **235**, 587–596.

Comunian, T.A. & Favaro-Trindade, C.S. (2016). Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. *Food Hydrocolloids*, **61**,442–457.

Coronel-Aguilera, C.P. & San Martín-González, M.F. (2015). Encapsulation of spray dried β-carotene emulsion by fluidized bed coating technology. *LWT - Food Science and Technology*, **62**, 187–193.

Coz-Bolaños, X., Campos-Vega, R., Reynoso-Camacho, R., Ramos-Gómez, M., Loarca-Piña, G.F. & Guzmán-Maldonado, S.H. (2018). Moringa infusion (*Moringa oleifera*) rich in phenolic compounds and high antioxidant capacity attenuate nitric oxide pro-inflammatory mediator in vitro. *Industrial Crops and Products*, **118**, 95–101.

Cuellar-Nuñez, M.L., Luzardo-Ocampo, I., Campos-Vega, R., Gallegos-Corona, M.A., González de Mejía, E. & Loarca-Piña, G. (2018a). Physicochemical and nutraceutical properties of moringa (*Moringa oleifera*) leaves and their effects in an *in vivo* AOM/DSS-induced colorectal carcinogenesis model. *Food Research International*, **105**, 159–168.

D'Archivio, M., Scazzocchio, B., Giovannini, C. & Masella, R. (2013). *Role of Protocatechuic Acid in Obesity-Related Pathologies*. *Polyphenols in Human Health and Disease*. Elsevier Inc.

Dadi, D., Emire, S.A., Hagos, A.D. & Eun, J.-B. (2019a). Development of microencapsulated bioactive product from *M. stenopetala* leaves extract by optimizing spray drying process parameters *Annals of Agricultural Science*, **20**, 191–206.

Dadi, D.W., Emire, S.A., Hagos, A.D. & Eun, J.-B. (2019b). Effects of spray drying process parameters on the physical properties and digestibility of the microencapsulated product from *Moringa stenopetala* leaves extract. *Cogent Food & Agriculture*, **5**, 1690316.

Dadi, D.W., Emire, S.A., Hagos, A.D. & Eun, J.B. (2019c). Effect of ultrasound-assisted extraction of *Moringa stenopetala* leaves on bioactive compounds and their antioxidant activity. *Food Technology and Biotechnology*, **57**, 77–86.

Darvishi, F., Destain, J., Nahvi, I., Thonart, P. & Zarkesh-Esfahani, H. (2012). Effect of additives on freeze-drying and storage of *Yarrowia lipolytica* lipase. *Applied Biochemistry and Biotechnology*, **168**, 1101–1107.

Devi, N., Sarmah, M., Khatun, B. & Maji, T.K. (2017). Encapsulation of active ingredients in

polysaccharide–protein complex coacervates. *Advances in Colloid and Interface Science*, **139**, 236–145.

Dhongade, H. kumar J., Paikra, B.K. & Gidwani, B. (2017). Phytochemistry and Pharmacology of *Moringa oleifera* Lam. *Journal of Pharmacopuncture*, **20**, 194–200.

Donsì, F., Sessa, M., Mediouni, H., Mgaidi, A. & Ferrari, G. (2011). Encapsulation of bioactive compounds in nanoemulsion- based delivery systems. *Procedia Food Science*, **1**, 1666–1671.

Drosou, C.G., Krokida, M.K. & Biliaderis, C.G. (2017). Encapsulation of bioactive compounds through electrospinning/electrospraying and spray drying: A comparative assessment of food-related applications. *Drying Technology*, **35**, 139–162.

Dykes, L. & Rooney, L.W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World*, **52**, 105–111.

Elwan, A.M., Salama, A.A., Sayed, A.M., Ghoneim, A.M., Elsaied, A.A., Ibrahim, F.A. & Elnasharty, M.M.M. (2018). Biophysical and biochemical roles of *Moringa oleifera* leaves as radioprotector. *Progress in Biophysics and Molecular Biology*, **140**, 142–149.

Eratte, D., Dowling, K., Barrow, C.J. & Adhikari, B. (2018). Recent advances in the microencapsulation of omega-3 oil and probiotic bacteria through complex coacervation: A review. *Trends in Food Science and Technology*, **71**, 121–131

Evageliou, V. & Saliari, D. (2017). Limonene encapsulation in freeze dried gellan systems. *Food Chemistry*, **223**, 72–75.

Fahey, J.W. (2005). *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life Journal*, 1–15.

Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J. & Muchenje, V. (2018). Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. *Food Research International*.

Fang, W., Nonappa, Vitikainen, M., Mohammadi, P., Koskela, S., Soikkeli, M., Westerholm-Parvinen, A., Landowski, C.P., Penttilä, M., Linder, M.B. & Laaksonen, P. (2018). Coacervation of resilin fusion proteins containing terminal functionalities. *Colloids and Surfaces B: Biointerfaces*, **171**, 590–596.

Ganesan, K. & Xu, B. (2018). A critical review on phytochemical profile and health promoting effects of mung bean (*Vigna radiata*). *Food Science and Human Wellness*, **7**, 11–33.

Gao, Z., Fang, Y., Cao, Y., Liao, H., Nishinari, K. & Phillips, G.O. (2017). Hydrocolloid-food component interactions. *Food Hydrocolloids*, **68**, 149–156.

Getachew, A.T. & Chun, B.S. (2016). Optimization of coffee oil flavor encapsulation using response surface methodology. *LWT - Food Science and Technology*, **70**, 126–134.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. & Saurel, R. (2007). Applications of

spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, **40**, 1107–1121.

Gómez, B., Barba, F.J., Domínguez, R., Putnik, P., Bursać Kovačević, D., Pateiro, M., Toldrá, F. & Lorenzo, J.M. (2018). Microencapsulation of antioxidant compounds through innovative technologies and its specific application in meat processing. *Trends in Food Science and Technology*, **82**, 135–147.

Gopalakrishnan, L., Doriya, K. & Kumar, D.S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, **5**, 49–56.

Govardhan Singh, R.S., Negi, P.S. & Radha, C. (2013). Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa oleifera* seed flour. *Journal of Functional Foods*, **5**, 1883–1891.

Gulu, N.B. (2017). *Functional and Rheological Properties of Bambara Groundnut Starch-Catechin Complex Obtained by Chemical Grafting*. (Thesis).

Gupta, S., Jain, R., Kachhwaha, S. & Kothari, S.L. (2018). Nutritional and medicinal applications of *Moringa oleifera* Lam.—Review of current status and future possibilities. *Journal of Herbal Medicine*, **11**, 1–11.

Hamany Djande, C.Y., Piater, L.A., Steenkamp, P.A., Madala, N.E. & Dubery, I.A. (2018). Differential extraction of phytochemicals from the multipurpose tree, *Moringa oleifera*, using green extraction solvents. *South African Journal of Botany*, **115**, 81–89.

Ibrahim, R.E., El-Houseiny, W., Behairy, A., Mansour, M.F. & Abd-Elhakim, Y.M. (2019). Ameliorative effects of *Moringa oleifera* seeds and leaves on chlorpyrifos-induced growth retardation, immune suppression, oxidative stress, and DNA damage in *Oreochromis niloticus*. *Aquaculture*, **505**, 225–234.

Indrawati, R., Sukowijoyo, H., Indriatmoko, Wijayanti, R.D.E. & Limantara, L. (2015). Encapsulation of brown seaweed pigment by freeze drying: Characterization and its stability during Storage. *Procedia Chemistry*, **14**, 353–360.

Islam, A.M., Phillips, G.O., Sljivo, A., Snowden, M.J. & Williams, P.A. (1997). A review of recent developments on the regulatory, structural and functional aspects of gum arabic. *Food Hydrocolloids*, **11**, 493–505.

Jahan, I.A., Hossain, M.H., Ahmed, K.S., Sultana, Z., Biswas, P.K. & Nada, K. (2018). Antioxidant activity of *Moringa oleifera* seed extracts. *Oriental Pharmacy and Experimental Medicine*, **18**, 299–307.

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry*, **175**, 556–67.

Jayawardana, B.C., Liyanage, R., Lalantha, N., Iddamalgoda, S. & Weththasinghe, P.

(2015). Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. *LWT - Food Science and Technology*, **64**, 1204–1208.

Jia, Z., Dumont, M.J. & Orsat, V. (2016). Encapsulation of phenolic compounds present in plants using protein matrices. *Food Bioscience*, **15**, 87–104.

Jiang, S., Altaf hussain, M., Cheng, J., Jiang, Z., Geng, H., Sun, Y., Sun, C. & Hou, J. (2018). Effect of heat treatment on physicochemical and emulsifying properties of polymerized whey protein concentrate and polymerized whey protein isolate. *LWT - Food Science and Technology*, **98**, 134–140.

Jideani, V.A. & Diedericks, C.F. (2014). Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: *Antioxidant-Antidiabetic Agents and Human Health*. InTech.

Jun-xia, X., Hai-yan, Y. & Jian, Y. (2011). Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. *Food Chemistry*, **125**, 1267–1272.

Karim, R.O., Kayode, R.M.O., Oyeyinka, S.A. & Oyeyinka, A.T. (2013). Proximate, mineral and sensory qualities of '*Amala*' prepared from yam flour fortified with Moringa leaf powder. *Food Science and Quality Management*, **12**, 10–23.

Khalid, S.A., Musa, A.M., Saeed, A.M., Abugroun, E.A., Sid Ahmed, E.O., Ghalib, M.B., Elnima, E.I., Alkarib, S.Y., Abdelsalam, T.M., Abdelgader, A., Phillips, G.O. & Phillips, A.O. (2014). Manipulating dietary fibre: Gum Arabic making friends of the colon and the kidney. *Bioactive Carbohydrates and Dietary Fibre*, **121**, 1276–1286.

Khalifa, I., Li, M., Mamet, T. & Li, C. (2019). Maltodextrin or gum Arabic with whey proteins as wall-material blends increased the stability and physiochemical characteristics of mulberry microparticles. *Food Bioscience*, **31**, 100445.

Khalifa, I., Zhu, W., Li, K. kai & Li, C. mei. (2018). Polyphenols of mulberry fruits as multifaceted compounds: Compositions, metabolism, health benefits, and stability—A structural review. *Journal of Functional Foods*, **40**, 28–43.

Khanvilkar, R.C., R. & A.K., S. (2016). Carrier materials for encapsulation of bio-active components of food. *International Journal of Pharmaceutical Sciences Review and Research*, **40**, 62–73.

Koo, S.Y., Cha, K.H., Song, D.G., Chung, D. & Pan, C.H. (2014). Microencapsulation of peppermint oil in an alginate-pectin matrix using a coaxial electrospray system. *International Journal of Food Science and Technology*, **49**, 733–739.

Kou, X., Li, B., Olayanju, J.B., Drake, J.M. & Chen, N. (2018). Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. *Nutrients*, **10**, 343–355.

Kozłowska, A. & Szostak-Węgierek, D. (2019). Flavonoids – Food sources, health Benefits,

and Mechanisms Involved. I*n: Merillon JM., Ramawat K. (eds) Bioactive molecules in food*. Springer cham.

Kuck, L.S. & Noreña, C.P.Z. (2016). Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*, **194**, 569–576.

Kurmukov, A.G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, **1**, 13–14.

la Rosa, L.A. de, Moreno-Escamilla, J.O., Rodrigo-García, J. & Alvarez-Parrilla, E. (2019). Phenolic Compounds. In: *Postharvest Physiology and Biochemistry of Fruits and Vegetables*. Pp. 253–271. Elsevier.

Labuschagne, P. (2018). Impact of wall material physicochemical characteristics on the stability of encapsulated phytochemicals: A review. *Food Research International*, **107**, 227–247.

León-González, A.J., Auger, C. & Schini-Kerth, V.B. (2015). Pro-oxidant activity of polyphenols and its implication on cancer chemoprevention and chemotherapy. *Biochemical Pharmacology*, **98**, 371–380.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. & Bertoli, S. (2016). *Moringa oleifera* seeds and oil: Characteristics and uses for human health. *International Journal of Molecular Sciences*, **17**, 1–14.

Li, J. & Nie, S. (2019). Food Hydrocolloids The functional and nutritional aspects of hydrocolloids in foods. *Food hydrocolloids*, **53**, 46–61.

Lin, M., Zhang, J. & Chen, X. (2018). Bioactive flavonoids in Moringa oleifera and their health-promoting properties. *Journal of Functional Foods*, **47**, 469–479.

Loughrill, E., Thompson, S., Owusu-Ware, S., Snowden, M.J., Douroumis, D. & Zand, N. (2019). Controlled release of microencapsulated docosahexaenoic acid (DHA) by spray–drying processing. *Food Chemistry*, **286**, 368–375.

Loyeau, P.A., Spotti, M.J., Braber, N.L. Vanden, Rossi, Y.E., Montenegro, M.A., Vinderola, G. & Carrara, C.R. (2018). Microencapsulation of *Bifidobacterium animalis subsp. lactis* INL1 using whey proteins and dextrans conjugates as wall materials. *Food Hydrocolloids*, **85**, 129–135.

Luna-Guevara, J.J., Ochoa-Velasco, C.E., Hernández-Carranza, P. & Guerrero-Beltrán, J.A. (2017). Microencapsulation of walnut, peanut and pecan oils by spray drying. *Food Structure*, **12**, 26–32.

Ma, Z.F., Ahmad, J., Zhang, H., Khan, I. & Muhammad, S. (2020). Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera*) as a potential functional food. *South African Journal of Botany*, **129**, 40–46.

Madene, A., Jacquot, M., Scher, J. & Desobry, S. (2006). Flavour encapsulation and controlled release - A review. *International Journal of Food Science and Technology*, **41**, 1–21.

Mahdavee Khazaei, K., Jafari, S.M., Ghorbani, M. & Hemmati Kakhki, A. (2014). Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. *Carbohydrate Polymers*, **105**, 57–62.

Mahfoudhi, N., Ksouri, R. & Hamdi, S. (2016). Nanoemulsions as potential delivery systems for bioactive compounds in food systems: preparation, characterization, and applications in food industry. *Emulsions*, 365–403.

Makita, C., Chimuka, L., Steenkamp, P., Cukrowska, E. & Madala, E. (2016). Comparative analyses of flavonoid content in *Moringa oleifera* and *Moringa ovalifolia* with the aid of UHPLC-qTOF-MS fingerprinting. *South African Journal of Botany*, **105**, 116–122.

Marques, G.R., Borges, S.V., Mendonça, K.S. de, Barros Fernandes, R.V. de & Menezes, E.G.T. (2014). Application of maltodextrin in green corn extract powder production. *Powder Technology*, **263**, 89–95.

Mbikay, M. (2012). Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. *Frontiers in Pharmacology*, **3**, 1–12.

Meng, F., Wang, S., Liu, H., Xu, X. & Ma, H. (2017). Microencapsulation of oxalic acid (OA) via coacervation induced by polydimethylsiloxane (PDMS) for the sustained release performance. *Materials and Design*, **116**, 31–41.

Milani, J. & Maleki, G. (2012). Hydrocolloids in food industry. In: *Food industrial processes - methods and equipment*. Intech open.

Moyo. (2012). Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology*, **11**, 2797–2802.

Moyo, B., Masika, P.J., Arnold, H. & Voster, M. (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, **10**, 12925–12933.

Moyo, B., Oyedemi, S., Masika, P.J. & Muchenje, V. (2012). Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science*, **91**, 441–447.

Moyo, B., Patrick, J.M., Arnold, H. & Voster, M. (2016). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, **10**, 12925–12933.

Muhoza, B., Xia, S., Cai, J., Zhang, X., Duhoranimana, E. & Su, J. (2019). Gelatin and pectin complex coacervates as carriers for cinnamaldehyde: Effect of pectin esterification

degree on coacervate formation, and enhanced thermal stability. *Food Hydrocolloids*, **87**, 712–722.

Mukunzi, D., Nsor-Atindana, J., XIaoming, Z. & Gahungu, A. (2011). Comparison of volatile profile of *Moringa oleifera* leaves from Rwanda and China using HS-SPME. *Pakistan Journal of Nutrition*, **10**, 602–608.

Nazzaro, F., Orlando, P., Fratianni, F. & Coppola, R. (2012). Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology*, **23**, 182–186.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S. & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, **1**, 1806–1815.

Nizori, A., Bui, L.T.T., Jie, F. & Small, D.M. (2018). Impact of varying hydrocolloid proportions on encapsulation of ascorbic acid by spray drying. *International Journal of Food Science and Technology*, **53**, 1363–1370.

Nobossé, P., Fombang, E.N. & Mbofung, C.M.F. (2018). Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Science and Nutrition*, **6**, 2188–2198.

Nouman, W., Anwar, F., Gull, T., Newton, A., Rosa, E. & Domínguez-Perles, R. (2016). Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. *Industrial Crops and Products*, **83**, 166–176.

Oguntibeju, O.O., Aboua, G.Y. & Omodanisi, E.I. (2019). Effects of *Moringa oleifera* on oxidative stress, apoptotic and inflammatory biomarkers in streptozotocin-induced diabetic animal model. *South African Journal of Botany*, **129**, 354–365

Oladipo, B. & Betiku, E. (2019). Process optimization of solvent extraction of seed oil from *Moringa oleifera*: An appraisal of quantitative and qualitative process variables on oil quality using D-optimal design. *Biocatalysis and Agricultural Biotechnology*, **20**, 101187.

Oluduro, A.O. (2012). Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. *Malaysian Journal of Microbiology*, **8**, 59–67.

Omodanisi, E.I., Aboua, G.Y. & Oguntibeju, O.O. (2017a). Therapeutic potentials and pharmacological properties of *Moringa oleifera* lam in the treatment of diabetes mellitus and related complications. *Tropical Journal of Pharmaceutical Research*, **16**, 1737–1746.

Omodanisi, E.I., Aboua, Y.G., Oguntibeju, O.O. & Lamuela-Raventós, R.M. (2017b). Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of *Moringa oleifera* in diabetes-induced nephrotoxic male wistar

rats. *Molecules*, **22**, 1–16.

Osamede Airouyuwa, J. & Kaewmanee, T. (2019). Microencapsulation of *Moringa oleifera* leaf extracts with vegetable protein as wall materials. *Food Science and Technology International*, **25**, 533–543.

Oyenihi, A.B. & Smith, C. (2019). Are polyphenol antioxidants at the root of medicinal plant anti-cancer success? *Journal of Ethnopharmacology*, **229**, 54–72.

Oyeyinka, A.T. & Oyeyinka, S.A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, **17**, 127–136.

Özcan, M.M. (2018). *Moringa spp*: Composition and bioactive properties. *South African Journal of Botany*, **129**, 25–31.

Padayachee, B. & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. *South African Journal of Botany*, **129**, 304–316.

Park, K. & Yeo, Y. (2007). Microencapsulation Technology. *Encyclopedia of Pharmaceutical Technology*, **1**, 2315–2327.

Paulo, F. & Santos, L. (2017). Design of experiments for microencapsulation applications: A review. *Materials Science and Engineering C*, **77**, 1327–1340.

Peixoto, J.R.O., Silva, G.C., Costa, R.A., Sousa Fontenelle, J. res L. de, Vieira, G.H.F., Filho, A.A.F. & Vieira, R.H.S. dos F. (2011). In vitro antibacterial effect of aqueous and ethanolic Moringa leaf extracts. *Asian Pacific Journal of Tropical Medicine*, **4**, 201–204.

Pellicer, J.A., Fortea, M.I., Trabal, J., Rodríguez-López, M.I., Gabaldón, J.A. & Núñez-Delicado, E. (2019). Stability of microencapsulated strawberry flavour by spray drying, freeze drying and fluid bed. *Powder Technology*, **347**, 179–185.

Perumal, S. & Klaus, B. (2003). Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera* Lam .) Leaves. *Journal of Agricultural and Food Chemistry*, **51**, 2144–2155.

Poornima, K. & Sinthya, R. (2017). Application of Various Encapsulation Techniques in Food Industries. *International Journal of Latest Engineering Research and Applications (IJLERA)*, **02**, 37–41.

Poozesh, S. & Bilgili, E. (2019). Scale-up of pharmaceutical spray drying using scale-up rules: A review. *International Journal of Pharmaceutics*, **562**, 271–292.

Poshadri, A. & Kuna, A. (2010). Microencapsulation technology: A review. *The Journal of Research ANGRAU*, **38**, 86–102.

Prabakaran, M., Kim, S.H., Sasireka, A., Chandrasekaran, M. & Chung, I.M. (2018). Polyphenol composition and antimicrobial activity of various solvent extracts from

different plant parts of *Moringa oleifera*. *Food Bioscience*, **26**, 23–29.

Pycia, K., Juszczak, L., Gałkowska, D., Witczak, M. & Jaworska, G. (2016). Maltodextrins from chemically modified starches. Selected physicochemical properties. *Carbohydrate Polymers*, **146**, 301–309.

Rahmam, S., Naim, M.N., Ng, E., Mokhtar, M.N. & Bakar, N.F.A. (2016). Encapsulation of bioactive compound from extracted jasmine flower using β-Cyclodextrin via electrospray. *IOP Conference Series: Earth and Environmental Science*, **36**.

Raja, R.R., Sreenivasulu, M., Vaishnavi, S., Navyasri, D.., Samatha, G. & Geethalakshmi, S. (2016). *Moringa Oleifera*-An Overview. *RA Journal Of Applied Research*, **2**, 620–624.

Rajabi, H., Ghorbani, M., Jafari, S.M., Sadeghi Mahoonak, A. & Rajabzadeh, G. (2015). Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and gelatin as wall materials. *Food Hydrocolloids*, **51**, 327–337.

Raman, J.K., Alves, C.M. & Gnansounou, E. (2018). A review on moringa tree and vetiver grass – Potential biorefinery feedstocks. *Bioresource Technology*.

Ramírez, M.J., Giraldo, G.I. & Orrego, C.E. (2015). Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. *Powder Technology*, **277**, 89–96.

Razis, A.F.A., Ibrahim, M.D. & Kntayya, S.B. (2014). Health benefits of *Moringa oleifera*. *Asian Pacific Journal of Cancer Prevention*, **15**, 8571–8576.

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A. & Segura-Carretero, A. (2015). Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves. *Industrial Crops and Products*, **66**, 246–254.

Rutz, J.K., Borges, C.D., Zambiazi, R.C., Crizel-Cardozo, M.M., Kuck, L.S. & Noreña, C.P.Z. (2017). Microencapsulation of palm oil by complex coacervation for application in food systems. *Food Chemistry*, **220**, 59–66.

Sagona, W.C.J., Chirwa, P.W. & Sajidu, S.M. (2019). The miracle mix of Moringa: Status of Moringa research and development in Malawi. *South African Journal of Botany*, **129**, 138–145

Sahay, S., Yadav, U. & Srinivasamurthy, S. (2017). Potential of *Moringa oleifera* as a functional food ingredient: A review. *International Journal of Food Science and Nutrition*, **2**, 31–37.

Saifullah, M., Shishir, M.R.I., Ferdowsi, R., Tanver Rahman, M.R. & Vuong, Q. Van. (2019). Micro and nano encapsulation, retention and controlled release of flavor and aroma compounds: A critical review. *Trends in Food Science and Technology*, **86**, 230–251.

Salminen, H., Ankenbrand, J., Zeeb, B., Badolato Bönisch, G., Schäfer, C., Kohlus, R. & Weiss, J. (2019). Influence of spray drying on the stability of food-grade solid lipid

nanoparticles. *Food Research International*, **119**, 741–750.

Santana, A.A., Cano-Higuita, D.M., Oliveira, R.A. De & Telis, V.R.N. (2016). Influence of different combinations of wall materials on the microencapsulation of jussara pulp (*Euterpe edulis*) by spray drying. *Food Chemistry*, **212**, 1–9.

Santos, C. dos, Buera, P. & Mazzobre, F. (2017). Novel trends in cyclodextrins encapsulation. Applications in food science. *Current Opinion in Food Science*, **16**, 106-113.

Sarabandi, K., Jafari, S.M., Mahoonak, A.S. & Mohammadi, A. (2019). Application of gum Arabic and maltodextrin for encapsulation of eggplant peel extract as a natural antioxidant and color source. *International Journal of Biological Macromolecules*, **140**, 59–68.

Saucedo-Pompa, S., Torres-Castillo, J.A., Castro-López, C., Rojas, R., Sánchez-Alejo, E.J., Ngangyo-Heya, M. & Martínez-Ávila, G.C.G. (2018). Moringa plants: Bioactive compounds and promising applications in food products. *Food Research International*, **111**, 438–450.

Schrooyen, P.M.M., Meer, R. Van Der & Kruif, C.G. De. (2001). Microencapsulation: its application in nutrition. *Proceedings of the Nutrition Society*, **60**, 475–479.

Silva, E.K., Zabot, G.L. & Meireles, M.A.A. (2015). Ultrasound-assisted encapsulation of annatto seed oil: Retention and release of a bioactive compound with functional activities. *Food Research International*, **78**, 159–168.

Šturm, L., Osojnik Črnivec, I.G., Istenič, K., Ota, A., Megušar, P., Slukan, A., Humar, M., Levic, S., Nedović, V., Kopinč, R., Deželak, M., Pereyra Gonzales, A. & Poklar Ulrih, N. (2019). Encapsulation of non-dewaxed propolis by freeze-drying and spray-drying using gum Arabic, maltodextrin and inulin as coating materials. *Food and Bioproducts Processing*, **116**, 196–211.

Sultana, B. & Anwar, F. (2008). Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chemistry*, **108**, 879–884.

Taheri, A. & Jafari, S.M. (2019). Gum-based nanocarriers for the protection and delivery of food bioactive compounds. *Advances in Colloid and Interface Science*, **269**, 277–295.

Tavares, L., Noreña, Z. & Pelayo, C. (2019). Encapsulation of garlic extract using complex coacervation with whey protein isolate and chitosan as wall materials followed by spray drying. *Food Hydrocolloids*, **89**, 360–369.

Teixeira, F.J., Santos, H.O., Howell, S.L. & Pimentel, G.D. (2019). Whey protein in cancer therapy: A narrative review. *Pharmacological Research*, **144**, 245–256.

Tirgar, M., Jinap, S., Zaidul, I.S.M. & Mirhosseini, H. (2015). Suitable coating material for microencapsulation of spray-dried fish oil. *Journal of Food Science and Technology*, **52**,

4441–4449.

Tolun, A., Altintas, Z. & Artik, N. (2016). Microencapsulation of grape polyphenols using maltodextrin and gum arabic as two alternative coating materials: Development and characterization. *Journal of Biotechnology*, **239**, 23–33.

Tomšik, A., Šarić, L., Bertoni, S., Protti, M., Albertini, B., Mercolini, L. & Passerini, N. (2019). Encapsulations of wild garlic (Allium ursinum L.) extract using spray congealing technology. *Food Research International*, **119**, 941–950.

Turasan, H., Sahin, S. & Sumnu, G. (2015). Encapsulation of rosemary essential oil. *LWT - Food Science and Technology*, **64**, 112–119.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y. & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Industrial Crops and Products*, **44**, 566–571.

Vos, P. de, Faas, M.M., Spasojevic, M. & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal*, **20**, 292–302.

Xie, H., Xiang, C., Li, Y., Wang, L., Zhang, Y., Song, Z., Ma, X., Lu, X., Lei, Q. & Fang, W. (2019). Fabrication of ovalbumin/κ-carrageenan complex nanoparticles as a novel carrier for curcumin delivery. *Food Hydrocolloids*, **89**, 111–121.

Xu, Y., Skotak, M. & Hanna, M. (2006). Electrospray encapsulation of water-soluble protein with polylactide. I. Effects of formulations and process on morphology and particle size. *Journal of Microencapsulation*, **23**, 69–78.

Yadav, K., Bajaj, R.K., Mandal, S. & Mann, B. (2019). Encapsulation of grape seed extract phenolics using whey protein concentrate, maltodextrin and gum arabica blends. *Journal of Food Science and Technology*, **57**, 426–434.

Yin, Y. & Cadwallader, K.R. (2019). Spray-chilling encapsulation of 2-acetyl-1-pyrroline zinc chloride using hydrophobic materials: Storage stability and flavor application in food. *Food Chemistry*, **278**, 738–743.

Yousefi, M. & Jafari, S.M. (2019). Recent advances in application of different hydrocolloids in dairy products to improve their techno-functional properties. *Trends in Food Science and Technology*, **88**, 468–483.

Zhang, H., Qi, R. & Mine, Y. (2019). The impact of oolong and black tea polyphenols on human health. *Food Bioscience*, **29**, 55–61.

Zhong, J., Wang, Y., Yang, R., Liu, X., Yang, Q. & Qin, X. (2018). The application of ultrasound and microwave to increase oil extraction from *Moringa oleifera* seeds. *Industrial Crops and Products*, **120**, 1–10.

Ziaee, A., Albadarin, A.B., Padrela, L., Femmer, T., O'Reilly, E. & Walker, G. (2019). Spray drying of pharmaceuticals and biopharmaceuticals: Critical parameters and experimental process optimization approaches. *European Journal of Pharmaceutical Sciences*, **127**, 300–318.

Zuidam, N.J. & Nedović, V.A. (2010). *Overview of microencapsulates for use in food products or processes and methods to make them*. In Zuidam, N.J. & Nedović, V.A. (eds) *Encapsulation Technologies for Active Food Ingredients and Food Processing*. Springer New York.

CHAPTER THREE
PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF *MORINGA oleifera* EXTRACTS FROM DIFFERENT SOLVENTS

Abstract

Moringa oleifera contains important biologically active compounds that possess phytochemicals with potent antioxidants qualities. In this study, the leaves (MoLP) and seeds (MoSP) of *M. oleifera* were extracted using three solvents; 60% ethanol (EtOH), acidified methanol (Ac. MeOH), and water (H_2O). The phytochemical composition of extracts was determined by measuring the total phenolic (TPC) and flavonoid (TFC) content in a spectrophotometer. Their antioxidant capacities were investigated using the 2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays. Also, the identification of specific constituents in MoLP and MoSP extracts was conducted using liquid chromatography-mass spectrometry (LC-MS). The different extraction solvents used in this study resulted in significant differences ($p < 0.05$) in TPC and TFC values obtained for MoLP and MoSP with the former being richer in bioactive compounds. In the MoLP extract, EtOH (24.0 ± 0.4) had the most TPC (mg gallic acid equivalent (GAE) per g) followed by Ac. MeOH (21.4 ± 0.9) and H_2O (16.4 ± 0.5) but the TFC (mg quercetin equivalent (QE) per g) in Ac. MeOH (16.4 ± 0.1) was more than EtOH (14.1 ± 0.2) and H_2O (9.3 ± 0.0). The MoSP extract had TPC values of 1.5 ± 0.0, 4.2 ± 0.1, and 18.9 ± 0.5 mg GAE/g and TFC values of 0.3 ± 0.0, 1.8 ± 0.1, and 1.5 ± 0.0 mg QE/g) for EtOH, Ac. MeOH and H_2O respectively. The TPC of the EtOH extract of the leaf shows strong positive correlation that is significant ($p < 0.05$) against ORAC scavenging activity of free radicals at R^2 = O.981. The LC-MS analysis of both MoLP and MoSP extracts identified at least twenty (20) compounds notably; rutin, quercetin, kaemferol, chlorogenic acid and quinic acid and/or their derivatives. Summarily, data from this study indicate that the MoLP extract contains more phytochemicals than MoSP which were suitably extracted more with the aid of the EtOH solvent and that can be exploited further for their antioxidant qualities in the current study on encapsulation.

