PHYSICOCHEMICAL, NUTRITIONAL AND DISSOLUTION PROPERTIES OF SOLID-DISPERSED *MORINGA OLEIFERA* LEAF POWDER EFFERVESCENT BEVERAGE GRANULES

by

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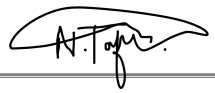
DECLARATION

I, Nontsikelelo Noxolo Tafu, declare that the contents of this dissertation/thesis represent my own unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

N.N Tafu  **20/08/2021**

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ABSTRACT

The aim of this study was to establish the physicochemical, nutritional, and dissolution properties of the solid-dispersed *Moringa oleifera* leaf powder (SDMOLP) with the view to present it as an easy-to-drink functional beverage. Solid dispersions of *Moringa oleifera* leaf powder (MOLP) were successfully prepared using polyethylene glycols (PEG4000 and PEG6000) as hydrophilic carriers and various solid dispersion methods, including melting, freeze-drying, solvent evaporation, and microwave irradiation methods, respectively. The resulting SDMOLPs were evaluated for their nutritional composition, functional and dissolution properties. The mineral composition was characterised using the Scanning Electron Microscopy-Energy Dispersive X-Ray Spectrometry (SEM-EDX) technique. Moreover, the characterisation of pure MOLP and SDMOLPs was carried out by performing SEM, scanning transfer electron microscopy (STEM), powder X-ray diffraction (PXRD), diffraction scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Fourier Transform Infrared spectroscopy (FTIR). A Vankel's dissolution apparatus with the United States Pharmacopeia (USP) basket method was employed for the dissolution study of the dispersions. SDMOLPs effervescent beverage granules were produced using a wet granulation method. A 5-point Hedonic test scale [1 (dislike very much) to 5 (like very much)] was used to determine the sensory and consumer acceptability of the beverage granules. Proximate analysis revealed that pure MOLP is rich in protein content (28.52%) and metabolisable energy (344.46 Kcal/100 g), with appreciable levels of fat 5.13%, moisture 8.44%, and ash 11.81%. While SDMOLP had protein content ranging from 12.42-14.23%; moisture, 2.65-4.03%; fat, 2.51-3.21%; and ash, 4.03-6.16%. All the SDMOLPs showed significantly higher ($p < 0.05$) solubility and water absorption capacity (WAC) that ranged from 56.86 to 63.29% and 468.86 to 686.37%, respectively. Pure MOLP had a solubility and WAC of 25.45% and 345.09%, respectively. The mineral composition indicated that all SDMOLPs were high in calcium (38.28-65.05%), magnesium (4.24-17.97%), phosphorous (1.73-13.52%), copper (2.33-27.05%), sulphur (6.00-36.98%), iron (0.15-3.88%), zinc (0.00-8.49%) and sodium (0.00-2.93%). PXRD results revealed that SDMOLPs were partially amorphous with strong diffraction peaks at 2θ values of 19° and 23°. The calorimetric and thermogravimetric curves showed that SDMOLPs were more thermally stable than pure MOLP. The FTIR spectrum of MOLP showed variable peaks at 3285 cm^{-1}, 2981 cm^{-1}, 2849.45 cm^{-1}, 1630.36 cm^{-1}, 1411.7 cm^{-1},1049.44 cm^{-1}, and 536.03 cm^{-1} attributable to O-H- bending and N-H group, asymmetric C-H stretching, symmetric C-H group, C=O stretch, C-N stretch, C-O-C symmetrical and N-H stretching, respectively. Significant peak shift and intensity reduction of O-H, N-H, and C=O functional groups in SDMOLPs evidenced the existence of intermolecular interactions between MOLP and PEG

carriers, indicating strong hydrogen bonding between the two components. The dissolution rate of SDMOLPs was significantly improved. The sensory evaluation results of the SDMOLP effervescent beverages showed that mean hedonic scores of sensory attributes ranged from neither like nor dislike to like moderately for appearance (3.19-3.92), colour (3.23-4.04), aroma (4.00-4.13), taste (3.08-3.77), texture (3.04-3.66) and overall acceptability (3.40-4.00). The novel MOLP solid dispersions could be used to produce functional easy-to-drink beverages.

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DEDICATION

Dedicated to Almighty God for His great grace and steadfast love. To my lovely parents, Mr. Amos and Mrs. Carol Tafu, for their endless love and support. To my siblings, Nokwazi and Andile Tafu, for being there for me, I love them so much. To my best friend, Victory, thank you for your unending support. Last but not least, my supervisor, Professor Victoria Jideani, for being my inspiration.

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity, however, repetition between chapters was unavoidable.

GLOSSARY

Terms/ Acronyms/Abbreviation	Definition/Explanation
$C_4H_6O_6$	Tartaric acid
$C_6H_8O_7$	Citric acid
CO_2	Carbon dioxide
DAFF	Department of agriculture, fishery and forestry
DSC	Differential Scanning Calorimetry
EDS	Energy Dispersive X-Ray Spectrometry
FDA	Food and drug administration
FTIR	Fourier Transform Infra-red
MANOVA	Multivariate analysis of variance
MOLP	*Moringa oleifera* leaf powder
$3NaHCO_3$	Sodium bicarbonate
$Na_3C_6H_5O_7$	Sodium citrate
$C_4H_4Na_2O_6$	Sodium tartrate
NADH/NADPH	Nicotinamide adenine dinucleotide phosphate
PCA	Principal component analysis
PEG	Polyethylene glycol
PEM	Protein Energy Malnutrition
PVP	Polyvinylpyrrolidone,
PXRD	Powder X-ray diffraction
SDMOLP	Solid dispersed *Moringa oleifera* leaf powder
ROS	Reactive Oxygen Species
SEM	Scanning electron microscopy
STEM	Scanning Transfer electron microscopy
TGA	Thermogravimetric analysis
USP	United States Pharmacopeia
UV/Vis	Ultraviolet-visible spectrophotometer
WAC	Water absorption capacity
WHO	World health organization

CHAPTER ONE
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

There has been a lot of interest in functional foods from both the food industry and consumers. Functional Food Ingredients' market value was US$ 76.2 Billion in 2020 and is forecast to grow by 5.4% per annum from 2020 to 2027 (Xiong *et al.*, 2020, Research and Markets, 2021). Recently, there has been an upsurge of interest in using *Moringa oleifera* leaf powder (MOLP) as a functional ingredient in food products. As a result of its high nutritional content, Moringa has been marketed as a superfood worldwide. *Moringa oleifera* is both a food plant and a medicinal plant. Its leaves are a rich source of protein (28%), essential amino acids, vitamins (A, B, C, and E), and minerals (Bamishaiye *et al.*, 2011; Jideani, 2014; Gopalakrishnan *et al.*, 2016; Ali *et al.*, 2019). In addition, the leaves being high in protein can be useful in combating the protein-energy malnutrition (PEM) faced by many underdeveloped and developing countries. Moreover, MOLP is a source of natural antioxidants that protect the body against free radicals and inhibit oxidation in the cells. The high antioxidant activity of this plant is attributed to the presence of phytochemical compounds, flavonoids, and phenolic compounds (Sermkaew & Plyduang, 2020). Compared to other vegetables and fruits, *Moringa oleifera* leaves contain a great deal more antioxidants than strawberries, carrots, and soybeans (Singh & Prasad, 2013). However, MOLP is still underutilised due to its poor solubility in aqueous systems. The poor solubility of MOLP is thought to be caused by the high content of various compounds in the leaves. This limits its industrial applications, therefore there is a need to improve its solubility to extend its industrial usage.

To enhance the solubility and dissolution rate of poorly soluble ingredients, several strategies have been developed. These include micronisation, complexation, solubilisation in the surfactant system, solid dispersion, to mention a few (Takeuchi *et al.*, 2004; Raj and Kumar, 2018; Lan *et al.*, 2019a; Chen *et al.*, 2020). However, this study will focus on solid dispersion technology. Solid dispersion technology is the dispersion of a poorly water-soluble ingredient in an inert hydrophilic carrier at a solid-state through kneading, melting, solvent evaporation, freeze-drying, microwave irradiation, spray drying, solvent evaporation, or neutralisation among others (Nikghalb *et al.*, 2012; Kumavat *et al.*, 2013; Park & Choi, 2015; Kwon *et al.*, 2019; Chen *et al.*, 2020). The result of these dispersions is a decrease in particle size, reduction of agglomeration and aggregation, increased wettability, and the conversion to an amorphous state, which is much more solubilized and dissolvable (Jha *et al.*, 2020). Solid dispersion can be achieved using various polymers; among them is

polyethylene glycol (PEG). Polyethylene glycols are widely used as polymers in many industries, including food and pharmaceuticals. Generally, in the production of solid dispersions, PEGs with molecular weights of 1500-20000 are utilised as hydrophilic carriers (Alshehri *et al.*, 2020). PEGs are normally well soluble in water and various organic solvents, but their solubility decreases as the molar mass increases. The melting points of PEGs are typically below 65°C [e.g., the melting ranges of PEG1000, PEG4000, and PEG6000 are 30−40, 50−58, and 55−63 °C, respectively] (Zawar & Bari, 2013; Alshehri *et al.*, 2020). The lower melting point of PEGs enables the production of solid dispersions with different methods, particularly the melting method. Additionally, these carriers can solubilise certain compounds/ingredients and improve wettability (Zawar & Bari, 2013), the result of which will be increased solubility and dissolution. Previously, Huang *et al.* (2020) studied the effect of superfine grinding technology on the physicochemical, functional, thermal, and structural properties of MOLP. However, to date, nothing has been reported on the solid dispersions of MOLP. Hence, this study seeks to reduce the particle size of MOLP by solid dispersion using polyethylene glycol (PEG) as a hydrophilic carrier. In this study, it is hoped that solid dispersions of MOLP-PEG will be successfully developed.

1.2 Statement of the Problem

Moringa oleifera leaf powder (MOLP) is a good source of protein, minerals, vitamins, and various phenolic compounds. However, MOLP is still largely underutilised as a functional ingredient in food systems due to its poor flowability, solubility, and dissolution (Ali *et al.*, 2018, 2019). These shortcomings limit its industrial usage, thus the need to improve its functionality. The mechanism for improved solubility and dissolution includes reducing the particle size, changing the crystalline state into an amorphous one, forming solid solutions and complexes, wetting, and solubilising the ingredient by the carrier (Biswal *et al.*, 2008a; Chuah *et al.*, 2014a,b; Park and Choi, 2015; Chen *et al.*, 2020; Mendonsa *et al.*, 2020; Monschke *et al.*, 2021). This study hopes to reduce the particle size of MOLP by solid dispersion using polyethylene glycol (PEG) as a hydrophilic carrier. It is also hoped that the MOLP-PEG solid dispersions will have improved solubility, functionality, and dissolution properties through the particle size reduction.

1.3 Research Objectives

1.3.1 Broad Objectives

This study aimed to produce solid-dispersed *Moringa oleifera* leaf powder granules using PEG as a hydrophilic carrier to present it as an easy-to-drink functional beverage for the food industry.

1.3.2 Specific Objectives

The specific objectives of this research included the following:

1. Produce solid dispersions of MOLP using PEG hydrophilic carrier through freeze-drying, melting, solvent evaporation, kneading, and microwave irradiation methods.
2. Determine the physicochemical, nutritional, mineral, functional, structural, thermal, compatibility, and crystallinity properties of solid-dispersed MOLP.
3. Establish the flow and dissolution properties of solid-dispersed MOLP.
4. Produce effervescent solid-dispersed MOLP beverage granules using the wet granulation method.
5. Establish physical, chemical, flow properties of the effervescent granules
6. Establish the consumer acceptability of the beverages.

1.4 Hypotheses

It is hypothesised that:

1. The new solid-dispersed MOLP will be produced using PEGs as hydrophilic carriers.
2. The new solid-dispersed MOLP will differ in thermal, crystallinity properties compared to pure MOLP.
3. The solid-dispersed MOLP will differ in solubility, flowability, and dissolution properties, compared to pure MOLP.
4. The development of solid-dispersed MOLP effervescent beverage granules will be possible using the wet granulation method.
5. The effervescent beverage granules will be acceptable to consumers.

1.5 Delimitations of the Research

The delimitations of this research include:

1. Only MOLP will be used in this study.
2. Polyethylene glycols are the only hydrophilic carriers that will be used in the development of solid-dispersed MOLP.
3. Only the wet granulation method will be used to formulate solid-dispersed MOLP effervescent granules.

1.6 Significance of the Research Study

Food trends change as the world changes. The functional food market has seen an increase in the consumer demand for healthy food and drinks due to consumers' increasing awareness about their diets. The production of beverage granules using *Moringa oleifera* could offer most people a more balanced nutrient intake since the plant has a high

nutritional value (28% protein). The significance of this study is that new knowledge about solid-dispersed MOLP and its effervescent beverage granules will be generated. *Moringa oleifera* is a resilient perennial plant with a large range of environmental and climatic tolerances and can thrive in various environments. Considering the predicted environmental and climatic changes that will have negative effects on agriculture, Moringa crops could serve as viable alternatives for rural consumers. Therefore, the promotion of its production, food products, and consumption can considerably improve the quality of life for marginalised populations. In addition to helping combat poverty, malnutrition, and food security, the Moringa tree can also combat other socio-economic problems. Since the plant is grown in rural areas of South Africa, it can be used to improve the livelihood, nutrition, and income of these communities.

Moreover, using this crop would potentially expand the trade of solid-dispersed MOLP effervescent beverage granules in Africa and worldwide as well as enhance South Africa's growth market for a new food beverage brand. Other sectors may also benefit by supplying other raw materials and services such as sugar, flavours, effervescent ingredients (acids and bases), and transportation. The economy will grow through the food and beverage trade, and international competitiveness will increase. In addition to creating jobs and raising incomes for farmers, beverage granule production would spur economic growth. This, in turn, will help to reduce unemployment, particularly among women and young people. *Moringa oleifera* is commonly cultivated by both men and women in arid regions and in sub-Saharan Africa where this crop is popular. Hence an increase in its production would also empower women and improve their socio-economic status. As a result, this research will contribute to the advancement of gender equality. This plant is primarily grown in rural areas of South Africa, which means this study will also create opportunities for rural people to grow and develop. People would gain from the developed beverage granules, especially those suffering from protein-energy malnutrition (PEM), vitamin and mineral deficiencies, and zinc deficiency. Finally, this study will also contribute to the national innovation system by increasing the number of Masters' students through joint research partnerships.

1.7 Expected Outcomes

This study will produce novel solid-dispersed MOLP and provide evidence for *Moringa oleifera* leaf powder as an easy-to-drink functional beverage. New knowledge with regards to solid-dispersed MOLP effervescent beverages will also be generated. At least one article will be published in an accredited journal from this study. Research output was presented in at least one international (6th International ISEKI-Food Association conference, Austria, held on 24-26 June 2021) and national (SaaFost Congress 2021, South Africa, held on 20-

22 September 2021) conferences. A Master's degree will also be obtained from this research study.

1.8 Ethical Statement

Ethical clearance was obtained from the Faculty of Applied Sciences at the Cape Peninsula University of Technology.

1.9 Thesis Overview

The thesis is divided into seven chapters and is arranged in article format, with each chapter representing a separate entity. The structure of the thesis is shown in Figure 1.1. Chapter one is the motivation and design of the study, stating the research problem, objectives, hypotheses, delimitations of the study, significance of the research, and expected outcomes. Chapter two is the literature review. A background on *Moringa oleifera* is stated, including its different edible parts, nutritional composition, secondary metabolites, food applications, medicinal and prophylactic properties. Solid dispersion technology, hydrophilic carriers, solid dispersion techniques, and dissolution are also outlined, emphasizing the application of solid dispersion in the food industry (functional ingredients). Furthermore, ready-to-drink beverages, effervescent beverage granules and effervescent technology are discussed.

Chapter three is the first research chapter of the thesis, focusing on the preparation of solid dispersions of MOLP using hydrophilic carriers (PEGs) and different solid dispersion techniques (melting, freeze-drying, solvent evaporation and microwave irradiation). Nutritional, mineral, functional, and structural properties of the solid-dispersed MOLP were evaluated. This chapter mainly includes the abstract, introduction, materials and methods, as well as the results, discussions, conclusions and references. Chapter four is the second research chapter of the thesis, outlining in detail the compatibility (FTIR), crystallinity, and thermal properties of the solid-dispersed MOLP produced in Chapter three. The characterisation of the novel solid-dispersed MOLP was carried out by performing FTIR, powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and thermogravimetric analysis. Chapter five examined the physical properties (bulk and tapped densities), UV-visible maximum absorbance, and *in vitro* dissolution of the MOLP solid dispersions. Chapter six is the last research chapter in which the new effervescent beverage granules are produced using the wet granulation method and evaluated in terms of densities (bulk and tapped), flow properties, colour, moisture content, pH, and sensory or consumer acceptability. Finally, Chapter seven gives the general summary of the entire work and conclusions. Recommendations for future work are also given in this chapter.

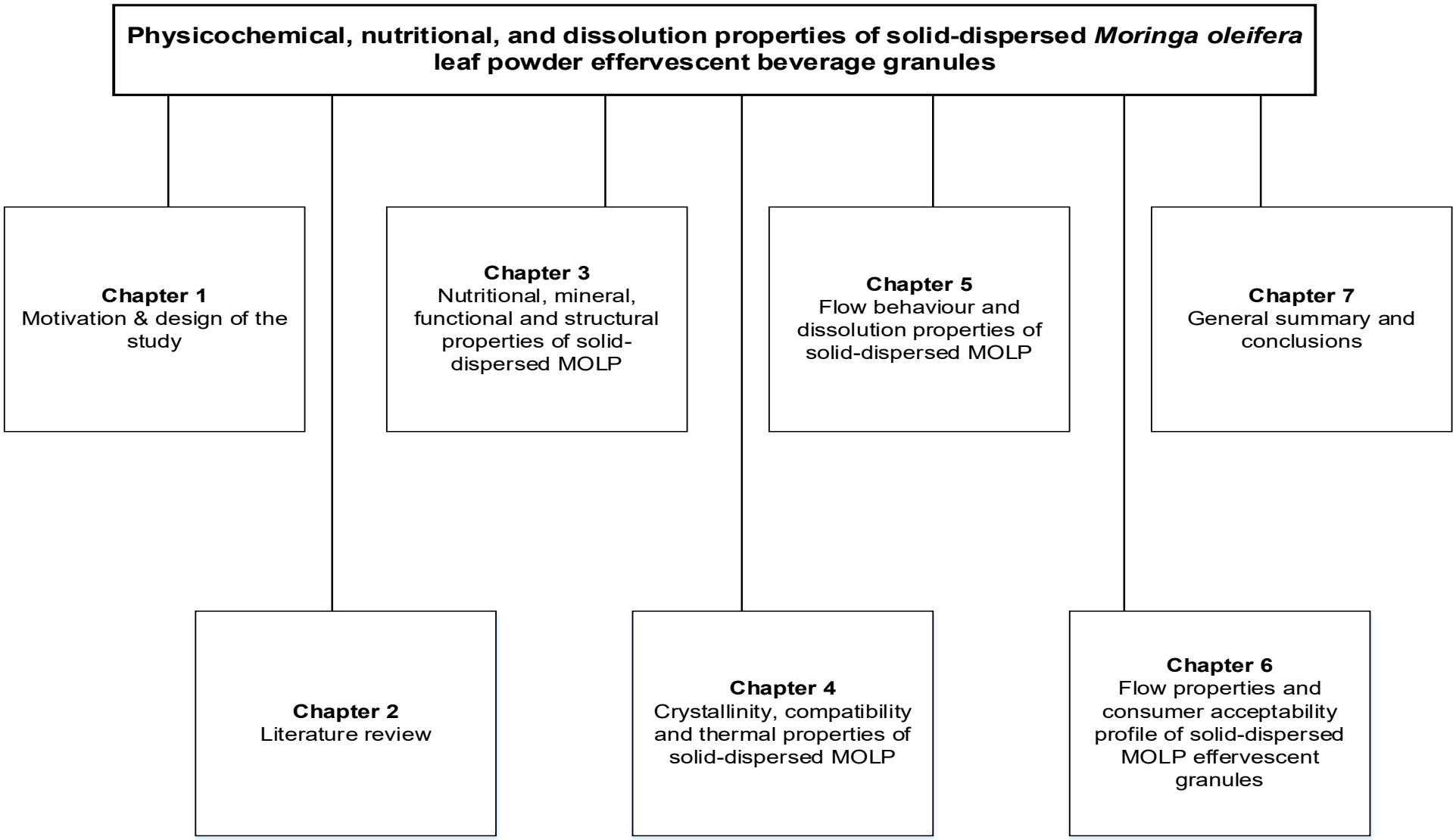

Figure 1.1 Thesis outline

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CHAPTER TWO
LITERATURE REVIEW

2.1 Background and description of *Moringa oleifera*

Moringa oleifera commonly referred to as "*Moringa*" is a rapidly growing and multifunctional plant that belongs to a Monogeneric family, *Moringaceae* (Ntila *et al.*, 2018; Ali *et al.*, 2019). The taxonomy of *Moringa oleifera* is shown in Figure 2.1. The family of plants *Moringaceae* consists of 13 species outlined in Table 2.1. Out of the 13 common species, only *Moringa oleifera* is the most widely known because extensive research and development have been accorded. *Moringa oleifera* stems natively from North India, the Himalayan Mountains stretching in the Indus and Pakistan, and is also found in the tropical and subtropical areas of Africa, the southeastern part of Asia, and South America (Gandji *et al.*, 2018; Liu *et al.*, 2018). *Moringa oleifera* is widely spread in a far-ranging distribution due to its strong drought resistance. Additionally, it is recorded that *M. oleifera* has been cultivated commercially throughout the entire world, including in Mexico, Hawaii, Cambodia, and the Caribbean Island (Fahey, 2005; Fahey, 2017; Mashamaite *et al.*, 2019) as a superfood.

Moringa oleifera in South Africa is cultivated in Gauteng, Limpopo, Mpumalanga, and KwaZulu-Natal provinces; most households have these trees in their backyards for leaf consumption, farm locations are presented in Figure 2.2. Although the provinces are in different climatic zones, they experience similar climatic conditions such as wet summers and drier winters. The climatic conditions were reported to have a significant effect on the yields of *M. oleifera* (Teclegeorgish *et al.*, 2021). Major production includes Ghana, Senegal, and Malawi, smaller production is in New Zealand and Fiji, and more recent production is in Nicaragua and Bolivia (Jideani & Diedericks, 2014). *Moringa* grows fast and has a high tolerance for the climate. It grows between 5 and 10 m in height within a year under low rainfall of at least 400 mm/annum (Mabapa *et al*., 2017; Tshabalala *et al*., 2019). Thus, it can grow well in extreme climatic conditions of the Limpopo and Mpumalanga provinces (South Africa) which most trees cannot withstand. Moringa is a medium-sized tree with a straight trunk (10-30 cm thick), whitish or grey, corky bark with longitudinal cracks (Jideani & Diedericks, 2014). It has a tuberous taproot whose presence helps the species' tolerance to drought conditions. The high drought resistance and tolerance to arid conditions due to the formation of huge tuberous roots make Moringa a vital famine food (Liu *et al.*, 2018). The tree is normally umbrella-shaped with a lax crown of graceful, airy foliage, whose feathery effect is due to the finely tripinnate division of the leaves (Jideani & Diedericks, 2014) (Figure 2.3). The leaves are densely crowded at the tops of the branchlets. *Moringa oleifera* is valued mainly for its edible fruits, leaves, flowers, roots, and seed oil, and is used extensively in traditional medicine and

throughout the world. The fact that *M. oleifera* is easily cultivable makes it a sustainable remedy for malnutrition. It also has the potential to boost food availability and alleviate poverty.

- Kingdom: Plantae
    - Order: Brassicales
        - Family: Moringaceae
            - Genus: *Moringa*
                - Species: *M. oleifera* Lam.

Figure 2.1 Taxonomy of *Moringa oleifera* Lam.