3.1 Introduction

Moringa Oleifera is a plant that is rich in nutrients, phytochemicals and bioactive compounds (Lin *et al.*, 2018; Oyeyinka and Oyeyinka, 2018; Padayachee and Baijnath, 2020). Because of the vast presence of important nutrients and biologically active compounds otherwise known as bioactives essential for human health, the plant has been termed the miracle tree (Fahey, 2005; Oyeyinka and Oyeyinka, 2018) and the mother's best friend (Chukwuebuka, 2015). *Moringa oleifera* is local to the sub-Himalayan tracts of India and has subsequently become naturalised in the lush areas of the Middle Eastern countries of the world (Anwar *et al.,* 2007; Moyo *et al.*, 2011). Both the leaf and seed of the *M. oleifera* plant are edible and has been established to contain important nutrient and bioactive compounds (Fahey, 2005; Jideani and Diedericks, 2014). For instance, the leaf powder has been used in the preparation of vegetable diets as well as in the fortification and enrichment of foods while the seed powder has been established to increase the protein, mineral, vitamin and antioxidant contents of foods when they are used as fortificants (Bolarinwa *et al.*, 2019). In addition to this, Özcan, (2018) reported the use of the plant's leaf in soup mixes to boost the production of breast milk among breast-feeding mothers in the Philippines.

Moringa oleifera leaf and seed powder contain important bioactives and phytochemicals, majorly polyphenols (flavonoids and phenolic compounds). This has heightened their antioxidant potential and thus makes the plant a suitable candidate for the development of functional foods, nutraceutical compounds as well as in the development of natural antioxidant that may serve as a replacement for existing synthetic antioxidant compounds (Cuellar-Nuñez *et al.*, 2018). In addition to this, several medicinal and biochemical studies show that the leaf and seed contain compounds that may help mitigate the effects of chronic and lifestyle diseases such as diabetics and inflammatory diseases (Omodanisi *et al.*, 2017; Oguntibeju *et al.*, 2019), cancer (Khan *et al.*, 2020) as well as corollary heart disease.

The first step in identifying and quantifying biologically active compounds present in this plant and their corresponding antioxidant activity is through extraction with suitable solvents (Dhanani *et al.*, 2017). This is crucial and essential to obtain optimum yield and activity. Furthermore, the type of extraction procedure, solvent composition and their inherent properties are factors that must be considered. Oladipo and Betiku, (2019) noted that solvent selection in extraction experiments play a crucial role in the quality of the final product. In addition to this, the amount of compounds quantified may vary with the kind of extraction solvent used because of variation in polarity (Rodríguez-Pérez *et al.*, 2015a). Different extraction solvents have been used in the past for *M. oleifera* leaf and seed, with

ethanol, methanol, water, acetone being the most used in different concentration and proportion (Medini *et al.*, 2014; Rodríguez-Pérez *et al.*, 2015a; Nouman *et al.*, 2016).

This chapter aimed to investigate the effect of three different solvents (60% ethanol, acidified methanol and water) on the phytochemical content, antioxidant activity and profile of *Moringa oleifera* leaf and seed powder extracts. The raw material and the solvent that gives the highest yield for the variables being tested in this chapter would then be taken forward for further use in this study for the encapsulation experiment.

3.2 Materials and methods

3.2.1 Chemicals and reagents

All chemicals and reagents used for the study were of analytical grade. They include; Folin-Ciocalteu‘s phenol solution, gallic acid, sodium carbonate, L-ascorbic acid, hydrochloric acid, TPTZ (2,4,6-tri[2-pyridyl]-s-triazine), iron (III) chloride hexahydrate, dimethylamino)-cinnamaldehyde, catechin hydrate standard, and acetate buffer ingredient were manufactured by Sigma Aldrich, USA Ethanol and methanol were purchased from United Scientific, South Africa.

3.2.2 Plant material and preparation

The methodology for the extraction of phytochemicals is summarized in the annotated flow diagram in Fig 3.1. *Moringa oleifera* leaf powder and seeds used were purchased from SupaNutri Cape town, South Africa. The seed kernels were dehulled, dried at 50 °C for 48 h to further lower the moisture present in the kernel and pulverized using a Hammer mill (Fritsch, Idar-Oberstein, Germany). The leaf powder was purchased in a pulverized form. The *M. oleifera* seeds were stored in a chiller set at 4-8 °C while the leaf powder sealed in an airtight sackbag was stored at room temperature before they were finally taken to the laboratory for extraction.

Sorting, dehulling and milling of *M. oleifera* seed was done at the Department of Food Science and Technology pilot plant of the Cape Peninsula University of Technology (CPUT) Bellville Campus, Western Cape South Africa. Extraction was carried out at the Food Science and Technology Department Research Laboratory and the Oxidative Stress Research Center CPUT. Antioxidant activity and free radical scavenging assays were conducted at the Oxidative Stress Research Center CPUT and LCMS analysis at the Central Analytical Facility of Stellenbosch University.

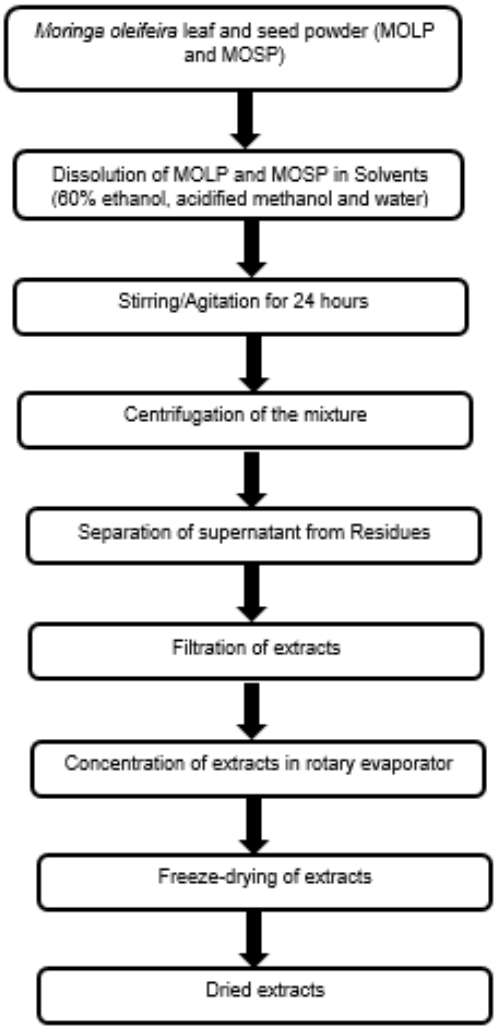


Fig 3.1 An annotated diagram of the extraction procedure of *M. oleifeira* leaf and seed powder

3.2.3 Preparation of *Moringa oleifera* leaf and seed powder extracts

The extraction of bioactive/phytochemical compounds present in *M. oleifera* leaf and seed powder was done using the method previously reported by Nouman *et al.* (2016) with modification. *Moringa oleifera* leaf powder (MoLP) and seed powder (MoSP) were each weighed and dissolved (10% w/v) in each of the extraction solvents – water, acidified methanol (1% HCl) and 60% ethanol employed in this study. The mixture was allowed to shake vigorously for 24 h in a laboratory shaker at 120 rpm and a temperature of 27 ℃. The mixture was then centrifuged in an Eppendorf centrifuge 5810 R (Hamburg, Germany) at 10000 rpm for 10 min at 4 ℃. The supernatant was decanted and the residue discarded. The supernatant was concentrated by removing ethanol using a rotary evaporator, BÜCHI

Labortechnik R-200 Rotavapor, (Flawil, Switzerland) set at a temperature of 40 ℃. The concentrated extracts were dried in a freeze drier Virtis genesis 25EL (Gardiner, New York, USA) freeze-dried for 48 h to obtain dried extract powder and stored at -4 ℃ until further analysis.

3.2.4 Phytochemical composition of *Moringa oleifeira* leaf and seed powder extract

3.2.4.1 Determination of total phenolic compounds (TPC)

The total phenolic compound contents of the extracts from the seed and leaf powder extract were determined using the Folin-Ciocalteu procedure according to Al-Owaisi *et al.* (2014) with minor modification. Briefly, dried 1 g of *M. oleifera* extract was rehydrated (10% w/v) and 25 µl of the extracts were transferred into respective wells in a 96-well clear plate after which a 125 µl of 0.2 N Folin-Ciocalteu reagent was added. The mixtures were then mixed with 100 µl of 7.5% w/v sodium carbonate (Na_2CO_3) solution. The mixture was allowed to stand for 2 h (under a dark atmosphere) and the absorbance measured using a Multiskan microplate reader (SpectraMax i3x Molecular devices, San Jose California, USA) with a wavelength of 765 nm (Gallic acid was used as the standard in this assay). The TPC was calculated by extrapolating from the gallic acid standard curve and results expressed as mg/g gallic acid equivalent (GAE) of dry extract.

3.2.4.2 Determination of total flavonoid contents (TFC)

The flavonoid content present in the samples was determined using the method described by Singleton *et al.* (1999) with slight modification. Briefly, dried *M. oleifera* extract was rehydrated and a 12.5 µl of the extract was transferred into a 96 clear well-plate with 237.5 µl of 2% HCl and then left to stand at room temperature (under a dark atmosphere) for 30 min. The absorbance was measured using a Multiskan microplate reader (SpectraMax i3x, Molecular devices, San Jose California, USA) at a wavelength of 360nm. Catechin was used as a standard and TFC calculated by extrapolating from the catechin standard curve with results expressed as mg/g catechin equivalent of dry extract.

3.2.5 Antioxidant activity of MoLP and MoSP extracts

3.2.5.1 Determination of oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance capacity (ORAC) of the extracts was determined using the method described by Ou *et al*. (2001) with slight modification. Briefly, 35 µl of the extract was transferred into a black 96 well-plate with 138 µl fluorescein (4×10^{-3} Mm) and the reaction started on the addition of 50 µl of AAPH (25 g/ml). The sample was pre-incubated at room temperature for 10 min and read kinetically over 2 h in a fluoroscan ascent with excitation wavelength set at 485 nm and emission wavelength set at 538 nm. Trolox was used as the standard and ORAC values were calculated by extrapolating from the trolox standard curve and expressed in µmol/g of trolox equivalent of dry extracts.

3.2.5.2 Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay was conducted using the method reported by Gullón *et al.* (2017) with little modification. Briefly, 10 µl of extract and 300 µl of FRAP reagent (30 ml acetate buffer, 300 mM pH 3.6, 3 ml Fe_2Cl_3, 3 ml of 10 mM TPTZ and 6 ml of H_2O) in a clear 96 well-plate. The plate was left to stand for 30 min at room temperature and the absorbance read in a Multiskan microplate reader (SpectraMax i3x, Molecular devices, San Jose California, USA at a wavelength of 593 nm. Vitamin C (Ascorbic Acid) was used as the standard in this experiment and the values measured and expressed as mg/g of ascorbic acid equivalent of the dry extract.

3.2.5.3 Determination of 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability

The DPPH (2-diphenyl-1-picrylhydrazyl) assay was used in determining the antioxidant activity and radical scavenging potentials of antioxidant compounds. The DPPH radical has a deep purple colour that turns brown or bleaches in the presence of a hydrogen donor (Nobossé *et al.*, 2018). In this study, the DPPH scavenging potential of the extracts was measured following the method of Herbello-Hermelo *et al.* (2018) with slight modification. Briefly, a 25 µl of each sample extract was pipetted into a 96 well-plate with a 275 µl of 0.01 Mm DPPH reagent in methanol. The plate was left to stand under a dark atmosphere for 30 min at room temperature and the absorbance read at 515 nm using a Multiskan microplate reader (SpectraMax i3x, Molecular devices, San Jose California, USA). Trolox was used as a standard, and the DPPH level expressed as µmol/g of trolox of dry extract.

3.2.5.4 Liquid chromatography-mass spectrometry (LC-MS) analysis

For sample preparation before analysis, a 2 g dried extract was dissolved in 15 ml extraction solvent (15 ml 50% MeOH in 0.1% formic acid), with ultrasonication and shaking overnight. Then, 2 ml of the sample was centrifuged at 14000 rpm before being transferred into a glass vial for subsequent analysis. A Waters Synapt G_2 quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 l/h, and the rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, one at low collision energy (4 V) and the other using a collision energy ramp (40−100 V) to obtain fragmentation data as well. Leucine enkephalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate. The separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column. An injection volume of 2 µl was used and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 100% solvent A for 1 min and changed to 28% B over 22 min in a linear way. It then went to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 ml/min and the column temperature was maintained at 55 °C.

The individual compounds present in the solvent extracts of *M. oleifera* leaf and seed powder was determined using liquid chromatography mass spectrometry (LC-MS). All compounds identified were done based on their retention time, parent element $(M-H)^-$, parent m/z $(M-H)^-$, ppm error and fragments. Identification and quantification of compounds was performed by checking values of m/z on an online database known as ChemCalc (https://www.chemcalc.org/?ionizations=&mf=), which gives possible molecular formula (MF) of compounds by matching them with their monoisotopic mass. The best match with the lowest ppm error value was selected for further evaluation. The MF of the compound previously obtained from ChemCalc were identified and quantified using MSDial and MSFinder (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan).

3.3 Statistical analysis

Experiments were conducted in triplicates were subjected to a One-Way Statistical Analysis of Variance (ANOVA) using SPSS (version 26, 2020). Means were separated using Duncan's least significance difference (LSD) and values were expressed as mean ± SD.

3.4 Results and discussion

3.4.1 The phytochemical composition of MoLP and MoSP extracts

The total phenolic content (TPC) and total flavonoid content (TFC) of MoLP and MoSP extracts of *M. oleifera* evaluated in this study are presented in Table 3.1. The MoLP extracts are generally composed of more phytochemicals than their MoSP counterparts, this has been established in previous works of literature (Nouman *et al.,* 2015). The EtOH used in this study significantly ($p < 0.05$) extracted more phenolic compounds while Ac MeOH was better suited for the extraction of flavonoids in the MoLP extract. In MoSP however, the aqueous extract overwhelmingly had a high TPC value that was about 5- and 12-fold significantly ($p < 0.05$) greater than those obtained for Ac MeOH and EtOH extracts respectively. Also, the TFC values in MoSP significantly ($p < 0.05$) differed in the order; Ac MEOH > aqueous > EtOH.

The TPC and TFC values reported for MoLP in this study are similar to the values previously reported by Vongsak *et al.* (2013). The higher TPC of EtOH extract of MoLP is consistent with the values obtained by Vongsak *et al.* (2013), where significantly high TPC values were reported for EtOH. Similarly, the high TPC in aqueous extract of MoSP is consistent with high values reported previously by Jahan *et al.* (2018) in a similar experiment where different solvents were used in the extraction of the bioactives. The variation of phytochemicals between the MoLP and MoSP extracts may be ascribed to the possible exposure of the leaf to nutrients and sunlight through photosynthesis than the seeds (Prabakaran *et al.*, 2018). The total flavonoid composition was found to be highest in Ac MeOH extract of MoLP. This may result from increased solubility of certain phytochemicals classified as flavonoids in acidified methanol over EtOH and water. The results presented in Table 3.1 also emphasized the role of the choice of solvent used for extraction as they affect the phytochemical composition (Dadi *et al.,* 2018). This is because the TPC and TFC of different extracts differs significantly with different solvents (Perumal & Klaus, 2003; Prabakaran *et al.*, 2018). The higher TPC obtained for EtOH extracts of MoLP in this study may also be ascribed to the solvent property as well as the solubility attribute of individual active compounds present in the raw plant (Medini *et al.,* 2014). The polarity properties of solvents and polyphenols used are also crucial to the extractibility of phenolic compounds

present in raw samples (Ravani *et al.*, 2018). Therefore, it can be deduced that the choice of solvent affects the phytochemical composition of MoLP and MoSP and as such must be carefully selected to maximize yield and prevent degradation during extraction.

Table 3.1 Bioactive content of *Moringa oleifera* leaf and seed powder extract

Sample	Solvent	Total phenolic Content mg GAE/g	Total Flavonoid Content mg QE/g
Leaf	60% Ethanol	24.00 ± 0.36[a]	14.07 ± 0.16[a]
	Acidified Methanol	21.36 ± 0.95[b]	16.38 ± 0.13[b]
	Aqueous	16.44 ± 0.54[c]	9.32 ± 0.05[c]
Seed	60% Ethanol	1.52 ± 0.040[d]	0.26 ± 0.026[d]
	Acidified Methanol	4.19 ± 0.106[e]	1.85 ± 0.141[e]
	Aqueous	18.93 ± 0.478[f]	1.50 ± 0.036[f]

Sample results are expressed as mean ± standard deviation at n = 4. Values with different superscript letters in the same column indicates significant differences when $p < 0.05$

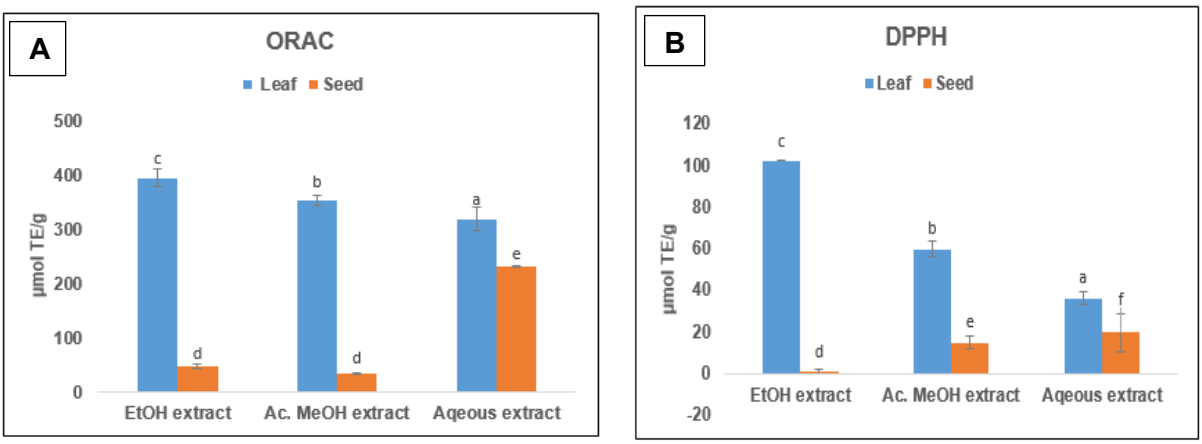
3.5 Antioxidant activity of MoLP and MoSP extracts

As expected, the antioxidant capacity determined by ORAC, FRAP and DPPH values, were higher for MoLP extracts compared to MoSP (Fig 3.2). The extracts obtained by EtOH (MoLP) demonstrated significantly ($p < 0.05$) superior antioxidant values than other respective solvents' extracts except in the MoSP where the water (ORAC and DPPH) and Ac MeOH (FRAP) extracts gave greater values. The ORAC values for the MoLP extracts ranged from 319.47 to 396.15 µmol TE/mg. In contrast, the aqueous extract of the seed exhibited the best ORAC antioxidant scavenging activity 231.57 TE/mg, while Ac. MeOH showed the least ORAC activity 34.53 µmol TE/mg.

The EtOH extract of MoLP had the highest ferric ion reducing ability with a FRAP value of 77.01 mg AAE/g while the aqueous extract had the lowest FRAP value of 44.66 mg AAE/g (Fig 3.2C). The FRAP results followed the same trend with the TPC values, FRAP value for EtOH was highest and the aqueous extract was lowest. There was significant difference ($p < 0.05$) in FRAP values for all extracts from the three solvents used for extraction. Generally, the values obtained indicates that *M. oleifera* had a reducing effect on

free radicals which can inhibit oxidative stress and damage. Hence, the extracts of *M. oleifera* leaf powder have good antioxidant reducing power. For DPPH, the values ranged from 35.95 to 102. 2 µmol TE/g (Fig 3.2B). The antioxidant activity through DPPH in extracts of leaves showed significant differences ($p < 0.05$) among the different extraction solvents. The scavenging activity of DPPH was highest in EtOH extract while the aqueous extract was the least. This followed the trend noticed earlier in the EtOH extracts with higher polyphenol contents.

Interestingly, on further analysis by the Pearson's correlation to ascertain the relationship of antioxidant capacity with phytochemical constituents, only the ORAC and FRAP values in the EtOH MoLP extract exhibited positive relationships with the TPC (Fig 3.3), the former reaching statistical significance at $p < 0.05$.



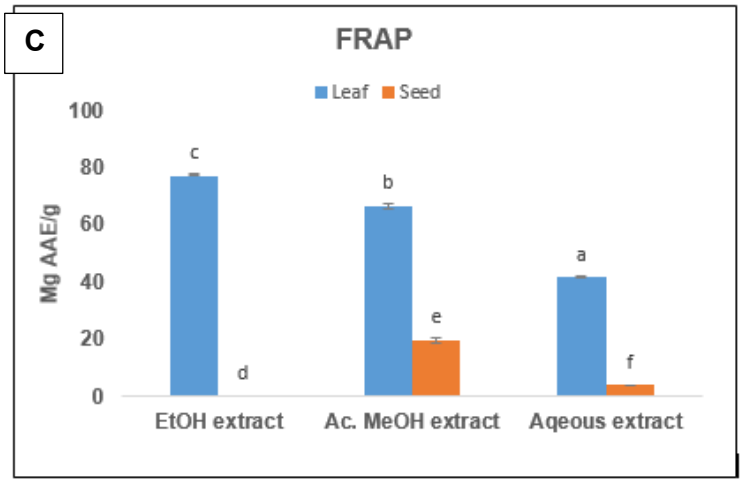


Figure 3.2 Antioxidant activity by ORAC (A), DPPH (B), and FRAP (C) of MoLP and MoSP extracts. ORAC: oxygen radical absorbance capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power; EtOH: hydroethanol; Ac MeOH: Acidified methanol; Aqeous-water.
(Bars denote: mean ± standard deviation; n = 3; letters significant differences among samples).

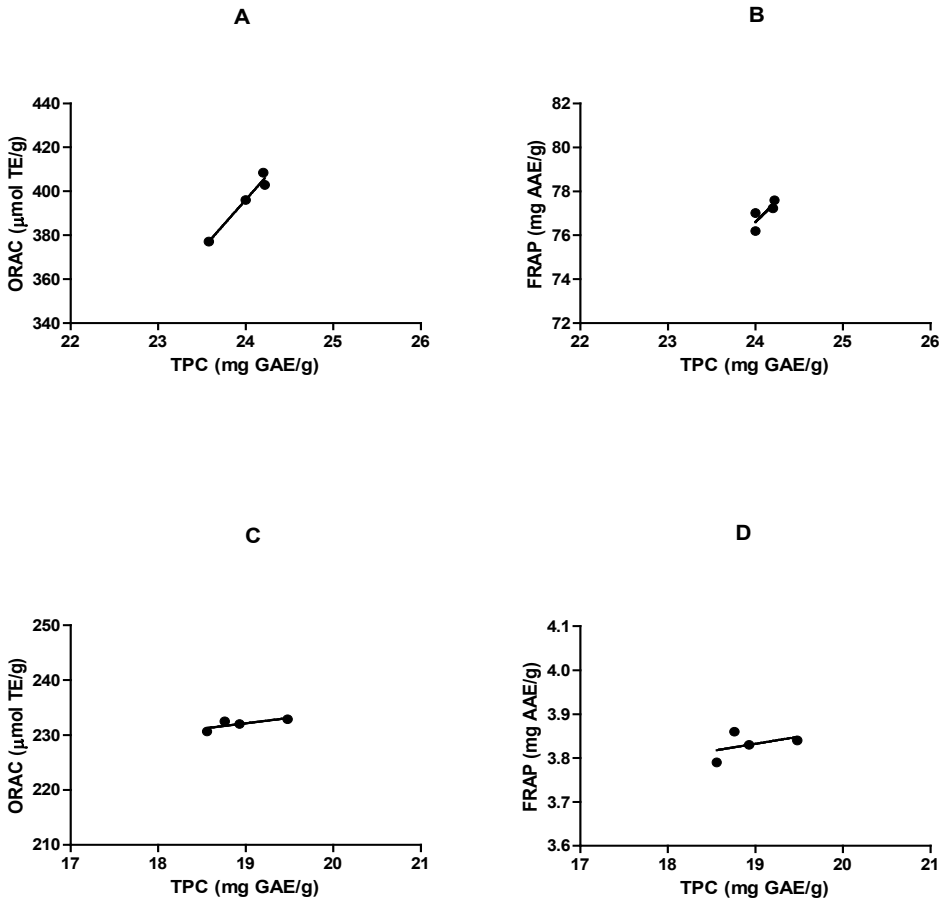


Fig 3.3 Correlation analysis of TPC against (A) ORAC (B) FRAP of leaf EtOH extracts of leaf (C) ORAC (D) FRAP of H_2O extract of *M. oleifera* seed powder (n=3)

The presence of antioxidants in *M. oleifera* extracts has previously been ascribed to the polyphenol compositions (Prabakaran *et al.*, 2018; Nobosse et al., 2018). Hence, the superior antioxidant value of EtOH (MoLP) extracts over MOSP. This is evident in the antioxidant activity of DPPH, radical scavenging activity of ORAC and reducing power of FRAP for EtOH (MoLP) extracts (Fig 3.2 A-C).

In the same manner, the aqueous extract of MoSP exhibited the high ORAC and DPPH powers when compared to EtOH and Ac MeOH, this is expected as they contain significantly higher TPC values. Generally antioxidant compounds have been reported to be responsible for scavenging of free radicals that result in oxidative imbalances in the body which causes oxidative stress (Jahan et al., 2018). In addition to this, the antioxidant activity of the extracts makes them an important natural antioxidant compound when included in foods (Singh et al., 2013). Hence, *M. oleifera* extracts from different solvents exhibited varying antioxidant activity.

3.6 LC-MS analysis

All extracts were subjected to LC-MS analysis to determine the specific identity of the phytochemical constituents and the result listed in Table 3.2. The chromatograms displaying the base peak and retention time for the identified compounds are shown in Figures 3.4 A-F The identification procedure was done using ChemCalc and metfrag. The parent m/z $[M - H]^-$ was retrieved from the base peak of the chromatograms with corresponding retention time. These values were imputed into ChemCalc where a possible list of the parent molecular formulae were generated. Some compounds were only found present in trace amounts while others were present in substantial quantity. A total of 20 compounds were detected as individual peaks and identified using MSDial/MSfinder (Table 3.2). The identified compounds can be generally classified as organic acids, phenolic acids, flavonoids and amino acids. The following specific compounds were identified and quantified; gluconic acid, quinic acid, malic acid, neochlorogenic acid (3CQA), chlorogenic acid (5CQA), rutin, p-coumaoryl quinic acid, Dicaffeoylquinic acid, tryptophan, niagirin, dihydro-resveratol, Quercetin O-glucoside, Kaempferol O-rutinoside, Kaempferol O-glucoside, Kaempferol O-acetyl glucoside, 3-Feruloylquinic acid and 5-Feruloylquinic acid. The compounds identified in MoLP extracts are more than those present in MoSP. In addition to this, EtOH and Ac.MeOH extracts of MoLP reflected the earlier results reported for the phytochemical composition. This is because more individual compounds were identified.

Some of these compounds have health benefits such as antioxidants (Govardhan Singh *et al.*, 2013; Padayachee and Baijnath, 2020), anti-carcinogenic (Anwar *et al.*, 2007; Sultana and Anwar, 2008), anti-inflammatory (Pero *et al.*, 2009; Jang *et al.*, 2017), anti-depressant and anti-diabetic potential (Ganatra Tejas *et al.*, 2012; Hamany Djande *et al.*, 2018) and could be included in foods that are low or deficient in these important healthy compounds.

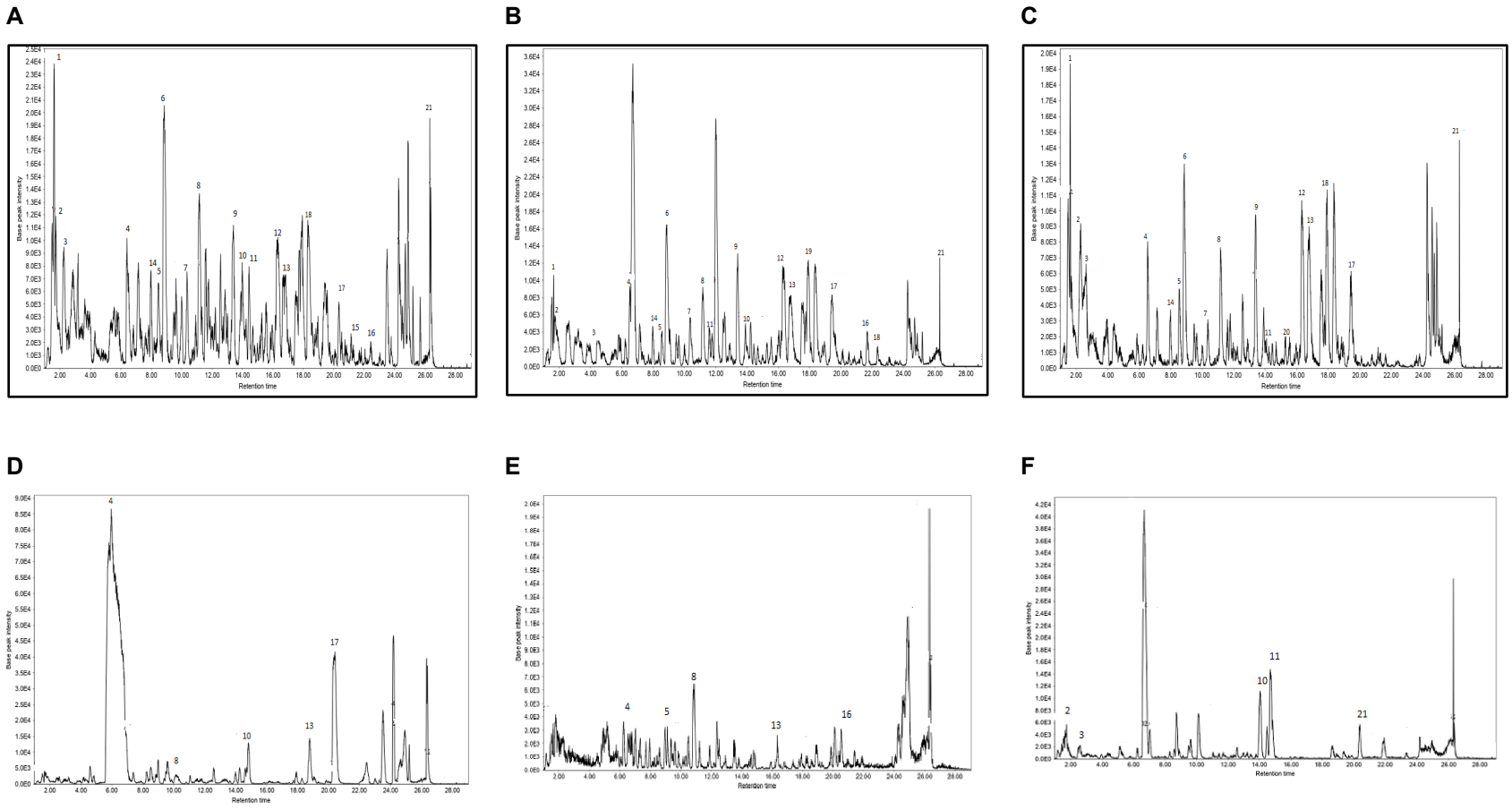


Figure 3.4 LCMS Chromatogram showing the peaks of different compounds in (A) EtOH (B) Ac. MeOH (C) H_2O extract of *M. oleifera* leaf powder (D) EtOH (E) Ac. MeOH and (F) H_2O extract of *M. oleifera* seed powder.