Table 2.1 Thirteen Moringa species and their origins[1]

Species	**Origin**
Moringa oleifera	India
M.drouhardi	Madagascar
M.cocanensis	India
M.arborea	Northeastern Kenya
M.hifrbrardtii	Madagascar
M. borziana	Kenya and Somalia
M. ovalifolia	Namibia and extreme southwestern Angola
M. peregrina	The Horn of Africa, Red Sea, Arabia
M. longituba	Kenya, Ethiopia, Somalia
M. stenopetala	Kenya, Ethiopia
M. pygmaea	Northern Somalia
M. rivae	Kenya, Ethiopia
M. ruspoliana	Kenya

[1]Adapted: Council National Research (2006); Jideani & Diedericks (2014) and Falowo *et al.* (2018)

Moringa oleifera species bear a lot of traditional names depending on their location and uses which include superfood tree, drumstick, tree of life, horseradish (Falowo *et al.*, 2018). Moringa is also often referred to as a “miracle tree” mainly due to its ability to treat more than 300 diseases (Lekgau, 2011; Makita, 2014; Oyeyinka & Oyeyinka, 2018). Consequently, to attest to its nutritive value, *Moringa oleifera* has been given the name *makgonatsohle*, Sepedi word (South Africa) which means the ability to do everything. It is also known as the mother's best friend, due to its nutritious edible parts that help revive malnourished children (Gopalakrishnan *et al.*, 2016). Some common names for *M. oleifera* are detailed in Table 2.2. *M. oleifera* leaves

are a good source of protein, β-carotene, vitamins, A, B, C, and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals, and various phenolic compounds (Moyo *et al.*, 2011a; Donkor *et al.*, 2013a; Vongsak *et al.*, 2013; Bello *et al.*, 2017; Falowo *et al.*, 2018; Bancessi *et al.*, 2019).

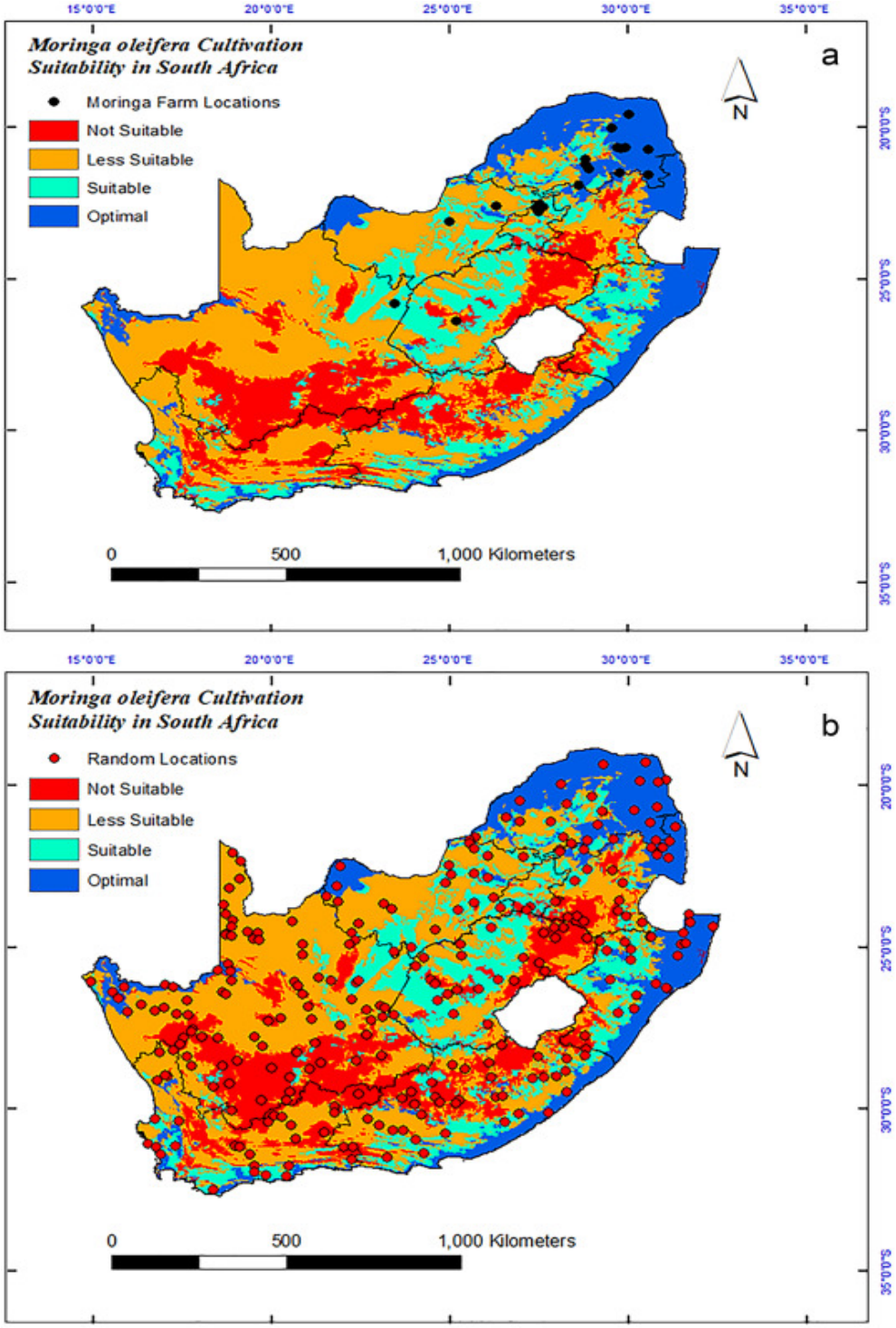

Figure 3.2 The location of some of the current Moringa farms (a) and random locations (b) on a Moringa oleifera cultivation suitability map in South Africa (Tshabalala *et al*., 2019)

Table 2.2 Moringa common names[1]

Country	Language	Name
	English	Moringa, horseradish tree, drumstick tree, sujuna, ben tree, ben oil tree Ben
	French	Ben ailé, ben oléifère, benzolive, arbre radis du cheval
	Spanish	Ben, árbol del ben, paraiso, morango, Moringa
	Portuguese	acácia branca, marungo, muringa, moringuiero; cedro (Brazil)
	Arabic	ruwag, alim, halim, shagara al ruwag (Sudan)
	Swahili	mzunze, mlonge, mjungu moto, mboga chungu, shingo
Germany	German	Behenbaum, Behenussbaum, flügelsaniger Bennussbaum, Pferderettichbaum
Italy	Italian	Sàndalo ceruleo Fon: kpatima, yovokpatin, kpano,yovotin
	Gun	èkwè kpatin, kpajima
Nigeria	Yoruba & Nago	èwè igbale, èwè ile, èwè oyibo, agun oyibo, ayun manyieninu, ayèrè oyibo
	Fulani	gawara, konamarade, rini maka, habiwal hausa
	Hausa	zogall, zogalla-gandi, bagaruwar maka, bagaruwar masar, shipka hali, shuka halinka, barambo, koraukin zaila, rimin turawa
	Ibo	Ikwe oyibo
Senegal		nebeday
Philippines		malunggay or malungai (Tagalog)
India		sujuna, sujuna, sajina, lopa, horseradish or drumstick tree
Haiti		benzolive (Haitian Creole)
South Africa	Sepedi	Makgonatsohle

[1]Source: Jideani & Diedericks (2014); Mashamaite *et al.*(2019)

2.1.1 Different edible parts of the Moringa plant

Moringa oleifera is a perennial tree. It is an important food and medicine plant that is esteemed as a functional plant, where a wide variety of nutritional, prophylactic, nutraceutical, and

therapeutic virtues have been attributed to its parts (Alegbeleye, 2018). In addition, *M. oleifera* is considered to be one of the most multiple used plants on the earth because almost every part of the tree is of value in one way or another. Moringa tree yields at least four different edibles, namely pod, leaves, seeds, and roots, illustrated in Figure 2.3. In many developing countries, different parts of *M. oleifera* are cooked in different ways and eaten as vegetables or dietary supplementary (Oyeyinka & Oyeyinka, 2018). Moreover, almost every part of this tree has already been employed to effectively combat a variety of ailments. As reported by researchers in different countries, all parts of Moringa are utilised as highly nutritive vegetables and combined into traditional food for human consumption in many countries, particularly in India, Pakistan, Hawaii, and some African nations such as Malawi, Nigeria, South Africa, and Ghana, among others (Daba, 2016). In addition, they also have a range of functional and nutraceutical properties.

The immature pods are the most valued and widely used of all three parts as it contains all the essential amino acids along with many vitamins and other nutrients. The tender pods have the general characteristics of a succulent string bean. It can be eaten raw or prepared like green peas or green beans. In India, they are usually added to curries and sometimes sliced, blanched, and canned (Jideani & Diedericks, 2014; Falowo *et al.*, 2018). While the mature pods quickly turn tough, as thick as a pencil, and are too fibrous to eat like the string beans. In that form, they are called drumsticks. However, they are cut into pieces to release the sweet frothy inside material, which is a well-known ingredient in pickles in India. The mature pods are usually fried and possess a peanut-like flavour (Dhakar *et al.*, 2011). Since the immature pods are eaten raw, and the mature ones are cooked as a substitute for beans, both are considered popular vegetables in the Philippines. Pods contain an abundance of dietary fibre, low lipid content, and a reasonable amount of unsaturated and essential fatty acids, especially oleic acid (Sánchez-Machado *et al.*, 2010). The rich nutrition makes the pods and seeds good food fortification in dairy food products. In addition, Pods have 30% of amino acid content, the leaves have 44% and flowers have 31% (Liu *et al.*, 2018). The high content of nutrition in pods and seeds of *M. oleifera* has been reported in many studies. There is about 9.98– 51.80 g crude protein, 17.26–20.00 g crude fibre, 3.36–18.00 g carbohydrate, 38.67–43.60 g fat, and 3.60–5.00 g ash per 100 g in *M. oleifera* seeds (Falowo *et al.*, 2018). The seeds are often referred to as peas and can be used from the time they appear until they turn yellow and their shells begin to harden. They can be cooked like green peas (Jideani & Diedericks, 2014). Hardened mature seeds are bitter and can be pressed, yielding 38–40% of non-drying, edible oil which is clear, sweet, and odourless and never becomes rancid and burns without smoke; its nutritional value resembles olive oil (Tsaknis *et al.*, 1999; Jideani & Diedericks, 2014; Su & Chen, 2020). Although the profiles of minerals and vitamins that exist

in *M. oleifera* pods and seeds are different from that of leaves and flowers, they are also abundant (Olagbemide & Philip, 2015). The rich nutrition makes the pods and seeds good food fortified in dairy products. It has been recorded that wheat flour added with *M. oleifera* seed powder can markedly improve the protein content in bread and cookies (Ogunsina *et al.*, 2011). In addition, the seed powder can be used for water treatment where the powder coagulates solids and removes 90–99% bacteria.

Figure 2.4 *Moringa oleifera* (a) the plant (b) flowers, (c) pods (d) seeds and (e) leaves (Liu *et al*., 2018).

Moringa flowers serve as a good source of nutrients such as protein, calcium, and potassium, making them ready for food or tea after being processed (Jideani & Diedericks, 2014; Kshirsagar & Bhogaonkar, 2017). The immature pods and flowers showed similar amounts of palmitic, linoleic, and oleic acids. According to Arise *et al.* (2015), fried flowers taste like a mushroom. They are traditionally eaten as a source of inspiration and positive energy to improve human welfare (Anya *et al.,* 2014). However, among the *Moringa oleifera* tree parts, Moringa leaves are still the most widely used part.

The fresh leaves are eaten as greens, salads, vegetable curries, pickles, and seasoning, displayed in Figure 2.4. The dried leaves are crushed or pound and sifted into leaf powder, which can be added to sauces and foods as a condiment. Despite its enormous properties and broad utilities, *Moringa oleifera* is still relatively unexploited, especially in countries outside of its origin.

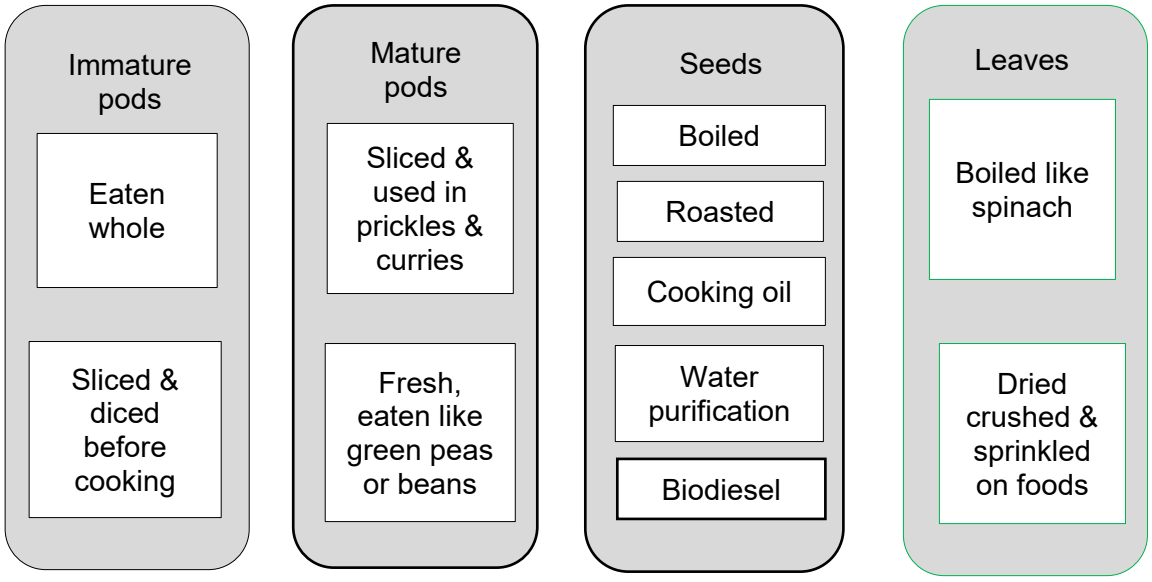

Figure 2.5 Some food uses of Moringa parts

2.1.2 Nutritional composition of *Moringa oleifera*

The composition of *Moringa oleifera* varies depending on climatic variations, crop management, whether it is cultivated or wild, the state of maturity of the plant at the time of harvesting, the type of post-harvest processing and depends on the growing area, i.e., the land where it is grown (Milla *et al.*, 2021). Table 2.3 shows the nutrient composition of *Moringa oleifera* edible parts. Over 90 nutrients, 46 antioxidants, 18 amino acids, and 36 anti-inflammatory compounds, all naturally occurring in the Moringa plant (Kim & Kim, 2019). Thus,

it is called the nutrient powerhouse. Recent reports of the analysed nutrient composition of the leaves, seeds, and powder of the plant show that they are rich in protein, essential amino acids, vitamins, minerals, and other bioactive compounds (Falowo *et al.*, 2018). Moringa leaves consist of a high content of protein (20-35 %) (Anwar *et al*., 2007; Mashamaite *et al*., 2019). Moringa leaves are known to overcome protein deficiency in developing countries as the leaves and other parts of the tree contain a high amount of crude proteins and amino acids compared with soybeans. Moringa leaves could greatly benefit people who do not get protein from meat. Additionally, the nutritional profile of the leaves of Moringa includes unsaturated fatty acids, including alpha-linoleic acid, which is uncommon in plant food (Gopalakrishnan *et al.,* 2016; Fahey, 2017; Liu *et al.*, 2018). *M. oleifera* leaf contains ~7.09% lipids (Su & Chen, 2020). Notably, unsaturated fatty acids account for more than half (57%) of the fatty acids in *M. oleifera* leaves, with -linoleic acid accounting for 44.57 percent (Moyo *et al.*, 2011). Unsaturated fats are the essential components of the human diet because they can decompose and esterify cholesterol, allowing normal cholesterol levels to be maintained and lowering the risk of atherosclerosis and other cerebrovascular diseases.

Table 2.3 Nutritional composition of fresh leaves, dry leaves of Moringa and MOLP (per plant material)[1]

Nutrients	**Fresh leaves (per 100 g)**	**Dry leaves (per 100 g)**	**MOLP (per 100 g)**
Calories (Cal)	92	329	205
Protein (g)	6.7	29.4	27.1
Fat (g)	1.7	5.2	2.3
Carbohydrates (g)	12.5	41.2	38.2
Fibre (g)	0.9	12.5	19.2
Vitamin A (mg)	0.1	6.8	16.3
Vitamin B (mg)	423		-
Vitamin B1 (mg)	0.06	2.02	2.64
Vitamin B2 (mg)	0.05	21.3	20.5
Vitamin B3 (mg)	0.8	7.6	8.2
Vitamin C (mg)	220	15.8	17.3
Vitamin E (mg)	448	10.8	113

[1]Source: Donkor *et al.* (2013) and Gopalakrishnan *et al.* (2016)

Nearly all vitamins, including vitamin B (folic acid, nicotinic acid, and pyridoxine), vitamin C, vitamin D, and vitamin E and vitamin A are plentiful in Moringa (Liu *et al.*, 2018a). These vitamins can be used to combat malnutrition. According to Zongo *et al*. (2013) and Falowo *et al.* (2018), a daily intake of 10 g powdered Moringa dry leaves by malnourished children could improve their weight gain and promote quick recovery. Another striking feature of *M. oleifera* leaf is its high mineral content, with ash content of up to 12.0%, which is significantly higher than that in soybean or cornmeal (Su & Chen, 2020). The leaves of this plant are rich in minerals that are crucial for body development and growth among which, potassium, calcium, zinc, iron, copper, and other minerals are considered as the essential minerals for human growth, as shown in Table 2.4. Table 2.5 gives the percentage of the recommended daily allowance of various nutrients. *M. oleifera* leaf powder [MOLP] (25 g daily) is reported to give a child the recommended daily allowance for protein (42%), calcium (125%), magnesium (61%), potassium (41%), iron (71%), vitamin A (272%), and vitamin C (22%) (Aslam *et al.*, 2005; Dhakar *et al.*, 2011; Singh *et al.*, 2018a). Gram for gram, *M. oleifera* leaves contain seven times the vitamin C in oranges, four times the calcium in milk, four times the β-carotene in carrots, twice the protein in milk, and three times the potassium in bananas (Table 2.6) (Jideani & Diedericks, 2014; Gopalakrishnan *et al.*, 2016; Singh *et al.*, 2018). In addition, *Moringa oleifera* leaf powder (MOLP) can be used as a substitute for iron tablets, hence it is utilised as a treatment for anaemia. Beef has only 2 mg of iron while MOLP has 28 mg of iron (Falowo *et al.*, 2018). A good dietary intake of zinc is essential for proper growth and MOLP show around 25.5–31.03 mg of zinc/ kg, which is the daily requirement of zinc in the diet (Aslam *et al.*, 2005; Moyo *et al.*, 2011).

Table 2.4 Macro- and micro elements of *Moringa oleifera* parts

Minerals (mg/100 g)	Pods	Seed	Fresh leaves	Dried leaves	MOLP
Ca	30	45	440	2185	2003
K	259	-	259	1236	1324
Mg	24	635	42	448	368
P	110	75	70	252	204
Fe	5.3	-	0.85	25.6	28.2
S	137	0.05	-	-	870
Cu	3.1	5.20	0.07	0.49	0.57

[1]Adapted: Dhakar *et al.* (2011)

The mineral element concentration in Moringa leaves affected by the climatic conditions and environment in which they grow. For instance, the effect of environment and elevation on mineral micronutrient concentration of leaves and other parts of the *Moringa oleifera* was studied in Ethiopia (Kumssa *et al.*, 2017). It was found that the concentrations of Ca, Fe, and Zn in Moringa leaves grown in mid-altitude areas during the rainy season were 24800, 578, and 24.3 mg/ Kg, respectively. While in low-altitude areas, the Ca, Fe, and Zn concentrations were 25700, 564 and 26 mg/ Kg, respectively, during the rainy season (Kumssa *et al.*, 2017). In addition, a study conducted in the Punjab province of Pakistan indicated that the Ca, Mg, and Zn concentration in the leaves and pods of *Moringa oleifera* varied significantly by region (Aslam *et al.*, 2005).

Table 2.5 Recommended daily allowance (RDA) of various nutrients[1]

	% RDA	
Nutritional component	Parent	Child
Protein	21	42
Ca	84	125
Mg	52	61
K	22	41
Fe	94	71
Vitamin A	143	272
Vitamin B	9	22

[1]Supplied to a nursing mother and a 1 to a 3-year-old child by Moringa leaf powder (6 tablespoons per day for a nursing mother; 1 tablespoon three times per day for a 1 to a 3-year-old child)

[2]Adapted from Dhakar *et al.* (2011)

Table 2.6 Comparison of the nutrient level of Moringa leaves with other nutrient-rich fruits, vegetables, and food products

Fresh leaves	**Dried leaves**
4 times the Vitamin A in carrots	10 times the Vitamin A in carrots
7 times the Vitamin C in oranges	½ times the Vitamin C in oranges
¾ times iron of spinach	25 times the iron in spinach
3 times the K in bananas	15 times the K in bananas
4 times the Ca in milk	17 times the Ca in milk
2 times the protein in yoghurt	9 times the protein in yoghurt

Sourced: Singh *et al.* (2018a)

Apart from the high content of protein, minerals, and vitamins, *Moringa oleifera* leaf powder is also a good source of essential amino acids. Moringa contains about 16-19 amino acids, of which ten are classified as essential amino acids, namely: tyrosine, methionine, leucine, histidine, valine, isoleucine, lysine, arginine, phenylalanine and tryptophan (Falowo *et al.*, 2018). *Moringa oleifera* leaves contain arginine and histidine, two amino acids especially important for infants (Mahmood *et al.*, 2010). These two essential amino acids are especially vital for infants who are unable to make enough protein for their growth requirements. The leaves have a 44% amino acid content (Milla *et al.*, 2021). These amino acids are the building blocks of proteins. It is quite rare for a vegetable to contain all of these amino acids but understandable for Moringa since it has 25-40% protein. The essential amino acids in *Moringa oleifera* fresh leaves, dry leaves are given in Table 2.7 and Figure 2.5. On a nutritional basis, the leaves of *Moringa oleifera* are extensively used to combat malnutrition among infants, pregnant women, and nursing mothers (Mishra *et al*., 2012; Gopalakrishnan *et al*., 2016; Falowo *et al*., 2018). In addition, 30% of children in sub-Saharan Africa are protein deficient. Therefore Moringa could be an extremely valuable food source (Aslam *et al.*, 2005; Amaglo *et al.*, 2010; Saini *et al.*, 2014a,b; Rani *et al.*, 2018).

Table 2.7 Amino acid content of *Moringa oleifera* (mg/ 100g)

Amino acid content (mg/100 g)	**Pods**	**Fresh leaves**	**Dried leaves**
Argenine	360	406.6	1325
Histidine	110	149.8	613
Lysine	150	342.4	1325
Tryptophan	80	107	425
Phenylalanine	40	310.3	1388
Methionine	140	117.7	350
Theroine	390	117.7	1188
Leucine	650	492.2	1950
Isoleucine	440	299.6	825
Valine	540	374.5	1063

Adapted: Dhakar *et al.* (2011)

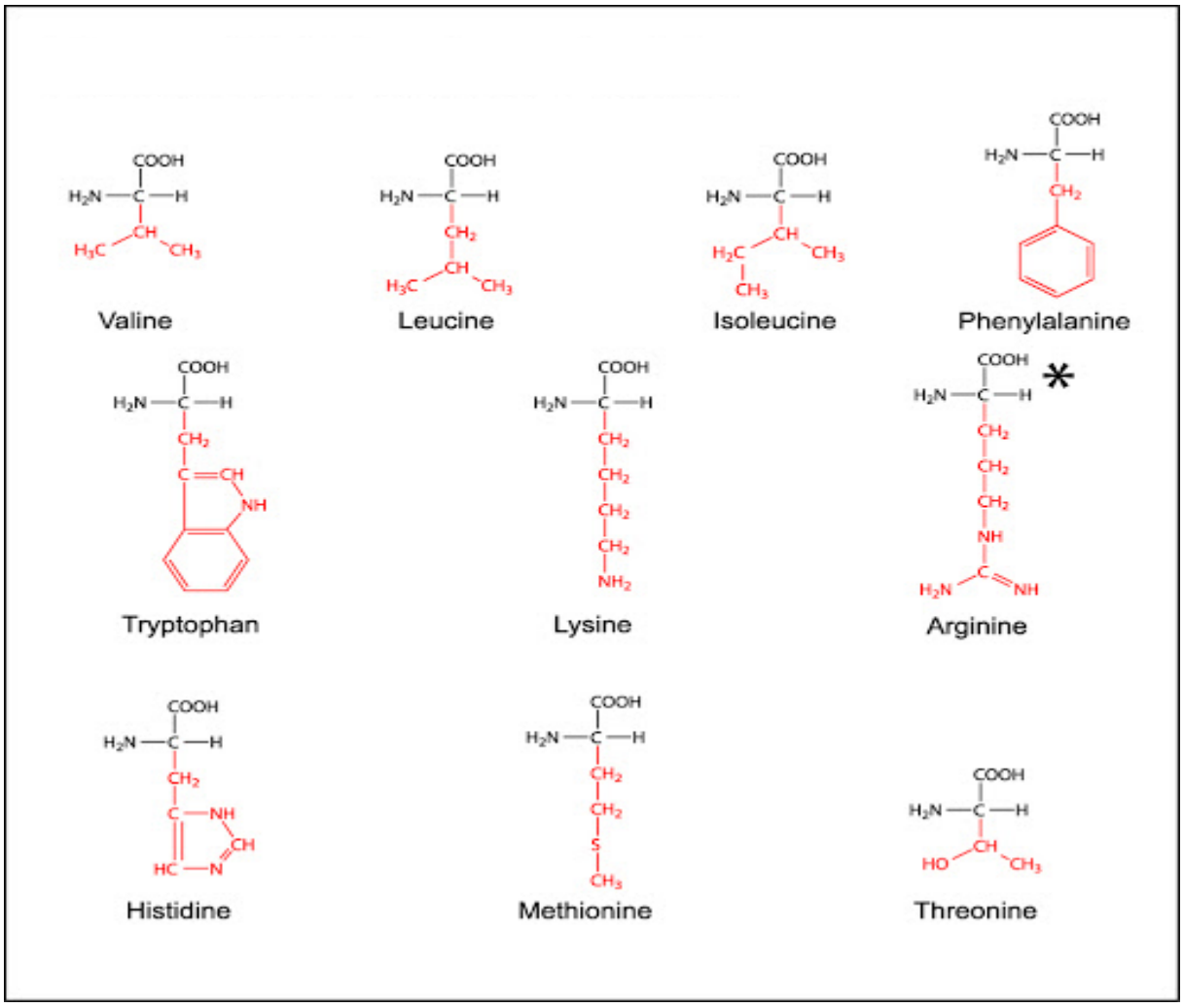

Figure 2.6 Structures of essential amino acids found in Moringa oleifera leaves

2.1.3 Secondary Metabolites of *Moringa oleifera* leaves

Moringa oleifera leaves are a good source of pharmacologically important compounds. Some of the identified secondary metabolite compounds with previously reported bioactivity are provided in Figures 2.6. Many compounds have been found in *M. oleifera* including glucosinolates, flavonoids, and phenolic acids (Milla *et al.*, 2021), carotenoids (Saini *et al.*, 2014a), isothiocyanates, tannins, and terpenes (Rani *et al.*, 2018), tocopherols (Saini *et al.*, 2014b), quercetin, vanillin, and kaempferol responsible for its multiple values (Brilhante *et al.*, 2017). Among the glucosinolates, benzyl 4-O-(α-L-rhamnopyranosyloxy)-glucosinolate is the most predominant (glucomoringin) (Milla *et al.*, 2021). Its leaves also include 11 phenolic acids such as gallic acid, ellagic acid, chlorogenic acid, ferulic acid, caffeic acid, o-coumaric acid, p-coumaric acid, gentisic acid, sinapic acid, syringic acid (Jideani & Diedericks, 2014; Saini *et al.*, 2014b; Brilhante *et al.*, 2017; Teclegeorgish *et al.*, 2021). The profile of these secondary

metabolites varies in different parts of *M. oleifera*. Chlorogenic acid, quercetin, and kaempferol are major compounds in *M. oleifera* leaf extracts, and the *M. oleifera* leaves also contain an abundance of phenolic acids, flavonoids, glucosinolates, and isothiocyanates (Liu *et al.*, 2018). Among the compounds, glucomoringin was found to be the most glucosinolates in all parts of *Moringa oleifera*. Karim *et al.* (2016); Liu *et al.* (2018) and Milla *et al.* (2021) detailed that glucomoringin derivate isothiocyanate of *Moringa oleifera* was found to exhibit anti-inflammatory activity, inhibition of carcinogen activation, antitumor cell proliferation, induction of apoptosis, and antibacterial activity. Hence, Moringa is known as a medicinal plant.

2.1.4 The utilisation of *Moringa oleifera* leaf powder in different parts of Africa

In the light of *Moringa oleifera'*s drought-tolerant characteristics and being a perennial tree, it has the potential to eradicate malnutrition and poverty in Africa. In addition, it also has the potential to improve nutrition, contribute towards food security, encourage rural development, and help to solve the problem of global warming in Africa. In African and Asian cuisine there are a lot of recipes based on *M. oleifera* leaves, seeds, flowers, and fruits. Moringa leaves are used to prepare dishes (egosi, khakhra) in Ghana, Nigeria, Ethiopia, East Africa, and Malawi (Sahay *et al.*, 2017a). While in South Africa, it has been used in green teas and amahewu (non-alcoholic beverages), and cereals. In the Philippines where Moringa is exceptionally popular, the boiled leaves are commonly fed to babies. Nutritionally, leaves are remarkable for methionine and cysteine. Both are essential to health and are among the hardest amino acids for the body to obtain through plant-based diets (Council National Research, 2006). In Cameroon, *Moringa* is consumed as a vegetable, is used to prepare soup but also to prepare dishes with meat and fish. Leaves can be used fresh or as dry powder. The powdered *Moringa oleifera* leaf in capsules has become a popular food supplement in Thailand (Wangcharoen & Gomolmanee, 2013). Fresh leaves are often used in the same way as spinach or to prepare salads, sauces, and soups (Babayeju & Gbadebo, 2014; Sahay *et al.*, 2017). In India, dried leaves are often milled and could be used to confer a spicy taste to dishes, also combined with other ingredients. Moringa can also be added to meat and fish to increase the taste. Since *Moringa oleifera* leaf powder is a functional food, it can be utilised to add functionality to other foods through fortification and supplementation. Functional foods may be whole, fortified, enriched or enhanced. Functional components of food include beta carotenes which are known to scavenge free radicals; calcium reduces the risk of osteoporosis; potassium reduces the risk of high blood pressure; flavonoids, fatty acids known to reduce coronary heart diseases and dietary fibre supports gastro intestine health (Naa *et al.*, 2013; Maghu *et al.*, 2017; Aderinola, 2018). The use of these plant leaves as an ingredient in foods to add functionality has increased recently. Many studies have shown the potential use of *Moringa oleifera* leaves

in food applications, summarised in Table 2.8. An alternative method for unleashing the great benefits of this nutritionally important plant is to incorporate it into a refreshing beverage. Naa *et al.* (2013) developed the fresh *Moringa oleifera* leaf beverage. The beverage did not appeal to consumers as a refreshing and attractive beverage due to its dark colour. However, nothing has been reported on the *Moringa oleifera* effervescent beverage granules. Hence, the prompt need to develop an easy-to-drink functional effervescent beverage of *Moringa oleifera.*

Table 2.8 Food uses of *Moringa oleifera* leaf powder

Food product	**References**
Soups	Gopalakrishnan *et al.* (2016)
Amala	Karim *et al*. (2015); Gandji et al., (2018)
Bread	Alam *et al*. (2014); A li *et al*., (2019); Chinma *et al.* (2014)
Herbal biscuits	Alam (2014)
Cake	Kolawole *et al.* (2013)
Capsules	Hekmat *et al*. (2015); Falowo *et al.* (2018)
Yoghurt	Hekmat *et al.* (2015)
Tea	Mahmood *et al*. (2010); Luhlaza (2011)
Buttermilk	Lemmens (2014); Otoluwa *et al*. (2014)
White cheese	Hassan *et al.* (2017)
Weaning foods	Babayeju *et al.* (2014); Liu *et al*. (2018); Arise *et al.* (2015)
Khakhras	Maghu *et al.* (2017)
Porridge	Ntila *et al.* (2018)
Soups: egosi and Efo Rio	Babayeju & Gbadebo (2014)
muffins	Srinivasamurthy *et al.* (2017)
Smoothie	Aderinola (2018)

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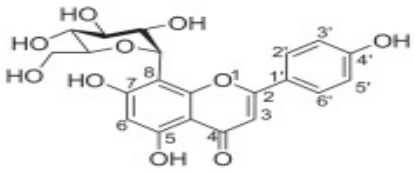
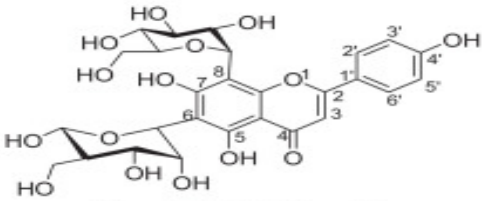
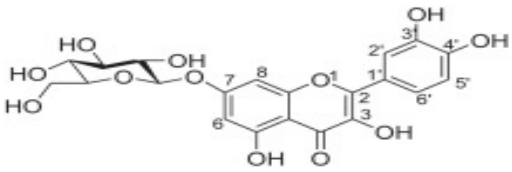


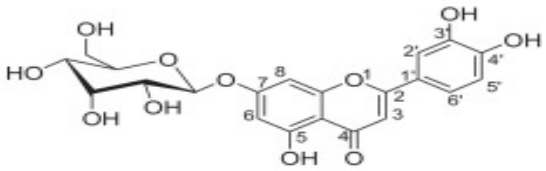
Apigenin-8-C-glucoside



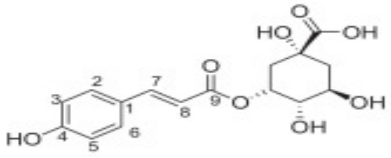
Apigenin-6-8-C-diglycoside



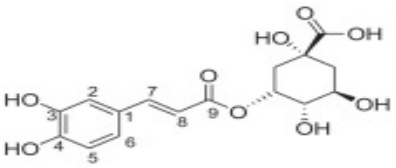
Quercetin-7-hexose



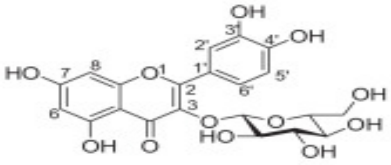
Luteolin-7-O-glucoside



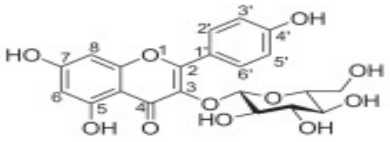
3-*p*-Coumaroylquinic acid



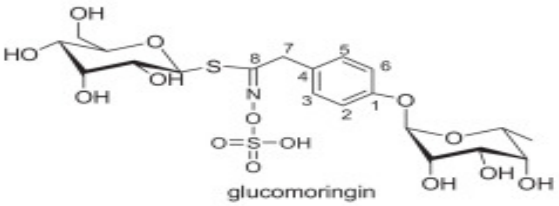
3-Caffeoylquinic acid



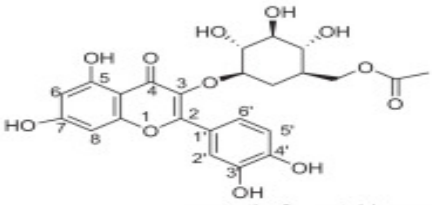
Quercetin-3-glucoside



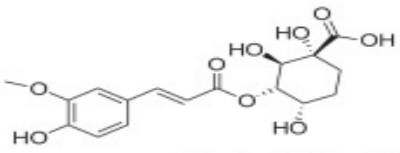
Kaempferol-3-glucoside



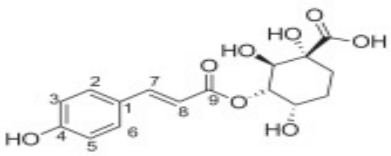
glucomoringin



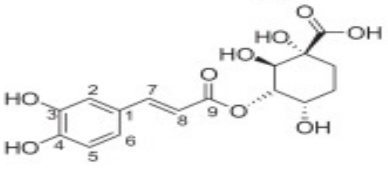
quercetin-3-acetyl-hexose



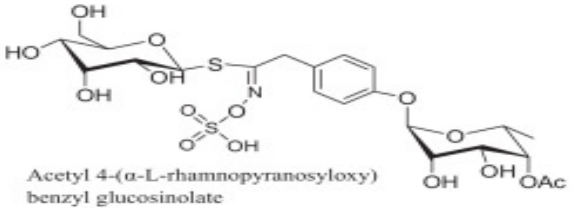
4-Feruloylquinic acid



4-*p*-Coumaroylquinic acid



4-Caffeoylquinic acid

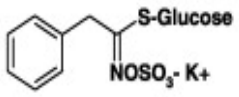


Acetyl 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate

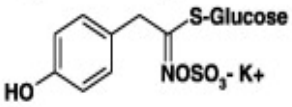
Glucosinolates

Benzylglucosinolate (Glucotropaeolin)

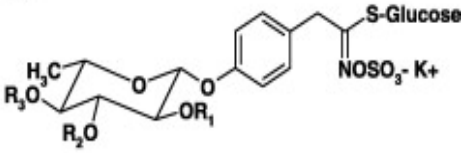
B



4-Hydroxybenzylglucosinolate (Sinalbin)



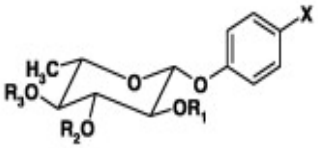
4-*O*-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate (Glucomoringin) (G2) (R1, R2, R3 = H)



4-*O*-(α-L-Acetyl-rhamnopyranosyloxy)-benzylglucosinolate (G3-G5) (R1 & R2 = H, R3 = Ac; R1 & R3= H, R2 = Ac; R1 = Ac; R2 & R3 = H)

Hydrolysis Products & Related Derivatives

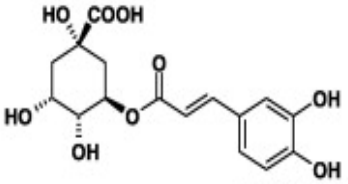
4-*O*-(α-L-Rhamnopyranosyloxy)-benzylisothiocyanate (R1 = R2 = R3 = H, X = N=C=S)



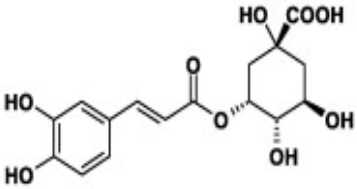
Niazirin (R1 = R2 = R3 = H, X = CN)
Niazirinin (R1 = R2 = H, R3 = Ac, X = CN)
Niazimin A/B (R1 = R2 = H, R3 = Ac, X = CH_2-NH-CO-OEt)
Niazinin A/B (R1 = R2 = R3 = H, X = CH_2-NH-(C=S)-OMe)
Niazicin A/B (R1 = R2 = H, R3 = Ac, X = CH_2-NH-(C=S)-OMe)
Niazimicin (R1 = R2 = R3 = H, X = CH_2-NH-(C=S)-OEt)
Niaziminin A/B (R1 = R2 = H, R3 = Ac, X = CH_2-NH-(C=S)-OEt)

Phenolics

3-Caffeoylquinic Acid (3-CQA) (Neochlorogenic Acid)



5-Caffeoylquinic Acid (5-CQA) (Chlorogenic Acid)

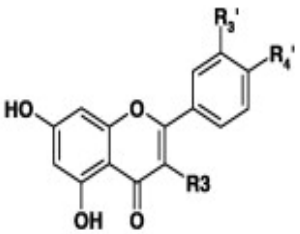


Major Flavonoids (K = Kaempferol, Q = Quercetin)

K 3-*O*-Rutinoside ((R3 = -GlcRha, R3′ = H, R4′ = OH) (F7)

K 3-*O*-Glucoside (R3 = -Glc, R3′ = H, R4′ = OH) (F9)

K 3-*O*-(6″-Malonylglucoside) (R3 = -GlcMal, R3′ = H, R4′ = OH) (F13)



Q 3-*O*-Rutinoside (R3 = -GlcRha, R3′ & R4′ = OH) (F4)

Q 3-*O*-Glucoside (R3 = -Glc, R3′ & R4′ = OH) (F6)

Q 3-*O*-(6″-Malonylglucoside) (R3 = -GlcMal, R3′ & R4′ = OH) (F8)

Figure 2.7 Structures of phytochemicals identified in *Moringa oleifera* (Amaglo *et al*., 2010; Teclegeorgish *et al*., 2021)

2.1.5 Medicinal and prophylactic properties of *Moringa oleifera* leaves

Apart from being highly nutritious, Moringa can also be treated as an important medicinal plant for treating many prolonged diseases, including cancer. More than 40 natural antioxidants with numerous other secondary metabolites of health importance are present in Moringa species (Devi *et al.*, 2017). Different parts of the plant in Moringa is well known for their medicinal properties like antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antidiabetic, antioxidant, antifungal, and antibacterial activities (Sreelatha & Padma, 2009; Moyo *et al.*, 2011b; Divi *et al.*, 2012; Devi *et al.*, 2017; Singh *et al.*, 2018a; Natsir *et al.*, 2019). Both old and young leaves possess oxidation resistance to protect major biomolecules from the oxidative damage of free radicals (Sreelatha & Padma, 2009). Figures 2.7 and 2.8 show the reactions of the hydroxyl radical with alpha-amino acids and polyunsaturated fatty acids (PUFAS), respectively. The substantially high antioxidant contents contribute directly to the anticancer potentials of *M. oleifera* leaves (Milla *et al.*, 2021). Additionally, the extracts of the leaves have been shown to have anti-proliferative effects, inhibiting the growth of cancer cells (Gopalakrishnan *et al.*, 2016). Based on the antioxidant abilities, extracts of *M. oleifera* leaves exhibit curative ability to both Type 1 and Type 2 diabetes in rats (Divi *et al.*, 2012). The antioxidant properties of *M. oleifera* leaves also account for many other natures, such as antiatherosclerotic, neuroprotectant, anti-inflammatory, cardioprotective, hepatoprotective, and antiulcer activity (Anwar *et al.*, 2007; Gopalakrishnan *et al.*, 2016; Gandji *et al.*, 2018; Liu *et al.*, 2018b; Murdiana *et al.*, 2018; Tshabalala *et al.*, 2019). Vitamin A in Moringa leaves can help prevent night blindness (Adeyemi & Elebiyo, 2014). Furthermore, Tete-Benissan *et al.* (2013) reported that *M. oleifera* leaves could also act as one good agent to improve immunity and help to build up the immune system preventing diseases such as HIV and AIDS.

An aromatic ring that can accommodate unpaired electrons of reactive oxygen species (ROS) is a common feature of antioxidant molecules. This is the case of vitamin E (lipophilic) and vitamin C (hydrophilic), which can donate electrons to hydroxyl or peroxyl radicals to form water or stable hydroxyl molecules, respectively (Figure 2.9). Interestingly, vitamin C can also regenerate vitamin E and other antioxidants after their oxidation by transferring an unpaired electron to them, while the regeneration of active vitamin C depends on the activity of nicotinamide adenine dinucleotide phosphate (NADH/NADPH-dependent reductases) (Vara & Pula, 2014). Therefore, the antioxidant potential of vitamin E and vitamin C has suggested their use to protect the cardiovascular system from degenerative diseases such as atherosclerosis (Vara & Pula, 2014).

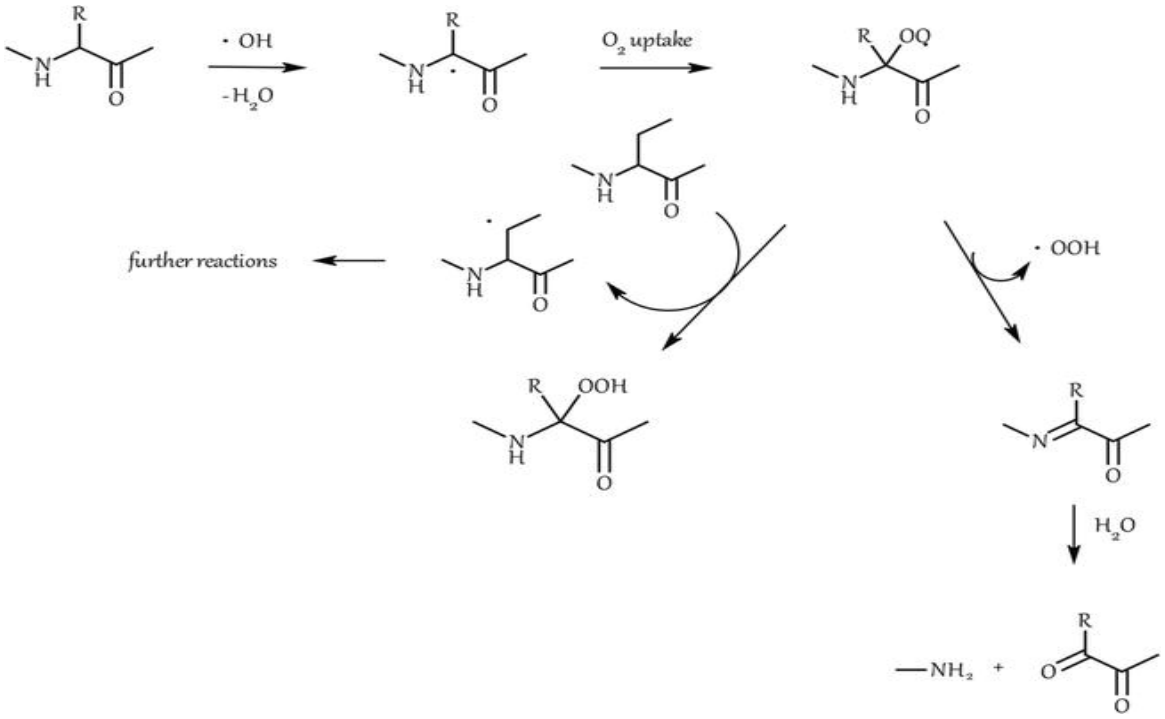


Figure 2.8 Reaction of hydroxyl radical with α-amino acids (Santos-Sánchez *et al*., 2012)

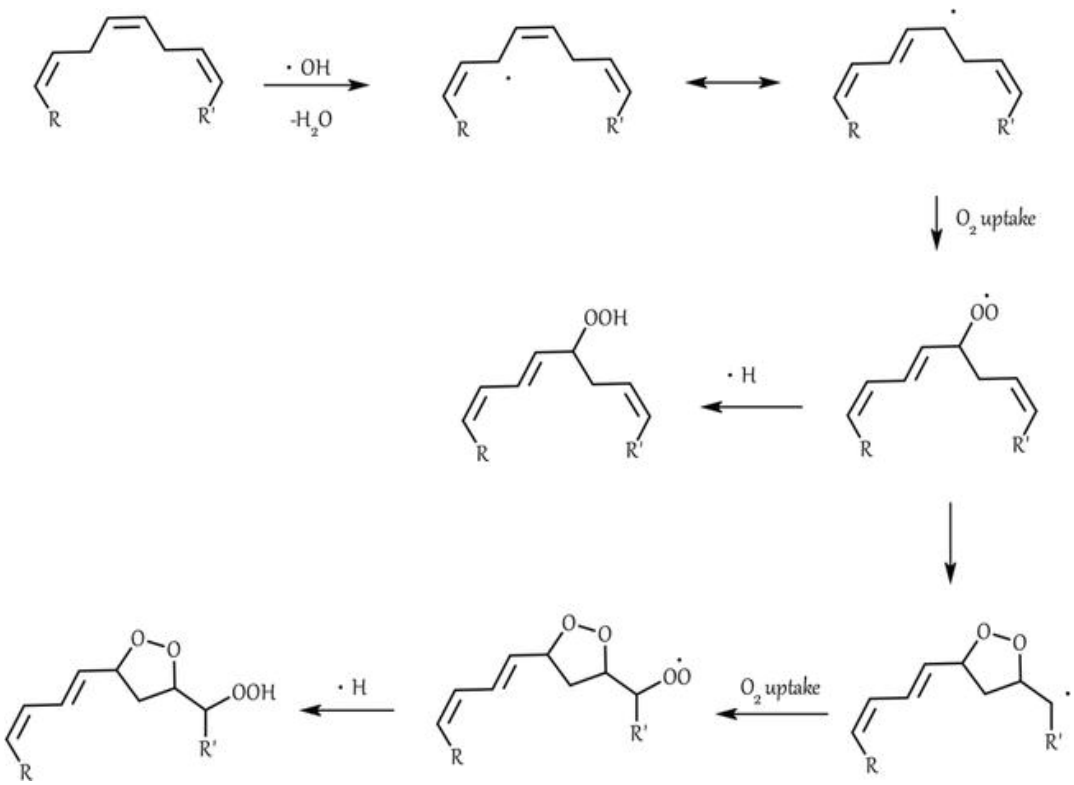


Figure 2.9 Hydroxyl radical with polyunsaturated fatty acids (Santos-Sánchez *et al*., 2012).