Table 3.2 Identification and Quantification of *Moringa oleifera* leaf and seed powder extracts (mg/Kg)

Peak Number	Compounds	Rt (min)	Molecular formula (MF)	Experimental m/z [M – H]$^-$	Fragment	Extract					
						EtOH Leaf	Ac. MeOH Leaf	H_20 Leaf	EtOH Seed	Ac. MeOH Seed	H_2O Seed
	Organic acids										
1	Quinic acid	1.70	$C_7H_{12}O_6$	191.054	135, 383	3211± 277	813 ± 44	1748 ± 90	ND	ND	ND
2	Malic acid	1.72	$C_4H_6O_5$	133.014	135	3028 ± 77	2324 ± 46	1794 ± 75	ND	58 ± 3.51	8 ± 1.2
3	Citric acid	2.26	$C_6H_8O_7$	191.021	147	3937 ± 143	2032 ± 95	4755 ± 168	ND	167 ± 15	721 ± 28
	Phenolic acids										
6	Neochlorogenic acid	8.80	$C_{16}H_{18}O_9$	353.087		15228 ± 84	10357 ± 75	7069 ± 203	ND	ND	ND
7	3-p-coumarylquinic acid	10.36	$C_{16}H_{18}O_8$	337.092	163, 293, 338	5139 ± 39	3916 ± 135	1935 ± 77	ND	ND	ND
8	cryptochlorogenic acid	11.16	$C_{16}H_{18}O_9$	353.087	173, 351	7503 ± 410	4546 ± 61	3484 ± 24	Traces	Traces	ND
14	Dicaffeoylquinic acid	8.01	$C_{25}H_{24}O_{12}$	515.142		3711 ± 105	1889 ± 103	1295 ± 35	ND	ND	ND
11	3-feruloylquinic acid	14.25	$C_{17}H_{20}O_9$	367.102	349	1412 ± 50	Traces	Traces	Traces	564 ± 6.8	267 ± 3.1
10	5-feruloylquinic acid	14. 05	$C_{17}H_{20}O_9$	369.028	368, 395	399 ± 6.5	Traces	Traces	Traces	1801 ± 53	472 ± 9.3
	Flavonoids										
12	Rutin	16.38	$C_{27}H_{29}O_{16}$	609.147	610, 611, 301	12140 ± 174	12854 ± 248	9554 ± 207	ND	Traces	ND
13	Quercetin 3-galactoside	16.64	$C_{21}H_{20}O_{12}$	463.087	464, 465	11775 ± 484	12217 ± 234	9478 ± 484	387 ± 33	291 ± 42	61 ± 10
9	Kaempferol O-rutinoside	13.38	$C_{27}H_{30}O_{15}$	593.148	521, 594	11408 ± 234	12012 ± 478	8735 ± 310	ND	ND	ND
18	Kaempferol 7-O-glucoside	18.33	$C_{21}H_{20}O_{11}$	447.095	448, 449	11469 ± 541	11009 ± 325	8034 ± 442	ND	ND	ND

17	Kaempferol O-acetyl glucoside	20.12	$C_{23}H_{22}0_{12}$	489.103	459, 490	15700 ± 280	18869 ± 315	10919 ± 85	ND	ND	ND
16	Myricetin	21.71	$C_{25}H_{24}0_{14}$	547.106		Traces	Traces	Traces	Traces	Traces	Traces
19	Astralagin 7-rhamnoside	17.92	$C_{27}H_{30}0_{15}$	593.155		Traces	ND	ND	ND	ND	ND
20	Brucine (alkaloid)	15.30	$C_{23}H_{26}N_2O_4$	393.174		ND	Traces	ND	ND	ND	ND
	Amino Acids										
4	Phenylalanine	6.53	$C_9H_{11}NO_2$	164.071		Traces	Traces	Traces	Traces	Traces	Traces
5	Trytophan	8.05	$C_{11}H_{11}N_2O_2$	203.082	204, 407	5487 ± 79	2269 ± 151	3152 ± 131	Traces	Traces	452 ± 62.7
21	Adenosine	26.5	$C_{10}H_{13}N_5O_4$	265.147		ND	Traces	ND	ND	ND	ND

ND: Compound not detected; Values are expressed as mean ± standard deviation (n=3)

3.6.1.1 Organic acids

In this study, three organic acids were identified tentatively among all samples (Fig 3.4). The compounds are quinic, malic and citric acids with m/z mass of 191,133 and 191 respectively and were eluted at a retention time of 1.64, 1.72 and 2.26 respectively at peak 1, 2 and 3 (Table 3.2). These compounds were previously reported to be present in *M. oleifera* leaf extract (Rodríguez-Pérez *et al.*, 2015; Hamany Djande *et al.*, 2018). Organic acids contribute substantially to the odour and taste of foods (Jiang *et al.*, 2020). These compounds were present in all leaf extracts while only malic and citric acid were present in the seed extracts. The quantity of these compounds varied with extraction solvent (Table 3.2) which further confirms reports from the phytochemical composition that was reported earlier in this chapter (Section 3.4.1). Quinic acid in peak 1 is an organic acid, and was previously identified in leaf extracts of *M. oleifera* by Mbikay, (2012), the compound is said to enhance the metabolism of tryptophan in the digestive tract and consequently repair damaged DNA material in the body (Pero *et al.*, 2009). In the current study, quinic acid was identified and quantified in the leaf but could only be identified in the seed extracts in trace level (Table 3.2).

Malic and citric acid, peak 2 and 3 respectively were identified and quantified in the leaf and seed extract of the plant although in minute quantities. These two compounds have been reported for their important use in food preservation (Rodríguez-Pérez *et al.*, 2015b). Of all the three organic acids identified and/or quantified, the ethanol extracts had the highest concentration of these compounds (Table 3.2).

3.6.1.2 Phenolic acids

The following phenolic acids were identified in the present study (Table 3.2); neochlorogenic acid (peak 6), 3-p-coumarylquinic acid (peak 7), cryptochlorogenic acid (peak 8), dicaffeoylquinic acid (peak 14), 3-feruloylquinic acid (peak 11) and 5-feruloylquinic acid (peak 10). These compounds vary with solvents used for the extraction. Phenolic acids present in EtOH were higher than Ac. MeOH and the aqueous extracts, this can be attributed to the higher TPC observed in the former. It is also important to mention that these compounds were more in MoLP than in MoSP which is expected as earlier values from phytochemical analysis lend credence to this. These compounds possess antioxidant properties and are effective against risks such as cardiovascular disorder, inflammatory disorder, as well as hyperglycaemia and other liver-related disorders (Amaglo *et al.*, 2010; Tajik *et al.*, 2017; Oguntibeju *et al.*, 2019) resulting from oxidative stress and damage. The phenolic compounds identified were previously reported by Nouman *et al.* (2016) in 7 different cultivars of *M. oleifera*. Although the quantities

detected in this previous study were significantly lower than values reported in the current study (Table 3.2). Beyond extraction solvent, process conditions, genetic attributes and agronomic conditions contributes to the differences in biochemical constituents of phenolic compounds.

3.6.1.3 Flavonoid compounds

Flavonoid compounds were also identified and quantified in the extracts of *M. oleifera* leaf and seed (Figure 3.4), the compounds identified include; rutin (peak 12), quercetin 3-galactoside (peak 13), kaempferol O-rutinoside (peak 9), kaempferol 7- o -glucoside (peak 18), kaempferol O-acetyl glucoside (peak 17), myricetin (peak 16), and astralagin 7-rhamnoside (peak 19). Other flavonoids identified tentatively in trace amounts but not captured on base peaks of chromatograms includes kaempferol 3-rhamnoside 7-galacturonide, apigenin 7-rhamnosyl-1 galacturonide, isorhamnetin 3-malonylglucoside and quercitrin. These compounds were previously identified by Coppin *et al.* (2013). Quercetin and kaempferol derivatives are the most dominant of the flavonoids identified in these samples, similar observations were previously reported by Rodríguez-Pérez *et al.*, (2015a). A high quantity of rutin, quercetin and kaempferol derivatives was reported for leaf extracts from EtOH and Ac. MeOH.The importance of flavonoids as viable antioxidant compounds has been reported previously (Sultana and Anwar, 2008; Coppin *et al.*, 2013; Kurmukov, 2013; Falowo *et al.*, 2018). Apart from their importance as antioxidants, their value as viable therapeutics and prophylactic against the problems posed by oxidative stress and inflammatory diseases has been studied (Coppin *et al.*, 2013). Although their presence in Ac. MeOH extracts of the leaf powder seems slightly higher than those reported for EtOH which further corroborates the higher TFC values earlier reported. The extracts of the leaf and seed can be further used in the development of functional foods beneficial to consumers. In all, flavonoid compounds were quantified and identified as present in the extracts of leaf and seed though in varied amount.

Other compounds identified includes phenylalanine (peak 4), adenosine (peak 21) and tryptophan (peak 5). These compounds have been identified in *M. oleifera* in previous studies by Rodríguez-Pérez *et al.*, (2015a). Tryptophan is an essential amino acid; essential amino acids are protein moieties which cannot be synthesised by the human body but are supplied only via the human diet. Oyeyinka and Oyeyinka (2018) reported that *M. oleifera* has an appreciable quantity of amino acids which further validates the importance of the miracle plant. Adenosine amino acid has been reported for its importance during metabolism as well as its neuro-modulating activity of the central nervous system (Ciruela *et al.*, 2010). Generally, all compounds identified from the seed and leaf extracts of *M. oleifera* indicate the presence of

important phytochemical compounds essential for consumer health against imbalances that may have resulted from oxidative stress. Discrepancies in the individual biologically active constituents of these extracts can be attributed to different extraction solvents, identification, and quantification procedure used.

3.7 Conclusion and recommendations

From this chapter, the extraction of bioactive compounds present in *M. oleifera* leaf and seed powder was carried out with effect of solvent on the extracted bioactive reported. It was established that *M. oleifera* leaf and seed powder extracts are rich in phytochemical compounds and subsequently resulting in corresponding antioxidant activity. The extraction solvent was reported to have significant impact on the phytochemical composition of the raw material. The EtOH extract of MoLP showed the best phytochemical composition and antioxidant activity while the aqueous extracts was best for MoSP. There was a strong positive significant correlation between the TPC of EtOH extracts and the ORAC using Pearson's correlation test (R^2 = 0.981) which was significant at 95% confidence level. The obtained data indicated that the bioactive compounds extracted can be used as inclusion in the production of functional foods and nutraceuticals in form of microcapsules with profound benefits for consumers. Overall, since the result obtained from this chapter indicated that the leaf extracts are rich in polyphenol content than the seed extracts and with chromatographic profiling validating this. Hence the leaf powder extracted with EtOH is proposed for further use in encapsulation in the subsequent chapter of the current study.

3.8 References

Amaglo, N.K., Bennett, R.N., Curto, R.B. Lo, Rosa, E.A.S., Turco, V. Lo, Giuffrida, A., Curto, A. Lo, Crea, F. & Timpo, G.M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chemistry*, **122**, 1047–1054.

Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L. & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry*, **51**, 6657–6662.

Chukwuebuka, E. (2015). *Moringa oleifera* "The Mother's Best Friend." *International Journal of Nutrition and Food Sciences*, **4**, 624.

Ciruela, F., Albergaria, C., Soriano, A., Cuffí, L., Carbonell, L., Sánchez, S., Gandía, J. & Fernández-Dueñas, V. (2010). Adenosine receptors interacting proteins (ARIPs): Behind the biology of adenosine signaling. *Biochimica et Biophysica Acta - Biomembranes*, **1798**, 9–20.

Coppin, J.P., Xu, Y., Chen, H., Pan, M.H., Ho, C.T., Juliani, R., Simon, J.E. & Wu, Q. (2013). Determination of flavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera*. *Journal of Functional Foods*, **5**, 1892–1899.

Cuellar-Nuñez, M.L., Luzardo-Ocampo, I., Campos-Vega, R., Gallegos-Corona, M.A., González de Mejía, E. & Loarca-Piña, G. (2018). Physicochemical and nutraceutical properties of moringa (Moringa oleifera) leaves and their effects in an in vivo AOM/DSS-induced colorectal carcinogenesis model. *Food Research International*, **105**, 159–168.

Dadi, D.W., Emire, S.A. & Hagos, A.D. (2018). Influences of different drying methods and extraction solvents on total phenolic and flavonoids , and antioxidant capacity of *Moringa stenopetala* leaves. *Journal of Pharmacognosy and phytochemistry*, **7**, 962–967.

Dhanani, T., Shah, S., Gajbhiye, N.A. & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, **10**, S1193–S1199.

Fahey, J.W. (2005). *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life Journal*, 1–15.

Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J. & Muchenje, V. (2018). Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. *Food Research International*, **106**, 317–334.

Govardhan Singh, R.S., Negi, P.S. & Radha, C. (2013). Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa oleifera* seed flour. *Journal of Functional Foods*, **5**, 1883–1891.

Gulu, N.B. (2017). *Functional and rheological properties of Bambara groundnut starch-catechin complex obtained by chemical grafting*, (Thesis)

Hamany Djande, C.Y., Piater, L.A., Steenkamp, P.A., Madala, N.E. & Dubery, I.A. (2018). Differential extraction of phytochemicals from the multipurpose tree, *Moringa oleifera*, using green extraction solvents. *South African Journal of Botany*, **115**, 81–89.

Herbello-Hermelo, P., Lamas, J.P., Lores, M., Domínguez-González, R., Bermejo-Barrera, P. & Moreda-Piñeiro, A. (2018). Polyphenol bioavailability in nuts and seeds by an *in vitro* dialyzability approach. *Food Chemistry*, **254**, 20–25.

Jiang, Z., Huang, Q., Jia, D., Zhong, M., Tao, J., Liao, G., Huang, C. & Xu, X. (2020). Characterization of organic acid metabolism and expression of related genes during fruit development of actinidia eriantha ‘ganmi 6.’ *Plants*, **9**, 332–347.

Jideani, V.A. & Diedericks, C.F. (2014). Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: *Antioxidant-antidiabetic agents and human health*. InTech.

Khan, F., Pandey, P., Jha, N.K., Jafri, A. & Khan, I. (2020). Antiproliferative effect of *Moringa oleifera* methanolic leaf extract by down-regulation of Notch signaling in DU145 prostate cancer cells. *Gene Reports*, **19**, 100619.

Kurmukov, A.G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, **1**, 13–14.

Lin, M., Zhang, J. & Chen, X. (2018). Bioactive flavonoids in *Moringa oleifera* and their health-promoting properties. *Journal of Functional Foods*.

Mbikay, M. (2012). Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. *Frontiers in Pharmacology*, **3**, 1–12.

Medini, F., Fellah, H., Ksouri, R. & Abdelly, C. (2014). Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. *Journal of Taibah University for Science*, **8**, 216–224.

Mukunzi, D., Nsor-Atindana, J., Xlaoming, Z. & Gahungu, A. (2011). Comparison of volatile profile of *Moringa oleifera* leaves from Rwanda and China using HS-SPME. *Pakistan Journal of Nutrition*, **10**.

Nobossé, P., Fombang, E.N. & Mbofung, C.M.F. (2018). Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Science and Nutrition*, **6**, 2188–2198.

Nouman, W., Anwar, F., Gull, T., Newton, A., Rosa, E. & Domínguez-Perles, R. (2016). Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. *Industrial Crops and Products*, **83**, 166–176.

Oguntibeju, O.O., Aboua, G.Y. & Omodanisi, E.I. (2019). Effects of *Moringa oleifera* on oxidative stress, apoptotic and inflammatory biomarkers in streptozotocin-induced diabetic animal model. *South African Journal of Botany*, **129**, 354–365.

Oladipo, B. & Betiku, E. (2019). Process optimization of solvent extraction of seed oil from *Moringa oleifera*: An appraisal of quantitative and qualitative process variables on oil quality using D-optimal design. *Biocatalysis and Agricultural Biotechnology*, **20**, 101187.

Omodanisi, E.I., Aboua, Y.G., Oguntibeju, O.O. & Lamuela-Raventós, R.M. (2017). Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of *Moringa oleifer*a in diabetes-induced nephrotoxic male wistar rats. *Molecules*, **22**, 1–16.

Owusu-Ansah, M., Gyingiri Achel, D., Adaboro, R.M., Asare, D.K. & Amoatey, H.M. (2011). Total phenolic content and antioxidant activity in leaf samples of twelve accessions of *Moringa oleifera* Lam. *International Journal of Chemical and Analytical Science*, **2**, 1226–1230.

Oyeyinka, A.T. & Oyeyinka, S.A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, **17**, 127–136.

Padayachee, B. & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. *South African Journal*

of Botany, **129**, 304–316.

Pero, R.W., Lund, H. & Leanderson, T. (2009). Antioxidant metabolism induced by quinic acid increased urinary excretion of tryptophan and Nicotinamide. *Phytotherapy Research*, **23**, 335–346.

Perumal, S. & Klaus, B. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry*, **51**, 2144–2155.

Prabakaran, M., Kim, S.H., Sasireka, A., Chandrasekaran, M. & Chung, I.M. (2018). Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of *Moringa oleifera*. *Food Bioscience*, **26**, 23–29.

Ravani, A., Prasad, R., Dumle, K. & Joshi, D.C. (2018). Profiling of polyphenols, antioxidants potential, nutrients and flavor compounds of *Moringa oleifera* pods (var. PKM - 1). *International Journal of Chemical Studies*, **6**, 1571–1575.

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A. & Segura-Carretero, A. (2015). Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves. *Industrial Crops and Products*, **66**, 246–254.

Sultana, B. & Anwar, F. (2008). Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chemistry*, **108**, 879–884.

Tajik, N., Tajik, M., Mack, I. & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *European Journal of Nutrition*, **56**, 2215–2244.

Vongsak, B., Sithisarn, P. & Gritsanapan, W. (2013). Bioactive contents and free radical scavenging activity of *Moringa oleifera* leaf extract under different storage conditions. *Industrial Crops and Products*, **49**, 419–421.

CHAPTER FOUR
OPTIMISATION OF THE MICROENCAPSULATION OF *MORINGA oleifera* LEAF POWDER EXTRACT USING MALTODEXTRIN AND / OR GUM ARABIC AS COATING MATERIALS

Abstract

Moringa oleifera leaf powder (MoLP) contains important bioactive compounds with health-promoting properties. However, these bioactive compounds (core or active) may degrade under normal storage and processing conditions. Encapsulation is a viable procedure that has been identified to offer enough protection for the core, this is made possible through the entrapment of the core in another material known as the coating or wall material. The efficiency of an encapsulation experiment is determined by the ability of the core to be fully entrapped in the coating material. Furthermore, encapsulation efficiency (EE) is dependent on the process or preparation variables prior to drying. Response surface methodology (RSM) makes use of statistical techniques and tools to predict optimum process conditions in encapsulation experiments. In this experiment, the coating to coating ratio (MD: GA), core to coating ratio (C: CR) and ultrasonication time were used to make microcapsules using the Box–Benkhen design of RSM. A total of 15 experimental runs conducted in duplicates were generated with three centre points. The overall regression model obtained was adequate and fits at 95% confidence level. The lack of fit of the experiment was not significant ($p > 0.05$). The coating to coating ratio and the core coating ratio had a significant effect on the encapsulation efficiency of the obtained microcapsules, while ultrasonication time was found not to influence the efficiency of encapsulation. The encapsulation efficiencies observed for the 15 experimental runs ranged from 64.25 – 88.79%. The optimal process conditions for the predicted maximum efficiency of 88.89% were coating ratio (MD: GA) of 7.5: 2.5, C: CR of 1:8.5 and ultrasonication time of 13.33 minutes. The model was validated for the predicted optimum condition and an EE of 84.90% was obtained. The obtained EE was not significantly different ($p > 0.05$) from the predicted EE. The obtained optimum process conditions for encapsulation can be further adapted for other plant extracts with properties and attributes similar to *M. oleifera* leaf powder extract.

4.1 Introduction

The surge in the demand for healthy and functional foods continues to create a paradigm shift in the global food markets (Paulo and Santos, 2017). This has led to the development of nutraceuticals and functional ingredients from biologically active compounds in plants. Biologically active compounds in plants are mostly unstable during processing, can deteriorate under normal storage conditions and tend to have astringent tastes or off-putting flavours. An important method that has been identified for solving these drawbacks is encapsulation. Encapsulation is a technology that helps in stabilising and protecting the encapsulated material, by creating a barrier which, masks astringent and unpleasant tastes, protects the bioactives from processing and gastro-intestinal fluids damage whilst aiding delivery, as well as enhancing the release properties of the encapsulated biologically active compounds (Poshadri and Kuna, 2010; Nedovic *et al.*, 2011; Ballesteros *et al.*, 2017). This is usually achieved by entrapping compounds in another material known as the carrier compounds or wall material (Poshadri and Kuna, 2010; Nedovic *et al.*, 2011).

A plant that has been established over the years to contain substantial quantities of biologically active and phytochemical compounds is *Moringa oleifera* (Anwar *et al.*, 2007; Razis *et al.*, 2014; Gupta *et al.*, 2018; Özcan, 2018; Padayachee and Baijnath, 2020). These active compounds are secondary metabolites (Jideani and Diedericks, 2014; Lin *et al.*, 2018), that have antioxidant properties comparable to plants such as strawberries (Oyeyinka and Oyeyinka, 2018). The major components of *M. oleifera* bioactive compounds are phenolic and flavonoid compounds (Rodríguez-Pérez *et al.*, 2015). These compounds have been reported for their role as antioxidant compounds and their potential in the development of functional foods and nutraceuticals (Nouman *et al.*, 2016; Saucedo-Pompa *et al.*, 2018). However, the active compounds are susceptible to damage by oxidation as well as when subjected to high temperatures during processing. Therefore, adequate protection is required for effective use and delivery. The encapsulation of phenolic compounds, as well as other bioactive compounds in the plant, has resulted in improved delivery, protection, retention and stabilization of these compounds (Cilek *et al.*, 2012; Chuyen *et al.*, 2019; Yadav *et al.*, 2019).

Efficient encapsulation of bioactive compounds depends on the choice of coating material, the appropriate blend of these coating materials and the ratio of core compounds/core to coating materials ratio, temperature etc. (Cilek *et al.*, 2012; Pellicer *et al.*, 2018, 2019; Yadav *et al.*, 2019). In addition, the stability of microcapsules developed is dependent on maximum entrapment and efficiency obtained during the encapsulation procedure (Ray *et al.*, 2016).

Therefore, an optimum condition for these factors must be developed to understand the interactions towards obtaining maximum encapsulation efficiency. Previous studies on the encapsulation of extracts of *M. stenopetala* (Dadi *et al.*, 2019) were optimized for the inlet temperature for spray drying, core to coating ratio as well as coating to coating ratio using maltodextrin and high methoxyl pectin. Other studies on encapsulation of grape polyphenols and sour cherry pomace extracts (Cilek *et al.*, 2012; Yadav *et al.*, 2019) have been optimised for encapsulation parameters using various methods using whey protein concentrate, gum Arabic and maltodextrin.

One of the methods often used in the design of an encapsulation experiment is known as the response surface methodology (RSM), and has previously been used for the design of encapsulation experiments (Mahdavee Khazaei *et al.*, 2014; Chuyen *et al.*, 2019; Dadi *et al.*, 2019). Response surface methodology is a combination of various mathematical and statistical techniques used to improve the efficiency of process conditions (Chuyen *et al.*, 2019; Oladipo and Betiku, 2019). The method is carried out by optimising response(s) of interest, from selected independent variables (Getachew and Chun, 2016). A mathematical model is generated to describe the process under investigation (Getachew and Chun, 2016). Chuyen *et al.* (2019) has used Box-Behnken design for optimising process variables for the retention of carotenoid and antioxidant activity in the encapsulation of carotenoid-rich oil in Gac-peel extracts, Dadi and Emire, (2019) also used this to optimise the development of encapsulated bioactives from *M. stenopetala*. This method was therefore employed in this study for process optimisation to obtain maximum efficiency.

Moringa oleifera leaf powder extract has been microencapsulated using vegetable protein by spray drying (Osamede Airouyuwa and Kaewmanee, 2019), however to the best of our knowledge, no previous study on the encapsulation of *M. oleifera* has been done using a combination of gum Arabic and maltodextrin as well as freeze-drying as the drying technique. Additionally, there are no previous studies on the optimisation of process parameters for the encapsulation of *M. oleifera*. Hence, this study aims to study the effect of coating material mixture ratio, core to coating ratio as well as ultrasonication time as variables on the encapsulation efficiency MoLP extract microcapsules.

4.2 Materials and Method

4.2.1 Materials

Moringa oleifera leaf powder was obtained from SupaNutri Graff-Reinet, South Africa; Maltodextrin from Sigma Aldrich, St. Louis USA, supplied through Sigma Aldrich South Africa; Gum Arabic from Thermofisher, Kandel Germany supplied through Industrial Analytical; ethanol from United Scientific, Goodwood, Cape town, South Africa. All other reagents used were of analytical grade. All experiments were carried out at the Oxidative Stress Research Centre, Cape Peninsula University of Technology, Bellville, South Africa.

4.2.2 Extraction of active compounds from MoLP

The extraction of active compounds from *M. oleifera* leaf powder (MoLP) was done using 60% ethanol. This mixture extract was selected based on the previous experiment (as detailed in Chapter 3) where 60% ethanol solution (EtOH) was found to be the best extraction solvent for the phytochemicals in MoLP out of all the solvents investigated. MoLP was dissolved in the solvent (10% w/v ratio) and the mixture agitated for 24 h in a laboratory shaking incubator, (Incotec shake, Labotec model 355, South Africa) at 120 rpm and temperature of 27 ℃. The mixture was then centrifuged using an (Eppendorf centrifuge 5810 R Hamburg, Germany) at 10000 rpm for 10 min at 4 ℃. The supernatant was decanted and residue discarded. The supernatant was concentrated by removing ethanol using a rotary evaporator (BÜCHI Labortechnik R-200 Rotavapor Flawil, Switzerland) set at a temperature of 40 ℃. The extract obtained was freeze-dried using a Virtis genesis 25EL freeze-drier (Gardiner, New York, USA) for 48 h to obtain dried extract powder and stored in a freezer at -4 ℃ until further analysis.

4.2.3 Preparation of coating materials and encapsulation of *Moringa oleifera* leaf powder extracts

Maltodextrin (MD) of dextrose equivalent (DE) 16.5 – 19.5 and gum Arabic (GA) *acacia* ash < 4% were used as coating material. The method described by Cilek *et al.* (2012) was employed but with modification. The coating materials (MD and GA) were dispersed separately in a tube containing distilled water to get a 10% total solid part of the mixture and allowed to mix overnight (18 h) using a standing shaker (LABOTEC model 205, Cape Town, South Africa) for complete hydration. The coating material solution was mixed using a high-speed homogeniser (IKA – WERIE, GMBH & Co. KG, Stanfen Germany) at 9500 rpm for 15 min, and allowed to

stand for 1 h. Freeze-dried ground *MoLP* extract was re-dissolved in distilled water by vigorous mixing, this was then mixed with the coating material solution using a high-speed homogeniser for 10 min at 9500 rpm after which it stood for 1 h. The total solid obtained after the mixing of the core and the coating material was 10%. The mixtures were subjected to ultrasonication for 10–20 min at 100 W power and 20 kHz frequency with a 50% pulse rate using a titanium probe (3.8 mm diameter) using an ultrasound sonicator (BANDELIN electronic GmBH & co. KG, Berlin Germany). All through this process, the samples were kept on an ice bath. After ultrasonication, the mixture stood for 5 h at room temperature before it was transferred into a Freeze dryer (Hanil Science Industrial Co. Ltd., South Korea) for 48 h to obtain dried MoLP extract microcapsules.

4.3 Experimental Design and Statistical Analysis

To optimize the process conditions for the preparation of MoLP microcapsules, the Box–Benkhen design (BBD) of response surface methodology (RSM) was applied. This is essential in determining the optimum condition for obtaining maximum encapsulation efficiency of the MoLP extract. In this experiment, there are three independent variable/factors namely; coating material ratio (X_1), core: coating ratio (X_2) and ultra-sonication time (X_3) with one response; encapsulation efficiency (Y_1) evaluated using Box–Benkhen design (BBD) (Table 4.1). The range of the factors evaluated is presented in Table 4.1 with coded levels -1, 0 and 1 representing the minimum, the center point and the maximum points of the factors respectively.