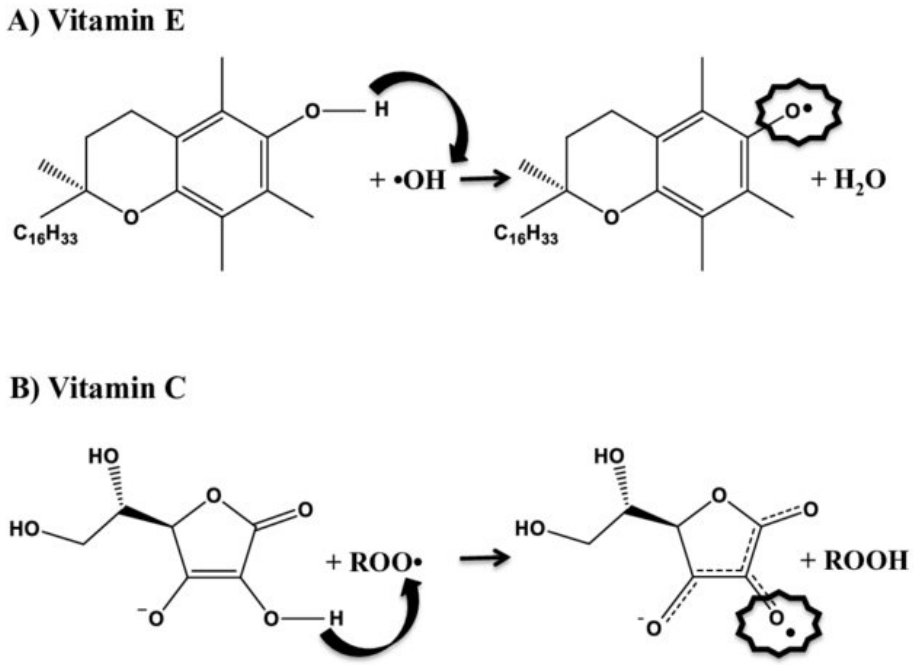


Figure 2.10 Structure and chemical mechanism of action for vitamins E as a detoxifier of hydroxyl radicals (A) and vitamin C as a detoxifier of peroxyl radicals (B). Natural antioxidants (Vara & Pula, 2014).

2.2 Solid Dispersion Technology

An ingredient's solubility is an important physical property that must be considered before applying it to any matrix. In addition, the poor solubility of an ingredient might limit its use, thus reducing its functionality. As a result, solubility enhancement technologies are an integral part of ingredient applications and have been well studied in both food and pharmaceutical applications. Commonly used techniques (displayed in Figure 2.10) for improving the solubility, dissolution, and bioavailability of poorly water-soluble ingredients and compounds include micronisation, nanonisation, complexation with cyclodextrins, nano-encapsulation, the addition of surfactants, and the use of solid dispersions (Ghareeb *et al.*, 2009; Punitha *et al.*, 2011; Jadhav *et al.*, 2012; Kumavat *et al.*, 2013; Pang *et al.*, 2015; Sarangi & Singh, 2017; Younis, 2017; Zhang, 2018). Although each of these strategies has been used to enhance dissolution and solubility of poorly water-soluble bioactive compounds and ingredients, each strategy has its substantial limitations, ranging from long preparation times to particle agglomeration and poor flow properties (Zawar & Bari, 2013). Solid dispersion, however, remains one of the most popular and successful methods for enhancing the solubility of products (Punitha *et al.*, 2011; Kumavat *et al.*, 2013; Zawar & Bari, 2013; Sarangi & Singh, 2017; Raj & Kumar, 2018) because of its simplicity and efficiency. In solid dispersion systems, a hydrophobic or poorly water-

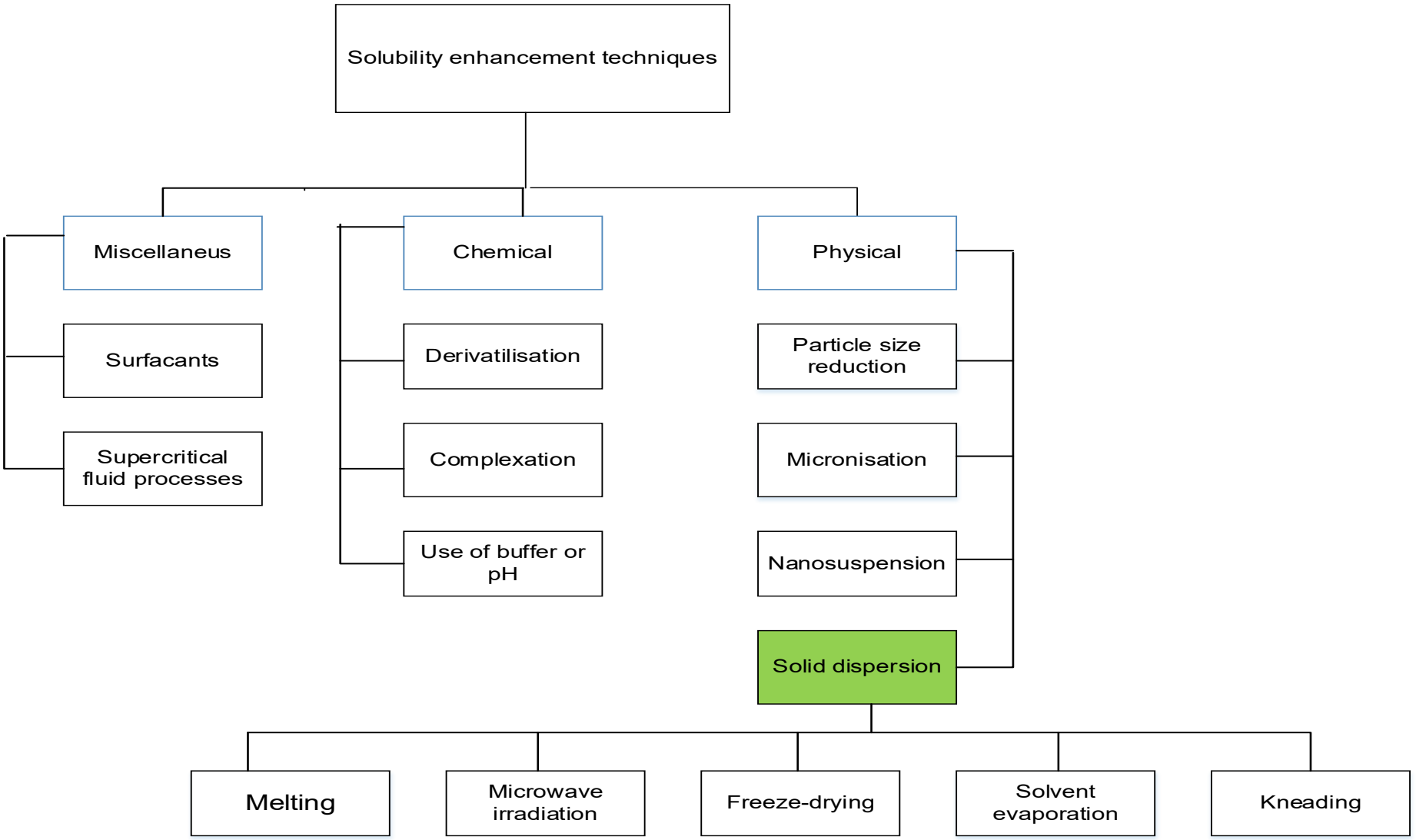


Figure 2.11 Various techniques for solubility and dissolution enhancement of poorly soluble compound

the soluble ingredient is molecularly dispersed in a hydrophilic polymer matrix to make the ingredient amorphous using solid dispersion methods such as melting, solvent evaporation, and freeze-drying, thereby improving its solubility, dissolution rates, and bioavailability (Yeo *et al.*, 2020). Selecting carriers is a significant factor in the success of developing efficient solid dispersion systems. Figure 2.11 illustrates how the ingredient's crystallinity is altered to an amorphous state during solid dispersions. There are several mechanisms by which ingredient solubility and dissolution rate can be enhanced with solid dispersion technology. Increasing the surface area of the ingredient by reducing the particle size to sub-microns is one of the most significant factors (Bolourchian *et al.*, 2013). It has been reported that reducing the particle size increases saturation solubility and dissolution. In addition to the reduction of particle aggregation and agglomeration, direct contact between the compound and its hydrophilic carrier lead to improved particle wettability (Zawar & Bari, 2013; Krstić *et al.*, 2020; Yu *et al.*, 2020). Moreover, changing the compound from crystalline to an amorphous state with higher energy can increase solubility (Zawar & Bari, 2013).

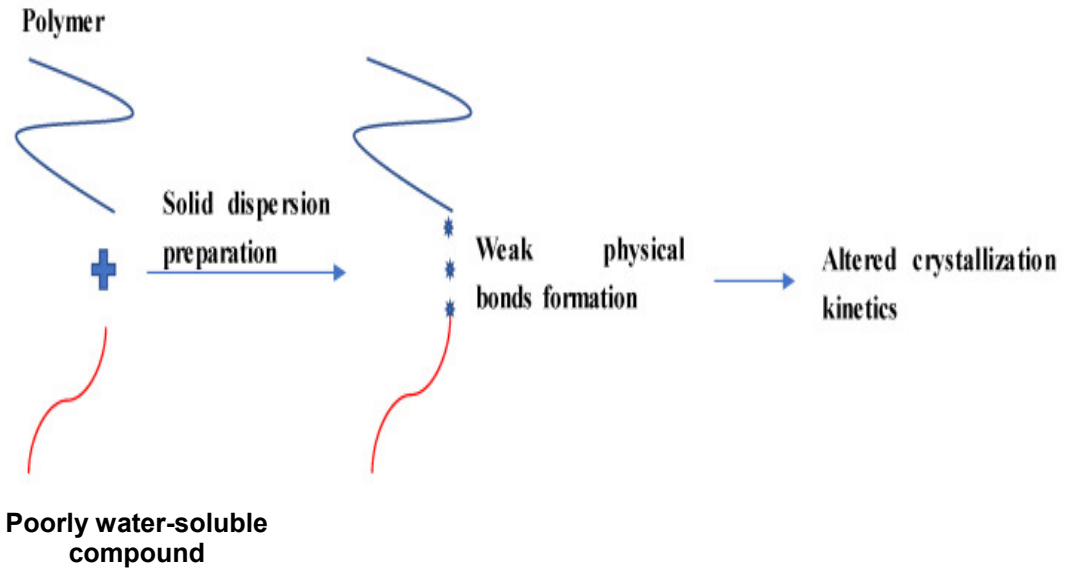


Figure 2.12 Illustration of typical molecular interactions in solid dispersions and their effects on ingredient/ compound crystallinity (Tran & Tran, 2020a)

2.2.1 Solid Dispersion Techniques

To form solid dispersion complexes between hydrophilic carriers and the poorly water-soluble ingredient, various techniques are used. These techniques include, but are not limited to, freeze-drying, melting, solvent evaporation, microwave irradiation, hot melt extrusion, spray drying and kneading, and are covered in the following section.

Freeze-drying

The freeze drying also (known as lyophilisation method) is an alternative to the solvent evaporation method in which the carriers and compound and are dissolved in a solvent and then the solution is frozen using an ultra-freezer or frozen in liquid nitrogen until it is completely frozen (Younis, 2017; Bindhani & Mohapatra, 2018). The frozen solution is further lyophilised. This method is used for thermally labile products that are unstable in aqueous solutions, but stable in a dry state for extended storage periods. The vital advantage of this method is that it reduces the risk of phase separation (Bindhani and Mohapatra, 2018), and during the formation of the solid dispersion, the compounds are subjected to minimal thermal stress.

Melting

The melting method is also referred to as the fusion method. This method was first used by Seikuchi and Obi in 1961, and its basic principle involves preparing a physical mixture of a compound and a hydrophilic carrier and heating it directly to 65^{o}C or using a water bath with a controlled temperature of 65^{o}C until it melts (Younis, 2017). The molten mixture is then solidified rapidly in an ice-bath with vigorous stirring. The formed solid mass is triturated and sieved (Younis, 2017). The melting method is popularly employed due to its simplicity and economic benefits.

Hot-melt extrusion method

Hot-melt extrusion (HME) is a method that is frequently used in the food industry. This manufacturing process has been useful in the preparation of solid dispersion in a single step. In this method, the compound and carrier are simultaneously melted, homogenized, and extruded through the die in the form of a rod, pellets, or powder (Bindhani & Mohapatra, 2018). The shapes of products at the outlet of the extruder can be controlled and do not require grinding in the final step. Solvents are not used; therefore, it is environmentally friendly, economical, and no residual solvent in the final product. The use of low temperature and short residence time is the most advantageous thing about this technique (Das *et al*. 2012). Other benefits include taste masking, stabilizing the ingredient, and enhancing the dissolution rate and bioavailability.

Solve evaporation

This method is one of the most widely used methods for increasing the solubility of poorly water-soluble compounds or ingredients and was pioneered by Tachibaria and Nakamura in 1965. In this method, the carrier and compound are dissolved in a volatile solvent to form a homogeneous mixture, which is then removed under vacuum (Tran *et al.*, 2019).

Furthermore, solvents can be removed using a variety of methods, including rotary evaporators, slow evaporation of solvent at low temperatures and vacuum oven. Ethanol, methanol, acetone, ethyl acetate, and methylene chloride are among the solvents used in this method. The solid dispersion that has formed is pulverised and sieved. The solvent method seems to be ideal for achieving particle size reduction. However, it is not often employed in the commercial market due to the high cost of production and selection of a non-toxic solvent.

Microwave irradiation

Microwaves, with their ability to penetrate any substance, allow the production of heat at any point in a sample at the same time. This is attributed to the presence of molecules characterized by a dipolar moment able to absorb microwave energy and convert it into heat. In recent years, the use of microwaves has become very attractive in organic chemistry (Zawar & Bari, 2013). Unlike conventional heating, e.g., conduction, convection, or radiation with infrared light, microwave irradiation offers several advantages: rapid volumetric heating, no overheating at the surface, addressable heating, energy-saving, and low operating efficiency cost. Besides, the main advantage of not using organic solvents is the absence of any risk originating from residual solvents (Hussain *et al.*, 2017). The application of microwaves represents a promising alternative to conventional preparative methods of solid dispersions of compounds as the microwave-induced method involves much shorter preparation times. It has been reported that microwave energy can influence the crystalline status of a compound and that the duration of exposure plays an important role in converting the compound to an amorphous state, thus improving its dissolution rate (Zawar & Bari, 2013; Hussain *et al.* 2017).

Kneading method

In this method, a carrier is permeated with water and transformed to paste. The compound is then added and kneaded thoroughly for 20 minutes or more, depending on the compound. Next, the kneaded mixture is dried using a vacuum oven. The obtained dry mass is then pulverized and passed through a sieve. When working on a laboratory scale, the kneading procedure can be carried out with a mortar and pestle, whereas extruders can be utilised when working on a larger scale. This method is suitable for thermo-labile compounds but not moisture-sensitive compounds (Mogal *et al.*, 2012). The kneading method is widely used because of its simplicity, large-scale use, and the fact that it is a relatively fast method.

Spray drying method

Spray drying is one of the most efficient technologies for manufacturing solid dispersions.

The compound is dissolved in a suitable solvent, and the required amount of carrier is dissolved in water to prepare the solution (Bindhani & Mohapatra, 2018). The two solutions are then mixed by sonication or another suitable method until the solution is clear. The formed solution is then spray dried to produce a solid dispersion form of free-flowing fine particles with an increased surface area. The powder is then screened through a sieve.

2.2.2 Hydrophilic Carriers- Polyethylene glycol (PEG)

Carriers play a major role in the formulation of solid dispersions. They can be hydrophilic, hydrophobic, or water-swellable. Based on their characteristics, they can either retard or enhance compound release. Furthermore, the dissolution characteristics of compounds are dependent on the nature of the carriers (Tekade & Yadav, 2020). The criteria for the selection of a carrier are as follows: the carrier must preferably be Generally Recognized as Safe (GRAS), water-soluble, soluble in a variety of solvents. It should be economical, inert, chemically compatible with compounds, and heat-stable (Dhirendra *et al.*, 2009; Janssens & Van den Mooter, 2009; Punitha *et al.*, 2011; Van Duong & Van den Mooter, 2016; Mendonsa *et al.*, 2020).

Hydrophilic carriers are water-soluble carriers. Various hydrophilic carriers employed in solid dispersions are displayed in Figure 2.12. This study, however, focuses on polyethylene glycol (PEG) polymer. PEGs which have an amorphous-semicrystalline structure, are extensively used in the preparation of solid dispersions because of their wetting, solubilising, and surface-active properties (Newa *et al.*, 2008). They have been reported to enhance the solubility, dissolution, and bioavailability of many poorly water-soluble compounds (Newa *et al.*, 2008; Hassnain *et al.*, 2012; Bolourchian *et al.*, 2013; Zhao *et al.*, 2014). These polymers are widely used in the food industry and pharmaceutical industries as food additives, carriers, and solubilising excipients because of their low melting point (55-63^{o}C), wide compatibility, hydrophilicity, the ability to dissolve rapidly, and the ability to dissolve in many organic solvents (Younis, 2017). Furthermore, PEGs are considered inert, indicating that they do not react with other materials, and are odourless, colourless, non-irritating, and do not evaporate easily. (Nikghalb *et al*., 2012). Therefore, they do not change the colour, odour, and taste of water and other solvents. Hence, they have many industrial, food, and pharmaceutical uses.

PEG as a hydrophilic carrier is used commonly in the preparation of solid dispersions formulation and is available in a range of molecular weights (200-300,000). However, PEGs with molecular weights of 1,500-20,000 are used to manufacture solid dispersions (Zawar & Bari, 2013). Each molecular weight of the polymer exhibits definite characteristics that are likely to affect the formulation of solid dispersions. PEGs have the general chemical structure: HO-(CH_2-CH_2-O)n-H, depicted in Figure 2.13a. The water

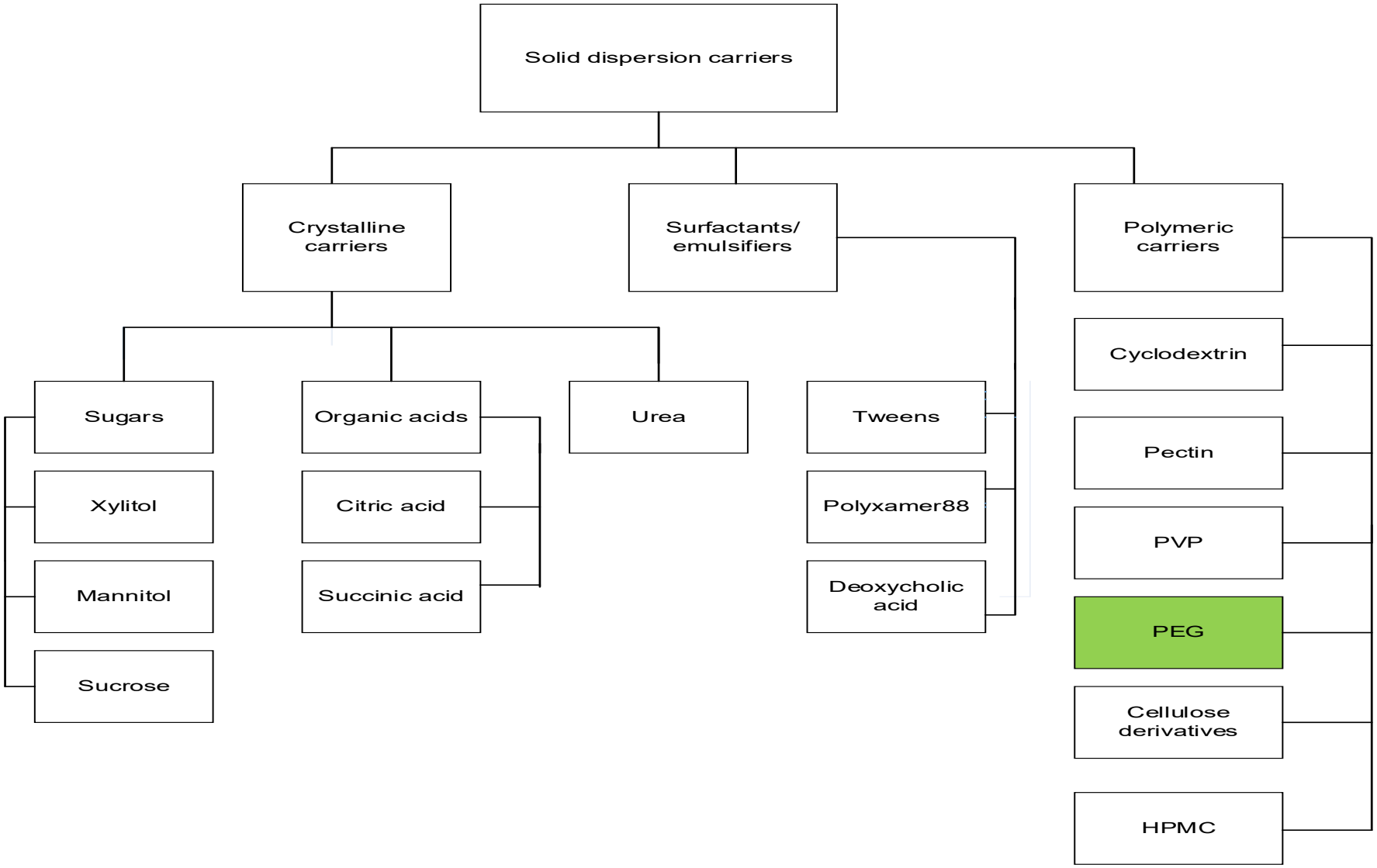


Figure 2.13 Different carriers used in solid dispersion technology

solubility of PEGs is generally good but decreases as molecular weight increases. In addition, PEGs have good solubility in many organic solvents, which makes them useful for the preparation of solid dispersion by both fusion and solvent methods. Generally, PEGs have melting points that are within the range of 65°C (e.g., PEG1000, PEG4000, PEG6000, and PEG20,000 are all 60–63°C) (Zawar & Bari, 2013; Sarangi & Singh, 2017). The low melting points are useful for preparing solid dispersions by the melting method. Figure 2.13b shows sites of hydrogen bond interactions between PEG polymer and compound during solid dispersions. Intermolecular interactions are usually formed between the hydroxyl (O-H) group of PEGs and the C=O group of the compound. This results in wetting and subsequently increased solubility and dissolution rate. In addition, crystallisation inhibition in solid dispersed ingredients can be attributed to interactions, such as hydrogen bonding between the ingredient or compound and the polymer (Shinde *et al.*, 2013).

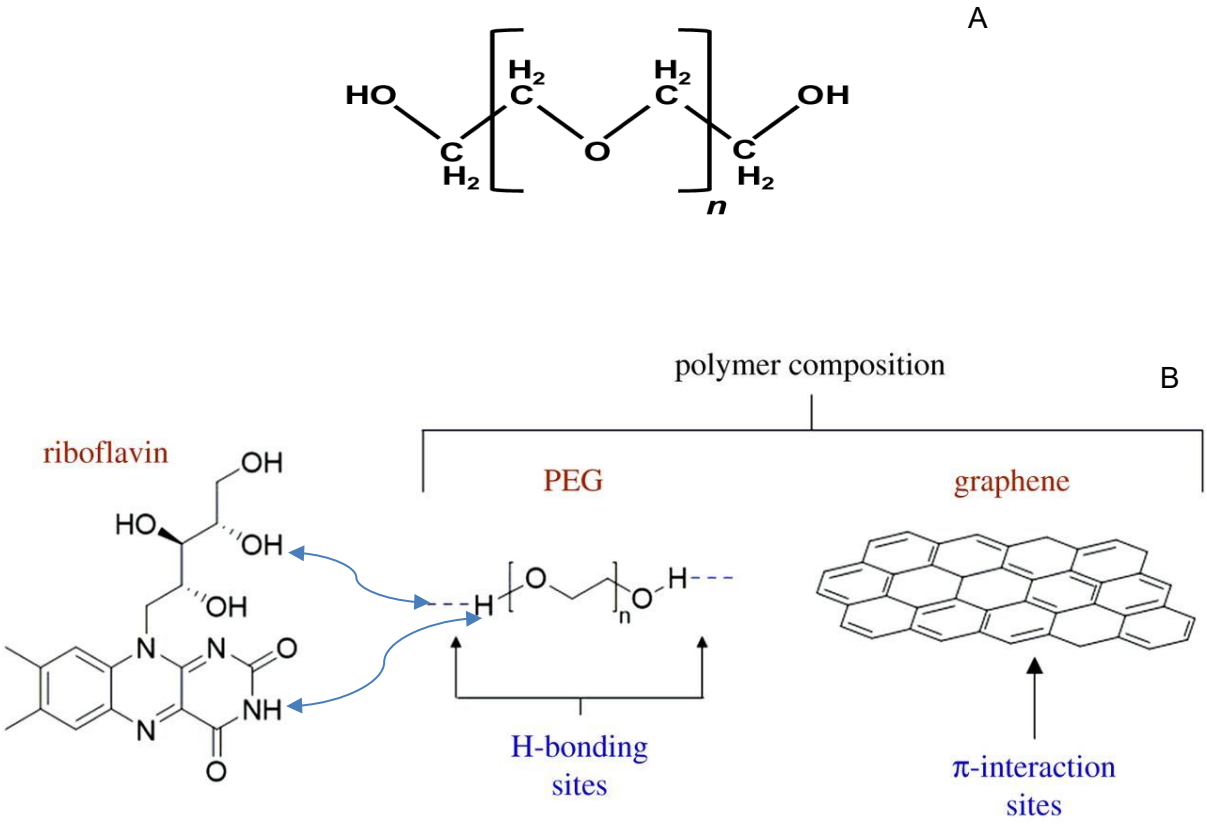


Figure 2.14 (A) Basic molecular structure of PEG and (B) Molecular structures of riboflavin and the polymer components, with emphasis on the bonding sites (B) (Hutchins, 2018).

Although optimal dispersion of the ingredient, as well as reduced particle size and carrier hydrophilicity, results in increased solubility, a variety of physical properties, including the

carrier's molecular weight, carrier form (amorphous, semi-crystalline, crystalline), and carrier hydrophilicity, can affect the preparation of solid dispersions (Van Duong & Van den Mooter, 2016). These variables have a significant impact on the compound's or ingredient's solubility, dissolution, and stability.

2.2.3 Application of solid dispersion technology in food systems

The application of solid dispersion of a compound in aqueous-soluble carriers was first described by Sekiguchi and Obi (1961). Since then, the use of solid dispersion technology has been investigated extensively to increase the solubility and dissolution of compounds in the pharmaceutical industry. Following the success of solid dispersions in the pharmaceutical industry, scientists are now using the technique in the food industry to control the delivery of functional foods like vitamins, flavours, and preservatives. Functional food is defined as “any modified food or food ingredient that may provide a health benefit beyond the conventional nutrients it contains” (Mcclements *et al.*, 2015). In addition to nutrition, functional ingredients are increasingly used in the development of functional food products as an opportunity to meet consumer demand for healthier and safer foods, thus improving human health by preventing the risk of nutrition-related diseases (Mcclements *et al.*, 2015). However, some biological properties of food ingredients are poorly soluble in foods and are often lost due to instability during food processing conditions. Incorporating bioactive compounds into commercial food products is also a challenge due to their poor stability and low solubility. Certain functional food ingredients' poor solubility and dissolution rate have greatly limited their use in food systems. In addition, the food and beverage industry is challenged by the problem of utilising natural and highly nutritious compounds due to their inability to dissolve and solubilise, especially in liquid systems (Lan *et al.*, 2019). Solid dispersion technology is being investigated and employed to overcome solubility, dissolution rate, and permeability challenges in the food, dietary supplement, and nutraceutical industries. Table 2.9 shows some of the active ingredients and bioactive compounds where solid dispersion technology was successfully implemented to improve their solubility, dissolution.

Table 2.9 List of food and beverage ingredients investigated for solid dispersion[1]

Food ingredient (Bioactive compounds)	Carriers	Solid dispersion method	Sources
Pea protein isolate	Gum Arabic, Maltodextrin	Spray drying	Lan *et al.* (2019)
Turmeric (Curcumin)	PVP K-30, PEG4000, and PEG6000	Spray drying	Chuah *et al.* (2014); Leimann *et al.* (2019)
	HPMC	Spray drying	Shin *et al.* (2019)
	PEG4000, PEG6000	Fusion/Melting	Kumavat *et al.* (2013)
***Rebaudioside* D (Stevia sweetner)**	Potassium sorbate	Spray drying	Mutilangi & Zhang (2013); Pang *et al.* (2015)
Phloretin	PVP K-30	Solvent evaporation	Han *et al.* (2015)
	Steviol glycoside	Solvent evaporation	Wang *et al.* (2020)
Beta Carotene	PVP, Sucrose fatty acid ester	Hot-melt technology	Ishimoto *et al.* (2019)
Epigallocatechin Gallate	HPMCP, soluplus	Freeze-drying	Cao, Teng & Selbo (2017)

2.2.4 Dissolution Study

Dissolution is a process of dissociating a solid substance in a solvent to form a chemically and physically homogeneous dispersion called solution (Pang *et al.*, 2015). In the pharmaceutical industry, it may be defined as the amount of material that goes into solution per unit time under standardised conditions of an interface between liquid and solid, solvent composition, and temperature (Mbamalu, 2015; Kandukoori, 2018). Dissolution testing is widely used as an analytical technique for evaluating the compound release characteristics as well as the consistency of the product. It was initially designed to quantify the amount and extent of drug release from solid oral dosage forms, including immediate-release tablets and capsules (Kandukoori *et al.*, 2018). Although this test is widely accepted for conventional drugs and formulations of active pharmaceutical ingredients (APIs), it remains a novelty for herbal remedies. Recently, dissolution tests have become important to verify the release of bioactive compounds in herbal products (Rajarajan *et al.*, 2009; Costa *et al.*, 2011; de Sousa *et al.*, 2011; Wang *et al.*, 2015; Bepe, 2017; Archer *et al.*, 2020; Sermkaew & Plyduang, 2020), effervescent beverage granules (Diyya & Thomas, 2018), solid-dispersed ingredients (Chuah *et al.*, 2014b; Muthu *et al.*, 2019), tea bags (Bepe, 2017), chewable tablets, powders, dietary supplements and semisolids (Azarmi *et al*., 2007). Herbal (medicinal) products have gained tremendous popularity in recent years. They may be incorporated into pharmaceutical or nutraceutical formulations as new formulations or "improved" versions of existing formulations. As such, there is a need for new approaches that will improve their quality and efficacy. Studying these materials' dissolution following relevant guidelines will likely result in the desired improvement. Herbal products are complex multi-component materials, so their dissolution tests may be conducted with several reference marker compounds to develop compendia and regulatory standards (Mbamalu, 2015).

The most common dissolution apparatus used throughout the world are (1) the basket method and (2) the paddle method, which are usually known as the United States Pharmacopeia (USP) Apparatus 1 and 2, respectively. These methods are simple and robust and are generally flexible enough to allow dissolution tests for a wide variety of products. For this reason, Apparatus 1 and 2 are recommended for dissolution method development. A typical PTWS 610 testing equipment is shown in Figure 2.14a. The equipment can be used as Figure 2.14b Rotating Basket Method and Figure 2.14c Rotating Paddle Method by switching corresponding shafts. Their schematic diagrams are illustrated in Figure 2.14d.

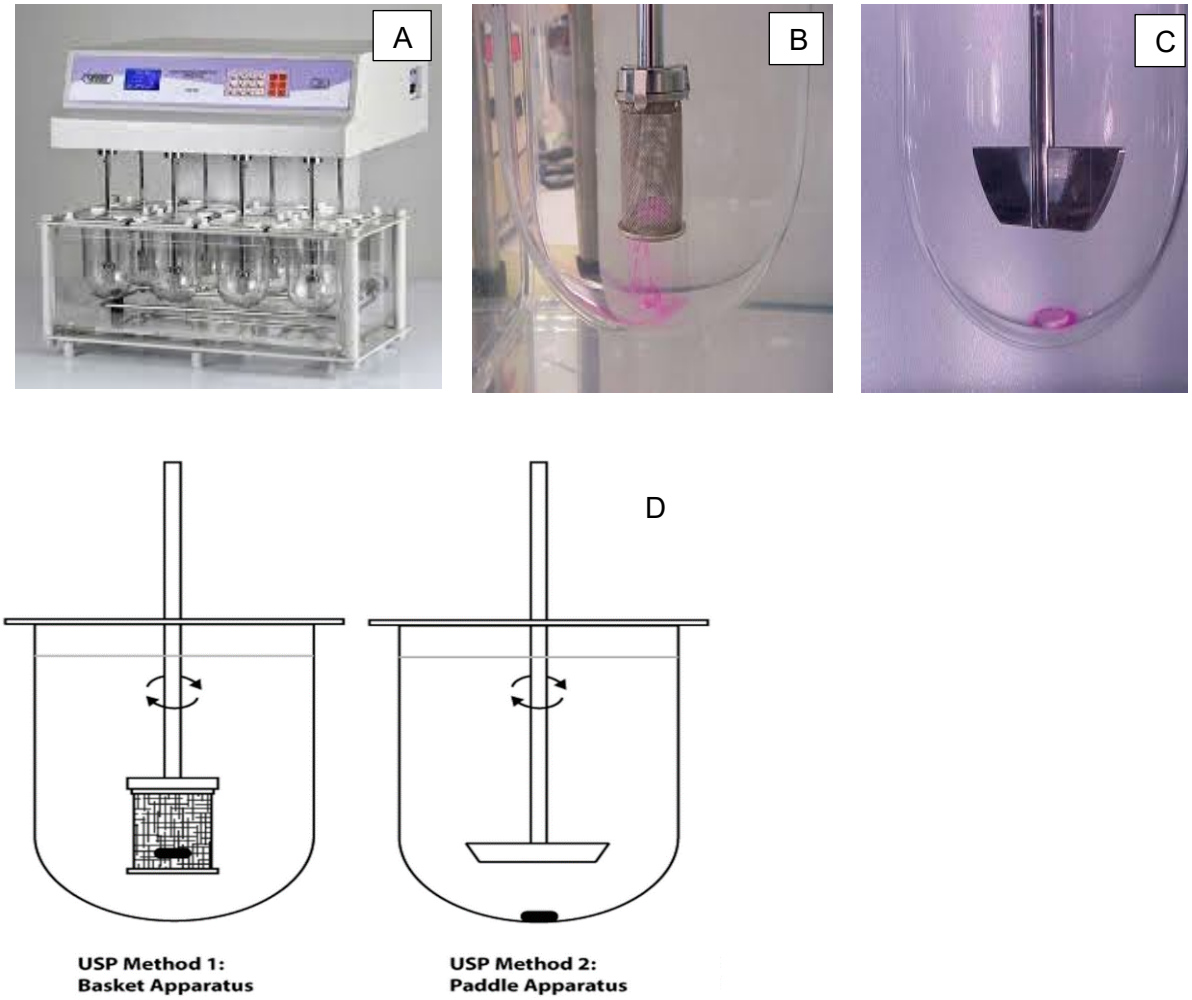


Figure 2.15 Dissolution apparatus. (A) six vessel dissolution test apparatus, (B) Rotating Basket Method and (C) Rotating Paddle Method (Vaghela *et al*., 2011) and (D) schematic diagrams of rotating basket apparatus and paddle apparatus.

2.2.5 Easy-to-drink Beverages

The term beverage includes a wide variety of compositions, including water, fruit juice, coffee, tea, milk, alcohol, energy drinks, soda, or any combination of them. There are four primary sectors of the global commercial beverage market: hot drinks, milk drinks, soft drinks, and alcoholic drinks. Hot drinks include tea, coffee, and hot chocolate. Soft drinks have five main subcategories such as bottled water; carbonated soft drinks; dilutable (squash, granules, and powders, cordials and syrups); fruit juices (100% fruit juice and nectars (25–99% juice content); still drinks, including ready-to-drink (RTD) teas, sports drinks, and other noncarbonated products with less than 25% fruit juice) (Sayed & Abdellatif, 2018). The high content of water leads to microbial spoilage or increased enzyme activity in many beverages.

Thus, preservation by way of drying is one of the preferred methods for them. Powdered or granulated drinks are the dried form of beverages produced with the aim of shelf-life prolongation and high quality. Nutrients in a powdered or granulated form degrade more slowly than those within a liquid. In addition, the granulated and powdered drinks have long-term stability, and the nutrients can be better preserved compared to liquid form (Çopur *et al.*, 2019). The handling, transportation, preparing easiness, and reduced volume are the other advantages of these products (Çopur *et al.*, 2019). The powdered or granulated drink is available for soft drinks such as coffee, milk, juice, effervescent powders and granules, and some mixtures (Nagashima *et al*., 2013; Çopur *et al*., 2019). At present, the demand for ready-to-drink beverages is increasing day by day. Easy-to-drink effervescent granules can meet consumer needs. Effervescent products containing functional ingredients such as probiotics, vitamins, minerals, phenolic compounds, antioxidants are added to any liquid product, which would provide a pleasant taste and facilitate the daily ingestion of such ingredients. Nagashima *et al.* (2013) developed effervescent probiotic products, while Hiola & Tungadi (2018) developed sweet corn milk effervescent granules as ready-to-drink beverages. More so, Murdiana *et al.* (2018) successfully produced *Moringa oleifera* extract-based effervescent tablets. The tablets were created as an anti-anemic dosage that pregnant women could take in place of iron tablets. The tablets, however, were discovered to be bitter; thus, a strong bitter masking-agent may be advantageous as the study's future directions. However, there is no reported information about the development of *Moringa oleifera* leaf powder effervescent beverage granules. Previous results showed that adding Moringa leaves powder in food and beverage products increased the nutritional and antioxidant content. Considering the health needs of consumers and the good properties of MOLP, more studies are needed to produce Moringa-based functional beverages. Moringa leaves with high antioxidants can be further processed to maintain the component of functional compounds. Hence, the present study seeks to develop MOLP functional effervescent beverage granules.

2.2.6 Effervescent Beverage Granules

An effervescent granule is a functional granule that is meant to be dissolved or dispersed in water before administration (Aslani & Jahangiri, 2013). Effervescent granules generally contain, in addition to active ingredients, a mixture of acids such as tartaric and citric acid, among others, and bases such as hydrogen carbonates or carbonates, including sodium carbonate or potassium carbonate, which release carbon dioxide (CO_2) when mixed in water. Effervescent granules are gaining popularity in various sectors including food, supplements, and pharmaceutical use, because of the ease with which they can be consumed. The use of traditional drinks in the form of effervescent is still rare, only limited to products of supplements

and vitamins. However, the technique has several advantages, including time efficiency and producing fresh taste due to the presence of carbonates which help improve the taste of the product (Giyatmi & Lingga, 2019). In addition, there are also numerous benefits over conventional granules, such as being easy to swallow, improved bioavailability, rapid absorption, convenience, better taste, and increased liquid intake (Patel & Siddaiah, 2018). Effervescent products can turn any liquid into functional beverages. Functional drinks have been developed to provide specific health benefits such as promoting heart health, improving immunity, and helping boost energy (Pandey *et al.*, 2013; Shamsi, 2017; Patel & Siddaiah, 2018). In addition, developing effervescent products which are easily transformed into functional beverages contributes largely to the environment as it minimises the use of plastics and other products. Powdered or granulated beverages provide an advantage over bottled ready-to-drink beverages because they take up less shelf space than liquid drinks (Shamsi, 2017a). Additionally, sachets and other light and moisture-resistant packaging options make it easier than ever to transport and protect powdered or granulated drinks. Furthermore, these beverages tend to have longer shelf lives as the nutrients in a powder form degrade slowly than those within a liquid. This makes effervescent products the preferred choice of many people.

2.2.7 Chemistry Behind Effervescence

Effervescence is the chemical reaction within an aqueous solution of acids and bases producing the release of carbon dioxide (Meyer, 2020). An underutilized formulation approach offers functional and consumer advantages over conventional food, dietary supplements, pharmaceuticals, and other market segments (Milward, 2001, Divya *et al.*, 2020). While the basic chemistry and process technology used to make effervescent products is not new, their complexity requires special formulation and manufacturing techniques. The chemistry that underlies the appeal of effervescent products is shown in Figure 2.17. An acid neutralises a carbonate salt, producing carbon dioxide gas, acid salt, and water. The CO_2 gas is the one that makes effervescent products appeal to people's senses. The most common reactions used in effervescent technology are either sodium bicarbonate and citric acid (1), or and sodium bicarbonate and tartaric acid (2), resulting in the release of carbon dioxide gas as depicted in reaction 1 and reaction 2, respectively (Divya *et al.*, 2020). This reaction occurs in the presence of water, even with a small amount as a catalysing agent, increasing the reaction rate. As water act as a catalysing agent for the reaction, so all the moisture-sensitive products or effervescent products are stored in a moisture-free environment (Ahmed *et al.*, 2014).

$C_6H_8O_7.H_2O + 3NaHCO_3\ (aq) \rightarrow Na_3C_6H_5O_7 + 4H_2O + 3CO_2\ (g)$ *reaction 1*

Citric acid + Sodium bicarbonate → Sodium citrate + Water + Carbon dioxide

$C4H_6O_6 + 2\ NaHCO_3 \rightarrow Na_2C_4H_4O_6 + 2H_2O + 2CO_2$ (g) *reaction 2*

Tartaric acid + Sodium bicarbonate → Sodium tartrate + Water + Carbon dioxide

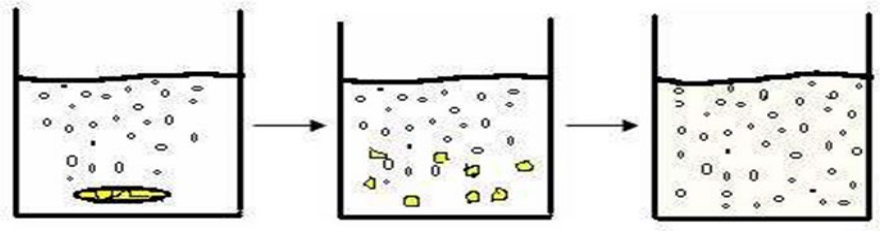


Figure 2.16 The mechanism of effervescent (Divya *et al*., 2020).

2.2.8 Acids and salts (bases) used in effervescent technology

Acid sources and alkaline sources have an important role in making effervescent products. Carbonate salt is a key material in effervescent formulations because it is the source of CO_2. The most commonly used carbonate salts are sodium carbonate and sodium bicarbonate, depicted in Table 2.10. Sodium bicarbonate is a white crystalline powder possessing a salty taste and non-hygroscopic. It is believed to have a higher proportion of CO_2 than sodium carbonate and can easily break down and release water in aqueous solutions (Milward, 2001). Sodium bicarbonate at relative humidity above 85% will quickly absorb water in the environment and will lead to decomposition and loss of carbon dioxide thus it is required to be stored in sealed storage (Dharmawan *et al.*, 2016). This salt is highly soluble in water, severe reaction, cheap, and widely available in the market in five particle size levels ranging from fine powder to free-flowing uniform granules, edible and is widely used in food products (Aslani & Fattahi, 2013). It is the weakest alkali sodium, possessing pH 8.3 in an aqueous solution and 0.85% concentration. It produces approximately 52% of carbon dioxide as a dissolving agent and CO_2 source in effervescent products (Dharmawan *et al.*, 2016). Products formulated with sodium bicarbonate tend to react or dissolve more quickly and be less stable than those using sodium carbonate. Some manufacturers have developed proprietary processes that improve the stability of products that utilise sodium bicarbonate. Calcium, potassium, and magnesium carbonate salts can also be used to formulate effervescent products (Milward, 2001; Sreelatha *et al.*, 2011). The other key component in effervescent compositions is the acid, as tableted in Table 2.11. Citric acid is an extensively used acid in effervescent formulations. It is commonly used as a food acid and is relatively inexpensive. This acid has high solubility, high acidity and is available in granular, anhydrous, and monohydrate forms.

Table 2.10 Carbonates used in effervescence formulation

	Sodium carbonate	**Sodium bicarbonate**
Chemical formula	Na_2CO_3	$NaHCO_3$
Molecular weight	106	84
Equivalent of acid	2	1
CO_2	41.5	52.4

In addition, it is also available in powder form and has a hygroscopic attribute (Dharmawan *et al.*, 2016). Therefore, handling and storage need special attention (Milward, 2001; Sreelatha *et al.*, 2011; Meyer, 2020). Furthermore, citric acid can be easily obtained, has good neutralising properties, and imparts a citrus-like flavour to products (Divya *et al.*, 2020). Fruit acids such as malic and tartaric acid are also suitable for effervescent products. Malic acid can be implemented into an effervescent formula for a smoother aftertaste. However, the price of malic acid is significantly higher than that of citric acid. Tartaric and fumaric acids are usually used in small amounts due to their low water solubility (Meyer, 2020). Even though they are more expensive than citric acid, they often moderate citric acid‘s sharp flavour. Several studies have reported that a combination of tartaric acid and citric acid is used as an effervescent base rather than either acid alone because when tartaric acid is used alone, chalky friable granules are produced, and citric acid alone results in a sticky mixture difficult to granulate during the process (Aslani & Jahangiri, 2013b; Diyya & Thomas, 2018; Jassim *et al.*, 2018a). In addition to acids, bases, and active ingredients, effervescent products contain other materials such as sweeteners, binders, and flavours.

Table 2.11 Acids used in effervescent formulations

	Citric acid	**Fumaric acid**	**Malic acid**	**Tartaric acid**
Chemical formula	$C_6H_8O_7$	$C_4H_4O_4$	$C_4H_6O_5$	$C4H_6O_6$
MW	192.1	116.1	134.1	150.1
Moles of acidity	3	2	2	2

Equivalent weight	64.05	58.05	67.05	75.05
solubility	68.6	1.1	55.8	>100

MW: molecular weight

2.2.9 Effervescent Granules Preparation Methods

Granulation methods like wet granulation and dry granulation are utilised to produce effervescent granules (Aslani & Jahangiri, 2013).

Wet granulation method

Wet granulation is a process of agglomeration that involves wet massing of the powder blend with a granulating liquid, wet sizing, and drying (Aslani & Jahangiri, 2013). The granulating fluid contains a volatile solvent to be removed by drying. Typical liquids used include water, ethanol, and isopropanol either alone or in combination (Hannes, 2016). The wet granulation method is one of the best methods used for producing effervescent beverage granules and tablets. This is the oldest method of granule preparation, although it suffers from reproducibility problems. However, wet granulation has witnessed various technical and technological innovations such as melt granulation, steam granulation, reverse granulation, and foam granulation. The significance and limitations of the recent wet granulation techniques and dry technologies are summarised in Table 2.12. Conventional wet granulation involves low shear granulation, fluid bed granulation, and high shear granulation.

Dry granulation method

The dry granulation process forms granules without using a liquid solution. Dry granulation is an efficient manufacturing option for many non-effervescent formulations and some less reactive effervescence. The most common dry granulation method is roller compaction, whereby a powder is compacted between two counter-rotating rollers and compressed into a ribbon (Gergely & Metz, 2012). As no liquids are used, the method does not involve costly and time-consuming drying steps (Shanmugam, 2015; Jannat *et al.*, 2016). Furthermore, any material that does not meet the required particle size can be recycled back in the process, minimising waste. As a result, this approach to granulation can be very cost-effective when the processibility and morphology of particles need to be improved. However, when it comes to manufacturing more reactive effervescent products, dry granulation is often not suitable as the effervescent components are compacted without passivation. The passivation is essential

to stabilise the reactive ingredients and maintain product quality during manufacture, shipping, and storage. Thus, more advanced technologies are required.

Table 2.12 Advantages and limitations of Wet granulation and Dry granulation methods

Method	Advantages	Limitations
Wet granulation	• Robust process suitable for most compounds. • Imparts Flowability to a formulation • Can reduce elasticity problems. • Binds active ingredient with an excipient, thus reducing segregation potential. • Coating surface with the hydrophilic polymer can improve wettability.	• Expensive • Time and energy-consuming process • Specialised equipment required • Stability issues moisture sensitive and thermolabile active ingredients with aqueous granulation
Wet granulation (non-aqueous)	• Suitable for the moisture-sensitive active ingredient • Vacuum drying techniques can remove/ reduce the need for heat.	• Expensive equipment • Needs organic facility • Solvent recovery issues • Health and environmental issues.
Dry granulation	• Eliminates exposure to moisture and drying. • Simplicity	• Dusty procedure. • Not suitable for all compounds. • Slow process

Source: Aslani & Jahangiri (2013); Jannat *et al.* (2016)

2.3. Conclusion

Moringa oleifera leaf powder is a prominent functional food ingredient rich in antioxidants, minerals, vitamins, and protein that can be used in the food and beverage industries. MOLP inclusion in foodstuffs could significantly address mineral and vitamin deficiencies, produce healthy foods, and increase MOLP market value. However, despite its numerous benefits, its poor solubility restricts its application. Therefore, the solid dispersion technology with the use of hydrophilic carriers can be employed to improve its solubility, dissolution rate, stability, and bioavailability, thereby expanding its use in high-water systems such as functional beverages.

When MOLP solid dispersions are prepared, amorphous solid dispersions may arise, which may enhance MOLP's functional properties. The formed MOLP solid dispersions will be more beneficial in beverages, nutraceuticals, and functional food formulations. This will further increase the use of MOLP, particularly in the food and beverage industry, as it has been identified as a superfood or functional crop that has been underutilized.