Table 4.1 - Process factors considered using Response Surface Methodology

Factor	Unit	Coded Factor levels		
		-1	0	1
Coating: coating ratio (MD:GA)	(w/w)	6:4	8:2	10:0
Core: coating ratio	(w/w)	1:6	1:8	1:10
Ultrasonication time	(min)	10	15	20

MD: GA (Maltodextrin: gum Arabic)

The three independent variables were imputed into the experimental design which returned 15 experimental runs carried out in duplicates. Process variable ranges were selected based on past experiments reported in literatures.The design consists of 15 experimental runs with 3

centre points and 12 model points. Process conditions responses were measured on the encapsulation efficiency by subjecting actual values from the laboratory to RSM for analysis. The responses were run, and the actual and predicted values are presented in Table 4.2. To determine the effects and interactions of the factors on the response, a quadratic polynomial equation (equation 4.1) was selected to model and predict the optimal process conditions for the three factors:

$$Y = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad \text{Equation (4.1)}$$

In equation (4.1), *Y* represents the response, A, B and C are the independent variables otherwise known as factors. A is the coating material ratio (Maltodextrin: Gum Arabic ratio), B is the core: coating ratio and C is the ultrasonication time. In the equation, β_o represents the interception point. The linear coefficients of the equation are β_1, β_2 and β_3 while the interaction and quadratic coefficients are β_{12}, β_{13} and $\beta_{23,}$ and β_{11}, β_{22}, and β_{33} respectively.

4.4 Determination of biochemical attributes of the encapsulated *Moringa oleifera* leaf powder extract

4.4.1 Total Phenolic Content (TPC) of *Moringa oleifera* leaf powder extract microcapsules

The total phenolic content of the MoLP extract microcapsules was determined using the method reported by Cilek *et al.* (2012). Folin Ciocalteau reagent was used in this assay and was reduced using sodium carbonate which was mixed with microcapsule extract. A colour change was noticed after they were mixed. A 100 mg of *M. oleifera* extract microcapsule powder was dissolved in a 10 ml ethanol-methanol mixture (50:50 v/v). The mixture was agitated for 5 min using a vortex and centrifuged for 5 min at 3000 rpm. The absorbance of the supernatant obtained was measured at 765nm using a Multiskan microplate reader (SpectraMax i3x Molecular devices, San Jose California, USA) and calibration curve R prepared. The total phenolic content was expressed as mg of Gallic acid extract per g (mg GAE/g). In the same way, the TPC of the *M. oleifera* microcapsule (MM) was determined and was expressed as mg of Gallic acid equivalent (mg GAE/g).

4.4.2 Surface phenolic content of *Moringa oleifera* leaf powder extract microcapsules

The phenolic content on the surface of the microcapsules was determined using the method of Saénz *et al.* (2009) with slight modification. One gram of MoLP extract microcapsule was dispersed in 10 ml of a mixture of ethanol and methanol at a ratio of (50:50 v/v). The mixtures were mixed for 10 s in a vortex. The mixture then centrifuged, and the surface phenolic content was determined using the Folin Ciocalteau reagent. The surface phenolic content was determined by measuring the absorbance of the sample using the Multiskan microplate reader, SpectraMax i3x Molecular devices, San Jose California, USA at a wavelength of 765 nm, with gallic acid as standard. A calibration curve R was prepared; the surface phenolic content of the microcapsules was expressed as mg of Gallic acid extract per g of the sample (mg GAE/g).

4.4.3 Encapsulation efficiency of *Moringa oleifera* leaf powder extract microcapsules

Encapsulation efficiency refers to the quantity of the encapsulated/entrapped active (phenolic) compounds divided by the total active (phenolic) compound in the system. To determine the quantity of phenolic content that was entrapped during this experiment, Equation 4.2 below was employed as previously described by Cilek *et al.* (2012).

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{TPC} - \text{SPC}}{\text{TPC}} \times 100 \qquad \text{Equation (4.2)}$$

Where TPC is total phenolic content and SPC the surface phenolic content.

4.4.4 Antioxidant (DPPH) activity of *Moringa oleifera* leaf powder extract microcapsules

The antioxidant activity of MoLP extract microcapsules was determined by the DPPH method described by Yadav *et al.* (2019). Briefly, a 100 mg microcapsule powder was dispersed in a 10 ml of 50:50 ethanol-methanol mixture. The microcapsule sample extract was then mixed with freshly prepared DPPH solution (6.925 mg/l) in methanol. The mixture stood for 30 min at room temperature after which the absorbance of the sample was measured at 515 nm using a Multiskan microplate reader (Spectramax, Molecular Devices, San Jose California, USA). A calibration curve was prepared using 100 µl of Trolox standard solution (100–1000 µM) along with DPPH solution. The DPPH scavenging activity was calculated using Equation 4.3.

$$\text{DPPH Scavenging activity} = \text{A0} - \frac{(\text{AM} - \text{AS})}{\text{A0}} \qquad \text{Equation (4.3)}$$

Where A_0 is the absorbance value for the control (DPPH only), A_M is the absorbance of DPPH mixed with the samples, and A_S is the absorbance of the samples without DPPH.

4.5 Statistical Analysis

Optimization experiment data are subjected to Design-Expert version 12.0 (Stat-Ease Inc., Minneapolis, USA). Other reported data are expressed as the means of duplicate runs using SPSS analysis software (Version 26.0, IBM SPSS Inc, Armonk, NY, USA).

4.6 Results and discussion

4.6.1 Encapsulation efficiency of *Moringa oleifera* leaf powder extract microcapsules

The encapsulation efficiency of the microcapsules was determined according to the method mentioned in section 4.4.3 and reported in Table 4.2. The total phenolic content (TPC) of freeze-dried *M. oleifera* powder prior to encapsulation was 24 mg GAE/g while TPC of the freeze-dried microcapsules of the 15 experimental runs ranged from 3.28 – 4.90 mg GAE/g. The surface phenolic content (SPC) of each run was determined and used in the calculation of the encapsulation efficiency using Equation 4.1. The SPC values for the runs ranged from 0.46 – 1.66 mg GAE/g. It is important to note that the more phenolic content deposited on the surface of the microcapsules the lower the EE. The EE of the 15 runs done in duplicate ranged from 64.25 – 88.79% (Table 4.2).

4.6.2 Regression and statistical analysis of the model

The range of parameters used in this experiment was based on values that have been reported by various researchers and the common factors and responses associated with the preparation. A preliminary run with the three independent variables was prepared using the earlier reported data. Earlier studies conducted on the microencapsulation of grape seed polyphenols and sour cherry pomace extracts, considered are coating material ratio, core-coating ratio and ultrasonication time as the independent variables with the response being encapsulation efficiency (Cilek *et al.*, 2012; Yadav *et al.*, 2019). In another study on the microencapsulation optimization condition for spray drying of *M. stenopetala* bioactives, Dadi *et al.* (2019) investigated the impact of coating to coating ratio and core:coating ratio as factors. The

conditions for the preparation of *M. oleifera* extract microcapsules in the present study were optimized with three factors and one response.

Table 4.2 – Box–Behnken design indicating independent and dependent variables for the encapsulation of *Moringa oleifera* leaf powder extracts microcapsules with maltodextrin: gum Arabic coatings

		Factor 1	Factor 2	Factor 3	Response (Actual)	Response (Predicted)
Std	Run	X_1 (w/w)	X_2 w/w	X_3(min)	Y (%)	Y (%)
13	1	10:0	1:10	15	75.680	77.46
9	2	10:0	1:8	10	78.090	77.59
17	3	10:0	1:8	20	76.965	75.74
14	4	10:0	1:6	15	64.250	64.20
3	5	8:2	1:10	10	88.270	87.00
11	6	8:2	1:6	20	70.090	71.36
16	7	8:2	1:6	10	65.455	66.01
15	8	8:2	1:10	20	82.525	81.97
4	9	6:4	1:10	15	85.010	85.06
8	10	6:4	1:8	10	79.425	80.65
12	11	6:4	1:8	20	82.320	82.82
1	12	6:4	1:6	15	68.505	66.73
10	13	8:2	1:8	15	88.785	87.14
7	14	8:2	1:8	15	86.745	87.14
5	15	8:2	1:8	15	85.880	87.14

X1 = MD: GA ratio, X2 = C: CR, X3 = UT, *Y* = EE; MD: Maltodextrin, GA: Gum Arabic C: Core, CR: Coating Ratio, UT: Ultrasonication Time, EE: Encapsulation Efficiency

The response determined in this study is the encapsulation efficiency while the preparation factors were coating: coating ratio (MD: GA), core: coating ratio and ultrasonication time. The coating: coating ratio (C: CR) is the ratio of the two coating or wall materials (maltodextrin and gum Arabic) used in this experiment, the use of different coating materials has been established to produce a microcapsule with high encapsulation efficiency (Cilek *et al.,* 2012). The core: coating ratio is the ratio of the core compounds to the coating material. The RSM model was

developed to predict the optimum condition for the preparation of *M. oleifera* microcapsules at 95% confidence level for encapsulation efficiency.

Microcapsule preparation was done in duplicates and the determination of encapsulation efficiency (total phenolic and surface phenolic content (Equation 4.1) was conducted in duplicate with analysis on each run done in triplicates. It is important to state that the encapsulation efficiency is always dependent on the total phenolic content and the surface phenolic content as earlier stated in Equation 4.1. The observed results (actual) and the corresponding predicted values for this experiment are presented in Table 4.2. The encapsulation efficiency of the actual results ranges from 64.250 – 88.75%. The regression model that shows the relationship between the factors (independent variables) and the response (dependent variable) is reported in Equation 4.4.

$$\textit{Encapsulation efficiency} = 87.14 - 2.53A + 7.90B + 0.00825C - 1.27AB - 1.00AC - 2.59BC - 5.58A^2 - 8.20B^2 - 2.26C^2 \quad \text{Equation (4.4)}$$

Where A is the coating: coating ratio (MD: GA), B is the core: coating ratio and C is the ultrasonication time (min). The statistical significance of the regression model for the encapsulation efficiency (EE) was evaluated using ANOVA and is presented in Table 4.3. The F-value for the developed model for EE is 28.64 while the p-value is 0.009 (Table 4.3), the p-value according to this model is significant. This is because a *p*-value less than or equal to 0.05 indicates a statistically significant model (Falowo *et al.*, 2019; Oladipo and Betiku, 2019). Generally, the p-value is an important indicator of probable errors as well as a form of validating the model. The results from the analysis of variance (ANOVA) for EE shows that the linear terms (independent variables) A and B are significant ($p < 0.05$) while the linear term C ($p > 0.05$) was not statistically significant. Of the three interactions, only ultrasonication time and core: coating ratio interaction shows statistical significance (Table 4.3).

To further validate the regression model, statistical parameters such as the R^2, adjusted R^2, predicted R^2, percentage coefficient of variance (CV %) and adequate precision were determined. In this experiment, the R^2 (0. 9810), was an indication that only 0.019 variations could not be accounted for in the encapsulation efficiencies in the developed regression model. This connotes that there is a desirable relationship between the observed and predicted values for the EE from the developed regression model (Chuyen *et al.*, 2019). This was further

corroborated by agreements between the adjusted (0.9467) and predicted (0.7598) R^2 values. It is believed that a predicted R^2 value within the 0.2 range of the adjusted R^2 value denotes a reasonable agreement of the linear model. Other values that establish the adequacy of the developed model and show good correlation include a CV of 2.42% which is within the recommended range (< 10%) and a standard deviation (SD) of 1.90 (Table 4.3).

Table 4.3 - Regression coefficients for the fitted polynomial model and analysis of variance for the experimental results of encapsulation efficiency

Source of variance	Sum of Squares	DF	Mean square	F-value	*P*-value	Quality of Fit	
						Parameter	Value
Model	933.91	9	103.77	28.64	0.009	R^2	0.9810
A – MD:GA ratio	51.38	1	51.38	14.18	0.0131	Adjusted R^2	0.9467
B – Core: coating ratio	499.04	1	499.04	137.72	<0.0001	Predicted R^2	0.7598
C – ultrasonication time	0.0544	1	0.0544	0.0150	0.9072	Adequate Precision	14.759
AB	6.44	1	6.44	1.78	0.2400	Standard Deviation	1.90
AC	4.04	1	4.04	1.11	0.3393	Mean	78.53
BC	26.94	1	26.94	7.43	0.0415	% CV	2.42
A^2	114.97	1	114.97	31.73	0.0024	PRESS	228.70
B^2	247.98	1	247.98	68.44	0.0004		
C^2	20.50	1	20.50	5.66	0.0633		
Residual	18.12	5	3.62				
Lack of fit	13.67	3	4.56	2.05	0.3448		
Pure error	4.45	2	22				
Cor total	952.03	14					

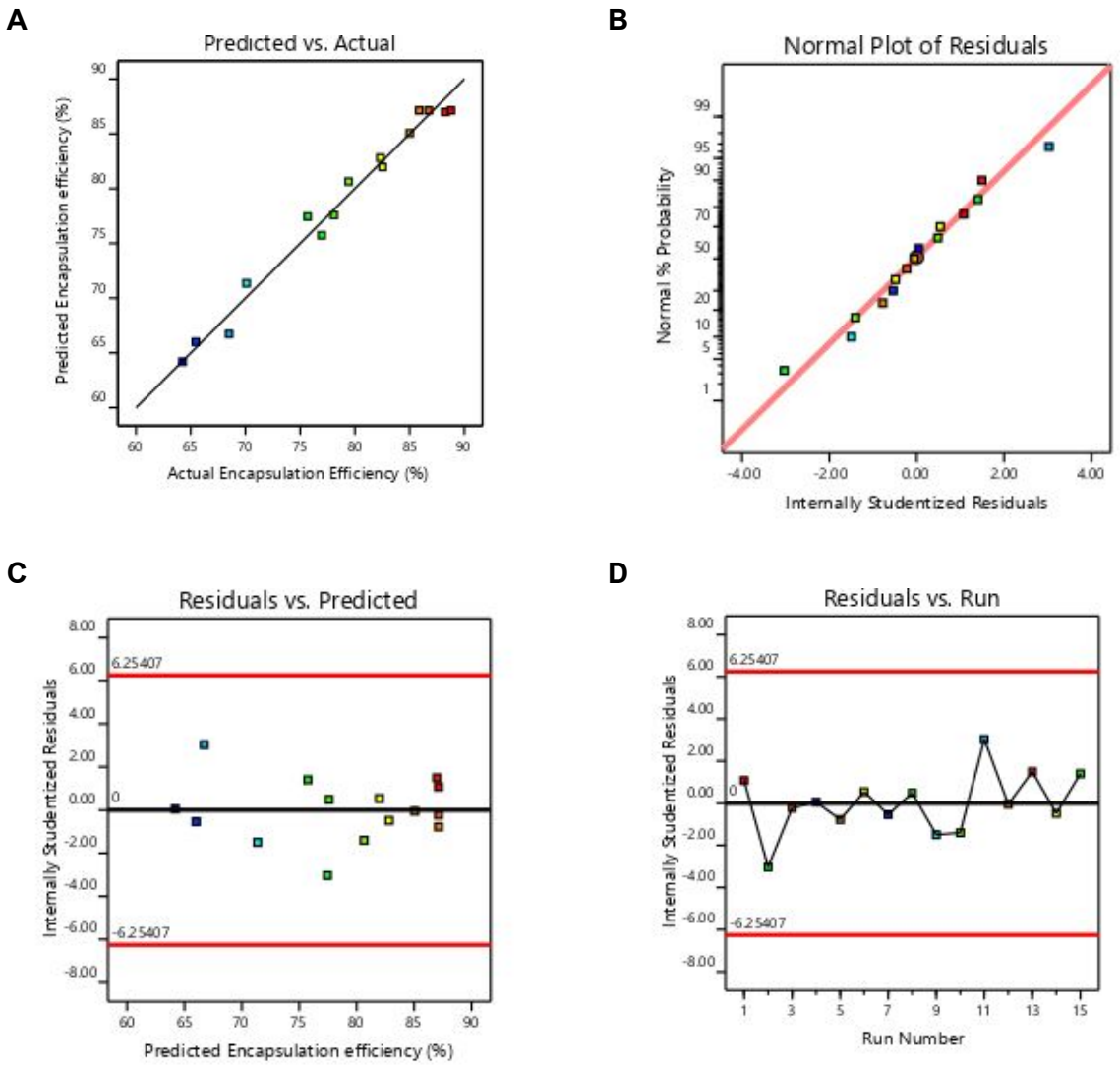


Figure 4.1 Model adequacy plot for Microencapsulation preparation condition (a) Predicted encapsulation efficiency vs actual encapsulation efficiency (b) normal residual plot (c) Residual vs predicted (d) outlier t plot

To further establish the accuracy of the model, diagnostic plots were generated as reported in Figure 4.1. The predicted EE versus the observed (actual) EE is shown in Fig 4.1A. The plot shows that the predicted and actual EE fits appropriately as points on both axes are near each other on the diagonal line, this shows that the response and the factors are closely fitted and shows a good estimation of the responses from the independent variables. Figure 4.1B shows the plot of the residuals. Residuals are differences or discrepancies between the predicted and the observed response. This parameter is a good way to measure if observed

response values mirror the ANOVA predictions of the regression model. As seen on the diagram, the points follow or are close to the diagonal line showing that the residuals agree with the normal percentage probability. Figure 4.1C presents the studentised residuals against the predicted EE. It can be observed that the residual points present are randomly scattered, this denotes that the initial variance remains constant for each of the responses.

Figure 4.1D presents the outlier t plot, for the experimental runs. The outlier t plot is the plot of residuals against the experimental runs for EE determination. Generally, the residuals are expected to be within ±3.00 as previously stated by (Oladipo and Betiku, 2019). The plot from this study properly fits into these criteria and no values were found outside the stated criteria which further validates the regression model. In all, the parameters and the statistical studies shows that the predicted and the observed response agrees and shows a good correlation with little or no variance.

4.7 Effect of interaction of process variables on encapsulation efficiency

4.7.1 Effect of core: coating ratio and coating: coating ratio on encapsulation efficiency

One of the important determinants of the efficiency of encapsulation of core compounds is the encapsulation efficiency. This parameter is pivotal in predicting the stability and controlled release properties of microcapsules (Dadi *et al.*, 2019). In addition to this, the encapsulation efficiency gives an insight into how much the core compounds are protected from external influences such as oxidation, thermal damage etc. Preparation variables and conditions exert effects on the outcome of an encapsulation experiment, more importantly, the efficiency of the process (Figure 4.2 A–C). This has been demonstrated in previous microencapsulation studies with variables such as coating material ratio, core: coating ratio and ultrasonication time (Cilek *et al.*, 2012; Yadav *et al.*, 2019).

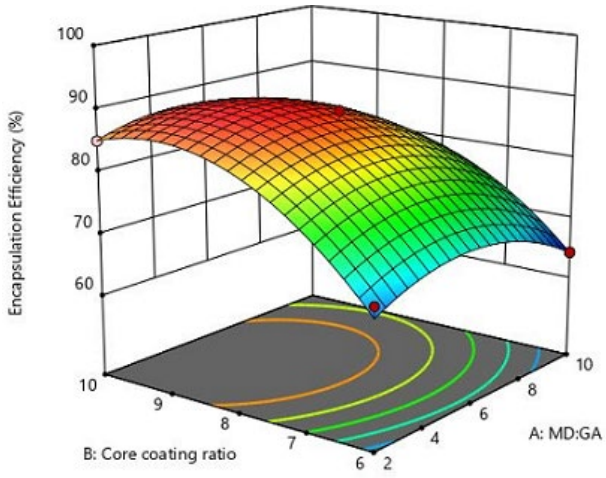
In the regression model, the interaction between the core: coating ratio and the coating: coating ratio has no significant effect ($p > 0.05$) on the encapsulation efficiency (Table 4.3). In addition to this, Fig 4.2A shows that the encapsulation efficiency at the core: coating ratio of 1:10, was higher than at 1:6. This is because there are fewer surface phenolic compounds present in samples with 1:10 core coating. Furthermore, it is expected that when more coating material is used, the ability to form a protective covering around the active compound is high which results in greater protection, hence higher efficiency. A similar observation was made by Cilek *et al.* (2012) and Yadav *et al.* (2019) in the encapsulation of phenolic compounds from sour cherry pomace and grape seed polyphenols, respectively.

Mahdavi *et al.* (2016) previously stated that when the coating material is increased with constant core material rates, a higher encapsulation efficiency is expected and vice versa. From Fig 4.2A, as coating material increased with the corresponding constant core material, there was an increase in the EE. It can also be deduced as coating: coating ratio moved from 10:0 to 6:4, there was a corresponding increase in encapsulation efficiency, however, efficiency was maximum at coating: coating ratio of 8:2 and core: coating ratio of 1:8. Therefore, it can be concluded that a higher amount of coating material around the core and a blend of gum Arabic with maltodextrin resulted in higher efficiency.

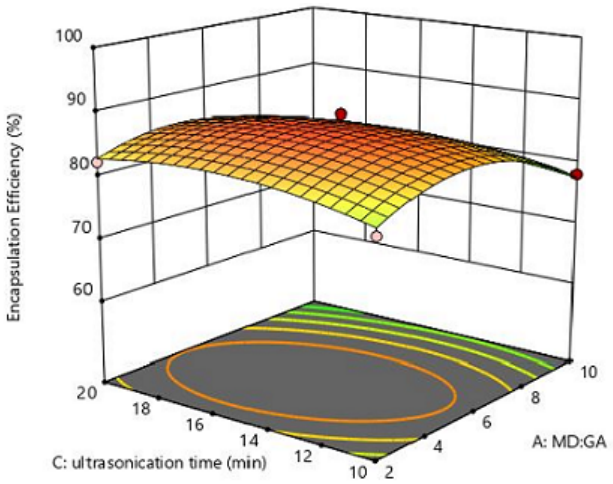
4.7.2 Effect of coating: coating ratio (Maltodextrin and Gum Arabic) and ultrasonication time on encapsulation efficiency

The effects of coating material ratio (MD and MD: GA) and ultrasonication time on the encapsulation efficiency of the microcapsules were also presented in Figure 4.2B. When MD alone was used, encapsulation efficiency was lower (64.25 – 78.09%) than samples where MD and GA were combined (65.46 - 88.79%). This may be because the use of two coating materials seems to exert a more protective effect on the active compounds than the use of single coating material (Dadi *et al.*, 2019).

A



B



C

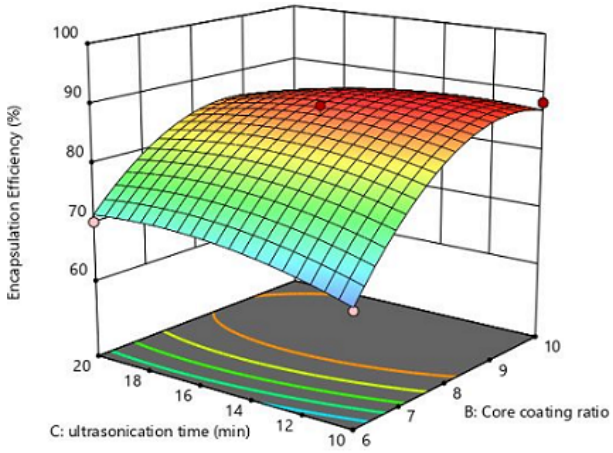


Fig 4.2: 3D Response surface plot for the effects of preparation variables on Encapsulation Efficiency (A) core coating and MD: GA ratio (B) ultrasonication time and MD: GA ratio (C) ultrasonication time and core coating ratio

This may also be ascribed to the inherent properties of the two different coating materials used. For example, while maltodextrin has been reported for its ability to form an amorphous glassy matrix around active compounds during encapsulation, gum Arabic has stabilizing and emulsifying properties as well as a film-forming characteristic that enhances the efficiency of the encapsulation process and gives good protection around the core (Ersus and Yurdagel, 2007; Cilek *et al.*, 2012; Mahdavi *et al.*, 2016). High EE reported in previous studies by Cilek *et al.* (2012) and (Ballesteros *et al.*, 2017) where a combination of maltodextrin and gum Arabic were used, agrees with the findings from this study. Furthermore, as the coating ratio increase with corresponding ultrasonication time, EE increased (Fig 4.2B). However, beyond the ultrasonication time of 16 min and MD: GA ratio of 7:3, equilibrium was reached and a slight decrease in EE sets in. This indicates that maximising EE could not be sustained after these points. The decrease in EE noticed beyond 16 min can be ascribed to continuous disruption of phenolic bonds at high energy density and high ultrasonication time which may result in presence of more surface phenolic compounds (Yadav *et al.*, 2019), hence decreasing the EE. Therefore, the maximum EE observed between the interaction among these variables was at 16 min and a coating: coating ratio of 7:3.

4.7.3 Effect of ultrasonication time and core coating ratio on encapsulation efficiency

Generally, ultrasonication is done to attain uniformity of the different components within the mixture not achieved during homogenizing. This uniformity and effective dissolution are attained by the action of sound energy or waves generated from electrical energy by the action of continuous cavitation thereby disrupting the bonds within samples for proper realignment. As ultrasonication time increased with a corresponding increase in core: coating ratio, EE increased (Fig 4.2C). Maximum encapsulation was reached after a core: coating ratio of 1:8 and an ultrasonication time above 15 min. After this point, EE begins to drop, increased ultrasonication can result in the collapse of internal structural properties of samples due to the influence of high cavitation energy for a prolonged period (Yadav *et al.*, 2019). This may result in the presence of more phenolic compounds on the surface of the microcapsules formed which may eventually defeat the whole essence of encapsulation. Although the interaction between core: coating ratio and ultrasonication time on the regression model seems insignificant ($p > 0.05$), there seems to be a direct relationship between the two, as the core: coating ratio increased from 10:0 to 6:4 until an equilibrium was reached. Hence, ultrasonication time and core: coating ratio contributes to EE.

4.8 Total phenolic content and surface phenolic content of *Moringa oleifera* leaf powder extract microcapsules

The total phenolic content of the microcapsule is the sum of phenolic compounds present in the microcapsule (enclosed and on the outer layer) while the surface phenolic content is the amount or quantity of phenolic compounds found on the surface of the microcapsules which were not entrapped. Determination of the TPC and SPC provides information on the EE (Table 4.4). The initial TPC of the *M. oleifera* leaf powder extract was 24 mg GAE/g, and after encapsulation, ranged from 3.28 – 4.90 mg GAE/g while the values obtained for SPC ranged from 0.46 – 1.66 mg GAE/g.

There was no significant difference ($p > 0.05$) for TPC values reported for all samples encapsulated with only MD. Although all samples were encapsulated at the different core: coating ratios, the TPC shows no significant differences. Microcapsules encapsulated with 8:2 coating: coating ratio did not particularly show similar trends as TPC values differ significantly except for run 5 and 8 with similar TPC values. Similarity amidst both runs may likely result from their similar core coating ratio. The samples that are regarded as the centre point all have similar TPC values with no significant differences ($p > 0.05$) noticed. This is expected as these runs were developed under similar preparation conditions. The SPC values for all runs followed a similar trend, it was observed that the higher the core: coating ratio, the higher the phenolic compounds noticed on the surface of the microcapsules (Table 4.4), which is an indication of a lower EE (Dadi *et al.*, 2019).

4.9 DPPH scavenging activity of MoLP extract microcapsules

The scavenging activity of DPPH of microcapsules was measured for microcapsules from all runs and ranged from 41.69 – 65.20 µmol TE/g (Table 4.4). The highest DPPH values were recorded for the samples that are described as the centre point (8:2, 1:8, and 15 min), this was expected as they have high TPC values. No specific trend was noticed for antioxidant activity. However, high antioxidant values were reported for samples coated with maltodextrin alone, similar observation was reported for grape seed polyphenols encapsulated with maltodextrin alone by Yadav *et al.* (2019). Other samples that gave lower antioxidant activity values may likely result from the deterioration that may have been caused by high ultrasound cavitation during mixing as high energy impact may result in degradation of the bioactive compounds in the microcapsules . In all, the microcapsules developed had good antioxidant / radical scavenging activities against DPPH.

Table 4.4 Total and surface phenolic content and antioxidant content of MoLP microcapsules

Run	TPC (mg GAE/g)	SPC (mg GAE/g)	DPPH (μmol TE/g)
1	4.90 ± 0.007^{e}	1.19 ± 0.033^{ef}	57.09 ± 0.42^{de}
2	4.49 ± 0.042^{de}	0.98 ± 0.058^{de}	59.08 ± 0.80^{e}
3	4.72 ± 0.110^{e}	1.09 ± 0.026^{ef}	58.60 ± 0.45^{d}
4	4.64 ± 0.301^{e}	1.66 ± 0.045^{g}	42.38 ± 0.51^{a}
5	4.78 ± 0.282^{e}	0.56 ± 0.115^{a}	41.69 ± 1.95^{a}
6	4.08 ± 0.017^{cd}	1.22 ± 0.010^{h}	53.23 ± 1.81^{c}
7	3.73 ± 0.098^{bc}	1.29 ± 0.077^{f}	51.15 ± 1.43^{b}
8	4.85 ± 0.054^{e}	0.86 ± 0.061^{cd}	48.94 ± 1.06^{b}
9	3.96 ± 0.242^{c}	0.46 ± 0.005^{a}	49.59 ± 1.44^{b}
10	4.71 ± 0.368^{e}	0.83 ± 0.129^{bcd}	49.84 ± 1.01^{b}
11	3.28 ± 0.036^{a}	0.98 ± 0.059^{ed}	50.06 ± 1.43^{ab}
12	3.37 ± 0.256^{ab}	1.17 ± 0.122^{ef}	50.55 ± 2.00^{b}
13	4.77 ± 0.168^{e}	0.57 ± 0.225^{a}	56.55 ± 1.24^{d}
14	4.76 ± 0.265^{e}	0.63 ± 0.059^{ab}	65.19 ± 0.03^{f}
15	4.72 ± 0.142^{e}	0.67 ± 0.089^{abc}	65.20 ± 0.01^{f}

Data are presented as mean ± standard deviation, different superscripts in the same column indicate significant differences at 95% confidence level using Duncan's test (where n = 6)

4.10 Optimization and validation of microencapsulation preparation conditions

According to the Response surface model, the predicted best preparation condition for MoLP extract microcapsules was found at a core: coating ratio of 1:85, coating: coating (MD: GA) ratio of approximately 7.5:2.5 and ultrasonication time of 13.33 min. After the optimal condition was confirmed in duplicate runs, experimentally observed values from the validation experiment showed an EE of 84.90% which is close to the predicted value of 88.89%, there were no significant differences ($p > 0.05$) noticed between the observed and the predicted EE. This shows that the combined use of MD and GA for the encapsulation of MoLP extract for use on a large scale under the predicted condition would provide enough protection for the core material at these predicted condition, validating the efficiency of the developed model that illustrates the microencapsulation preparation conditions.

4.11 Conclusion

Optimisation of preparation conditions for MoLP extract microcapsules using maltodextrin and gum Arabic resulted in optimum encapsulation. The regression model developed fitted and was acceptable at a 95% confidence level in predicting the encapsulation efficiency. The optimal efficiency of encapsulation can be obtained at a coating: coating (MD: GA) ratio of 7.5: 2.5, core: coating ratio of 1: 8.5 and ultrasonication time of 13.33 min. This preparation condition was predicted to give an optimised EE value of 88.89% which was validated with an EE of 84.90% obtained for the validation experiment. The optimized preparation condition can be used subsequently in microencapsulation experiments on a large or commercial scale. The conditions for the preparation of this microcapsule will be further explored in Chapter 5 to develop microcapsules of *M. oleifera* leaf powder extracts from maltodextrin gum Arabic mixtures, and maltodextrin and gum Arabic and to determine their properties.