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CHAPTER THREE
PHYSICAL, FUNCTIONAL AND STRUCTURAL PROPERTIES OF SOLID-DISPERSED *MORINGA OLEIFERA* LEAF POWDER

Abstract

Moringa oleifera leaf powder (MOLP) is a rich source of antioxidants, protein, minerals, vitamins, and various phytochemicals. However, despite its many benefits, MOLP has low solubility in water. To improve the solubility of MOLP, solid-dispersed *Moringa oleifera* leaf powders (SDMOLPs) were developed through melting, solvent evaporation, freeze-drying, and microwave irradiation methods using polyethylene glycols (PEG4000 and PEG6000) (1:1) as hydrophilic carriers. The solid dispersions were evaluated for percentage yield, particle size distribution, colour, proximate composition, water absorption capacity (WAC), solubility, and structural properties. Furthermore, structural properties and mineral composition were determined by scanning electron microscopy (SEM) with energy dispersive x-ray spectroscopy (EDX) and scanning transmission electron microscopy (STEM). The percentage yield of SDMOLP which varied significantly ($p < 0.05$), ranged from 62.9-99.7%. All SDMOLPs had the same particle size distribution >150 m. Pure MOLP was lighter (L*), greener (-a*), and yellower (+b*) than solid dispersions in terms of colour characteristics. Significant differences ($p < 0.05$) existed in colour between MOLP and solid dispersions. Proximate analysis revealed that MOLP is rich in protein content 28.5% and metabolisable energy (344.46 Kcal/100g), with appreciable levels of fat 5.13%, moisture 8.44%, and ash 11.81%. While SDMOLP had protein content ranging from 12.42-14.23%; moisture, 2.65-4.03%; fat, 2.51-3.21%; and ash, 4.03-6.16%. Furthermore, all solid dispersions showed significantly higher ($p > 0.05$) solubility that ranged between 56.9% and 63.3% than the pure MOLP (25.5%). Elemental composition indicated that SDMOLP samples were high in calcium (38.3-65.1%), magnesium (4.2-17.9%), phosphorous (1.7-13.5%), copper (2.3-27.1%), sulphur (6.0-36.9%), iron (0.15-3.8%), zinc (0.0-8.5%) and sodium (0.0-2.9%). Conclusively, the improved solubility of solid-dispersed MOLPs may make them useful as functional ingredients in foods, dietary supplements, or nutraceutical formulations.

3.1 Introduction

Moringa oleifera crop can grow well in the humid tropics or hot drylands, survive in less fertile soils, and be little affected by drought. Several studies have indicated that the leaves of this plant have immense nutritional value (Bamishaiye *et al.*, 2011; Witt, 2014; Murdiana *et al.*, 2018; Ali *et al.*, 2019), and can be used to combat malnutrition, especially among infants and

nursing mothers (Sultana, 2020). *Moringa oleifera* leaves contain a rich concentration of multi-vitamins, micro and macro minerals, fatty acids, phenolics, antioxidants, and essential amino acids for body development (Gopalakrishnan *et al.*, 2016b; Ali *et al.*, 2019). These leaves also have medicinal properties and health benefits. Moringa has been used for treating more than 300 conditions due to its antioxidant, anticancer, anti-inflammatory, antidiabetic, antifungal, antidepressant, and antimicrobial properties, among others (Hagan *et al.*, 2018).

While the vast potential of *Moringa oleifera* leaf powder (MOLP) has been reported, MOLP is still largely underutilised as a functional ingredient in food systems. The major problem associated with MOLP is its inadequate solubility in liquid systems (Ali *et al.*, 2018), which finally limits its utility in the food and beverage industry. Solubility is an important prerequisite for a high protein ingredient to be an effective nutritional ingredient in high moisture food applications, such as protein fortified beverages. It is, therefore, important to develop an appropriate formulation to improve the solubility of MOLP. Several methods have been developed to enhance the solubility of poorly water-soluble ingredients including solid dispersion (Punitha *et al.*, 2011b; Jadhav *et al.*, 2012; Bolourchian *et al.*, 2013; Chuah *et al.*, 2014a; Cao *et al.*, 2017; Vadlamudi and Dhanaraj, 2017; Zhai *et al.*, 2017; Hassouna *et al.*, 2019; Leimann *et al.*, 2019), micronisation (Vadlamudi and Dhanaraj, 2017; Tran *et al.*, 2019), solubilisation using co-solvents, salt formation and milling (Punitha *et al.*, 2011). Solid dispersion technique is a promising means that has been used to improve the solubility of plant-based ingredients like curcumin (Kumavat *et al.*, 2013; Muthu *et al.*, 2019; Deshkar & Satpute, 2020) *Rebaudioside A* (Lan *et al.*, 2019b), *Rebaudioside D* (Pang *et al.*, 2015) and *Kaempferia parviflora* extract (Weerapol *et al.*, 2017). The fundamental concept of this technique involves the dispersion of poorly water-soluble ingredients in a hydrophilic carrier matrix through spray-drying, kneading, freeze-drying, solvent evaporation, microwave irradiation, and/or melting methods that create a dispersed state with improved solubility (Pang *et al.*, 2015). The melting method is a process to prepare solid dispersions by mixing ingredients and carrier, then heating them directly until they melt. The molten mixture is then solidified. In the solvent evaporation method, the ingredient and carrier are dissolved in a common solvent, and then the solvent is removed to form the solid mass. Nevertheless, the solvent evaporation method is popular in solid dispersions.

The solid dispersion technique is usually employed with polymeric carriers, such as polyethylene glycol (PEG) derivatives, polyvinylpyrrolidone (PVP), cyclodextrins, among others (Yu et al., 2011; Jadhav et al., 2012; Pang et al., 2015; Younis, 2017; Xiong et al., 2019). PEG as a hydrophilic carrier is commonly used in the preparation of solid dispersion formulations and is available in a range of molecular weights. Each molecular weight of the polymer has definite characteristics that may lead to various characteristics of solid dispersion

formulations. However, studies on the development of herbal products using the solid dispersion technique are still limited. Solid dispersion containing MOLP is not available to date. Therefore, the objective of this study was to develop MOLP-PEG solid dispersions using different methods (freeze-drying, melting, solvent evaporation, and microwave irradiation).

3.2 Materials and Methods

3.2.1 Source of materials and equipment

MOLP was procured from Supa Nutri PTY Ltd in Cape Town, South Africa. The polyethylene glycol polymers (PEG200, PEG400, PEG4000, and PEG6000) were purchased from Spellbound Company, South Africa. Chemicals used in this study were of analytical grade.

The major equipment used in this study included Scanning Electron Microscopy (Zeiss MERLIN FE-SEM) and electron dispersive X-ray (SEM-EDX) which were provided by the Department of Geology at Stellenbosch University. The air oven, domestic microwave (LG), centrifuge (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc., USA), colour spectrophotometry (counter lab), freeze-dryer, and magnetic stirrer were provided by the Food Science and Technology department. Figure 3.1 outlines analyses carried out in this chapter.

3.3 Preparation of Solid Dispersions

Five different methods were employed to form solid dispersions of MOLP using PEG carriers (PEG200, PEG400, PEG4000 and PEG6000). These methods include solvent evaporation, freeze-drying, melting, and microwave irradiation, discussed in sections 3.3.1 to 3.3.5.

3.3.1 Preparation of solid dispersion by the solvent evaporation method

The solvent evaporation method of solid dispersion adapted from Raj & Kumar (2018) and Sapikal *et al*. (2018) with slight modifications was used. Equal amounts (50 g) 1:1 ratio of MOLP and PEGs were accurately weighed. The MOLP and carrier were dissolved in an adequate (60 ml) amount of absolute methanol with constant magnetic stirring at 200 rpm for 20 min. The solvent was then rapidly evaporated with the aid of mild heat up to about 50°C in the air oven for 24 h until complete evaporation and then kept at room temperature for 48-72 h in a desiccator until a dry cake was obtained. Obtained solid dispersion was ground and sieved through a 125 µm sieve. Samples were stored in a desiccator until further use.

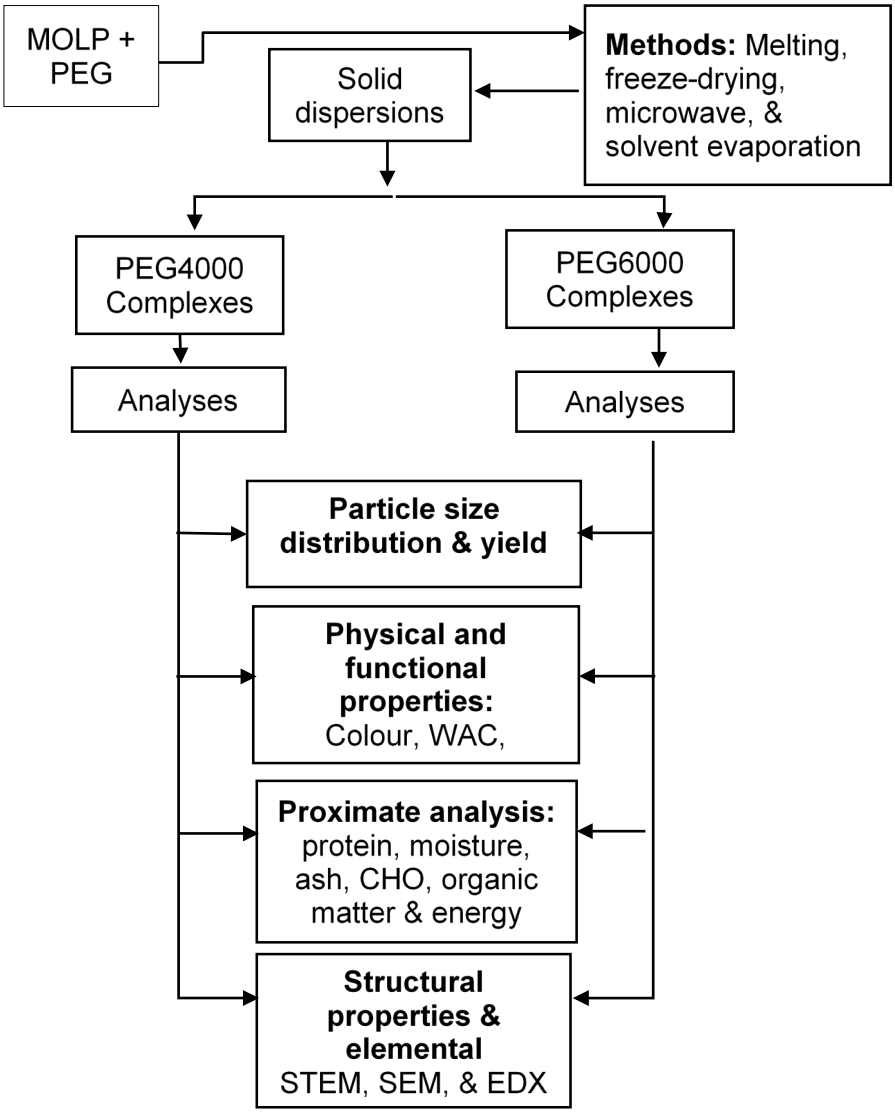

Figure 3.1 Chapter three outline

3.3.2 Preparation of solid dispersion by freeze-drying method

The freeze-drying method adapted from Fitriani *et al*. (2016) with some modifications, was used. MOLP and PEG were mixed with a 1:1 (50 g each) ratio. MOLP dissolved in 5 mL of 96% ethanol, whereas PEG was dissolved in 20 mL of distilled water. The solution was mixed on a magnetic stirrer and then homogenised by gradually introducing the beaker containing MOLP to the beaker containing the PEG under stirring at 500 rpm on a magnetic stirrer for 30 minutes. Once homogeneous, the mixture was dried using a freeze-dryer (Christ Alpha 1-2 LD Plus, France) for 2 days at -50°C. Obtained solid dispersions were triturated and passed through a 150-125 µm sieve, stored in centrifuge tubes, and placed in a desiccator until further evaluation.

3.3.3 Preparation of solid dispersion by melting method

The melting method described by Mogal *et al.* (2012) and Tran *et al.* (2019) with slight modifications, was used. Amounts (50 g) 1:1 of MOLP and PEG were accurately weighed separately into 400 mL glass beakers. The carrier was initially melted using a magnetic stirrer at 58°C for 10 min and the MOLP was added to the molten base to obtain a molecular dispersion. The molten mixture was thoroughly mixed and stirred for 30 min. The melted mass was rapidly cooled and solidified in an ice bath under constant stirring. On solidification, the solid mass was pulverized and sifted through a 150-125 µm sieve. The obtained product was stored in centrifuge tubes and kept in a desiccator until use.

3.3.4 Preparation of solid dispersion by microwave method

The microwave irradiation method adapted from Zawar & Bari (2013), Alshehri *et al.* (2017), Hussain *et al.* (2017), and Kalaiselvi *et al.* (2018), with some modifications, was used. Equal amounts (50 g) 1:1 of MOLP and PEG were mixed in a 400 mL glass beaker and subjected to microwave for 2 min at 360 W in a domestic microwave (LG model). The obtained solid dispersions were cooled and ground using a mortar and pestle and then passed through a 150-125 µm sieve. All samples were stored in centrifuge tubes at room temperature (20^{o}C) until further use.

3.4 The yield of solid dispersions

The yield (%) of SDMOLP was determined by dividing the weight of SDMOLP obtained after the mixing process with the weight of pure MOLP and carrier used in the formulations (equation 3.1).

$$\% \text{ yield} = \frac{\text{SDMOLP weight}}{\text{MOLP+Carrier weight}} \times 100 \qquad \textit{Equation 3.1}$$

3.5 Particle Size Distribution

The particle size of MOLP and SDMOLP samples was determined using a set of eight Endicott test sieves (MINOR, Endicott Ltd., England) ranging from 600 to 75 microns' sieve sizes arranged in decreasing order of pore size. About 50 g of each sample was sieved for 15 min on an Endecott's sieve shaker (Endicott Ltd.). The sample powder retained on each sieve and in the receiver pan was weighed and expressed as the percentage of total sample powder (Oladunmoye *et al.*, 2010).

3.6 Proximate analyses of *Moringa oleifera* leaf powder and solid dispersions

The moisture and ash content in pure MOLP and SDMOLP were determined using the moisture content analyzer (Denver instrument IR-30) and muffle furnace method, respectively (AOAC, 2005). Protein content was determined using a Nitrogen analyzer (Leco-TruSpec-N) furnace set at 950^{o}C, and the protein factor used was 6.25. Fat content was determined using a soxhlet system. The carbohydrates were determined by difference (AOAC, 2005).

%Carbohydrate =100 – (%moisture + %fat + %ash + %crude protein).

The organic matter content was estimated by subtracting the percentages of moisture and ash content from one hundred.

% Organic matter = 100 – (% moisture + % ash)

The caloric value (Kcal) of the sample was calculated using the “Atwater factor” by multiplying the values of the crude protein, lipid, and carbohydrate by 3.99, 9.1, 3.99, respectively, and taking the sum of the product.

3.7 Colour measurement of pure *Moringa oleifera* leaf powder and solid-dispersed *Moringa oleifera* leaf powder

A spectrophotometer (Counter Lab) was used to determine the colour attributes of solid-dispersed MOLP, set to 10° and D65 standard observer. The spectrophotometer was calibrated with white and black plates, followed by calibration on zero. A sample equivalent to 4 g was poured into a petri dish with a diameter of 30 mm, and the reflectance was measured with (L*) lightness, (a*) redness/greenness, and (b*) yellowness/blueness. Measurements were done in triplicate. The total colour difference amongst the samples was calculated by the colour difference equation as shown in equation 3.2.

$$\Delta E = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]} \qquad \textit{Equation. 3.2}$$

Where L*: lightness; a*: redness/greenness; b*: yellowness/blueness

3.8 Functional properties of MOLP and solid-dispersed MOLP

3.8.1 Determination of Water Absorption Capacity

Water absorption capacity (WAC) was determined using a method described by Köhn *et al.* (2015) with some modifications. About 3 g of a sample (MOLP, solid dispersions) was weighed into pre-weighed graduated 50 mL conical centrifuge tubes and mixed with 30 mL of distilled

water with continuous stirring for 5 min. The suspension was allowed to stand at 30 ± 2°C for an hour. The resulting slurry was centrifuged at 3000 rpm for 5 min. Thereafter, the supernatant was decanted, and the tubes were weighed and dried in an oven for 5 h. After drying, the tubes were weighed again, and the water retained per gram sample was calculated using equation 3.3.

$$\text{WAC (\%)} = \frac{\text{Weight of bound water (g)}}{\text{Weight of sample (g)}} \times 100 \qquad \textit{Equation 3.3}$$

3.8.2 Determination of Solubility

The solubility of pure MOLP and solid-dispersed MOLP was determined by slightly modifying the method of Beacht *et al*. (2016). A sample equivalent to 3 g was accurately weighed and dispersed in 30 mL of distilled water in a centrifuge tube. The solution was mixed at high speed of 1000 rpm for 20 min using a vortex. The dispersed powder was then centrifuged at 3000 rpm for 5 min. The supernatant was further filtered using a 0.45 µm filter membrane and the aliquots of the supernatant were carefully pipetted and transferred to a pre-weighed porcelain dish and then oven-dried at 105°C in an air oven for 5 h. The solubility (%) of the samples was therefore calculated by taking the weighted difference using equation 3.4.

$$\text{Solubility (g/100g)} = \frac{\text{Weight of dried sample and dish} - \text{weight of the dish}}{\text{Weight of powder sample}} \times 100 \qquad \textit{Equation 3.4}$$

3.9 Structural properties of *Moringa oleifera* leaf powder and solid-dispersed *Moringa oleifera* leaf powder

3.9.1 Scanning Electron Microscopy (SEM)

The morphology of MOLP and SDMOLP samples was characterised using scanning electron microscopy (SEM). Powder samples were transferred onto double-sided conductive carbon tape and mounted onto an aluminum SEM tub. To improve conductivity, the tubs were coated with a thin layer of gold using a 10 nm (Leica EM ACE200) sputter-coater. Thereafter, the sample was loaded into a Zeiss MERLIN Field Emission Scanning Electron Microscope (Carl Zeiss Microscopy, Munchen, Germany) at the Electron Microbeam Unit of Stellenbosch University's Central Analytical Facility (CAF). A Zeiss Inlens Secondary Electron (SE) Detector, Zeiss Backscatter Electron (BSE) Detectors, and Zeiss Smart SEM software were used to generate images, the acceleration voltage and working distance were 2.0 kV and 6.8 mm, respectively.

Scanning transmission electron microscopy analysis

The scanning transmission electron microscopy (STEM) (A Zeiss EVO40 XVP) equipped with a STEM detector was used to further evaluate the morphology of MOLP and SDMOLP samples.

3.9.2 Electron Dispersive X-ray Spectroscopy (EDX) analysis- Elemental composition

SEM-Energy Dispersive X-ray spectroscopy (SEM-EDS) with EDS Oxford Instruments® XMax 20 mm_2 detector was used to analyse the morphology and the elemental composition of the pure MOLP and SDMOLP. Operating conditions of 20 V accelerating voltage and 8.5 mm were applied. The mineral phases were studied using Philips diffractometer (PW1840), HT generator (PW1729), and Chart recorder (PW8203A).

3.10 Data Analysis

Multivariate analysis of variance (MANOVA) was used to determine the significant differences ($p < 0.05$) in attributes among samples. Duncan's multiple range test was used to separate means where a significant difference existed (IBM SPSS version 26, 2020).

3.11 Results and Discussion

3.11.1 Solid Dispersion Observations

In the preliminary study, solid dispersion complexes of MOLP with polyethylene glycol (PEG200, PEG400, PEG600, PEG4000, and PEG6000) were prepared using kneading, solvent evaporation, melting, microwave irradiation, and freeze-drying solid dispersion methods. On the other hand, solid dispersions made using PEG200, PEG400, and PEG600, on the other hand, were not successful. These solid dispersions were watery and did not solidify after drying, perhaps due to the low molecular weight (MW) of the PEGs. PEGs with low MW do not result in successful dispersions because they are typically available in a liquid state which only wets the compound. Therefore, the results obtained for PEG 200, 400, and 600 in MOLP solid dispersion preparations concur with the ones reported by Biswal *et al*. (2008); Thangamuthu (2009) and Nikghalb *et al*. (2012), that PEGs with MW of 1,500-20,000 are preferred for successful solid dispersions. Thus, the higher the MW, the more viscous and crystalline the PEG polymer is. MOLP solid dispersions, on the other hand, were successfully synthesized utilising PEG4000 and PEG6000 carriers in all procedures. When these solid dispersions were wet, they were dark to olive green, but after drying, solidification, and powdering, they turned green to light green.

The solid dispersions prepared through melting, microwave, and solvent evaporation

methods were hard rock, crystalline and glassy solid mass (Figure 3.2), suggesting that PEGs may have recrystallized upon cooling. In contrast, freeze-dried solid dispersions appeared highly porous and easily grindable; this may be attributed to the lyophilisation process. PEG 4000 and 6000 are hydrophilic, and semi-crystalline carriers are potentially used as solid dispersion carriers. The physical characteristics of PEGs (4000 and 6000) in MOLP solid dispersions observed in this study are similar to the previously reported solid dispersions of Ibrufen (Newa *et al.*, 2008), simvastatin (Bolourchian *et al.*, 2013), enteroxib (Das *et al.*, 2011), epigallocatechin (Cao *et al.*, 2017), and *Kaempferia parviflora* extract (Weerapol *et al.*, 2017). Solid dispersion MOLP-PEG4000 and MOLP-PEG6000) were further characterised for compatibility or interaction (FTIR), crystallinity properties (PXRD), structural properties (SEM), proximate and elemental composition, and functional properties.

Figure 3.2 Pictorials of solid-dispersed *Moringa oleifera* leaf powder prepared through melting method using (A) PEG6000 and (B) PEG4000. (C) pulverised solid dispersion with PEG6000.

3.11.2 Percentage yield of solid dispersions

The yield (%) of the SDMOLP samples is depicted in Table 3.1. The yield of various SDMOLP was within the range of 62.88 to 99.72%. The results revealed that the yield was highest in solid dispersions prepared by the melting method (both PEG6000 and PEG4000) with 99.72% and 99.30%, respectively. Followed by solid dispersions prepared through microwave method (both PEG6000 and PEG4000) with yields of 98.60% and 97.30%, respectively, and freeze-drying solid dispersions (PEG6000 and PEG4000) with 71.60% and 71.59%, respectively. However, the yield of the solvent evaporation solid dispersions was the lowest of all the

formulations, with values 63.90% and 62.88% for PEG6000 and PEG4000, respectively. All the solid dispersions had high yields. Since yield (%) represents the recovery of the SDMOLP from the apparatus, this could mean that melting and microwave products were easily recovered from the container and were relatively more rigid. Similar results are reported in previous studies where solid dispersions prepared by the melting method exhibited higher yields than that for solvent evaporation (Samar, 2015; Polimerleri *et a*l., 2020). This could be because there was no solvent or heat drying involved during melting solid dispersions, as only an ice bath was used for solidification. The lowest yield in solvent evaporation may be attributed to the method of preparation that employed an air oven (±50°C) that imposed maximum loss of materials during collection from the oven. In addition, the product was to the sides of the container more than others. There was no difference in the yield of solid dispersions prepared by the melting method (PEG6000 and PEG4000). More so, there was no significant difference in the yields of the melting method (both PEG6000 and PEG4000) and microwave method (both PEG6000 and PEG4000) solid dispersions. However, a significant difference was observed between the yields of solid dispersions prepared through melting and solvent evaporation methods. Another difference in yields of microwave and solvent evaporation solid dispersions was observed. The yields of solid dispersions (PEG4000 and PEG6000) prepared by the solvent evaporation method did not differ significantly. Moreover, no significant difference among freeze-dried solid dispersion yields. All the solid dispersion yields agree with the values reported in previous studies (Kumavat et al., 2013; Nayak, 2015; Samar, 2015; Sapikal et al., 2018; Polimerleri et al., 2020).

Table 3.1 Yield (%) of solid-dispersed *Moringa oleifera* leaf powder[1]

	Yield (%)	
Method	**PEG4000**	**PEG6000**
Freeze-drying	71.6	71.6
Melting	99.3	99.7
Solvent evaporation	62.9	63.9
Microwave	97.3	98.6

[1]PEG4000: Polyethylene glycol with a molecular weight of 4000. PEG6000: Polyethylene glycol with 6000 molecular weight.

3.11.3 Particle size distribution of *Moringa oleifera* leaf powder and solid dispersions

The cumulative particle size distribution curves of MOLP and SDMOLP samples are shown in Figure 3.4. The percentage of MOLP particles passing through 75, 106, 150, 250, 300, 425,

500 and 600 μm standard sieves were 26.12, 58.63, 85.78, 87.02, 87.98, 97.47, 99.55 and 99.67%, respectively. While that of SDMOLP samples passing through the same standard sieves were in the range 19.32-29.16, 61.71-69.15, 90.35-99.32, 96.14-99.68, 99.40-99.74, 99.60-99.82, 99.70-99.90, and 99.80-99.95%, respectively and significantly varied ($p < 0.05$) among all solid dispersions across all sieve sizes. Mean particle sizes were 176.2 and 116.2 μm for MOLP and solid-dispersed MOLP, respectively. The value for MOLP is in line with the previous work presenting that MOLP particle size ranges from 50 to 100 microns (Ali *et al*., 2019). However, Okoye *et al.* (2013) reported a slightly higher mean particle size (192 μm) for MOLP. The differences could be attributed to different fibre contents and milling methods. MOLP contains fibres (soluble and insoluble dietary), which are difficult to mill to some extent. According to Huang *et al*. (2020), MOLP contains a total dietary fibre that ranges between 25.60 and 29.80 g/100 g. Moreover, the relatively high moisture content might influence its size reduction. It must further be noted that the degree of fineness in milling operation depends on the type and efficiency of the applied machine. The wider particle size distribution of MOLP conformed to the particle size distribution curve (Figure 3.4). Similar tendencies were observed in rice bran dietary fibre (Zhao *et al.*, 2018) and *Dendrobium Officinale* powder (Meng *et al.*, 2017), while contrary results were obtained in sugar beet pulp (Huang *et al.*, 2018) and downy rose-myrtle powder (Gao *et al.*, 2019). The discrepancies may be attributed to the types of raw materials and the grinding method.

In the solid dispersions, more material was retained in the small aperture sieves (150, 106, and 75 μm) while screens with the largest apertures caught the least due to the spherical shape and fineness of SDMOLP samples. The particle size analysis results showed that the mean particle size of SDMOLP samples decreased significantly ($p < 0.05$) compared to the pure MOLP. This, therefore, suggests that the solid dispersion techniques reduced the size of the SDMOLP. The small particle size obtained for SDMOLP samples concurs with previous studies for solid dispersions (Siahi-Shadbad *et al.*, 2014; Ghule *et al.*, 2018; Monschke *et al.*, 2021). Thus, suggesting that the solid dispersion techniques improve the solubility, wettability, dissolution, and bioavailability of the ingredient and increase the surface area of the material through decreased particle size. Therefore, one of the reasons for the higher solubility of the solid dispersions compared to pure MOLP maybe be explained by particle size reduction during the solid dispersion process. Furthermore, particle distribution serves as an indication of flowability (Ali et al., 2018, 2019); SDMOLP exhibited good flow properties than pure MOLP. Powders with broad particle sizes tend to have poor flowability than those with a narrow size distribution. Both the MOLP and SDMOLP were polydisperse but still in good agreement with the specification for food powders that must at least have a particle size of 180 μm. According to the particle size reduction, powders are classified into two types, including (1) polydisperse

or uniform and (2) monodisperse or non-uniform (Chaloupkova *et al.,* 2016; Ali *et al*., 2018). The polydisperse is a bulk powdered material with a range of particle sizes. Most of the powders, especially natural polymers, are polydisperse types, while monodisperse consists of only one size, which is rare (Murdiana *et al.*, 2018).

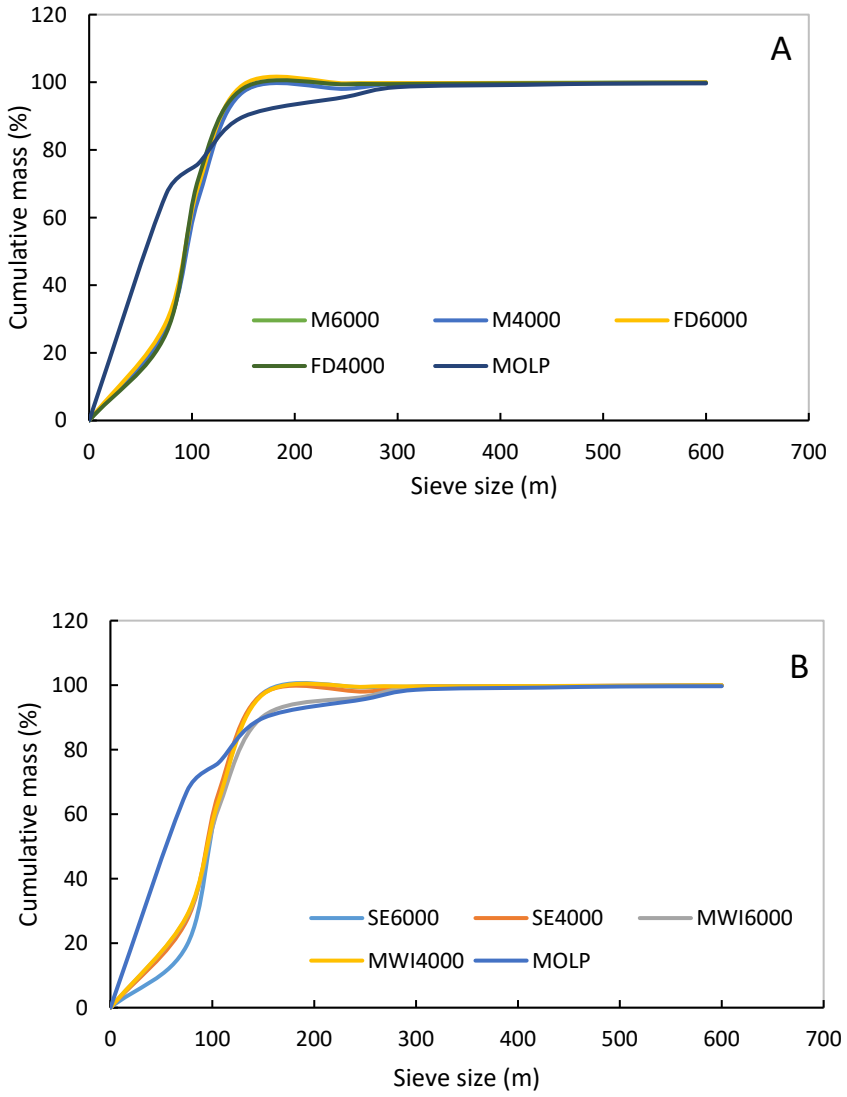

Figure 3.3 Cumulative particle size distribution of MOLP and SDMOLPs prepared by (A) melting (M6000 and M4000), freeze-drying (FD6000 and FD4000) methods, and (B) solvent evaporation (SE6000 and SE4000), and microwave irradiation methods (MWI6000 and MWI4000) methods.

3.11.4 Colour Characteristics of MOLP and SDMOLP

The colour attributes of pure MOLP were lightness (L*), greenness (-a*), redness (+a*),

blueness (-b*), yellowness (+b*) (Table 3.3). Lightness is the luminous intensity of colour measured on a scale of 0 to 100, with 0 indicating black and 100 indicating white. Hue is how the colour of an object is perceived, and chroma is the vividness or dullness of colour, and it suggests the richness or saturation of a colour (Murevanhema & Jideani, 2015). On the colour scale, a* describes the redness or the greenness of colour, with positive a* indicating redness and negative a* indicating greenness. The b* measures blueness when negative and yellowness when positive.

The lightness of MOLP was 53.44, as shown in Table 3.5. The greenness (-a*) was -3.41, and the yellowness (b*) was 21.6. These colour values agreed with the ones obtained for the pure MOLP in previous studies (Ali *et al*., 2014). The solid dispersions prepared with PEG6000 carrier exhibited lightness (L*) ranging from 54.20 to 58.49. There was no significant difference in lightness among solid dispersions. Moreover, there was no significant difference ($p > 0.05$) observed in lightness between MOLP and freeze-dried (FD6000) solid dispersions, MOLP and melting (M6000), and MOLP and microwave irradiation (MWI6000) solid dispersions (Table 3.5). The greenness (-a*) of PEG6000 solid dispersions was in the range of -1.82 (freeze-drying method) to 0.98 (melting method). The freeze-dried solid dispersion was greener compared to other solid dispersions, followed by microwave solid dispersions (-0.52). Other solid dispersions (solvent evaporation and melting method, 0.36 and 0.98, respectively) were less green. When compared to the pure MOLP, there was a significant difference ($p < 0.05$) between MOLP and all the solid dispersions (PEG6000). A significant difference ($p < 0.05$) in greenness was also observed among the solid dispersions. The change in the greenness of solid dispersions is attributed to chlorophyll degradation, which might be due to solid dispersion preparation conditions. Furthermore, drying causes changes in the food properties, including discolouration. The yellowness of solid dispersions ranged from 17.61 (solvent evaporation method) to 21.62 (freeze-drying method). The yellowness of solid dispersions was similar to that of pure MOLP.

Solid dispersions prepared with PEG4000 were lighter than pure MOLP. The lightness of PEG4000 solid dispersion ranged from 52.94 to 56.00 (Table 3.5). There was no significant difference ($p > 0.05$) in lightness among solid dispersions. The greenness (-a*) of the solid dispersions ranged from -1.85 (freeze-drying method) to 0.53 (microwave method). A slight difference in greenness among solid dispersions was observed. The yellowness (b*) of solid dispersions ranged from 14.82 (melting method) to 21.14 (melting method) (Table 3.5). There was no significant difference ($p > 0.05$) in yellowness between MOLP and freeze-dried solid dispersions. However, more lightness and greenness were observed in other solid dispersions, this may be due to chlorophyll degradation to pyropheophytin and pheophytin during drying (Ali *et al*., 2014).

Table 3.2 Colour attributes of MOLP and solid-dispersed MOLP[1,2]

Samples	L*	a*	b*
Pure MOLP	53.4±0.6[a]	-1.72±1.7[a]	26.2±5.8[a]
PEG4000			
Melting	55.2±0.7[b]	-0.12±0.4[b]	14.8±1.0[b]
Solvent evaporation	55.4±0.61[b]	-0.85±0.9[b]	15.9±3.2[b]
Freeze-drying	56.0±0.4[b]	-1.85±1.1[a]	21.1±1.0[c]
Microwave irradiation	52.9±0.6[b]	0.53±1.5[c]	14.9±3.6[b]
PEG6000			
Melting	54.2±1.1[b]	0.98±0.9[c]	18.17±2.3[bc]
Solvent evaporation	54.8±0.3[b]	0.36±0.5[c]	17.61±2.5[bc]
Freeze-drying	58.4±0.5[b]	-1.82±0.7[a]	21.6±1.3[c]
Microwave irradiation	56.0±0.6[b]	-0.52±1.6[b]	20.42±4.0[c]

[1]Values are the mean ± standard deviation of three replicates. Means within a column followed by the same superscript are not significantly [p > 0.05] different.
[2]MOLP: Moringa oleifera leaf powder. PEG: Polyethylene glycol. L*: lightness; a*: red/ green; b*: yellow/blue; MOLP: Moringa oleifera leaf powder; PEG: Polyethylene glycol

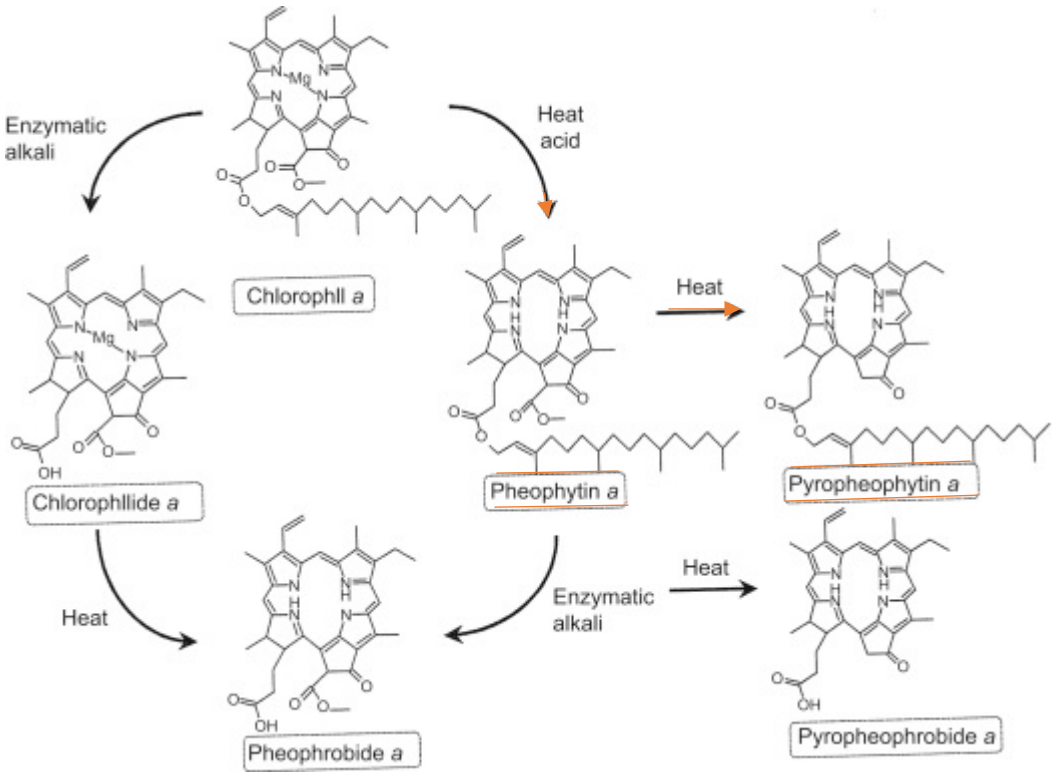

Figure 3.4 Main transformations of the chlorophyll molecule during food processing, storage, or cooking (Roca *et al*., 2016)

Pyropheophytins (PPP) are chlorophyll pigment breakdown compounds from the thermal degradation of leaves (Li *et al.*, 2015). Heat and long storage time break down chlorophyll into pheophytins then into PPP (Figure 3.4). The pure MOLP was lighter, greener, yellower, more saturated, and had a higher hue compared to the solid dispersions. The relatively lower degree of lightness in the MOLP can be attributed to duller colour and low green pigmentation from chlorophyll.

Colour differences among the solid dispersions

A colour difference (ΔE) of 1 is the threshold at which a trained observer would notice the difference between two colours (Sharma, 2005). It is known as a just-noticeable difference (JND). The difference between the two colours can be perceivable but still deemed acceptable. A ΔE between 4 and 8 is acceptable and above 8 is unacceptable and likely to be rejected by consumers (Sharma, 2005). Table 3.6 gives the colour difference between MOLP and the solid dispersions. The solid dispersions showed perceivable and acceptable colour differences with ΔE ranging between 1.06 and 6.48, suggesting that the dispersions could be used interchangeably in products without a noticeable difference.

Table 3.3 Colour differences between pure *Moringa oleifera leaf* powder and solid dispersions[1]

Method	**PEG4000 (∆E)**	**PEG6000 (∆E)**
MOLP-Melting	11.56	8.53
MOLP-Solvent evaporation	10.52	8.97
MOLP-Freeze-drying	5.68	6.82
MOLP- Microwave irradiation	11.51	6.47
Melting-Freeze-drying	6.45	6.18
Melting-Solvent evaporation	1.28	1.06
Melting- Microwave irradiation	2.70	3.28
Freeze-drying-Solvent evaporation	5.40	5.83
Freeze-drying-Microwave irradiation	7.36	3.01
Solvent evaporation- Microwave irradiation	2.91	3.18

[1]∆E: Colour difference; MOLP: *Moringa oleifera* leaf powder; PEG: Polyethylene glycol

The solid dispersions had ΔE above 1, meaning the consumer could perceive the difference. Still, the values are below 8, thus making the difference acceptable. However, the pure MOLP and some solid dispersion (MOLP-Melting, MOLP-Solvent evaporation, and MOLP-Microwave) comparisons for PEG6000 and PEG4000 had ΔE above 8. Their colour differences were very apparent. Except for MOLP and freeze-drying method (for both PEG4000 and PEG6000, 5.68 and 6.82, respectively), ΔE is below 8, suggesting their colour differences may be acceptable.

3.11.5 Proximate compositions of *Moringa oleifera* leaf powder and solid dispersions

Moisture content

The proximate composition for MOLP and SDMOLPs is presented in Table 3.4. The average moisture content of MOLP was 8.44%. The observed moisture value reported in this study is in agreement with the value (8.20%) reported by Okiki (2015) but lower compared to 10% recommended for dried vegetables. Comparatively, significantly lower moisture content was reported for MOLP, ranging from 6.60 to 6.88% in the previous studies (Mikore & Mulugeta, 2017). However, other studies reported variations in moisture content of MOLP, showing relatively high values than the one obtained in this study, between 9.53 and 11.76% (Bamishaiye *et al.*, 2011; Korsor *et al.*, 2017). The differences in moisture content from those reported in previous studies could be due to the drying technique (sun, oven, shade, etc.) and the drying temperature. The moisture content of MOLP in this study is in the expected range, below 10%. High moisture content in leaves makes them highly perishable and susceptible to microbial spoilage during storage. The low moisture of MOLP would prevent the growth of microorganisms and prolong storage life.

The solid dispersions (SDMOLP) revealed moisture content that ranged from 2.65-4.03%. The lowest moisture content (2.65%) was recorded for solvent evaporation-PEG6000 solid dispersion, while the highest value 4.03% was observed in freeze-drying-PEG4000 solid dispersion. There was no significant difference in moisture content among solid dispersions. However, the moisture content of MOLP was significantly higher ($p < 0.05$) than the solid dispersions. The observed relatively low moisture content in the solid dispersions (SDMOLP) could be attributed to the effect of processing techniques and the added drying stage either by an oven, microwave, or freeze-drying, which removed excess moisture. Lower moisture content is desirable in food powders as it indicates that the microorganism's activity would be reduced and thereby increase shelf life.

Protein

The crude protein of MOLP was 28.53%. The proximate analysis showed that MOLP is highly

rich in protein. The observed high protein content in MOLP was in accord with previous findings of 28.00% by Bamishaiye *et al.* (2011) and Okiki *et al*. (2015) and comparable to the reported value for MOLP (28.9 g/100 g) (Devisetti *et al.*, 2016). However, the result was lower than the value 32.18% reported by Moyo *et al.* (2011) and Msabapa (2017), but significantly higher than 22.67% and 24.20% as previously reported by Ali *et al.* (2018). Other similar studies concerning the proximate analysis of MOLP have reported different values for the protein content of MOLP, ranging from 16-40%. These compared favourably with some leafy vegetables commonly consumed in Nigeria, such as *Amaranthus caudatus* (20.59%), cassava leaves (*Manihot utilisima*), 24.88%, *Piper guineeses Schum* (black pepper) (Bolanle *et al.*, 2014) 29.78%, and *Talinum triangulare* 31.00% (Bamishaiye *et al.*, 2011; Bolanle *et al.*, 2014, Okolie *et al.*, 2015). The differences in protein values from those reported in previous studies may be due to agro-climatic conditions during cultivation. Appreciable protein content in MOLP makes it a good source of supplementary protein for man and livestock. MOLP constitutes a good source of protein in regions where animal protein is rare. Thus, it is used to combat malnutrition with regards to protein deficiency.

Lower protein content for solid dispersions (SDMOLP) ranged from 12.43 to 14.23%, with the highest value recorded for melting solid dispersion (PEG6000). Devisetti *et al.* (2016) also reported a low protein content for pre-treated MOLP. No significant difference ($p > 0.05$) in protein contents among SDMOLP samples. However, the protein content in MOLP was comparatively higher ($p < 0.05$) than solid dispersions. Therefore, the lower protein content observed in the solid-dispersed MOLP samples ($p \leq 0.05$) may be due to the leaching of protein during PEG treatment. The interaction between MOLP and PEG gave a significantly different effect on protein content. Moreover, the observed significant decrease in protein contents of solid dispersions can be attributed to the solid dispersion processing techniques and conditions. In addition, the heat might have denatured most of the essential amino acids and protein molecules, further confirming that heat affects the protein content of foods. However, the protein content of solid dispersion is still higher than the ones for leafy vegetables. Thus, indicating their potential for use as a source of good quality. Subject to a high level of intake and amino acid supplementation, it is conceivable that these vegetable proteins could meet quite a large proportion of animal protein requirements. More so, according to Foodstuffs, Cosmetics and Disinfectants Act: Regulations: Labelling and advertising of foodstuffs in South Africa, solid-dispersed MOLP were high in protein because they have protein content not less than 10 g/100 g solids (Gazette, 2002).

Table 3.4 Proximate compositions of pure *Moringa oleifera* leaf powder and solid dispersions of MOLP[1,2]

Samples	Protein (%)	Moisture (%)	Ash (%)	Fat (%)	CHO (%)	OM (%)	Energy (Kcal)
Pure MOLP	28.53 ± 1.6[a]	8.44 ± 1.6[a]	11.81 ± 1.2[a]	4.89 ± 0.8[a]	46.33 ± 2.4[a]	79.75 ± 1.4[a]	344.46 ± 9.2[a]
PEG4000							
Melting	14.23 ± 1.6[b]	3.56 ± 2.9[b]	6.14 ± 0.0[b]	2.85 ± 0.0[b]	73.22 ± 3.[b]	90.30 ± 0.2[b]	404.52 ± 0.6b
Solvent evaporation	12.98 ± 0.8[b]	3.28 ± 0.8[b]	4.03 ± 0.2[b]	2.43 ± 0.1[b]	75.41 ± 1.1[b]	90.69 ± 0.4[b]	386.57 ± 8.1[ab]
Freeze-drying	12.43 ± 0.3[b]	4.03 ± 0.3[b]	5.48 ± 0.06[b]	3.15 ± 0.0[ab]	74.91 ± 0.2[b]	90.49 ± 0.3[b]	377.45 ± 1.9[ab]
Microwave	12.90 ± 1.1[b]	2.73 ± 1.1[b]	5.82 ± 0.6[b]	2.59 ± 0.0[b]	75.96 ± 0.4[b]	91.45 ± 0.9[b]	378.11 ± 3.[ab]
PEG6000							
Melting	13.23 ± 0.6[b]	3.07 ± 0.6[b]	5.66 ± 0.02[b]	2.84 ± 0.1[b]	75.30 ± 2.4[b]	91.27 ± 0.2[b]	374.89 ± 7.27[ab]
Solvent evaporation	12.92 ± 1.0[b]	2.65 ± 1.0[b]	6.16 ± 0.0[b]	2.27 ± 0.0[b]	76.00 ± 0.5[b]	91.19 ± 0.3[b]	375.53 ± 1.3[ab]
Freeze-drying	12.70 ± 0.6[b]	3.95 ± 0.6[b]	5.72 ± 0.0[b]	2.95 ± 0.0[b]	74.68 ± 0.4[b]	90.33 ± 0.1[b]	375.12 ± 2.0[ab]
Microwave	12.52 ± 0.0[b]	3.10 ± 0.0[b]	5.89 ± 0.2[b]	2.75 ± 0.[b]	75.72 ± 0.1[b]	91.01 ± 0.2[b]	377.66 ± 1.4[ab]

[1]Values are expressed as mean ± standard deviation. Means in a column not sharing the same letter are significantly different ($p < 0.05$).

[2]MOLP: *Moringa oleifera* leaf powder. PEG: Polyethylene glycol. CHO: Carbohydrate. OM: organic matter

Ash content

The ash content is generally recognized as a measure of quality for assessing the functional properties of foods (Selladurai, 2018). The ash content of MOLP was 11.88%. The result is in good agreement with the results (11.60%, 12.0%) obtained by Okiki *et al*. (2015). Ash in food contributes to the residue that remains after all the moisture has been removed and organic components (fat, vitamins, protein, organic acid, carbohydrates, etc.) have been incinerated. The ash content thus determines a measure of mineral concentration in dried leaf powder. In line with prior research, the results show that MOLP contains high mineral concentrations. The high ash content of MOLP reflects the mineral content preserved in the food material. Therefore, the results suggest a high deposit of mineral elements in the leaves, further indicating that MOLP could be a good source of minerals as previously reported in the literature (Isitua *et al.*, 2015; Sahay *et al.*, 2017b; Singh *et al.*, 2018b, 2019). Other earlier findings had reported a slightly lower ash content of MOLP that ranged between 8.05 and 10.38% (Okiki, 2015; Sultana, 2020). Variations in MOLP ash contents might be attributed to differences in location, variety, environmental/soil type, and temperature at which the leaves were dried. The ash content of MOLP in this study was comparatively higher than that in soybean (5.60%) or cornmeal (Sharma *et al.*, 2015; Su & Chen, 2020). On the other hand, SDMOLPs exhibited ash contents that ranged from 4.03-6.16%. The highest value was recorded for PEG6000 solvent evaporation solid dispersion while the lowest was obtained for PEG4000 solvent evaporation dispersions. No significant difference existed in ash contents of SDMOLPs. However, a significant difference ($p < 0.05$) between MOLP and SDMOLP was observed. The ash contents of SDMOLPs were similar and comparable to soybean values of 5.60% (Su & Chen, 2020).