4.12 References

Akhavan Mahdavi, S., Jafari, S.M., Assadpoor, E. & Dehnad, D. (2016). Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. *International Journal of Biological Macromolecules*, **85**, 379–385.

Anwar, F., Latif, S., Ashraf, M. & Hassan, A. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research*, **659**, 651–659.

Ballesteros, L.F., Ramirez, M.J., Orrego, C.E., Teixeira, J.A. & Mussatto, S.I. (2017). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. *Food Chemistry*, **237**, 623–631.

Chuyen, H. V, Roach, P.D., Golding, J.B., Parks, S.E. & Nguyen, M.H. (2019). Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technology*, **344**, 373–379.

Cilek, B., Luca, A., Hasirci, V., Sahin, S. & Sumnu, G. (2012). Microencapsulation of phenolic compounds extracted from sour cherry pomace: Effect of formulation, ultrasonication time and core to coating ratio. *European Food Research and Technology*, **235**, 587–596.

Dadi, D., Emire, S.A., Hagos, A.D. & Eun, J.-B. (2019a). Development of microencapsulated bioactive product from *M. stenopetala* leaves extract by optimizing spray drying process parameters *Annals of Agricultural Science*, **20**, 191–206.

Ersus, S. & Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. *Journal of Food Engineering*, **80**, 805–812.

Falowo, O.A., Oloko-Oba, M.I. & Betiku, E. (2019). Biodiesel production intensification via microwave irradiation-assisted transesterification of oil blend using nanoparticles from elephant-ear tree pod husk as a base heterogeneous catalyst. *Chemical Engineering and Processing - Process Intensification*, **140**, 157–170.

Getachew, A.T. & Chun, B.S. (2016). Optimization of coffee oil flavor encapsulation using response surface methodology. *LWT - Food Science and Technology*, **70**, 126–134.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, **40**, 1107–1121.

Gupta, S., Jain, R., Kachhwaha, S. & Kothari, S.L. (2018). Nutritional and medicinal applications of *Moringa oleifera* Lam.—Review of current status and future possibilities. *Journal of Herbal Medicine*, **11**, 1–11.

Jideani, V.A. & Diedericks, C.F. (2014). Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: *Antioxidant-Antidiabetic Agents and Human Health*. InTech.

Lin, M., Zhang, J. & Chen, X. (2018). Bioactive flavonoids in *Moringa oleifera* and their health-promoting properties. *Journal of Functional Foods*, **47**, 469–479

Mahdavee Khazaei, K., Jafari, S.M., Ghorbani, M. & Hemmati Kakhki, A. (2014). Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. *Carbohydrate Polymers*, **105**, 57–62.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S. & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, **1**, 1806–1815.

Nouman, W., Anwar, F., Gull, T., Newton, A., Rosa, E. & Domínguez-Perles, R. (2016). Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. *Industrial Crops and Products*, **83**, 166–176.

Oladipo, B. & Betiku, E. (2019). Process optimization of solvent extraction of seed oil from *Moringa oleifera*: An appraisal of quantitative and qualitative process variables on oil quality using D-optimal design. *Biocatalysis and Agricultural Biotechnology*, **20**, 101187.

Osamede Airouyuwa, J. & Kaewmanee, T. (2019). Microencapsulation of *Moringa oleifera* leaf extracts with vegetable protein as wall materials. *Food Science and Technology International*, **25**, 533–543.

Oyeyinka, A.T. & Oyeyinka, S.A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, **17**, 127–136.

Özcan, M.M. (2018). *Moringa spp*: Composition and bioactive properties. *South African Journal of Botany*, **129**, 25–31.

Padayachee, B. & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. *South African Journal of Botany*, **129**, 304–316.

Paulo, F. & Santos, L. (2017). Design of experiments for microencapsulation applications: A review. *Materials Science and Engineering C*, **77**, 1327–1340.

Pellicer, J.A., Fortea, M.I., Trabal, J., Rodríguez-López, M.I., Carazo-Díaz, C., Gabaldón, J.A. & Núñez-Delicado, E. (2018). Optimization of the microencapsulation of synthetic strawberry flavour with different blends of encapsulating agents using spray drying. *Powder Technology*, **338**, 591–598.

Pellicer, J.A., Fortea, M.I., Trabal, J., Rodríguez-López, M.I., Gabaldón, J.A. & Núñez-Delicado, E. (2019). Stability of microencapsulated strawberry flavour by spray drying, freeze drying and fluid bed. *Powder Technology*, **347**, 179–185.

Poshadri, A. & Kuna, A. (2010). Microencapsulation technology: A review. *The Journal of Research ANGRAU*, **38**, 86–102.

Ray, S., Raychaudhuri, U. & Chakraborty, R. (2016). An overview of encapsulation of active compounds used in food products by drying technology. *Food Bioscience*, **13**, 76–83.

Razis, A.F.A., Ibrahim, M.D. & Kntayya, S.B. (2014). Health benefits of *Moringa oleifera*. *Asian Pacific Journal of Cancer Prevention*, **15**, 8571–8576.

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A. & Segura-Carretero, A. (2015). Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves. *Industrial Crops and Products*, **66**, 246–254.

Saénz, C., Tapia, S., Chávez, J. & Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chemistry*, **114**, 616–622.

Sarabandi, K., Jafari, S.M., Mahoonak, A.S. & Mohammadi, A. (2019). Application of gum Arabic and maltodextrin for encapsulation of eggplant peel extract as a natural antioxidant and color source. *International Journal of Biological Macromolecules*, **140**, 59–68.

Saucedo-Pompa, S., Torres-Castillo, J.A., Castro-López, C., Rojas, R., Sánchez-Alejo, E.J., Ngangyo-Heya, M. & Martínez-Ávila, G.C.G. (2018). Moringa plants: Bioactive compounds and promising applications in food products. *Food Research International*, **111**, 438–450

Yadav, K., Bajaj, R.K., Mandal, S. & Mann, B. (2019). Encapsulation of grape seed extract phenolics using whey protein concentrate, maltodextrin and gum arabica blends. *Journal of Food Science and Technology*, **57**, 426–434.

CHAPTER FIVE

CHARACTERISATION OF *MORINGA oleifera* LEAF POWDER EXTRACT ENCAPSULATED IN MALTODEXTRIN AND/OR GUM ARABIC COATINGS

Abstract

Moringa oleifera leaf powder (MoLP) leaf extract microcapsules were developed with maltodextrin (MD), gum Arabic (GA) and maltodextrin-gum Arabic (MD-GA) as a coating and their properties assessed. The bulk and tap density of the microcapsules were 0.177, 0.325 and 0.297 g/ml and 0.126, 0.295 and 0.259 g/ml respectively for GA, MD and MDGA microcapsules. Flowability properties of microcapsules indicated an intermediate flow property except for GA microcapsules which exhibited poor flow properties. The moisture content of the microcapsules ranged from 1.47 to 1.77% with no significant differences ($p > 0.05$) observed. All the microcapsules had high water solubility ranging from 86.35 for GA microcapsules to 98.74% for MD microcapsules and 90.51% for MDGA. Thermo Gravimetric Analyses (TGA) revealed that encapsulation enhanced the thermal stability of the active compounds as the maximum thermal degradation temperature of the microcapsules was observed at 341 ^{o}C while the maximum degradation of the non-encapsulated extracts was observed at 320 ^{o}C. The crystalline pattern of the microcapsules and MoLP extracts using the X-ray diffraction analysis revealed microcapsules and dried extract to have an amorphous nature. This was further validated by the surface morphology analysis which indicates the presence of amorphous, irregular, and flake-like attributes, except for MDGA microcapsules with slightly spherical microcapsules and indented surfaces. The Fourier Transform Infra-Red (FTIR) spectra of the microcapsules show the presence of C-O and O-H aromatic rings as well as amine groups. New spectra were noticed after encapsulation at 1177, 1382 and 1411cm^{-1} for MDGA, MD and GA microcapsules respectively which connotes a slight modification in the chemical structural pattern after encapsulation. Storage stability tests performed over 28 days at 4, 25 and 40 °C showed that the stability of microcapsules differs significantly ($p < 0.05$) with coating material type and temperature, as microcapsules were most stable at 4 ^{o}C. The simulated *in vitro* gastrointestinal release profile of polyphenols shows a significantly high ($p < 0.05$) release percentage of MDGA microcapsules over MD and GA microcapsules, although higher TPC values were reported for MD and GA microcapsules. The encapsulation efficiency of the microcapsules differs significantly ($p < 0.05$) and ranged from 72.87 for GA to 85.66% MDGA microcapsules. Antioxidant activity through DPPH does not differ significantly among microcapsules from different carriers with values ranging from 34.62 – 36.16 µmol TE/g while the FRAP values ranged from 16.90 – 20.28 mg AAE/g.

5.1 Introduction

Moringa oleifera Lam is a well-known plant that originates from the Himalayan and middle eastern regions of the world (Anwar *et al.*, 2007; Ferreira *et al.*, 2008). The plant has been reported for its richness in important bioactive and phytochemical compounds that have the potential for use in the development of functional foods. The presence of these bioactive compounds has heightened its use as a viable inclusion in foods for fortification and enrichment and to improve the overall functional importance of foods (Saucedo-Pompa *et al.*, 2018). Furthermore, bioactive compounds in the plant has been established as a potent substances against free-radical molecules that cause oxidative stress which results from an imbalance between free radicals and antioxidants in the body (Moyo *et al.*, 2012; Ma *et al.*, 2020; Padayachee and Baijnath, 2020). Hence, their inclusion in foods is believed to be pivotal in the development of functional foods.

The stability and storage life of bioactive compounds present in the plants have been a cause for concern over the years as they undergo degradation and loss of activity when they are exposed to certain environmental and processing conditions. The reason for this may be attributed to the presence of unsaturated bonds in the bioactive compounds which makes them prone to oxidative damage at high temperature and exposure to light. An effective method that has been proposed as a potential solution to these drawbacks is encapsulation. This method is important for retaining and stabilising active compounds in food, shielding sensitive and beneficial phytochemical compounds from degradation that might occur from environmental conditions, prevention of reaction of active compounds with food products as well as controlling their rate of release (Zuidam and Nedović, 2010; Aguiar *et al.*, 2016; Mahfoudhi *et al.*, 2016; Castro-Rosas *et al.*, 2017; Gómez *et al.*, 2018; Ozkan *et al.*, 2019). In addition, encapsulation is believed to improve the bioavailability and dissolution properties of bioactive compounds in food ingredients (Gómez *et al.*, 2018).

In an encapsulation experiment, the choice of coating material and encapsulation procedure plays a major role. According to Nedovic *et al.* (2011), coating materials for encapsulation experiments are selected based on their properties and the impact they have on the desired properties of the final products including; efficacy in protecting active compounds, cost, and the release rate of the active compound. The use of these biopolymers such as polysaccharides (maltodextrin), gums (gum Arabic), and protein (various legume protein extracts and concentrates) alike in the encapsulation of biologically active compounds shields them from the effect of light and oxygen that may result in degradation (Cilek *et al.*, 2012). These coating materials have been used previously in the encapsulation of sour cherry pomace extract (Cilek

et al., 2012), grape seed polyphenols (Yadav *et al.*, 2019), anthocyanins in *Ipomoea batatas* (Nawi *et al.*, 2015) and natural anthocyanins from bilberry (Akhavan Mahdavi *et al.*, 2016), etc. The choice of maltodextrin and gum Arabic has been ascribed to their stabilising, emulsifying, film-forming property as well as their low viscous properties in high concentration (Ersus and Yurdagel, 2007).

In addition to the choice of coating material, the choice of a suitable encapsulation and drying technique is crucial. Freeze drying is one of the methods that has been used in the encapsulation of active compounds in recent times. It has been used for drying of heat-sensitive materials (Ballesteros *et al.*, 2017) and its use has been found to aid the retention of the original properties of the compounds of interest. Freeze drying has also been used in the encapsulation and drying of the following; grape seed polyphenol (Yadav *et al.*, 2019), non-dewaxed propolis (Šturm *et al.*, 2019), sour cherry pomace extract (Cilek *et al.*, 2012), saffron petal anthocyanin (Mahdavee Khazaei *et al.*, 2014), polyphenols in spent coffee ground (Ballesteros *et al.*, 2017a), polyphenols in fruits (Ramírez *et al.*, 2015) amongst others.

To determine the efficacy of an encapsulation experiment, certain properties that include physical and functional, as well as structural are measured, such parameters are measured with the aid of; Fourier transform infrared spectroscopy (to identify molecular and structural components of the microcapsules), thermogravimetric analyses (to understand the behaviour of microcapsules at different temperature), SEM (to determine the morphological properties of microcapsules) amongst others. Currently, no study has been carried out on the encapsulation of *M. oleifera* leaf powder extracts using gum Arabic and maltodextrin as the coating material. The objective of this chapter is to determine the physical and functional properties of encapsulated *M. oleifera* leaf powder extract with maltodextrin and gum Arabic. Furthermore, the storage stability and the *in vitro* release property in a simulated gastro-intestinal model was determined.

5.2 Materials and Methods

Moringa oleifera leaf powder was obtained from SupaNutri Graff-Reinet, South Africa, Maltodextrin from Sigma Aldrich Germany, supplied through Sigma Aldrich South Africa, Gum Arabic from Thermofisher, Kandel Germany supplied through Industrial Analytical, ethanol from United scientific, Goodwood Cape town, South Africa. The physical and functional properties of *Moringa oleifera* leaf powder extract microcapsules were carried out at the physical property laboratory of the Food Science and Technology Department, Oxidative stress Research Centre,

Chemistry and Textiles Department of the Cape Peninsula University of Technology, Western Cape, iThemba laboratory, and Stellenbosch University South Africa. All reagents used were of analytical grade and all equipment used for testing was operated under perfect conditions.

5.2.1 Extraction of active compounds from *Moringa oleifera* leaf Powder (MoLP)

The extraction of active compounds from *M. oleifera* leaf powder (MoLP) was done using the procedure outlined in section 3.2.3 and section 4.2.2.

5.2.2 Preparation of coating material for encapsulation

The method described by Cilek *et al.* (2012) was adopted with some modifications. Maltodextrin (MD) of dextrose equivalent (DE) 16.5 – 19.5 and gum Arabic (GA) *acacia* ash < 4% were used as the coating materials. The coating materials were measured separately in the following proportion MD (1:0), GA (1:0) and MDGA (7.5: 2.5) were dispersed separately in a tube containing distilled water and allowed to mix overnight (18 h) using a standing shaker, (LABOTEC model 205, Cape Town, South Africa) for complete hydration. The coating material solutions were then mixed using a high-speed homogeniser, (IKA – WERIE, GMBH & Co. KG, Stanfen Germany) at 9500 rpm for 15 min, and was allowed to stand for 1 h prior to the addition of the core.

5.2.3 Preparation of MoLP extract microcapsules

Microcapsules of *M. oleifera* leaf powder extract were produced following the method reported by Cilek *et al.* (2012) with modification. This involves the use of optimum process and preparation condition obtained in Chapter 4. This condition makes use of a coating: coating ratio of 7.5:2.5 ((MDGA), a core: coating ratio of 1: 8.533 and ultrasonication time of 13.33 min. 100% MD and GA coatings were also used (Control) in the development of microcapsules using the same core: coating ratio and ultrasonication time that was stated above in section 5.2.2.

5.2.4 Freeze drying

The freeze drying of microcapsules were done after the preparation. The prepared mixtures were allowed to stand for 4–6 h prior to freeze drying. The mixture was then dried for 48 h in a Virtis genesis 25EL (Gardiner, New York, USA).

5.3 Encapsulation efficiency, total phenolic and surface phenolic content

Encapsulation efficiency refers to the quantity of the encapsulated/entrapped active (phenolic) compounds divided by the total active (phenolic) compound in the system. To determine the quantity of phenolic content that was entrapped during this experiment, Equation 5.1 below was employed as previously described by Cilek *et al.* (2012). The total and surface phenolic contents were determined following the Folin-Ciocalteu method earlier described in Section 4.4.1 & 4.4.2

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{TPC} - \text{SPC}}{\text{TPC}} \times 100 \qquad \text{Equation (5.1)}$$

Where TPC is total phenolic content and SPC the surface phenolic content.

5.4 Determination of different properties of encapsulated *Moringa oleifera* leaf powder extracts

5.4.1 Bulk and tapped density

Bulk and tapped density measurement was done using the method reported by Dadi and Emire, (2019). 5 g of MoLP microcapsule was placed in a 25 ml measuring cylinder, the volume occupied by the microcapsules was then recorded while for tapped density, the measuring cylinder with 5 g MoLP microcapsules was tapped steadily on a laboratory tabletop, until a consistent volume was obtained. The bulk density is expressed mathematically as the ratio of the weight of the microcapsule to the volume occupied in the graduated cylinder in g/ml.

$$\text{Bulk Density} = \frac{weight\ of\ microcapsule}{volume\ occupied} \qquad \text{Equation (5.2)}$$

5.4.2 Flowability

The flow properties of the microcapsules was evaluated using the method described by Mahdavi *et al.* (2016) with slight modifications. This was done using the Hausner's ratio, Carr's index and the angle of repose. The angle of repose was measured by using a fixed laboratory funnel and a digital angle ruler (DXN-360, DigiX New, Japan). The Hausner's ratio and Carr's index were determined using the initial data obtained from the bulk and tapped density values in the equation below:

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density} \qquad \text{Equation (5.3)}$$

$$Carr's\ index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \times 100$$ Equation (5.4)

Angle of repose (◦) = $\tan^{-1}$ (H/R) Equation (5.5)

Where H is the height of the pile and R is the radius at the base.

5.4.3 Colour

A colour analyser, (ColorFlex HunterLab, Reston VA, USA), was used to determine the colour of the microcapsule. This was done in terms of lightness (L^*), redness to greenness (a^*), and yellowness to blueness (b^*). The method described by Airouyuwa and Kaewmanee, (2019) was used with little modification. The microcapsule was placed in the colour analyser and colour measurement was taken. This experiment was done in triplicate and the results of this experiment were reported in terms of mean values of all replicate samples ± standard deviation.

5.4.4 Moisture content analysis

The moisture content of the microcapsule samples was determined by weighing 1 g of the sample in a hot air oven set at 105 ℃ for 3 h (AOAC, 2000, method 985.26). This was done in triplicate for precision.

$$Moisture\ content = \frac{Wi - Wf}{Wi} \times 100$$ Equation (5.6)

Where *Wi* is the initial weight and *Wf* is the final weight of the sample

5.4.5 Hygroscopicity of the *Moringa oleifera* leaf powder extract microcapsules

Hygroscopicity is an indication of the amount of moisture the MoLP microcapsule was able to absorb from the storage environment. The hygroscopicity test was done using the method described by Sun *et al.* (2019) with slight modification. 1 g of MoLP microcapsule samples was placed in Petri dishes, the Petri dishes were placed in containers that were sealed and contained a saturated solution of Sodium chloride (NaCl) at a working temperature of 25 ℃ and relative humidity of 75%. After fourteen days, the samples were weighed and the hygroscopicity was determined as the mass of moisture absorbed per 100 g of the dry MoLP microcapsules.

$$Hygroscopicity = \frac{W2 - W1}{W1} \times 100$$ Equation (5.7)

Where *W1* is the weight of the initial sample and *W2* is the weight of the final sample obtained after the hygroscopicity treatment.

5.4.6 Water absorption and solubility Index

The water absorption index (WAI) of the MoLP extract microcapsules was determined using the method reported by Dadi *et al.* (2020) with slight modifications. A 1 g sample was placed in a 50 ml centrifuge tube of known weight. 25 ml of distilled water was then added, after which the mixture was mixed vigorously using a mechanical shaker at 35 ˚C for 30 min. The resulting mixture was then centrifuged at 4000 rpm for 4 min. The supernatant was decanted and kept apart while the hydrated gel was weighed to determine the WAI using the equation below:

$$\text{Water absorption index} = \frac{\text{weight of hydrated gel}}{\text{weight of initial sample}} \qquad \text{Equation (5.8)}$$

The water-solubility Index (WSI) of the MoLP extract microcapsules was determined by pouring the supernatant from the WAI determination, in an aluminum pan of known weight. The supernatant was then dried overnight in an oven set at 105 ℃. The WSI was expressed as the ratio of dried supernatant to the ratio of the initial sample multiplied by 100%.

$$\text{Water solubility index} = \frac{\text{weight of dried supertant}}{\text{weight of initial sample}} \times 100\% \qquad \text{Equation (5.9)}$$

5.4.7 Thermal behaviour (TGA)

The thermogravimetric analysis (TGA) was performed using a Shimadzu thermogravimetric analyser (Model TGA 50, Shimadzu Corporation, Kyoto, Japan). A 5 mg sample was placed in a small aluminum plate and heated from 30 to 800 °C at a heating rate of 10 °C/min and a flow rate of 50 cm^3/min under a nitrogen atmosphere. The data obtained were plotted and weight loss was measured.

5.4.8 Surface morphology (Scanning electron microscopy)

The morphological attributes of MoLP extract microcapsules were examined using a scanning electron microscope (SEM, Hitachi Ltd., Tokyo Japan). In a method earlier described by Lei *et al.*, (2018), 5 mg of microcapsules were placed on stubs of copper and then coated with gold to a thickness of 20 nm. Samples were evaluated by SEM at a voltage of 15kV and SEM images recorded at different magnifications.

5.4.9 Structural property by Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared spectroscopy was used to identify interactions that may have taken place between the wall material and the core compound. The FT-IR spectra for all the samples were obtained using a Jasco FT-IR 4000 spectrometer. The approach used by Lei *et al.* (2018) was used, samples to be analysed were mixed with solid potassium bromide (KBr) powder after which the transmittance was recorded at wavelengths of 400 – 4000 cm^{-1}.

5.4.10 Structural elucidation of microcapsules using X-ray diffraction

The crystalline or amorphous structure of the extract, carrier and microcapsules were determined using the XRD – D8, - BRUKER AXS (Germany) advanced diffractometer. The radiation was generated at a tube voltage and current of 40 kV and 40 mA respectively, with a $2\text{-}\Theta$ angle of 5 to 80^{o} and a measurement time of 0.5 sec/step.

5.5 Storage stability test

To evaluate the storage stability test of the microcapsules and the *M. oleifera* extracts, all samples were stored at 4, 25 and 45 ˚C for 4 weeks. The method reported by Dadi *et al.* (2020) was adopted and used to determine the loss in the total phenolic content weekly. The TPC was determined using the Folin-Ciocalteu method in six experimental replicates for precision. The percentage TPC loss was calculated after 4 weeks.

5.6 *In vitro* simulated release profile

The release and *in vitro* digestion property of MoLP extract microcapsules was done using the method reported by Saikia *et al.* (2015) and Dadi *et al.* (2019) for the stomach and intestinal stage. The *in vitro* release profile was divided into two stages: the gastric and intestinal phases. The conditions in all these phases were designed to imitate the *in vivo* digestion phases in

humans. The total phenolic content (TPC) of the product obtained at the end of each phase was analysed using the Folin-Ciocalteu method. The release rate was determined by comparing with the TPC of the microcapsules prior to digestion.

5.6.1 The gastric phase

The simulated gastric fluid was prepared using a 3.2 g porcine that was derived from porcine stomach mucosa with activity ranging from 800 to 2500 Units/mg and 2 g sodium chloride. Both were mixed with 7 ml hydrochloric acid (HCl) and distilled water was added to the solution to bring the total liquid to 1000 ml. The pH of the prepared solution was then adjusted to 1.2 by using 0.2 N hydrochloric acid (HCl). Microcapsules (0.2 g) was then weighed into a 40 ml centrifuge tube and 2.4 ml of the gastric fluid solution added. The resulting mixture was sealed and placed in a shaking water at 80 rpm and 37 °C for 120 min. The solution was then allowed to cool and then it was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was decanted and then filtered through a 0.45 µm filter, it was then neutralized by adjusting the pH to 7 using 0.2 N NaOH solution. The TPC was then analysed and expressed in mg/g of Gallic acid equivalent of dried microcapsules.

5.6.2 The intestinal phase

The simulated intestinal phase (SIP) of digestibility study was done following the method reported by Saikia *et al.* (2015) with slight modification. In preparing the simulated intestinal fluid, a stock solution, 6.8 g of sodium monobasic phosphate was mixed with 250 ml distilled water. A 77 ml of 0.2 N NaOH solution was then added to the mixture, 500 ml of additional distilled water was added. Pancreatic enzyme 10 g was added to the already prepared mixture (stock solution) after which the solution was topped up to 1000 ml. Microcapsules (0.2 g) and 4.8 ml of the prepared simulated intestinal fluid were mixed in a 50 ml centrifuge tube. The mixture was sealed and allowed to stand in a static water bath at 37 ± 0.5 ˚C for 120 min. The resultant solution was cooled, centrifuged at 4000 rpm at 4 ˚C for 10 min. The solution, with a pH of 7.5 was then acidified with 0.2 N HCl to inactivate the enzyme, after 15 min, the mixture was then neutralized to a pH of 6.8 using a 0.2 N NaOH. The TPC of the resultant mixture was then analysed and expressed in mg/g of Gallic acid equivalent of dried microcapsules.

5.7 Statistical Analysis

Experiments were conducted in triplicates and were subjected to a One-Way Statistical Analysis of Variance (ANOVA) using SPSS (version 26, 2020). Means were separated using Duncan's Multiple Range Test (DMRT) and values were expressed as mean ± SD

5.8 Results and discussion

5.8.1 Total phenolic and flavonoid content and Encapsulation efficiency of the microcapsules

The total phenolic content (TPC) of the microcapsules (Table 5.1) ranged from 5.13 – 5.51 mg GAE/g. The GA microcapsules exhibited the highest TPC (5.51 mg GAE/g) which is significantly ($p < 0.05$) higher than MD and MDGA, respectively. This is likely due to the lower encapsulation efficiency and high surface polyphenols present in this sample which makes the active compounds more conspicuous on the surface than in the other samples (Table 5.1). The MD and MDGA microcapsules had a TPC of 5.28 and 5.13 mg GAE/g respectively, with significant differences ($p > 0.05$) observed. This is similar to the previous observation of Dadi *et al.* (2020) when maltodextrin and maltodextrin-high pectin blend were used in the encapsulation of *M. stenopetala* extracts. Although similar TPC values were obtained for MD and MDGA microcapsules, the significantly ($p < 0.05$) higher TPC obtained for GA shows that coating materials affect the TPC.

The surface polyphenol content (SPC) is the portion of the TPC retained on the surface of the microcapsules. For SPC, the same trend observed for TPC was observed as GA microcapsules had the highest SPC (1.49 mg GAE/g) while MDGA had the lowest (0.73 mg GAE/g) (Table 5.1). All samples were significantly ($p < 0.05$) different indicating that coating material differences affect the ability of core materials to be fully entrapped, which subsequently affects encapsulation efficiency. Hence, different coating materials and their combination play a crucial role in the retention of TPC, and the low presence of phenolic compounds on the surface of the microcapsules produced.

The total flavonoid content (TFC) ranged from 2.91 – 3.75 mg QE/g (Table 5.1). The GA microcapsules exhibited the highest yield which is in tandem with the TPC results. There were no significant ($p > 0.05$) differences between the TFC of GA and MDGA while the TFC of MD was significantly ($p < 0.05$) different from both GA and MDGA.

Encapsulation efficiency (EE) is measured as the ratio of the difference in the TPC and SPC to the TPC (expressed as a percentage). The encapsulation efficiency of microcapsules is largely dependent on the number of phenolic compounds found on the surface (Cilek *et al.*, 2012; Akhavan Mahdavi *et al.*, 2016; Yadav *et al.*, 2019). Although, Dadi *et al.* (2019a) opined that this may be due to the combination of other factors which has earlier been demonstrated in Chapter 4. The encapsulation efficiency of microcapsules in this study ranged from 72.87 – 85.66%. The MDGA microcapsules exhibited the highest EE while GA microcapsules had the lowest EE.

Table 5.1 Phytochemical composition, encapsulation efficiency and antioxidant activity of microcapsules of *Moringa oleifera* leaf powder extract

Parameters	Samples		
	MDGA	**MD**	**GA**
TPC (mg GAE/g)	5.13 ± 0.08[a]	5.28 ± 0.14[a]	5.51 ± 0.15[b]
SPC (mg GAE/g)	0.73 ± 0.05[a]	1.12 ± 0.10[b]	1.49 ± 0.28[c]
TFC (mg QE/g)	3.60 ± 0.16[b]	2.91 ± 0.15[a]	3.75 ± 0.24[b]
Encapsulation efficiency (%)	85.66 ± 0.96[a]	78.70 ± 2.37[b]	72.87 ± 5.10[c]
DPPH (μ mol TE/g)	35.46 ± 2.44[a]	34.62 ± 3.01[a]	36.16 ± 1.85[a]
FRAP (mg AAE/g)	20.38 ± 1.31[b]	16.90 ± 1.60[a]	19.24 ± 1.67[b]

Data are presented as mean ± standard deviation of 6 runs, different superscripts in the same row indicate significant differences at 95% confidence level using Duncan's test. TPC: Total phenolic content, SPC: Surface phenolic content, TFC: Total flavonoid content (where n = 6)

This has been ascribed to the presence of fewer surface polyphenols in MDGA microcapsules (Table 5.1). The EE obtained for the MDGA microcapsules is close to the predicted value earlier reported in Chapter 4 for optimization.

Factors that affect the EE of microcapsules include the inherent property of coating materials used, as different coating materials possess different entrapment potentials (Yadav *et al.*, 2019), which may be responsible for the differences observed. In addition to this, the combined activity of gum Arabic and maltodextrin in MDGA may be responsible for the high encapsulation efficiency observed. It has previously been reported that a combination of coating materials improves the efficiency and stability of microcapsules produced (Yadav *et al.*, 2019). In this respect, gum Arabic and maltodextrin possess stabilising, emulsifying and film forming properties that are crucial to the EE (Cilek *et al.*, 2012; Yadav *et al.*, 2019). In addition to this, EE increase with the number of soluble solids present in the infeed mixture (Rajabi *et al.*, 2015), which may also impact its release ability in the digestive tract. In all, the MDGA microcapsule was found to have the best EE of all three coating materials used.

5.8.2 Antioxidant activity of the microcapsules

The antioxidant activity of the microcapsules was measured using the DPPH scavenging activity and the ferric reducing antioxidant power (FRAP). The DPPH values ranged from 34.62 – 36.16 μmol TE/g while the FRAP values ranged from 16.90 – 20.28 mg AAE/g (Table 5.1). The coating

materials did not significantly ($p > 0.05$) affect the DPPH scavenging activity in all three samples. This is similar to the previous observation of Cilek *et al.* (2012), who observed that DPPH scavenging activity of microcapsules produced from different coating materials (MD and GA) with the same ultrasonication time for sour cherry pomace did not differ. The GA microcapsule had the highest DPPH scavenging, this is expected as the microcapsules had the highest TPC and SPC which may have been responsible for this. Overall, all microcapsules of the MoLP extracts had good antioxidant activities which would make it an important ingredient in the production of functional foods in the food industry.

The FRAP values of the MDGA and MD microcapsules (Table 5.1) are not significantly different ($p > 0.05$) but were both significantly different ($p < 0.05$) from the GA microcapsules. This observation is consistent with findings from for the encapsulation of *M. stenopetala* leaf bioactives (Dadi *et al.,* 2020). The similar trend in FRAP values maybe attributed to the low drying temperature of the freeze-drier which may not result in the degradation of the phytochemical content during drying.

5.8.3 Bulk and tapped density of MoLP microcapsules

The bulk and tapped densities of a powder are crucial parameters in determining its properties and behaviours during packaging, storage, distribution, and transportation (Luna-Guevara *et al.*, 2017; Dadi *et al.*, 2020). Hence, both parameters are used in the food industry to predict and determine the quantity of powder that will fit into a given packaging container. It is important to mention that all samples were produced under the same freeze-drying conditions with different coating materials. The bulk density of the various microcapsule samples is presented in Table 5.2 with values ranging from 0.177 to 0.325 g/ml. There were significant differences ($p < 0.05$) in the bulk density among all microcapsules indicating that coating material composition affected the bulk density. The MDGA microcapsules had a bulk density value of 0.297 g/ml compared to the MD and GA with bulk densities of 0.325 g/ml and 0.177 g/ml respectively. The microcapsules from MD and MDGA possess relatively high bulk densities translating to ease of storage with less space requirement. Previous reports show that high bulk densities indicate the reduced presence of air in the microcapsules, thereby decreasing the chances of damage caused by oxidative instability (Edris *et al.*, 2016; Chuyen *et al.*, 2019).

The bulk densities observed are consistent with findings by Dadi *et al.* (2020) where freeze-dried microcapsules from maltodextrin and maltodextrin pectin blends were significantly different from each other. In the current study, microcapsules produced from GA had the lowest bulk density which may be ascribed to the viscous property of gum Arabic, this indicates that a

larger storage container is required for GA microcapsules. Similar observation was made when maltodextrin, maltodextrin-gum Arabic, and gum Arabic were used as coating materials in the encapsulation of eggplant extracts by Sarabandi *et al.* (2019).

Table 5.3 Bulk density, tapped density, and flowability of MoLP extract microcapsules

Parameters	Samples		
	MDGA	**MD**	**GA**
Bulk density (g/ml)	0.297 ± 0.007[a]	0.325 ± 0.003[b]	0.177 ± 0.004[c]
Tapped density (g/ml)	0.259 ± 0.002[a]	0.295 ± 0.003[b]	0.126 ± 0.003[c]
Hausnier ratio	1.146 ± 0.029[a]	1.103 ± 0.060[a]	1.407 ± 0.058[b]
Carr's index (%)	12.550 ± 2.246[a]	9.230 ± 0.255[a]	28.833 ± 2.947[b]
Angle of Repose (◦)	34.540 ± 0.759[a]	32.010 ± 0.320[b]	34.980 ± 0.686[a]

Data are presented as mean ± standard deviation, different superscripts in the same row indicate significant differences at 95% confidence level using Duncan's test. MDGA: Maltodextrin and gum Arabic coated mixture sample (optimized sample from chapter 4); MD: maltodextrin coated sample; GA: Gum Arabic coated sample. (where n = 3)

The differences were ascribed to the relatively high viscosity of GA, which may have resulted in the formation of large microcapsule particles. The differences may also be attributed to the inherent chemical composition of the coating materials, their molecular weight, and the internal structural bond exhibited by the coating materials (Akhavan Mahdavi *et al.*, 2016). The irregular and amorphous structure of the microcapsules resulting from the freeze-drying process may have contributed to significant differences noticed among all three samples with different wall materials. The values obtained show that wall material affects the bulk density of all developed microcapsules.

Tapped density values ranged from 0.126 to 0.295 g/ml (Table 5.2), with significant differences ($p < 0.05$) between all three samples indicating that coating material composition affected the tapped density. As stated earlier, the structural pattern, molecular weight of wall material, and their inherent viscous properties contribute to the tapped density of microcapsules produced. The results reported for the three samples follow the same trend reported by Sarabandi *et al.* (2019) where the highest tapped density was reported for MD microcapsules.

The combination of GA and MD resulted in a slight decrease in tapped density indicating the influence of coating material combination on this characteristic of MDGA coated microcapsules which were significant ($p < 0.05$). The GA microcapsule had the lowest tapped

density value which may be ascribed to its viscous property. Hence, tapped density values reported indicated that coating material has a significant effect on the density of produced microcapsules which may, in turn, affect the space required for packaging and oxidative stability due to the presence of airspaces.

5.8.4 Flowability

Flowability is an important parameter used in predicting and determining the condition of processing, formulation, packaging, and transportation of products in the food industry (Sarabandi *et al.*, 2019). To evaluate the flow properties of the produced microcapsules, the Hausner's ratio, Carr's index, and angle of repose were measured, as a single parameter cannot be used in predicting the flow properties of granular particles such as microcapsules (Dadi *et al.*, 2019b).

The Hausner's ratio values ranged from 1.103 to 1.407, Carr index values within the range of 9.23 to 28.83%, and the angle of repose ranged from 32.01 to 34.98^{o} (Table 5.2). The values reported in the current study are within the ranges reported by Premi and Sharma, (2017). It is believed that if the Hausner's ratio, Carr's index, and the angle of repose values are greater than 1.25, 25%, and 45, respectively, the resultant product can be described as poor flowing material. The results presented in Table 5.2 show that the MD and MDGA microcapsules have medium or fair flow properties (Gallo *et al.*, 2011; Mahdavi *et al.*, 2016). This may be attributed to the amorphous structural pattern of the microcapsules as well as the low moisture content which may have affected the cohesive and frictional forces among the microcapsules. There were no significant differences ($p < 0.05$) for the Hausner ratio and Carr's index value of both MDGA and MD microcapsules. The Hausner's ratio and Carr's index value reported for the GA microcapsules established that GA microcapsules have a poor flow property, although the angle of repose value seems lower than the benchmark for such classification.

5.8.5 Colour measurement

Measuring of colour properties of microcapsules was carried to determine the lightness (L^{*}) luminosity, (a^{*}) redness to greenness, and the saturation (yellowness to blueness) of the sample (b^{*}). As presented in Table 5.3, lightness values ranged from (63.13 – 69.66). There were significant differences ($p < 0.05$) among the lightness of all microcapsules developed from different coating materials indicating that the coating material type and combination influence the lightness of all microcapsules formed. In addition to this, a high coating to core ratio may have accounted for the light colour reported as the coating material may have masked the green

pigment/colour of the MoLP extract (Dadi *et al.*, 2019a). The pure white colour of maltodextrin may have accounted for its highest lightness colour among all samples.

Table 5.3 Colour characterization of *Moringa oleifera* leaf powder extract microcapsules

Samples	L^*	a^*	b^*	Hue angle	Chroma
MDGA	66.24 ± 0.276^a	4.09 ± 2.050^d	29.75 ± 4.804^e	8.23 ± 4.67^a	30.09 ± 4.57^a
MD	69.66 ± 0.372^b	3.24 ± 0.447^d	29.95 ± 2.329^e	6.17 ± 0.71^a	30.12 ± 2.34^a
GA	63.13 ± 0.225^c	5.42 ± 1.363^d	27.03 ± 2.894^c	11.53 ± 3.85^a	27.61 ± 2.64^b

Data are presented as mean ± standard deviation, different superscripts in the same column indicate significant differences at 95% confidence level using Duncan's test. MDGA: Maltodextrin and gum Arabic coated mixture sample (optimized sample from chapter 4); MD: maltodextrin coated sample; GA: Gum Arabic coated sample. (where n = 3)

The high maltodextrin concentration in the wall material for the MDGA microcapsules may have resulted in its higher lightness value exhibited over the GA microcapsules.

The degree of redness to greenness (a*) among all samples ranged from 3.24 – 5.42, there were no significant differences ($p > 0.05$) among all samples tested. The values reported indicate that the microcapsules tended towards redness than greenness which indicated that the colour of the extract was well masked by the coating material used for the encapsulation procedure which further supports the high encapsulation efficiency of the procedure. The high a* value reported for gum Arabic coated samples may be attributed to its yellow-like colour and appearance. A similar observation was made for GA-coated samples by Sarabandi *et al.* (2019) for microcapsules developed from eggplant extract. The b* values ranged from 27.03 – 29.95 indicating that all samples tended towards yellow than blue, the coating material type or combination did not significantly ($p > 0.05$) influence the yellowness of MD and MDGA microcapsules, this may be ascribed to the similarity in the coating material used and the higher concentration of maltodextrin present in MDGA.

The GA microcapsules were significantly different ($p < 0.05$) among all samples for b* which aligns with the value reported for L* for this sample as it exhibits the least value for lightness. The low-temperature drying (freeze-drying) may have resulted in the preservation of the original colour of the prepared mixture before the drying process of the prepared feed solution. In all, the type of wall material and their combination only affected the lightness but did not impact the physical outlook of the microcapsules.

5.8.6 Moisture content

The moisture content is an essential attribute of microcapsules that can affect the shelf-life and stability of microcapsules, this parameter has also been reported to affect the flowability and stickiness of the products (Dadi *et al.*, 2020). High moisture content can result in changes in the glassy nature of the coating material during storage which may result in the leaching and deterioration of the entrapped core material (Kang *et al.*, 2019). The moisture content of the microcapsules using different coating materials ranged from 1.47 – 1.77% (Table 5.4.). The GA microcapsules have the highest moisture content, this is similar to the observation of Nawi *et al.* (2015) where MDGA, MD and GA were used in the encapsulation of anthocyanins from *Ipomea batatas*. The values reported for moisture for all samples indicated that different coating materials used did not significantly impact the moisture content of the microcapsules. Similar observations were made by Kuck and Noreña, (2016) and Dadi *et al.*, (2020) in a study on the encapsulation of grape phenolic extract using gum Arabic and polydextrose (a modified starch similar to maltodextrin) and encapsulation of *M. stenopetala* extract respectively. The moisture content reported in the current study is lower than the values reported by all these authors for freeze-dried microcapsules and within the ranges reported by Premi and Sharma, (2017) for microcapsules of *M. oleifera* seed oil coated with maltodextrin and gum Arabic. The values indicate that developed microcapsules have the potential to be stored for a longer period limiting spoilage associated with microbial degradation.

5.8.7 Hygroscopicity

Hygroscopicity is the ability of the powder to absorb moisture from the surrounding atmosphere (Mahdavi *et al.*, 2016). This parameter is essential as it affects the stability of stored powdered products (Nawi *et al.*, 2015; Dadi *et al.*, 2020). The hygroscopicity values of the microcapsules ranged from 11.13 – 15.86% (Table 5.4). The hygroscopicity of the MD microcapsules were significantly higher ($p < 0.05$) than MDGA, this implies that coating had a significant effect on how much moisture the microcapsules absorbed during storage. This observation follows similar trends with values reported by Dadi *et al.* (2020). The MD microcapsules have been reported to possess high moisture absorbing capacities (Tengse *et al.*, 2017; Lee *et al.*, 2018), which may have accounted for the high hygroscopicity reported for them. The MDGA microcapsules were the most desirable as it has a lower hygroscopicity which may contribute to the stability of the product over time. The lower hygroscopicity reported for the MDGA microcapsules may have resulted from possible chemical interaction and structure of the combined coating material.

Table 5.4 Moisture content, hygroscopicity, water absorption, and solubility index of microcapsules

Parameters	Samples		
	MDGA	**MD**	**GA**
Moisture content (%)	1.53 ± 0.176[a]	1.47 ± 0.243[a]	1.77 ± 0.351[a]
Hygroscopicity (%)	11.13 ± 0.917[a]	15.86 ± 2.217[b]	14.35 ± 1.590[b]
Water Solubility Index (%)	90.51 ± 2.189[a]	98.74 ± 1.054[b]	86.35 ± 4.777[c]
Water Absorption Capacity (g)	0.15 ± 0.036[a]	0.17 ± 0.017[a]	0.23 ± 0.026[b]

Data are presented as mean ± standard deviation, different superscripts in the same row indicate significant differences at 95% confidence level using Duncan's test. MDGA: Maltodextrin and gum Arabic coated mixture microcapsule (optimized sample from chapter 4); MD: maltodextrin coated microcapsule; GA: Gum Arabic coated microcapsule. (where n=3)

The hygroscopicity for GA microcapsules did not differ significantly ($p > 0.05$) from the MD microcapsules, which may affect their storage stability. Since MDGA microcapsule had the lowest hygroscopicity, it is preferred over MD and GA microcapsules as low hygroscopicity improves storage stability and decrease their proneness to microbial and oxidative degradation that may result from moisture absorption from the environment.

5.8.8 Water Solubility and Absorption index (WSI & WAI)

The solubility properties of microcapsules are related to their reconstitution attribute (Tonon *et al.*, 2008; Rezende *et al.*, 2018). The values for water solubility index (Table 5.4), ranged from 86.35 – 98.74%, indicating that all microcapsules exhibited high solubility properties in water. There were significant differences ($p < 0.05$) among all the samples, which implies that the coating material type and combination influence the solubility properties of the microcapsules. The MD microcapsules had the highest WSI (98.74%) while the GA microcapsules had the lowest (86.35%). The high solubility index from MD samples can be attributed to the high solubility property of maltodextrin (Cano-Chauca *et al.*, 2005). The MDGA microcapsule value difference may be ascribed to the blending of two different coating materials which may have resulted in intermolecular and chemical interaction of the carriers. A similar observation was made when maltodextrin-gum Arabic mixture was used as a coating in the encapsulation of

eggplant extract, by Sarabandi *et al.* (2019). In addition to this, the MDGA microcapsule has a lower WSI value than the value reported for MD microcapsule. This observation follows similar trends reported in two recent studies where similar coating materials were used as carriers (Sarabandi *et al.*, 2019; Dadi *et al.*, 2020). The reported values for the samples indicated that encapsulation has the potential to increase the solubility properties of plant extracts in water. Since MD microcapsules had the highest solubility, it is preferred in terms of solubility in water over the MDGA and GA microcapsules.

Similarly, the water absorption index (WAI) was determined to measure the ability of the microcapsules to take up water. The results of WAI (Table 5.4), range from 0.15 – 0.23 g. The MDGA microcapsule had the least WAI while the GA microcapsules had the highest WAI. There was no significant difference ($p > 0.05$) between MDGA and MD microcapsules. This may be ascribed to the high ratio of maltodextrin to gum Arabic in the former, this result shows that the interaction between the two coatings did not show noticeable changes in the ability of the two samples to retain water. Although the GA microcapsules were observed to have a much higher WAI, this is likely due to the high molecular weight and the hydrophilic nature of gum Arabic which is typical of many natural gums that are long-chain polysaccharides (Song *et al.*, 2011).

5.8.9 Thermal Behaviour using thermogravimetric analysis

The thermogravimetric analysis (TGA) of the dried MoLP extracts, the coating materials (MD and GA) and the microcapsules were done to elucidate the thermal stability of the products (Figure 5.1A-D). Generally, in thermal behaviour analysis, the temperature at which the highest weight loss is noticed is been described as the maximum degradation temperature (Shetta *et al.*, 2019). As seen in Fig 5.1A and in comparison, with Fig 5.1B-d, the major structural changes and weight loss caused by temperature is related to the coating material used. Pure gum Arabic and maltodextrin presented a two-step weight loss, the first weight loss of 7.3 and 7% respectively, were noticed around 63 and 70 °C respectively similar to the observations of Rodrigues da Cruz *et al.* (2019) for pure gum Arabic. The second weight loss of 47 and 54% occurred around 290 and 282 °C respectively. Similar observations were made for pure gum Arabic by Rodrigues da Cruz *et al.* (2019) and maltodextrin by Ballesteros *et al.* (2017).

The TGA plot of MDGA, GA and MD microcapsules indicates a three-step loss of compounds present in all samples (Fig 5.1 B-D). The first weight loss of 5, 3 and 7.9% in MDGA, MD and GA respectively occurred around 115, 108 and 97 °C, these losses can be attributed to moisture or water loss from the microcapsules at these high temperatures (Ballesteros *et al.*, 2017). The presence of OH bond was confirmed by the FTIR analysis at around 3200 cm^{-1},

which may be surface moisture present in the microcapsule. The second stage loss of about 59% (MDGA), 56% (MD) and 57% (GA) was around 341 °C. The decomposition here may be ascribed to the degradation of phenolic compounds that were entrapped in the coating material. The third stage loss was noticed around 743, 764 and 738 ℃ with losses of 18.83, 18.52 and 14.76% for MDGA, MD and GA respectively which may have signalled the destruction of all the core material portions with complex antioxidant attributes.

For the non-encapsulated MoLP extracts, there was a three-step weight loss. The first loss of about 6.5% occurred around 70 °C, which may likely be due to gradual evaporation of surface moisture present in the freeze-dried extract as well as the presence of heat labile bioactive compounds that deteriorate below boiling temperature (100 °C). There was a significant weight loss between 135 and 320 °C of about 45% which may have resulted from thermal decomposition of polyphenols and other bioactive compounds present in the extracts with different structural patterns. A similar observation was made for the second stage weight loss reported for eucalyptus extracts by Gullón *et al.* (2017). The observation between encapsulated and non-encapsulated samples shows that coating of extracts had a protective and significant effect on the core. This indicates that encapsulation of MoLP extract enhances thermal stability, and is in agreement with Ballesteros *et al.* (2017b) who observed that encapsulated extract of spent coffee ground was more thermally stable than spent coffee ground extracts. The last weight loss in MDGA, MD and GA occurred around 753, 764 and 738 °C respectively for the microcapsules.

In general, the degradation/weight loss that occurred in all encapsulated samples were similar and shows more thermal stability than in non-encapsulated MoLP extracts. In addition to this, all microcapsules showed similar stability at high temperature, with MDGA and MD microcapsules being more thermally stable. This may be attributed to the protective effect brought about using combined coating material as well as the high encapsulation efficiency reported.

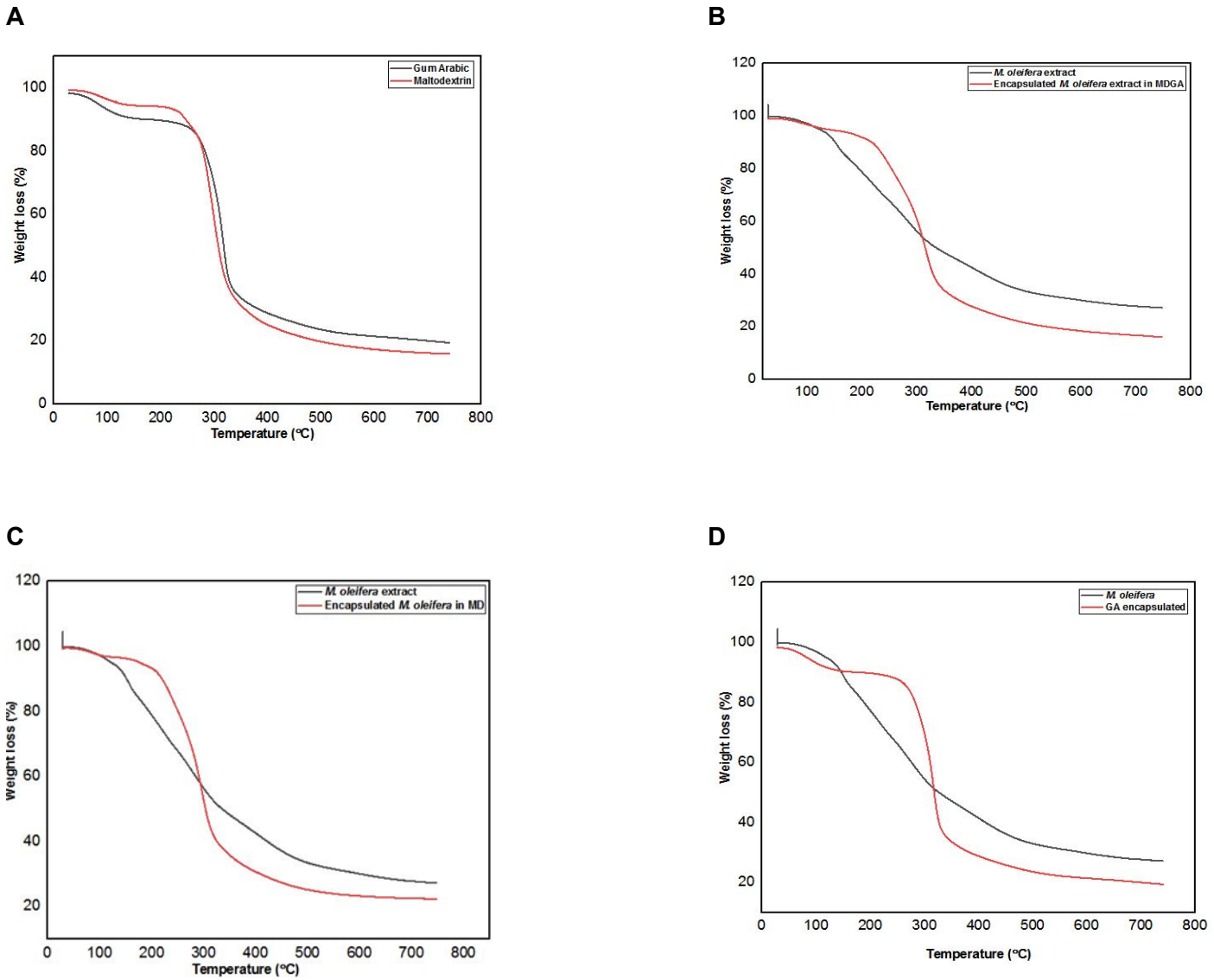


Figure 5.1A-D TGA (A) plot of pure coating materials (B) MDGA encapsulated and *M. oleifera* leaf powderextract (c) MD encapsulated, and *M. oleifera* leaf powder extract (D) GA encapsulated and *M. oleifera* leaf powder extract.

5.8.10 Structural Properties of *M. oleifera* leaf powder microcapsules

5.8.10.1 Particle morphology of Moringa oleifera leaf powder extract microcapsules

Scanning electron microscopy (SEM) was used to observe the surface morphology of *M. oleifera* leaf powder extract microcapsules. Particle morphology influences the storage stability and protection offered to the extract by the coating materials (Tolun *et al.*, 2016). Fig 5.2–5.4 shows the particle morphology of microcapsules prepared from different coating materials. There were slight structural differences among all three microcapsule samples observed.

Figure 5.2 presents the particle morphology of microcapsules developed from MDGA coating material. Fig 5.2A–C at high magnification (x1500) showed some spherical and slightly irregular-shaped microcapsules with little dented surfaces, with some differences in shapes and sizes. The particle size for microcapsules of MDGA microcapsules ranged from 10 – 50 µm, the large particle size may be due to low process temperature and combination of different coating materials (Rezende *et al.*, 2018). The slightly dented surface of the microcapsules can be attributed to the coating material ratio as this contains two different coatings with different characteristics and attributes.

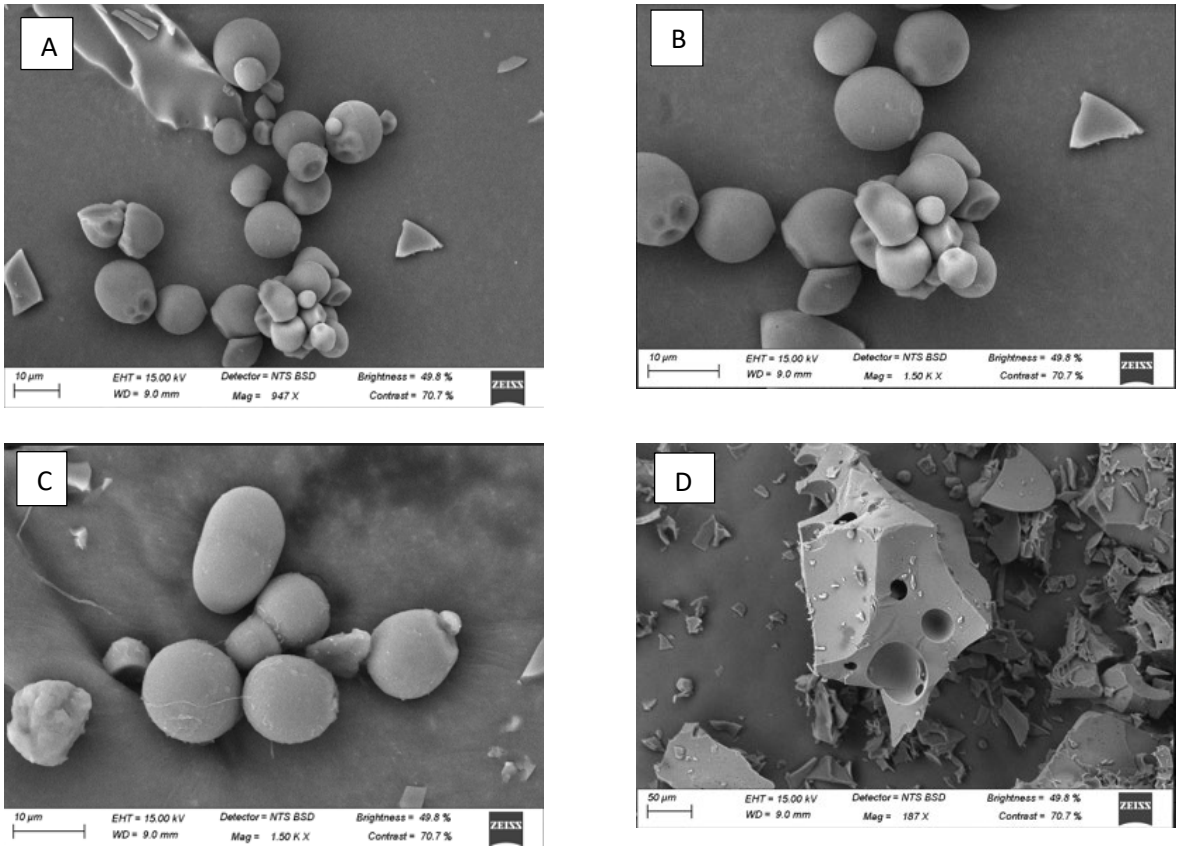


Fig 5.2 Morphological structures of MoLP coated with Maltodextrin-gum arabic [A] surface morphology (x947) [B] surface morphology (x1500) [C] surface morphology (x1500) [D] cross sectional view at (x187).

Similar observations were reported by Tolun *et al.* (2016) for microcapsules of grape polyphenols developed with maltodextrin – gum Arabic blends. The slightly spherical shape may likely result in increased protection from external influences such as light, high temperature, oxygen etc. which may cause degradation and deterioration of the microcapsules. The mixture and interaction of MD and GA were desirable as it resulted in the formation of a slightly spherical surface attribute for the microcapsules, although X-ray diffraction studies show that the properties of this microcapsule are more amorphous than crystalline. The cross-sectional areas of the developed microcapsules from MDGA at lower magnification (Fig 5.2D) shows a structure of microcapsules with slight cracks and some hollowing that may influence the stability of the microcapsules over a long period of storage in a high humid environment (Premi and Sharma, 2017).

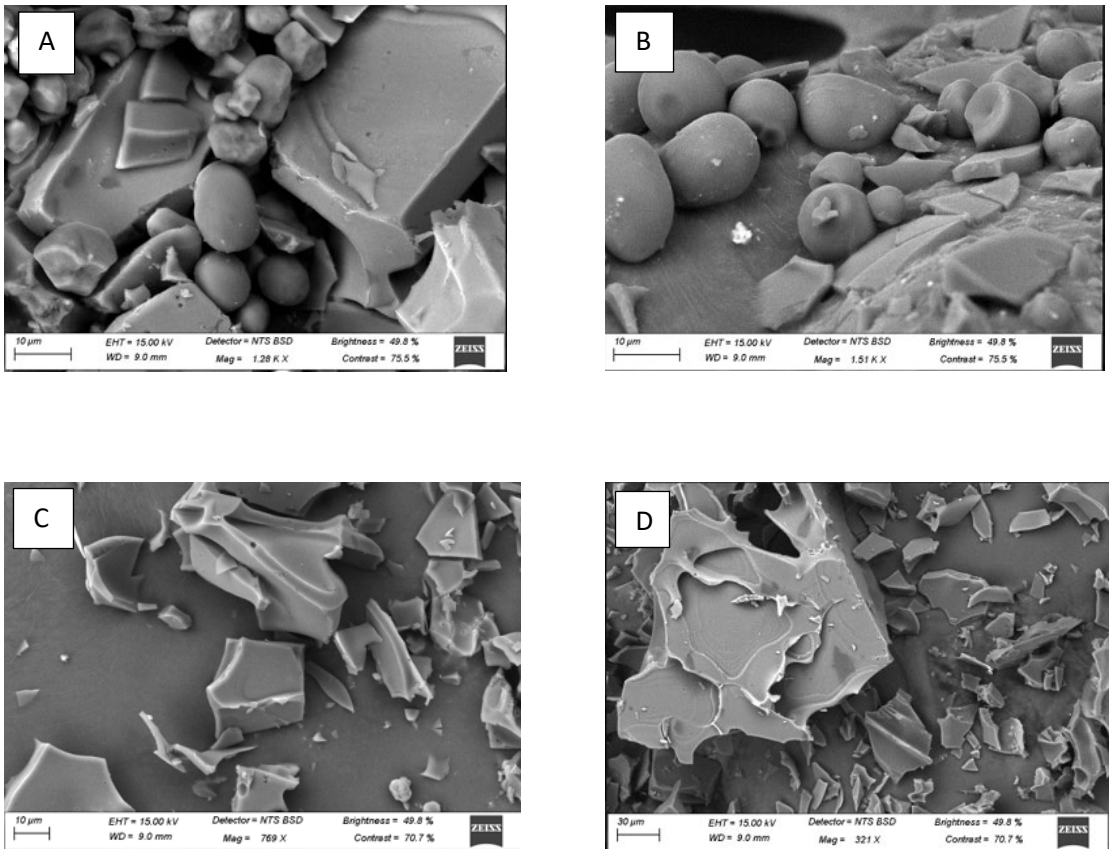


Figure 5.3 Morphological structures of MoLP extract coated with maltodextrin [A] surface morphology (x1000) [B] surface morphology (x1500) [C] surface morphology at (x769) [D] surface morphology (X321)

The rough cross-sectional appearance is an indication that entrapment of active compounds occurred, and that coating material may shield them against light, high temperature, oxygen etc. that may alter stability and shelf-life (Chranioti *et al.*, 2015; Pellicer *et al.*, 2019).

Figure 5.3 A–D shows the outer particle morphology of MoLP extract microcapsules developed from maltodextrin coating alone. The appearance shows an amorphous, flake and glass-like structure typical of many microcapsules developed from maltodextrin (Khalifa *et al.*, 2019; Pellicer *et al.*, 2019). This appearance is slightly different from the MDGA microcapsules, as the appearance of MDGA may likely be due to the interaction that occurred during their preparation. Similar surface morphology was observed in freeze-dried MD microcapsules by Chranioti *et al.* (2015). Generally, most microcapsules developed using freeze-drying are characterised by an amorphous glass-like appearance with dented and somewhat shrivelled surfaces which has been ascribed to the effect of low-temperature drying.

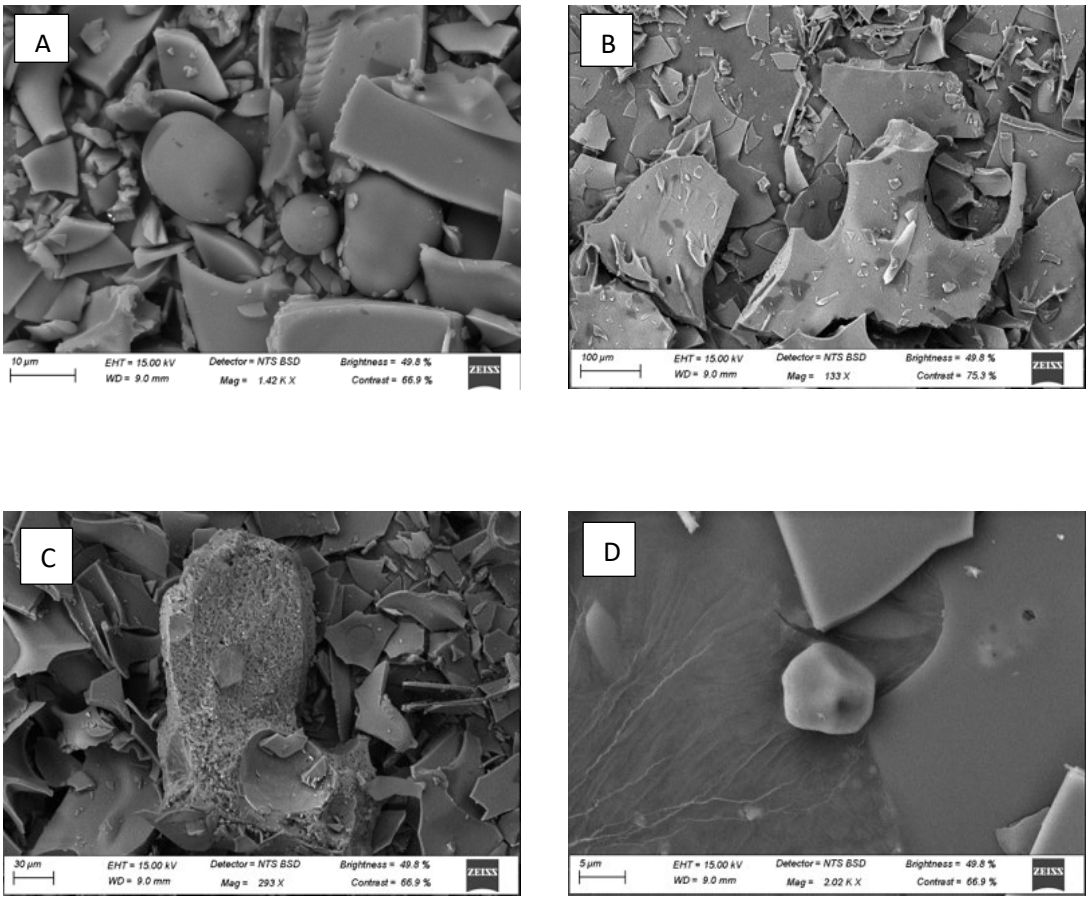


Figure 5.4 Morphological structures of MoLP extract coated with Gum Arabic [A] surface morphology (x1420) [B] surface morphology (x133) [C] surface morphology at (x293) [D] surface morphology (X2002)

Figure 5.4 A-D presents the particle morphology of GA microcapsules produced from pure gum Arabic coating, at different magnifications, the appearance indicates slightly disordered, amorphous microcapsules with some dented surface and minimal pore-like appearance (Fig 5.4C), that may have resulted from freeze-drying (Ballesteros *et al.*, 2017). Similar in appearance to these microcapsules reported is the characteristic morphology of microcapsules of beetroot extracts coated with gum Arabic by Chranioti *et al.* (2015). The appearance of all microcapsules only differs slightly, although the MDGA microcapsules seem to have a surface and particle morphology better than as they appear to have a physical outlook that indicated that the core compound has been efficiently masked which further validates data obtained for the high encapsulation efficiency.

5.8.10.2 Fourier transform Infra-Red spectroscopy measurement.

Fourier transform infrared spectroscopy was used to elucidate the presence of some characteristic functional groups in the microcapsules as well as to study the potential interaction between the coating materials and the core compound. The spectrum of maltodextrin, MoLP extracts, gum Arabic and encapsulated MoLP extract is presented in Fig 5.5 – 5.7. In Figs 5.5 and 5.6, three absorption bands were noticed in maltodextrin (B), which shows broad band at a wavelength of 3288.90 cm^{-1}. This falls within the region described as an O-H stretching and normal polymeric O-H stretch (Gulu *et al.,* 2019). This broad band characterised the presence of water which is mostly indistinctive and vibrate in several ways such as symmetric, and asymmetric stretching and bending. The wavelength at 1369.77 cm^{-1} shows the presence of a CH_2 asymmetrical bending indicating the presence of an alkane functional group (Velmuragan *et al.,* 2017). The 1016.15 cm^{-1} is within the characteristic wavelength of a primary alcohol with a C–O and C-O-H stretching and bending (Coates, 2006). These peaks indicate the presence of starch molecules in the microcapsules, this is not farfetched as the coating materials are characterised by the presence of long-chain polysaccharides. Similar peaks within the stated functional groups have been identified in previous studies (Smrčková *et al.*, 2013; Kang *et al.*, 2019).

The spectra of MoLP extract (Fig 5.5–5.7), shows broad band at approximately 3226 cm^{-1} which corresponds to O-H stretching vibration present in phenolic acid groups (Cruz-Espinoza *et al.,* 2012; Siripireddy and Mandal, 2017). This band also indicates the presence of alcohols and hydrogen bonding which connotes the presence of water molecules (Cruz-

Espinoza *et al.,* 2012). A similar peak has been reported at this wavelength (3226 cm^{-1}) which was ascribed to the presence of bioactive polyphenols (Siripireddy and Mandal, 2017). The peak around 2924 cm^{-1} indicates the presence of a C–H stretching band and CH_2 vibration of aliphatic hydrocarbons which are typical of the carboxylic acid and carbonyl group (Kang *et al.,* 2019). This further validates the presence of organic acids in the extracts of MoLP. A peak similar to this was previously reported within the same region for MoLP extract (Panwar and Mathur, 2019; Pal *et al.*, 2018). Another notable peak at approximately 1667 cm^{-1} was identified, this peak shows the characteristic of a C=C aromatic stretching ring found in phenolic compounds (Gullón *et al.,* 2017). Two peaks were identified at 1599 and 1511 cm^{-1} respectively, which can be ascribed to the presence of amide II stretches, C=O and C=C aromatic stretching (Matinise *et al.*, 2017).

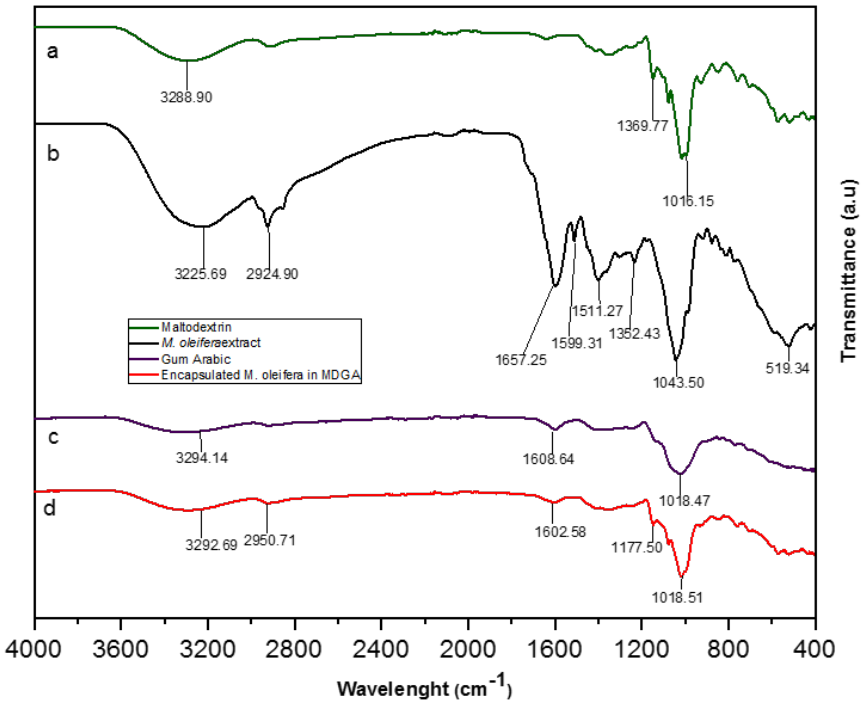


Figure 5.5 FTIR spectra of (A) maltodextrin (MD coating (B) *M. oleifera* leaf powder extracts (C) gum Arabic and (D) MDGA microcapsule.

The peak at 1352 and 1043 cm^{-1} can be ascribed to the presence of amines, C-O and C-O-H bending respectively (Kang *et al.,* 2019). The peak observed at 519 cm^{-1} may likely be due to the presence of aliphatic halogens (iodides) (Coates, 2006). Generally, the peaks in the extracts further confirmed the presence of organic and phenolic acid-like compounds previously identified in LCMS analysis in Chapter 3.

There were three spectra observed in Fig 5.5C for pure gum Arabic. The broad peak at 3294 cm^{-1} represents an O–H broad band, while the peak at 1608 cm^{-1} represents the presence of C=O stretching and N-H bending (Kang *et al.*, 2019). The peak noticed at 1018 cm^{-1} indicates the presence of a C–C and C-O stretching band (Kang *et al.,* 2019; Zanetti *et al.*, 2019).

For the MDGA, MD and GA microcapsules (Fig 5.5 – 5.7), peaks present in the core and coating materials were present while new peaks were formed, further confirming that encapsulation occurred. For instance, in the MDGA microcapsule, a new peak with medium-strength intensity was formed at 1177 cm^{-1}. This peak is within the ranges that have been described as the C–O aromatic ringed amines group (Coates, 2006; Matinise *et al.*, 2017; Zanetti *et al.*, 2019). The broad peaks around 3292 and 2950 cm^{-1} can be ascribed to O–H, COOH and C-H bands which are characteristic peaks of certain bioactive compounds that may be present in the entrapped core (Matinise *et al.*, 2017). Some previously identified peaks in the coating materials were present in the microcapsules formed although little shifts and adjustments in their bands were noticed. This shift may likely be associated with the disruption experienced during high-frequency mixing caused by ultrasound cavitation. Therefore, encapsulation successfully occurred as peaks present in MoLP extract were found present in the microcapsules.

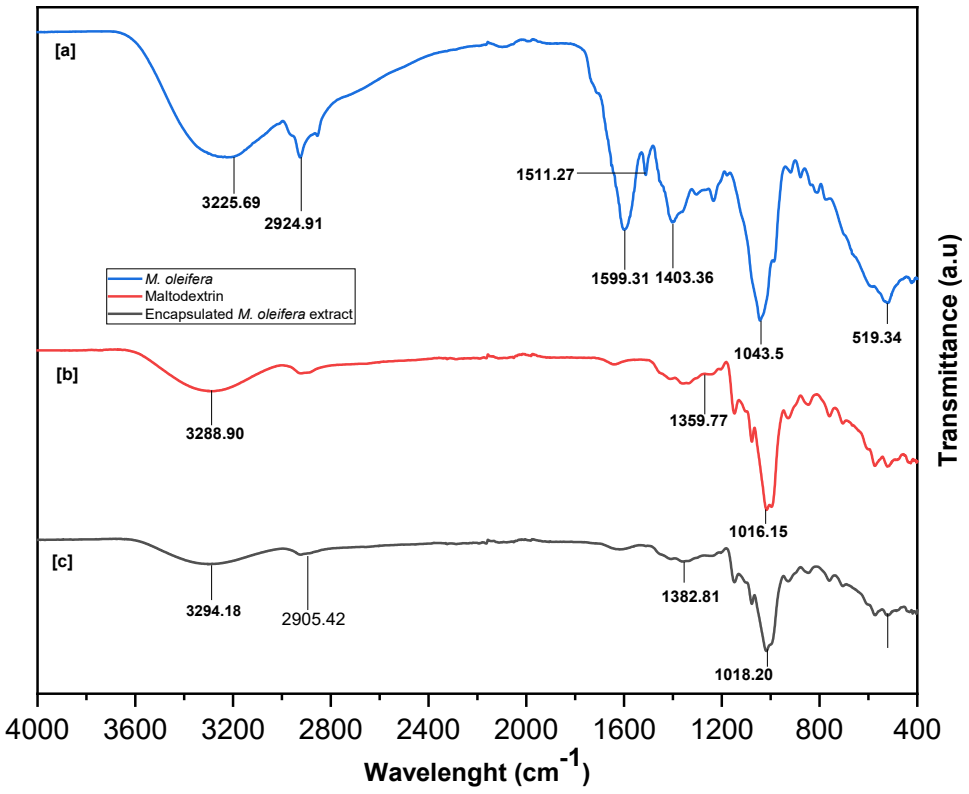


Figure 5.6 FTIR spectra of (A) *M. oleifera* leaf powder extracts (B) maltodextrin (MD) (C) MD microcapsule

In the MD microcapsule Fig 5.6C, a new peak was observed at a wavelength of 1382 cm^{-1}, this may likely occur via stretching and extension of the peak around 1400 cm^{-1} in *M. oleifera* extract.

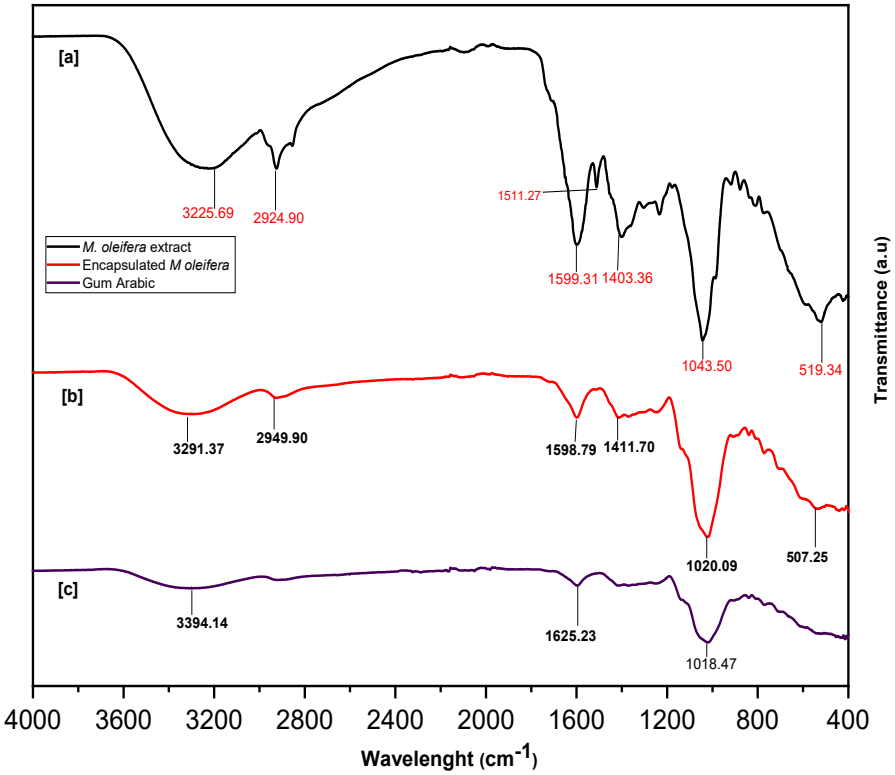


Figure 5.7 FTIR spectra of (A) *M. oleifera* leaf powder extracts (B) GA microcrocapsule (C) gum Arabic (GA) coating.

This peak is within the range described for primary, secondary and tertiary bending of the O–H bond and phenols, this peak may also be attributed to the presence of aromatic C=O, C=N, NH and C=C aromatic stretching vibrations (Matinise *et al.*, 2017). This further shows that encapsulation may result in the modification of chemical structures of a newly formed product, further confirming that encapsulation of the core took place.

The FTIR spectra for GA microcapsules are presented in Fig 5.5C and 5.7B. Some of the peaks present in the MoLP extracts were found present. For instance, the peaks around 3300 cm^{-1} may indicate the presence of an O–H stretching band (Kang *et al.,* 2019). The peaks around 2900–2950 cm^{-1} may indicate the presence of a C-H stretching band (Gulu *et al.,* 2019). The peak around 1400–1599 cm^{-1} indicates the presence of carboxylic and phenolic acids (Velmuregan *et al.,* 2017). The peak found at 1020 cm^{-1}, may have resulted

from the presence of the C–O and the primary amine C–N functional groups (Muhoza *et al.,* 2019). A new peak was observed for GA microcapsules at 1411 cm^{-1}, and may be a clear indication that encapsulation occurred (Muhoza *et al.,* 2019).

In all, the new peaks obtained in the microcapsules all indicated that encapsulation of the active compound took place in all coating materials with a minimal structural modification that may have resulted from high energy mixing caused by ultrasound cavitation. Overall, FTIR analysis confirmed that encapsulation of the core material took place as new peaks and bands were observed after the procedure.

5.8.10.3 X-ray Diffraction Analysis

X-ray diffraction analysis measures the structural pattern, characteristics and crystallinity of constituent compounds present in a powder (Gulu *et al.*, 2019). In addition to this, the measured crystallinity may affect the stability of the formed microcapsules (Kang *et al.*, 2019). The XRD analysis of MoLP extract, MDGA, MD and GA microcapsules, and pure maltodextrin and gum Arabic carriers are presented in Fig 5.8 A-F. The XRD patterns showed diffused peaks that are broad which indicates that all samples are amorphous and disordered with noise around, a characteristic attribute of many microcapsules coated with maltodextrin and gum Arabic (Ballesteros *et al.*, 2017; Kang *et al.*, 2019).

Moringa oleifera leaf powder extract (Fig 5.8A) shows two broad diffused peak 2 θ around 21° and 39°. The diffused pattern of these peaks indicates that the extract is amorphous with very low crystallinity. The peak around 20° was much more conspicuous than the dispersed and low intense peak seen around 39°. For the encapsulated extracts, MDGA, MD and GA respectively (Fig 5.8B-D), all samples presented broad diffused peaks that are conspicuous around 19, 21 and 22°, this may be indicative of total entrapment of the core in the coating material. The dense, noisy pattern of the diffractogram shows that the microcapsules are amorphous which may have resulted from the absence of the attribute needed for defined crystallinity during the process of freeze-drying (Cano-Chauca *et al.*, 2005). All peaks reported around 20° indicated the presence of the core material in the coating materials. The peaks obtained for MD and GA microcapsules are similar to the diffused peaks reported earlier for mango extract microcapsule coated with MD and GA respectively by Cano-Chauca *et al.* (2005). Broad diffused peaks were also observed around 34° for MDGA, 35° for MD and 40° for GA microcapsules.

Both MD and GA coatings had two broad peaks around 19° and 20° respectively (Fig 5.8E–F). This observation was consistent with broad peaks obtained for both coating materials in the study of Ballesteros *et al.* (2017).

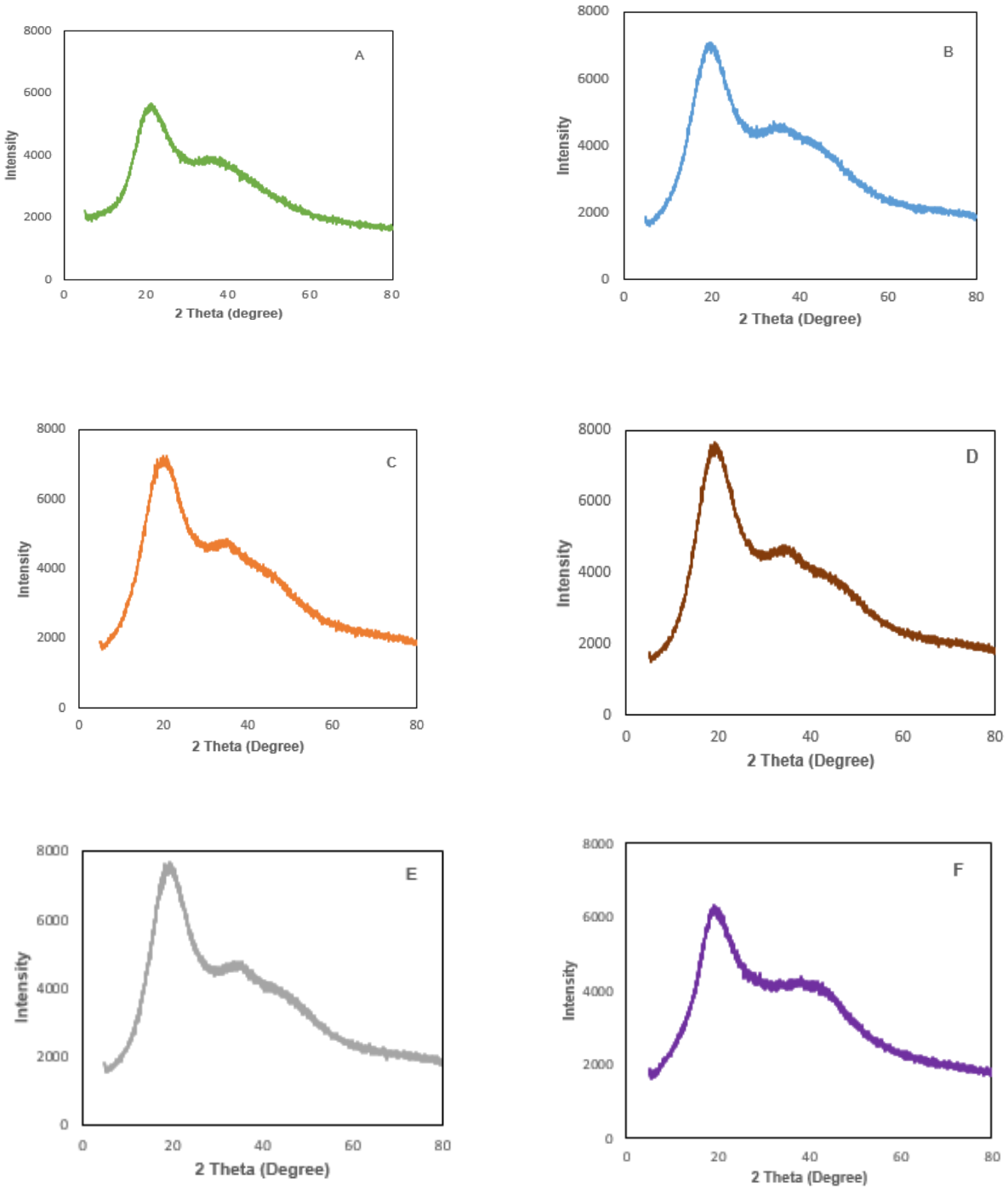


Figure 5.8 X-ray diffractogram of (A) *M. oleifera* leaf powder extract (B) *M. oleifera* leaf powder extract microcapsules in MDGA (C) *M. oleifera* leaf powder extract microcapsule in MD (D) *M. oleifera* leaf powder extract microcapsule in GA (E) MD coatings (F) GA coatings. (MDGA: Maltodextrin-gum Arabic coated microcapsules; MD: Maltodextrin coated microcapsules; GA: Gum Arabic coated microcapsules)

Other broad, diffused but less conspicuous peaks were observed around 35° and 42° respectively for pure maltodextrin and gum Arabic coatings. In all, the XRD pattern shows that the extract and the microcapsules are structurally amorphous may aid the release of the active compound digestibility, the water solubility as well as the storage stability of the microcapsules.

5.8.11 Storage stability test

The stability of the microcapsules produced with different coating materials were studied for 28 days at different temperatures (4, 25 and 40 °C respectively) for their ability to retain TPC. This was done to determine the appropriate storage condition for these microcapsules based on the protective effect of the coating materials (non-encapsulated *M. oleifera* leaf powder extract was used as a control). At 40 °C, there was a rapid decrease in the total phenolic content of all the samples in the first 21 days (Fig 5.9). This is in tandem with the observation of Dadi *et al.* (2020) where the rate of decrease experienced was faster in the first 20 days of 90-day storage. After 28 days, there was an approximately 50% loss in the TPC present in MoLP extracts at 40 °C, whereas there was a total loss of 26.88%, 40.14% and 42.45% observed in the encapsulated samples (MDGA, MD and GA respectively) (Fig 5.9 A-D). The significant difference ($p < 0.05$) observed between the encapsulated samples and the non-encapsulated extracts can be ascribed to the protective effect of the coating material. Since the primary role and goal of encapsulation is to shield the core compounds as well as retain their activity from degradation caused by environmental factors. The storage at this temperature (40 °C) shows that encapsulation resulted in the fulfilment of this crucial goal. It is also important to mention that the use of combined coating materials (MDGA) resulted in decreased degradation (26.88%) during 28 days of storage when compared with the other samples. The quantity of TPC lost in all microcapsules are significantly lower than those reported for *M. oleifera* extracts coated with tragacanth gum stored at 35 °C for 35 days reported by (Castro-López *et al.*, 2020), indicating that the coating materials used in our study resulted in increased stability. The increased retention of TPC noticed in MDGA over this period may result from the lower amount of surface polyphenols present in these microcapsules as well as the high encapsulation efficiency of this combined coating material. The lower loss of TPC noticed in MDGA is similar to the observation of Sansone *et al.* (2011) and Dadi *et al.* (2020), where high retention of TPC was observed after long term storage, these authors ascribed this to higher encapsulation efficiency obtained when two coating materials are combined and used as carriers. There was no significant difference ($p > 0.05$) noticed between the quantity of TPC lost after 28 days between MD and GA microcapsules. The rate of loss observed indicated that with time, there is a reduction in the amount of the

TPC present in stored products. The stability of the microcapsules produced with different coating materials were studied for 28 days at different temperatures (4, 25 and 40 °C respectively) for their ability to retain TPC. This was done to determine the appropriate storage condition for these microcapsules based on the protective effect of the coating materials (non-encapsulated *M. oleifera* leaf powder extract was used as a control). At 40 °C, there was a rapid decrease in the total phenolic content of all the samples in the first 21 days (Fig 5.9). This is in tandem with the observation of Dadi *et al.* (2020) where the rate of decrease experienced was faster in the first 20 days of 90-day storage. After 28 days, there was an approximately 50% loss in the TPC present in MoLP extracts at 40 °C, whereas there was a total loss of 26.88%, 40.14% and 42.45% observed in the encapsulated samples (MDGA, MD and GA respectively) (Fig 5.9 A-D). The significant difference ($p < 0.05$) observed between the encapsulated samples and the non-encapsulated extracts can be ascribed to the protective effect of the coating material. Since the primary role and goal of encapsulation is to shield the core compounds as well as retain their activity from degradation caused by environmental factors. The storage at this temperature (40 °C) shows that encapsulation resulted in the fulfilment of this crucial goal. It is also important to mention that the use of combined coating materials (MDGA) resulted in decreased degradation (26.88%) during 28 days of storage when compared with the other samples. The quantity of TPC lost in all microcapsules are significantly lower than those reported for *M. oleifera* extracts coated with tragacanth gum stored at 35 °C for 35 days reported by (Castro-López *et al.*, 2020), indicating that the coating materials used in our study resulted in increased stability. The increased retention of TPC noticed in MDGA over this period may result from the lower amount of surface polyphenols present in these microcapsules as well as the high encapsulation efficiency of this combined coating material. The lower loss of TPC noticed in MDGA is similar to the observation of Sansone *et al.* (2011) and Dadi *et al.* (2020), where high retention of TPC was observed after long term storage, these authors ascribed this to higher encapsulation efficiency obtained when two coating materials are combined and used as carriers. There was no significant difference ($p > 0.05$) noticed between the quantity of TPC lost after 28 days between MD and GA microcapsules. The rate of loss observed indicated that with time, there is a reduction in the amount of the TPC present in stored products.

At 25 °C, a loss of 27.05% was noticed in the TPC of the MoLP extract while only a loss of 11.11, 16.41 and 22.53% of TPC present in MDGA, MD and GA respectively, after 28 days.

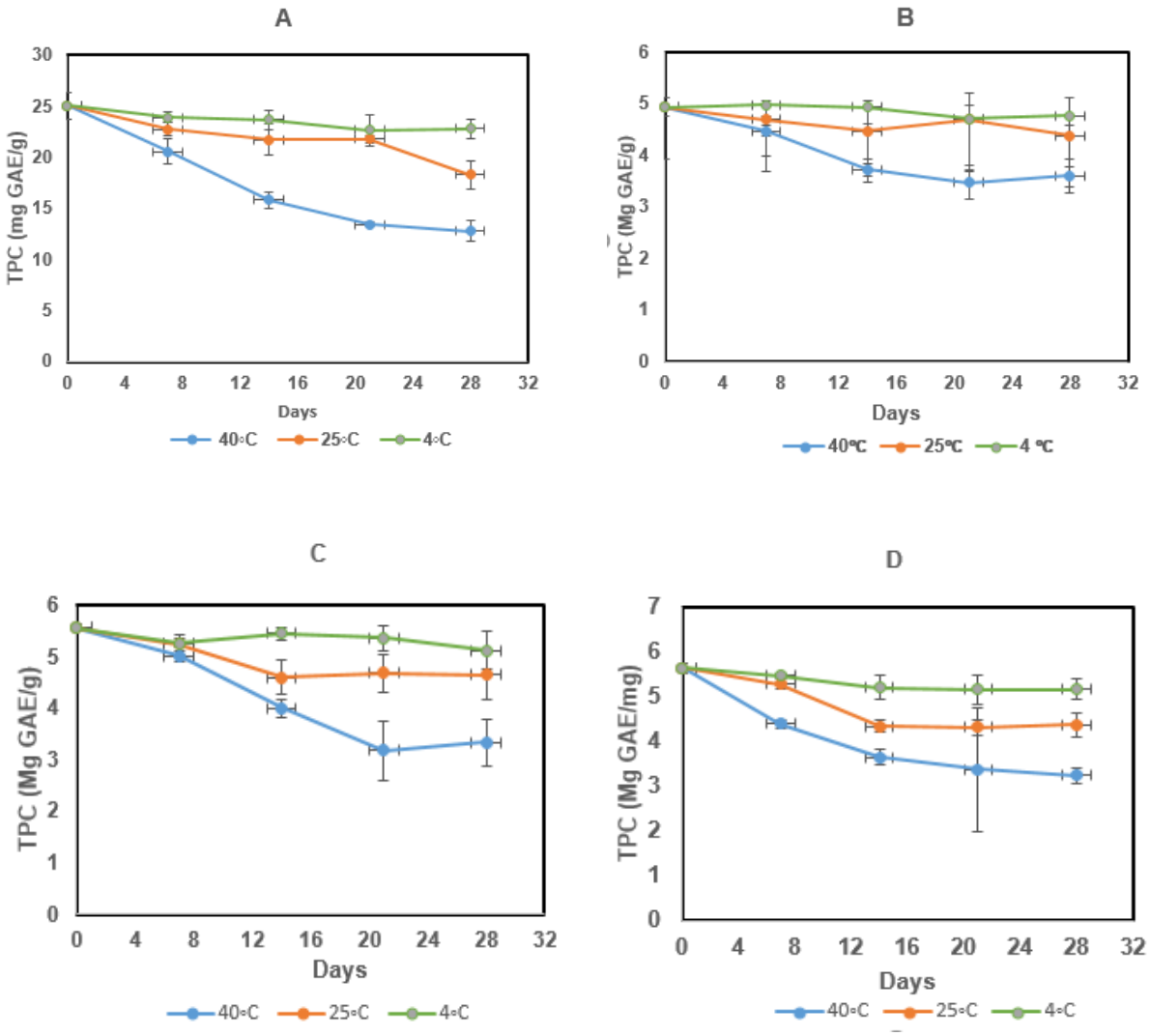


Figure 5.9 Total polyphenol contents at different temperatures for (A) *M. oleifera* leaf powder extract (B) MDGA microcapsules (C) MD microcapsules (D) GA microcapsules

This further shows the importance of encapsulation as an important procedure in conserving important compounds that have beneficial importance during storage. A lesser quantity of TPC was lost in the encapsulated samples in comparison with the dried extracts stored under the same temperature condition. Maltodextrin-gum Arabic coated microcapsules recorded the lowest loss at this temperature which indicates that coating material and their property influenced the storage stability of microcapsules developed from them over the storage period.

At 4 °C, minimal losses in TPC were recorded after 28 days, only 4.40, 7.84 and 8.46% losses were reported for MDGA, MD and GA respectively indicating that at low

temperature, minimal losses of phenolic content occurs. The differences noticed between the TPC of samples with different coatings also showed that the stability of stored microcapsules is dependent on the coating materials which is dependent on how much protection is offered to the active/core compounds (Osamede Airouyuwa and Kaewmanee, 2019). In addition to this, only 10% of the initial TPC present in the extract powder was lost at 4 °C implying that this temperature or lower temperatures enhance the storage stability of dried extracts. In all, the stability of the microcapsules during storage was affected by temperature and the coating material used. Furthermore, a progressive decline in the polyphenols was noticed with respect to time (days) for all samples which were similar to a previous observation by Osamede Airouyuwa and Kaewmanee, (2019). This study shows that low temperature storage at 4 °C will minimize loss and that the combined use of coating material (MDGA) offers increased stability and as such is recommended.

5.8.12 *In vitro* digestibility studies

The *in vitro* release profile of the developed microcapsules in the simulated gastric and intestinal phase (SGP and SIP) are presented in Fig 5.10 A and B. The release of the phenolic compounds entrapped in the coating material was determined to understand the ability of the digestive tract to absorb the core materials, the absorbed phenolic compound is therefore the amount released for use in the site of action against the activities of free radicals. In addition to this, the stability and bioaccessibility of phenolic compounds present in an entrapped matrix affect their activity at their site of action (Castro-López *et al.*, 2020). The activities of microcapsules in the gastric tract are also dependent on the coating material property and their proneness to the digestibility activities of the digestive enzymes as well as the condition of the pH of the gastric environment (Saikia *et al.*, 2015). This is important for the maximum release of the fraction of the active components entrapped in the coating.

The amount of the TPC of the microcapsules was significantly different ($p < 0.05$) among all coatings used in this study for both the gastric and intestinal phases. Similarly, the amount of phenolic compounds released in the gastric phase was significantly higher ($p < 0.05$) than those released in the intestinal phase. This may be attributed to the relatively unstable attribute of high molecular weight polyphenols in the acidic environment of the human stomach (Awika *et al.*, 2003). A similar observation was reported previously on the high release of TPC in SGP than SIP (Saikia *et al.*, 2015; Dadi *et al.*, 2020).

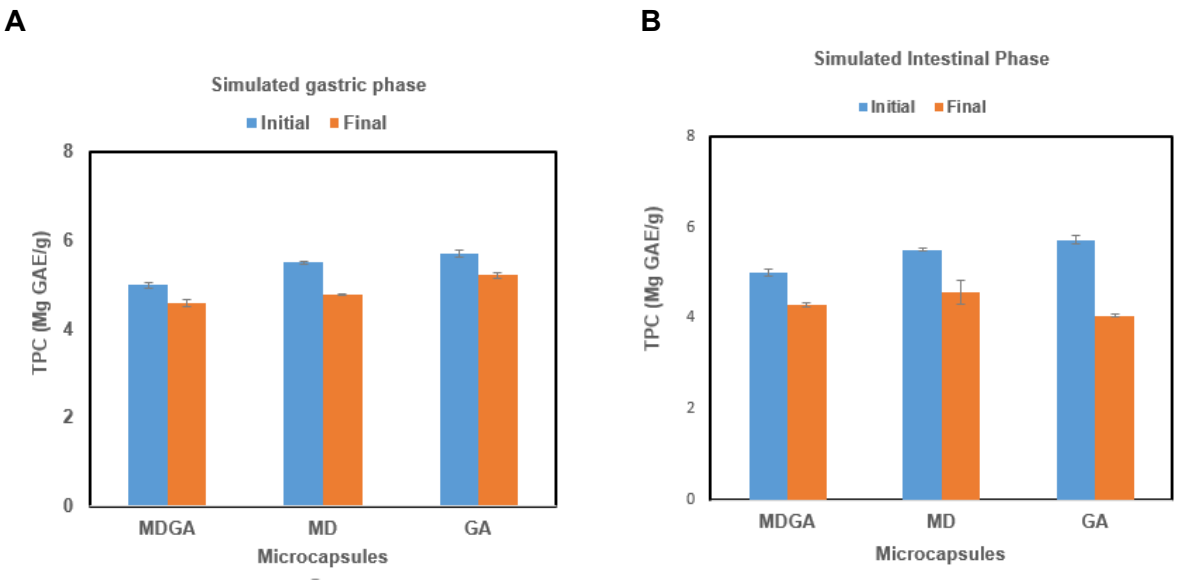


Figure 5.10 *In vitro* digestibility study graph (A) TPC Simulated gastric phase (B) TPC Simulated intestinal phase. (where n = 6)

This implies that the microcapsules are more soluble in the gastric environment than in the intestinal phase, and hence release well under the condition of the gastric mucosa.

GA microcapsules gave the highest TPC for SGP, this was due to the presence of more initial TPC and SPC (Table 4.4) in these samples when compared with others. MDGA microcapsule had the lowest TPC content, this is likely due to the high encapsulation and low polyphenol contents observed on the surface of the initial microcapsules prior to release profile analyses. The MD microcapsules were observed to have the highest TPC in the intestinal phase. This may likely be due to the high solubility of maltodextrin at neutral pH which resulted in the near-complete dissolution of the microcapsules (Saikia *et al.*, 2015).

In comparison with the initial TPC content obtained before the release profile assay, the release rate obtained in the gastric phase for MDGA, MD and GA are; 91.90% 86.93% and 91.27% respectively, with MDGA having a significantly ($p < 0.05$) higher release rate than MD microcapsules but comparable release rate with GA microcapsules (Figure 5.10A).

Furthermore, the release rate of the microcapsules in the intestinal phase for MDGA, MD and GA are 85.95%, 83.03% and 70.93% respectively showing that MDGA had a higher release rate in the intestinal phase like the gastric phase. The release rate here was significantly ($p < 0.05$) higher than GA microcapsules but does not differ significantly ($p < 0.05$) from MD. The above release rates may be attributed to the ability of the microcapsules to break up under the acidic environment of the gastric phase than the neutral pH environment of the SIP.

Saikia *et al.* (2015) and Dadi *et al.* (2020) earlier stated that the release property of microcapsules is dependent on the coating material make-up and property, as well as the pH of the digestive environment. This property involves their solubility and swelling property, swelling property has been reported to affect the disintegration property of microcapsules (Castro-López *et al.*, 2020). Hence, the higher release rate was observed in the gastric phase than in the intestinal phase. Generally, the release rate for all microcapsules was high which may also be attributed to the amorphous nature of the powder as well as the hydrophilic nature of the coating materials used.

5.9 Conclusion

The *M. oleifera* leaf powder extract was encapsulated in MD, GA and MDGA and then freeze-dried. The encapsulation procedure was further confirmed by the FTIR, TGA and the storage stability analysis to ascertain the stability and efficacy of the process. The XRD and SEM analysis indicated that the microcapsules were amorphous with slightly irregular structural patterns. The encapsulation efficiency was impacted by the type of coating materials used. The combination of MD and GA resulted in improved encapsulation efficiency, storage stability as well as high release percentage in the gastric and intestinal site of action than others. In addition to this, all microcapsules exhibited good stability at very high temperatures using thermogravimetric analysis study compared to the dried *M. oleifera* leaf powder extracts which further proves the effectiveness of encapsulation to enhance stability. The gastro-intestinal release profile of total phenolic compounds was affected by the coating materials used as well as the pH in the stomach and the intestinal area. The developed microcapsules can form an inclusion in traditional foods because of its bioactive contents and can improve the functional/nutraceutical nature of such foods. The ability of the coating material to mask the green colouration of the extracts effectively which was evident from the colour analysis further shows that the sensory attribute of food products that may likely be fortified with these capsules may not be affected in terms of colour. The MDGA coating mixture offered the best potential for the microcapsules in food applications based on the various analysis conducted in this chapter.

5.10 References

Aguiar, J., Estevinho, B.N. & Santos, L. (2016). Microencapsulation of natural antioxidants for food application – The specific case of coffee antioxidants – A review. *Trends in Food Science and Technology*, **58**, 21–39

Akhavan Mahdavi, S., Jafari, S.M., Assadpoor, E. & Dehnad, D. (2016). Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. *International Journal of Biological Macromolecules*, **85**, 379–385.

Anwar, F., Latif, S., Ashraf, M. & Hassan, A. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research*, **659**, 651–659.

AOAC. (2000). Analytical Methods. *Toxicology and Industrial Health*, **15**, 715–717.

Awika, J.M., Dykes, L., Gu, L., Rooney, L.W. & Prior, R.L. (2003). Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. *Journal of Agricultural and Food Chemistry*, **51**, 5516–5521.

Ballesteros, L.F., Ramirez, M.J., Orrego, C.E., Teixeira, J.A. & Mussatto, S.I. (2017a). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. *Food Chemistry*, **237**, 623–631.

Ballesteros, L.F., Ramirez, M.J., Orrego, C.E., Teixeira, J.A. & Mussatto, S.I. (2017b). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. *Food Chemistry*, **237**, 623–631.

Cano-Chauca, M., Stringheta, P.C., Ramos, A.M. & Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science and Emerging Technologies*, **6**, 420–428.

Castro-López, C., Gonçalves, C., Ventura-Sobrevilla, J.M., Pastrana, L.M., Aguilar-González, C.N. & Martínez-Ávila, G.C.G. (2020). *Moringa oleifera*-storage stability, *In vitro*-simulated digestion and cytotoxicity assessment of microencapsulated extract. *Processes*, **8**, 770–783.

Castro-Rosas, J., Ferreira-Grosso, C.R., Gómez-Aldapa, C.A., Rangel-Vargas, E., Rodríguez-Marín, M.L., Guzmán-Ortiz, F.A. & Falfan-Cortes, R.N. (2017). Recent advances in microencapsulation of natural sources of antimicrobial compounds used in food - A review. *Food Research International*, **102**, 575–587.

Chranioti, C., Nikoloudaki, A. & Tzia, C. (2015). Saffron and beetroot extracts encapsulated in maltodextrin, gum Arabic, modified starch and chitosan: Incorporation in a chewing gum system. *Carbohydrate Polymers*, **127**, 252–263.

Chuyen, H. V, Roach, P.D., Golding, J.B., Parks, S.E. & Nguyen, M.H. (2019). Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technology*, **344**, 373–379.

Cilek, B., Luca, A., Hasirci, V., Sahin, S. & Sumnu, G. (2012). Microencapsulation of phenolic compounds extracted from sour cherry pomace: Effect of formulation, ultrasonication time and core to coating ratio. *European Food Research and Technology*, **235**, 587–596.

Coates, J. (2006). Interpretation of Infrared Spectra, A Practical Approach. In: *Encyclopedia of Analytical Chemistry*. Pp. 10815–10837. Chichester, UK: John Wiley & Sons, Ltd.

Cruz-Espinoza, J.E., Orduña-Díaz, A., Rosales-Perez, M., Zaca-Morán, O., Delgado-Macuil, R., Gayou, V.L. & Rojas-López, M. (2012). FTIR analysis of phenolic extracts from *Moringa oleifera* leaves. *Journal of Biometrics & Biostatistics*, **11**, 2802.

Dadi, D.W., Emire, S.A. & Hagos, A.D. (2018). Influences of different drying methods and extraction solvents on total phenolic and flavonoids , and antioxidant capacity of *Moringa stenopetala* leaves. *Journal of Pharmacognosy and phytochemistry*, **7**, 962–967.

Dadi, D.W., Emire, S.A., Hagos, A.D. & Eun, J.-B. (2019b). Effects of spray drying process parameters on the physical properties and digestibility of the microencapsulated product from *Moringa stenopetala* leaves extract. *Cogent Food & Agriculture*, **5**, 1690316.

Dadi, D.W., Emire, S.A., Hagos, A.D. & Eun, J.B. (2020). Physical and Functional Properties, Digestibility, and Storage Stability of Spray- and Freeze-Dried Microencapsulated Bioactive Products from *Moringa stenopetala* Leaves Extract. *Industrial Crops and Products*, **156**, 112891.

Edris, A.E., Kalemba, D., Adamiec, J. & Piaotkowski, M. (2016). Microencapsulation of *Nigella sativa* oleoresin by spray drying for food and nutraceutical applications. *Food Chemistry*, **204**, 326–333.

Ersus, S. & Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. *Journal of Food Engineering*, **80**, 805–812.

Ferreira, P.M.P., Farias, D.F., Oliveira, J.T.D.A. & Carvalho, A.D.F.U. (2008). *Moringa oleifera*: Bioactive compounds and nutritional potential. *Revista de Nutricao*, **21**, 431–437.

Gallo, L., Llabot, J.M., Allemandi, D., Bucalá, V. & Piña, J. (2011). Influence of spray-drying operating conditions on *Rhamnus purshiana* (*Cáscara sagrada*) extract powder physical properties. *Powder Technology*, **208**, 205–214.

Gómez, B., Barba, F.J., Domínguez, R., Putnik, P., Bursać Kovačević, D., Pateiro, M., Toldrá, F. & Lorenzo, J.M. (2018). Microencapsulation of antioxidant compounds through innovative technologies and its specific application in meat processing. *Trends in Food Science and Technology*, **82**, 135–147.

Gullón, B., Gullón, P., Lú-Chau, T.A., Moreira, M.T., Lema, J.M. & Eibes, G. (2017). Optimization of solvent extraction of antioxidants from *Eucalyptus globulus* leaves by response surface methodology: Characterization and assessment of their bioactive properties. *Industrial Crops and Products*, **108**, 649–659.

Gulu, N.B., Jideani, V.A. & Jacobs, A. (2019). Functional characteristics of Bambara groundnut starch-catechin complex formed using cyclodextrins as initiators. *Heliyon*, **5**, e01562.

Kang, Y.R., Lee, Y.K., Kim, Y.J. & Chang, Y.H. (2019). Characterization and storage stability of chlorophylls microencapsulated in different combination of gum Arabic and maltodextrin. *Food Chemistry*, **272**, 337–346.

Khalifa, I., Li, M., Mamet, T. & Li, C. (2019). Maltodextrin or gum Arabic with whey proteins as wall-material blends increased the stability and physiochemical characteristics of mulberry microparticles. *Food Bioscience*, **31**, 100445.

Kuck, L.S. & Noreña, C.P.Z. (2016). Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*, **194**, 569–576.

Lee, J.K.M., Taip, F.S. & Abdulla, H.Z. (2018). Effectiveness of additives in spray drying performance: a review. *Food Research*, **2**, 486–499.

Lei, M., Jiang, F.C., Cai, J., Hu, S., Zhou, R., Liu, G., Wang, Y.H., Wang, H.B., He, J.R. & Xiong, X.G. (2018). Facile microencapsulation of olive oil in porous starch granules: Fabrication, characterization, and oxidative stability. *International Journal of Biological Macromolecules*, **111**, 755–761.

Luna-Guevara, J.J., Ochoa-Velasco, C.E., Hernández-Carranza, P. & Guerrero-Beltrán, J.A. (2017). Microencapsulation of walnut, peanut and pecan oils by spray drying. *Food Structure*, **12**, 26–32.

Ma, Z.F., Ahmad, J., Zhang, H., Khan, I. & Muhammad, S. (2020). Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera*) as a potential functional food. *South African Journal of Botany*, **129**, 40–46.

Mahdavee Khazaei, K., Jafari, S.M., Ghorbani, M. & Hemmati Kakhki, A. (2014). Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. *Carbohydrate Polymers*, **105**, 57–62.

Mahfoudhi, N., Ksouri, R. & Hamdi, S. (2016). Nanoemulsions as potential delivery systems

for bioactive compounds in food systems: preparation, characterization and applications in food industry. *Emulsions*, 365–403.

Matinise, N., Fuku, X.G., Kaviyarasu, K., Mayedwa, N. & Maaza, M. (2017a). ZnO nanoparticles via *Moringa oleifera* green synthesis: Physical properties & mechanism of formation. *Applied Surface Science*, **406**, 339–347.

Matinise, N., Fuku, X.G., Kaviyarasu, K., Mayedwa, N. & Maaza, M. (2017b). ZnO nanoparticles via *Moringa oleifera* green synthesis: Physical properties & mechanism of formation. *Applied Surface Science*, **406**, 339–347.

Mohd Nawi, N., Muhamad, I.I. & Mohd Marsin, A. (2015). The physicochemical properties of microwave-assisted encapsulated anthocyanins from *Ipomoea batatas* as affected by different wall materials. *Food Science and Nutrition*, **3**, 91–99.

Moyo, B., Oyedemi, S., Masika, P.J. & Muchenje, V. (2012). Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science*, **91**, 441–447.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S. & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, **1**, 1806–1815.

Osamede Airouyuwa, J. & Kaewmanee, T. (2019). Microencapsulation of *Moringa oleifera* leaf extracts with vegetable protein as wall materials. *Food Science and Technology International*, **25**, 533–543.

Ozkan, G., Franco, P., Marco, I. De, Xiao, J. & Capanoglu, E. (2019). A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. *Food Chemistry*, **272**, 494–506.

Padayachee, B. & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. *South African Journal of Botany*, **129**, 304–316.

Pal, S., Mondal, S., Maity, J. & Mukherjee, R. (2018). Synthesis and characterization of zno nanoparticles using *Moringa Oleifera* leaf extract: Investigation of photocatalytic and antibacterial activity. *International Journal of Nanoscience and Nanotechnology*, **14**, 111–119.

Panwar, A. & Mathur, J. (2019). Zone based phytochemical variability in different genotypes of *moringa oleifera* Lam. Using FTIR spectroscopy. *International Journal of Scientific and Technology Research*, **8**, 128–133.

Pellicer, J.A., Fortea, M.I., Trabal, J., Rodríguez-López, M.I., Gabaldón, J.A. & Núñez-Delicado, E. (2019). Stability of microencapsulated strawberry flavour by spray drying,

freeze drying and fluid bed. *Powder Technology*, **347**, 179–185.

Premi, M. & Sharma, H.K. (2017). Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (*Moringa oleifera*) oil. *International Journal of Biological Macromolecules*, **105**, 1232–1240.

Rajabi, H., Ghorbani, M., Jafari, S.M., Sadeghi Mahoonak, A. & Rajabzadeh, G. (2015). Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and gelatin as wall materials. *Food Hydrocolloids*, **51**, 327–337.

Ramírez, M.J., Giraldo, G.I. & Orrego, C.E. (2015). Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. *Powder Technology*, **277**, 89–96.

Rezende, Y.R.R.S., Nogueira, J.P. & Narain, N. (2018). Microencapsulation of extracts of bioactive compounds obtained from acerola (*Malpighia emarginata* DC) pulp and residue by spray and freeze drying: Chemical, morphological and chemometric characterization. *Food Chemistry*, **254**, 281–291.

Rodrigues da Cruz, M.C., Andreotti Dagostin, J.L., Perussello, C.A. & Masson, M.L. (2019). Assessment of physicochemical characteristics, thermal stability and release profile of ascorbic acid microcapsules obtained by complex coacervation. *Food Hydrocolloids*, **87**, 71–82.

Saikia, S., Mahnot, N.K. & Mahanta, C.L. (2015). Optimisation of phenolic extraction from *Averrhoa carambola* pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chemistry*, **171**, 144–152.

Sansone, F., Mencherini, T., Picerno, P., D'Amore, M., Aquino, R.P. & Lauro, M.R. (2011). Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. *Journal of Food Engineering*, **105**, 468–476.

Sarabandi, K., Jafari, S.M., Mahoonak, A.S. & Mohammadi, A. (2019). Application of gum Arabic and maltodextrin for encapsulation of eggplant peel extract as a natural antioxidant and color source. *International Journal of Biological Macromolecules*, **140**, 59–68.

Saucedo-Pompa, S., Torres-Castillo, J.A., Castro-López, C., Rojas, R., Sánchez-Alejo, E.J., Ngangyo-Heya, M. & Martínez-Ávila, G.C.G. (2018). Moringa plants: Bioactive compounds and promising applications in food products. *Food Research International*, **111**, 438–450.

Shetta, A., Kegere, J. & Mamdouh, W. (2019). Comparative study of encapsulated peppermint and green tea essential oils in chitosan nanoparticles: Encapsulation, thermal stability, *in-vitro* release, antioxidant and antibacterial activities. *International*

Journal of Biological Macromolecules, **126**, 731–742.

Siripireddy, B. & Mandal, B.K. (2017). Facile green synthesis of zinc oxide nanoparticles by *Eucalyptus globulus* and their photocatalytic and antioxidant activity. *Advanced Powder Technology*, **28**, 785–797.

Smrčková, P., Horský, J., Šárka, E., Koláček, J., Netopilík, M., Walterová, Z., Kruliš, Z., Synytsya, A. & Hrušková, K. (2013). Hydrolysis of wheat B-starch and characterisation of acetylated maltodextrin. *Carbohydrate Polymers*, **98**, 43–49.

Song, J.E., Phenrat, T., Marinakos, S., Xiao, Y., Liu, J., Wiesner, M.R., Tilton, R.D. & Lowry, G. V. (2011). Hydrophobic interactions increase attachment of gum arabic- and PVP-coated Ag nanoparticles to hydrophobic surfaces. *Environmental Science and Technology*, **45**, 5988–5995.

Šturm, L., Osojnik Črnivec, I.G., Istenič, K., Ota, A., Megušar, P., Slukan, A., Humar, M., Levic, S., Nedović, V., Kopinč, R., Deželak, M., Pereyra Gonzales, A. & Poklar Ulrih, N. (2019). Encapsulation of non-dewaxed propolis by freeze-drying and spray-drying using gum Arabic, maltodextrin and inulin as coating materials. *Food and Bioproducts Processing*, **116**, 196–211.

Sun, X., Cameron, R.G. & Bai, J. (2019). Microencapsulation and antimicrobial activity of carvacrol in a pectin-alginate matrix. *Food Hydrocolloids*, **92**, 69–73.

Tengse, D.D., Priya, B. & Kumar, P.A.R. (2017). Optimization for encapsulation of green tea (*Camellia sinensis* L.) extract by spray drying technology. *Journal of Food Measurement and Characterization*, **11**, 85–92.

Tolun, A., Altintas, Z. & Artik, N. (2016). Microencapsulation of grape polyphenols using maltodextrin and gum arabic as two alternative coating materials: Development and characterization. *Journal of Biotechnology*, **239**, 23–33.

Tonon, R. V., Brabet, C. & Hubinger, M.D. (2008). Influence of process conditions on the physicochemical properties of açai (*Euterpe oleraceae* Mart.) powder produced by spray drying. *Journal of Food Engineering*, **88**, 411–418.

Yadav, K., Bajaj, R.K., Mandal, S. & Mann, B. (2019). Encapsulation of grape seed extract phenolics using whey protein concentrate, maltodextrin and gum arabica blends. *Journal of Food Science and Technology*, **57**, 426–434.

Zanetti, M., Mazon, L.R., Meneses, A.C. de, Silva, L.L., Araújo, P.H.H. de, Fiori, M.A. & Oliveira, D. de. (2019). Encapsulation of geranyl cinnamate in polycaprolactone nanoparticles. *Materials Science and Engineering C*, **97**, 198–207.

Zuidam, N.J. & Nedović, V.A. (2010). *Encapsulation technologies for active food ingredients and food processing*. *Encapsulation Technologies for Active Food Ingredients and Food Processing*. Springer New York.

CHAPTER SIX

GENERAL SUMMARY AND CONCLUSION

In this study, the characterization and encapsulation of *M. oleifera* extracts were demonstrated. The primary aim of the study was to evaluate the efficacy of incorporating extracts from *M. oleifera* plant into coating materials and determining optimum preparation conditions based on their encapsulation efficiency, to protect the bioactive compounds present therein. The following objectives were achieved during the study:

1. Extraction, phytochemical profiling, and antioxidant activity of *M. oleifera* extracts using different solvents.
2. Optimisation condition for the preparation of microcapsules for maximum encapsulation efficiency of *M. oleifera* leaf powder extract using maltodextrin and/or gum Arabic as coating materials.
3. Effect of coating materials (Maltodextrin, gum Arabic and Maltodextrin gum Arabic mixture) on some properties of encapsulated *M. oleifera* leaf powder extract.

In achieving these objectives, three different solvents were used in the extraction of *M. oleifera* leaf powder bioactives using the conventional solvent extraction method that involves mechanical shaking. The use of 60% ethanol in the extraction of the leaf powder and H_2O in the extraction of the seed powder was found to be beneficial as higher phytochemical and antioxidant composition and activity respectively were observed. The individual compounds identified further established the better extraction capacity of these solvents as more compounds were found in samples extracted with these (EtOH & H_2O) solvents. In addition to this, the *M. oleifera* leaf powder extract was higher in phytochemicals (TPC and TFC) and antioxidant composition and activity, than the seed powder extract which resulted in the further encapsulation of the leaf powder extract in chapter 4.

The second objective was accomplished by optimising the microencapsulation preparation condition for maximum efficiency. Coating: coating ratio (MD: GA), core: coating ratio and ultrasonication time were the factors in the model design, while encapsulation efficiency was the response. An optimum preparation condition was obtained at coating material ratios (MD: GA) of 7.5: 2.5, core coating ratio of 1: 8.5 and ultrasonication time of 13.33 minutes. The predicted encapsulation efficiency from the model was 88.90% after which the model was validated and an efficiency of 84.90% was obtained which was not significantly different from the predicted response. The regression model was significant while lack of fit was not significant. The adjusted R^2 0.9467 value was within the range (± 0.2) of the predicted value 0.7598 which further underlines the reliability of the model.

The third and last objective was achieved by examining and characterizing the effect of different coating materials on the properties of microcapsules of the MoLP extract. This

was done by determining the physical, thermal, and structural properties of the microcapsules produced. The bulk and tapped densities suggested that the MDGA and MD microcapsules can fit into packaging material spaces more effectively than the GA microcapsules. The low moisture content of all microcapsules produced indicated a possible long shelf life without microbial or chemical deterioration. The structural composition of the microcapsules confirmed encapsulation as new FTIR peaks were formed in all microcapsules around 1177 to 1400 cm^{-1} confirming encapsulation. The FTIR analysis also showed that the microcapsules were dominated by the presence of carbon, hydrogen, oxygen and some amide and amine bonds which were present in the coating material and the extracts, respectively. The X-ray diffraction analysis and the scanning electron microscopy analysis indicated that all microcapsules were amorphous, although SEM analysis of the MDGA microcapsules indicated a slightly spherical shape. Thermogravimetric analysis indicated good stability of the microcapsules at high temperature with the maximum degradation temperature noticed above 300 ℃. The storage stability test resulted in better stability of encapsulated MoLP extracts over non-encapsulated extracts with minimal losses obtained at 4 ℃ over 28 days than at 25 and 40 ℃ respectively. The *in vitro* release profile showed that the release of TPC in the gastric phase was higher than in the intestinal phase. In addition to this, the percentage release of TPC from the MDGA microcapsules showed that it possessed a better release property than the others. The scavenging activity of DPPH indicated that there was no significant difference ($p > 0.05$) among the antioxidant activity of the microcapsules. The following conclusions can be drawn from this study:

- The use of different solvents for the extraction of *M. oleifera* bioactives resulted in significant differences in the phytochemical and antioxidant composition of extracts as well as varying quantities of individual compounds present in the extracts.
- Optimisation experiments for the preparation conditions of microcapsules for maximum encapsulation efficiency resulted in fewer experiments for the determination of different attributes and resulted in high encapsulation efficiency that protected the core compounds.
- The thermal properties and the storage stability of the microcapsules showed that encapsulation enhanced stability at high temperatures which makes their inclusion in food processed at high temperatures important.
- The MDGA microcapsules had overall enhanced properties over MD and GA microcapsules.
- Overall, encapsulation proved to be an effective procedure in the protection of important bioactives present in *M. oleifera* leaf powder extracts, against factors that may result in deterioration.