Fat content

The fat content of MOLP was 5.13%. This result agrees with previous results that reported fat values to range from 4.03 to 9.51% (Sultana, 2020). This observation was also similar to the findings of other investigators who reported the fat content of MOLP as 4.00% (Okiki, 2015). Moreover, the MOLP fat content obtained in this study was comparable to that of Kenaf leaves (5.70%), as Yee et al. (2020) reported. The fat content obtained in this study is moderate when compared to those from other green leafy vegetables such as *Cardiospermum halicacabum, Premna latifolia, Delonix elata, Argyreia pomacea, Mollugo pentaphylla* and *Pisonia grandis* (2.18-4.75%) (Selladurai, 2018). Dietary fats function in the increase of palatability of food by absorbing and retaining flavours. Accordingly, Okiki *et al.* (2015) postulated that a diet providing 1-2% of its calorific energy as fat is said to be sufficient for human beings, as excess fat consumption is implicated in certain

cardiovascular disorders such as cancer and aging. Therefore, MOLP will provide 5% fat of its calorific energy. Moreover, *Moringa oleifera* leaves contain more dietary polyunsaturated fatty acids (PUFAs) than saturated fatty acids (SFAs). Higher content of PUFAs and a lower amount of SFAs are desirable; thus, the inclusion of PUFAs in the diet is recommended. They can prevent the occurrence of diseases, thereby promoting good health (Moyo *et al.*, 2011; Isitua *et al.*, 2015; Sultana, 2020, Grassland, 2020). The fat content of SDMOLPs was in the range of 2.29 to 3.21%. The highest fat content was observed for freeze-dried solid dispersions (PEG4000 and PEG6000), whereas the lowest values were observed for solvent evaporation dispersions (PEG6000 and PEG4000). According to the Gazette (2002) advertising and labelling Act (Act 54 of 1972), food solids must contain 3 g per 100 g fat to be declared low fat (Gazette, 2002). Hence, the solid dispersions are low in fat. The fat content significantly ($p < 0.05$) decreased in solid dispersions, and processing techniques could explain this. Moreover, fat content in SDMOLPs exhibited a negative correlation with carbohydrates. This suggests that low-fat content had decreased moisture content, which increased total carbohydrates. Thus, high carbohydrate content for SDMOLP was observed in this study. The low-fat content of SDMOLPs was similar to the values (2.18-4.75%) for leafy vegetables such as *Cardiospermum halicacabum, Premna latifolia, Delonix elata, Argyreia pomacea, Mollugo pentaphylla and Pisonia grandis* (Selladurai, 2018). However, the fat contents of SDMOLP observed in this study were comparatively higher than 0.38-1.91% reported for sweet potato leaves (Oduro *et al.*, 2008). Low fat is desirable in foods as it plays a significant role in avoiding obesity.

Carbohydrates

Carbohydrates are valuable components, which are the primary energy sources for the human body. The carbohydrate content for MOLP was calculated as 46.10%. MOLP is high in carbohydrates and has a high caloric value, which can help to meet the body's caloric needs. Carbohydrates are an important part of a balanced and healthy diet, accounting for half of our daily calorie intake. This carbohydrate value of MOLP is similar to those (47.25 and 48.97%) reported by Sultana (2020) and comparable to the ones 54.61 and 57.61% obtained by Valdez-Solana *et al.* (2015). The slight variations in carbohydrates of MOLP obtained in the study and others could be explained through different soil types and organic matter. The carbohydrate content was in the range of 73.16-77.20% among solid dispersions. The carbohydrate content of SDMOLP was significantly higher ($p <0.05$) than the MOLP. The total carbohydrate content was highest in solvent evaporation (PEG4000) solid dispersions while lowest in melting (PEG4000) solid dispersions. There was a

significant increase ($p < 0.05$) in the carbohydrate content of solid dispersions with a corresponding decrease in the protein and fat content. There was no significant ($p > 0.05$) difference among the solid dispersions. The results obtained in this study were comparable with previously reported values of cassava (Chila cultivar) which ranged between 84.32 and 86.57% (Chisenga *et al.*, 2019). Carbohydrates correlated negatively with other proximate contents, protein, fat, fibre, ash, and moisture contents. The negative correlation implies that protein, fat, and moisture contents were the major components impacting carbohydrates, and a decrease in these molecules would lead to a significant increase in total carbohydrates. The findings are also true for the pure MOLP, which was observed to possess a relatively low carbohydrate content than SDMOLP due to its high protein, fat, ash, and moisture contents. Carbohydrates are the principal sources of energy. One of the main functions of the soluble carbohydrate in the body is for energy supply, thus MOLP can be eaten as a vegetable or fortified in foods. The carbohydrate content of SDMOLP is high, therefore suggesting that it could be a good supplement as well as a source of energy and structural materials. Generally, carbohydrates play an essential role in the bulk of the diets as they provide energy to cells such as the brain, muscles and blood (Selladurai, 2018).

The energy value of MOLP was 344.46 Kcal/100 g, while that of SDMOLP was in the range of 374.89-404.52 Kcal/100g. The energy values obtained in this study were similar to previously reported values of 353.03-368.17 Kcal/100 g for MOLP (Okiki, 2015; Sultana, 2020). The energy contents observed in SDMOLPs were relatively ($p < 0.05$) higher than that of MOLP. The slight differences could be due to differences in carbohydrate content. The high energy content in MOLP and SDMOLPs can contribute significantly to the daily caloric requirement of the body. The contribution of energy from plants is important since a sufficient supply of energy in the diet is required for the protein to be utilised (Okiki *et al.*, 2015). The solid dispersion exhibited high organic matter ranging between 90.30 and 91.45%, while that of MOLP was 79.75%.

3.12 Functional Properties of *Moringa oleifera* and Solid Dispersions

3.12.1 Water absorption capacity

The water absorption capacity (WAC) of MOLP and solid dispersions are shown in Figure 3.5 and Table 3.5. MOLP exhibited a high WAC of 345.09%, due to the presence of higher amounts of protein, carbohydrates, and dietary fibre in the leaves. According to Huang *et al.* (2020), MOLP contains a total dietary fibre that ranges between 25.60 and 29.80 g/100 g. Dietary fibre binds and absorbs water in several ways such as through 1) polar interactions involving anions and cations; 2) ionic interactions; 3) hydrogen bonds; 4) hydrophobic interactions; and 5) trapping of water involving capillary action (Chaplin, 2003).

Interactions of protein with water are essential to properties such as hydration and solubility. The WAC is considered a necessary attribute of food ingredients for formulating various value-added food products, including bakery products and beverages (Rafe *et al.*, 2017). The MOLP's WAC agrees with the values reported previously (335%, 158.00%, 415%, 255.50%, and 350%) (Sultana, 2020). Devisetti *et al.* (2016) reported a slightly higher WAC value (434.3 mg/100g) for MOLP than the one obtained in this study. The minor variation may be attributed to the variation of location, time, and position of leaves on the tree during collection and the proximate composition. The polar amino acid residues of proteins have an affinity to water molecules and differences in WAC may be due to the change in contents of these amino acids in MOLP arising from the loss of hydrophilic amino acids during drying and grinding. Comparatively, Osungbaro *et al*. (2012) reported the WAC of cassava leaf flour as 221.80%. MOLP's WAC is comparable to that of cassava leaf flour, suggesting that it may have a physiological role similar to that of cassava leaf flour.

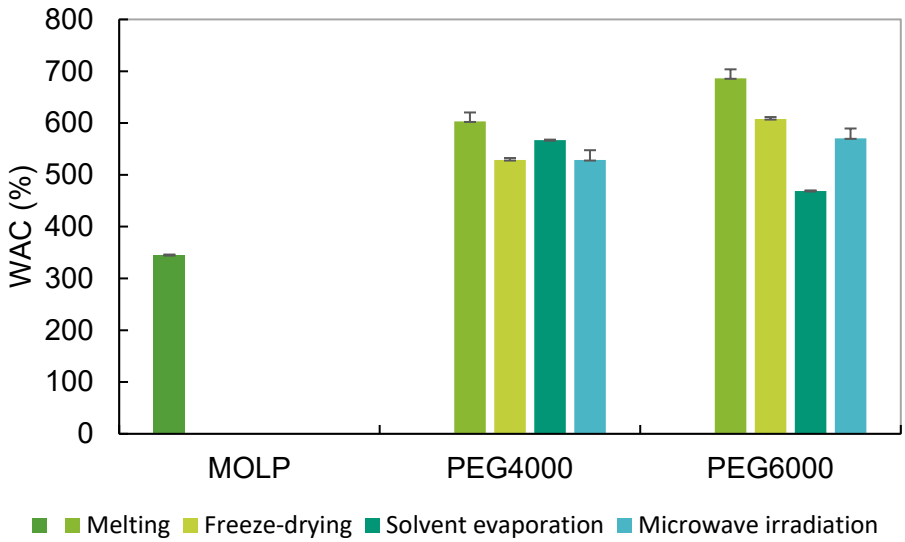

Figure 3.5 Water absorption capacity of MOLP and solid-dispersed MOLP prepared by melting, freeze-drying, solvent evaporation, and microwave methods using PEG4000 and PEG600 carriers

On the other hand, the WAC of solid-dispersed MOLPs (SDMOLPs) ranged from 468.86% solvent evaporation with PEG6000 to 686.37% melting method with PEG6000. SDMOLP formed by melting (PEG 6000 and PEG40000) had significantly greater WACs than solid dispersions prepared by solvent evaporation and microwave. Freeze-dried SDMOLPs, on

the other hand, showed a considerably greater WAC than solvent evaporation (PEG6000) and microwave dried SDMOLPs ($p < 0.05$) (PEG4000). The slight variances in WACs between the eight solid dispersions could be attributable to structural differences and variations in the protein or fibre compositions due to different production methods. Furthermore, as demonstrated by the SEM results of this work, different drying processes may produce significant structural changes in SDMOLP samples, particularly in solvent evaporation and microwave samples. Therefore, the lower WAC of these two solid dispersions could be attributable to the drying processes, which would have lowered the ability of proteins and fibres to absorb water. WACs of SDMOLPs (468.86-686.37%) were significantly higher ($p < 0.05$) than pure MOLP. Hydrophilic components like PEG in SDMOLP could explain this variation. Furthermore, the water absorbed by SDMOLPs may be linked to protein molecules. Proteins are amphiphilic molecules having both hydrophilic (polar) and hydrophobic (nonpolar) properties, allowing them to interact with water in foods. The key determinants impacting WAC include polar amino acid residues of proteins with a high affinity for water molecules, as well as carbohydrate content. Moreover, PEG with optimal molecular weight is more capable of interacting with proteins, which induces the conformational change of proteins through more stable binding sites and stronger interactions with long-chain PEG (Wu *et al.*, 2014). PEG can form strong hydrogen bonds with MOLP via the O-H hydroxyl group (of PEG) and the N-H amide group (MOLP). Enhanced PEG–protein interactions are likely due to the change of hydrophilicity to amphiphilicity of PEG with increasing PEG molecular weight (MW_{PEG}). Butt & Butool (2010), reported that variations in WAC of different foods/flours can be due to different concentrations of protein, their conformational characteristics, and their degree of interaction with water. Increased solubility, owing to solid dispersion techniques and the inclusion of hydrophilic carriers with a solubilizing effect, may potentially explain the high WAC seen in SDMOLPs. This can further be explained by a greater interaction between charged residues and ions derived from the PEG dissociation when these are present in solution, they provide a more extended chain that favours solubility. High WAC is associated with better reconstitution abilities of powder (Ssepuuya *et al.*, 2018). WAC of powders and flours represent their ability to be associated with water (reconstitute) under limited water conditions. The WAC is therefore important for the quality and texture of food products because it stabilises the food against undesirable effects.

Table 3.5 Functional properties of pure *Moringa oleifera* leaf powder and solid-dispersed *Moringa oleifera* leaf powder[1]

Samples	Solubility (%)	WAC (%)
Pure MOLP	25.64 ± 0.6[a]	345.09 ± 3.3[a]
PEG4000		
Melting	56.51 ±0.0[b]	603.98 ±17.4[b]
Solvent evaporation	58.12 ±1.6[b]	566.94 ± 3.9[b]
Freeze-drying	61.17 ±0.1[b]	528.52 ±10.8[b]
Microwave	56.45 ± 1.2[b]	530.40 ±19.0[b]
PEG6000		
Melting	56.90 ±1.8[b]	686.37 ±21.0[b]
Solvent evaporation	53.48 ± 4.3[b]	468.86 ± 10.6[ab]
Freeze-drying	63.60 ±0.3[b]	607.65 ± 11.1[b]
Microwave	61.30 ±1.4[b]	570.45 ± 29.7[b]

[1]Values are expressed as mean ± standard deviation. Means in a column not sharing the same letter are significantly different ($p < 0.05$).
[2]MOLP: *Moringa oleifera* leaf powder. PEG: Polyethylene glycol. WAC: water absorption capacity.

3.12.2 Solubility

Solubility is the most reliable criterion for evaluating powders in an aqueous solution. Table 3.5 and Figure 3.6 detail the solubility of pure MOLP and solid-dispersed MOLP. The solubility of MOLP was 25.64%. The low solubility of MOLP agrees with the report (25.3 mg/100g) of Ali *et al.* (2018) and Devisetti *et al.* (2016), stating that MOLP has poor solubility and dissolution. The low solubility of MOLP can be attributed to the texture of the powder, which was observed to be fluffy, less dense, and thus making it hard to dissolve in water. Furthermore, fibres present in MOLP might not have been broken into tiny pieces during milling, thus resulting in lower solubility. The crude fibres (28-29%) in MOLP powder are primarily cellulose, hemicellulose, and lignin, representing dietary fiber components (Lehman *et al.*, 2018). likely, these components were not degraded into small or superfine molecules during grinding, as confirmed by the particle size distribution of MOLP. In addition, the high protein, minerals, vitamins, and phenolic compounds found in MOLP may also account for its low solubility.

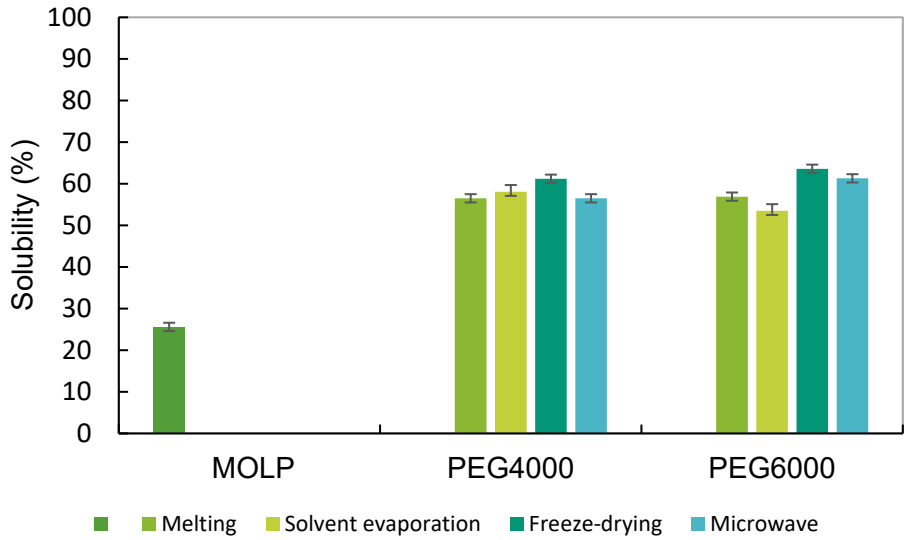

Figure 3.6 Solubility of MOLP and solid-dispersed MOLP prepared through melting, solvent evaporation, freeze-drying, and microwave irradiation using PEG4000 and PEG6000 carriers

On the other hand, the solubility of solid dispersed MOLP varied significantly between 55% and 64%. Solid dispersion of PE6000 by freeze-drying exhibited the highest solubility in the water among all solid dispersions with a solubility of 64%, which is approximately twice as much as that of pure MOLP. Compared to MOLP, all solid dispersions demonstrated improved ($p < 0.05$). The superior solubility profile observed for all dispersions is attributed to the amorphisation of *Moringa oleifera* leaf powder by solid dispersion techniques and wetting characteristics of the hydrophilic carriers (PEG4000 and PEG6000) with MOLP. This result is in agreement with the studies reported by Newa *et al.* (2008), Zawar & Bari (2013) and Kumavat *et al.* (2013) where they concluded that the solubility of Ibuprofen, *Repaglinide, Curcumin,* respectively increased when PEGs were added during solid dispersion processing using freeze-drying, melting, solvent evaporation and microwave irradiation methods. PEG is a hydrophilic polymer used in many biochemical and industrial applications such as food, cosmetics, and pharmaceutical products. It is soluble in most solvents, thus resulting in solid dispersions that are highly soluble. Kumavat *et al.* (2013) also confirmed that PEG as a carrier and coating agent increased the solubility of Curcumin solid dispersions. This might be attributed to improved wetting in the presence of PEG4000 and PEG6000 or intermolecular hydrogen bonding interactions between the functional groups of PEGs and MOLP (O-H and N-H) (as confirmed by the FTIR study). Fibres present

in SDMOLP might have been broken into tiny pieces as a result of the high atomisation of the material resulting in increased solubility. Furthermore, the higher solubility of SDMOLPs could be due to the decrease in total dietary fibre (TDF) content after grinding. This was in turn attributed to the degradation of hemicellulose, cellulose, and lignin into some small molecular substances (Caparino *et al.*, 2012; Huang *et al.*, 2020). Thus, suggesting that the cellular structure was destroyed by grinding and treatment, and the damage increased as the particle size decreased. This was subsequently linked to surface activity and wetness, resulting in less agglomeration and, hence, greater surface area and PEG solubilising action. When the SDMOLP came in contact with water, the polymer particles might have hydrated rapidly (because of the high hydrophilic potency of PEG4000 and PEG6000) into water. This hydration contributed to the increased wettability of the MOLP and to the local enhancement of the MOLP solubility. As a result, PEG has been shown to be effective in enhancing the solubility of solid-dispersed MOLP.

3.13 Morphology of *Moringa oleifera* leaf powder and solid-dispersed *Moringa oleifera* complexes

3.13.1 SEM and STEM morphologies of MOLP and SDMOLPs

Scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) were used to determine the morphology of MOLP and SDMOLP. The STEM was used to provide further insight into the morphology and particle size distribution of the pure MOLP and the solid dispersion complexes. STEM analysis also gives more detailed images at higher resolutions and confirms the presence of specific molecules on the particle surface.SEM and STEM micrographs of MOLP and solid-dispersed MOLP are shown in Figures 3.7-3.11. MOLP micrograph (Figure 3.7) showed a slightly rough surface morphology with a heterogeneous distribution of spherical-like shaped and irregular shaped particles. These results are in good agreement with those reported by Huang *et al.* (2020). The greatest heterogeneity was consistent with the proximate composition: high protein, ash, fat content, and elemental compositions, reported in this study. The STEM micrograph with High-angle annular dark-field scanning transmission (HAADF) at 20 000 X magnification clearly illustrated that MOLP particles do not have a well-defined shape (irregularly shaped). However, they appear to have spherical clusters with spiked crystals (branched structures with various tips grown out of the core). Furthermore, it was observed that MOLP particles have non-uniform morphologies with broad size distributions. This concurs with the SEM results obtained in this study. Tiny granular quasi-spherical and spiky morphologies were observed. Similar structures (spiked crystals and druse) for MOLP SEM were reported in previous studies (Dachana *et al.*, 2010). The morphologies of MOLP were

associated with protein, pigment, and other diverse substances.

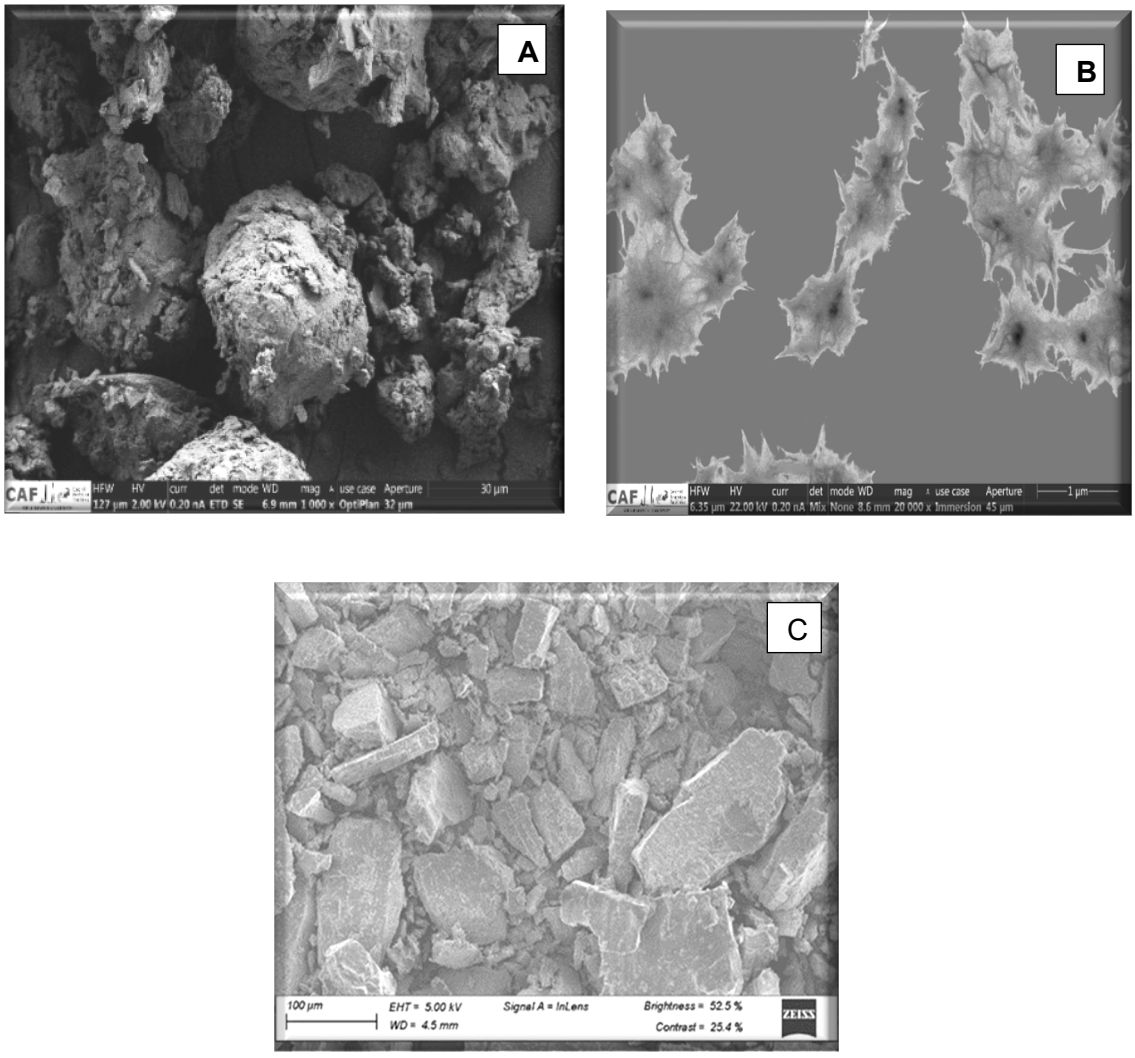

Figure 3.7 (A) SEM (magnifcaction:1000) and (B) STEM (magnification: 20 000) microstructures of pure MOLP and (C) SEM structure of pure polyethylene glycol carrier (PEG6000) 100 µm

Moreover, the spiked spherical cluster crystals were also associated with calcium oxalate which is found predominantly in plants. There are no previous studies reported for STEM images of MOLP. However, the STEM images corresponded to the SEM images of MOLP in this study and previous studies.

3.13.2 SEM and STEM morphologies of solid-dispersed MOLPs

The SEM microstructures of solid dispersions Figures 3.8 to 3.11 appeared as less

spherical-like shaped, more crystalline and irregular shaped with slightly smooth surfaces and non-uniform particle sizes. This confirms the successful interaction between MOLP and PEG. The solid dispersions prepared through the freeze-drying methods (both PEG6000 and PEG4000) (Figure 3.8) displayed highly porous and rougher structures. This can be explained through the lyophilisation process, where the sublimating ice crystal leaves smaller pores. Palzer *et al.* (2012) reported a similar observation for the freeze-dried dairy powder. Solvent evaporation solid dispersions with PEG6000 (Figure 3.9) appeared smoother, resembled angular bricks, compact, and regular-shaped with evenly and homogeneously distributed particles than PEG4000 particles. This could be attributed to the molten mass of the carriers, suggesting that MOLP was well dispersed into the carrier, thus resulting in a solid dispersion that looked like a matrix. Whereas the SEM morphological characteristics of microwave irradiation solid dispersions (both PEG4000 and PEG6000) (Figure 3.10 A and C) revealed that the sample surfaces were crystalline, porous, rougher, and the texture was also crumbly due to the susceptibility of electromagnetic waves as the temperature increases during microwave irradiation. Furthermore, internal pressure increases due to high vapour pressure inside the cells and thus accelerates cell rupture, resulting in porosity (Gasmalla *et al.*, 2017). The results are comparable to the ones reported by Fazaeli *et al.* (2012), which depicted that the SEM microstructure of microwave dried mint leaves was more porous, open, and uniform than that of hot air-dried.

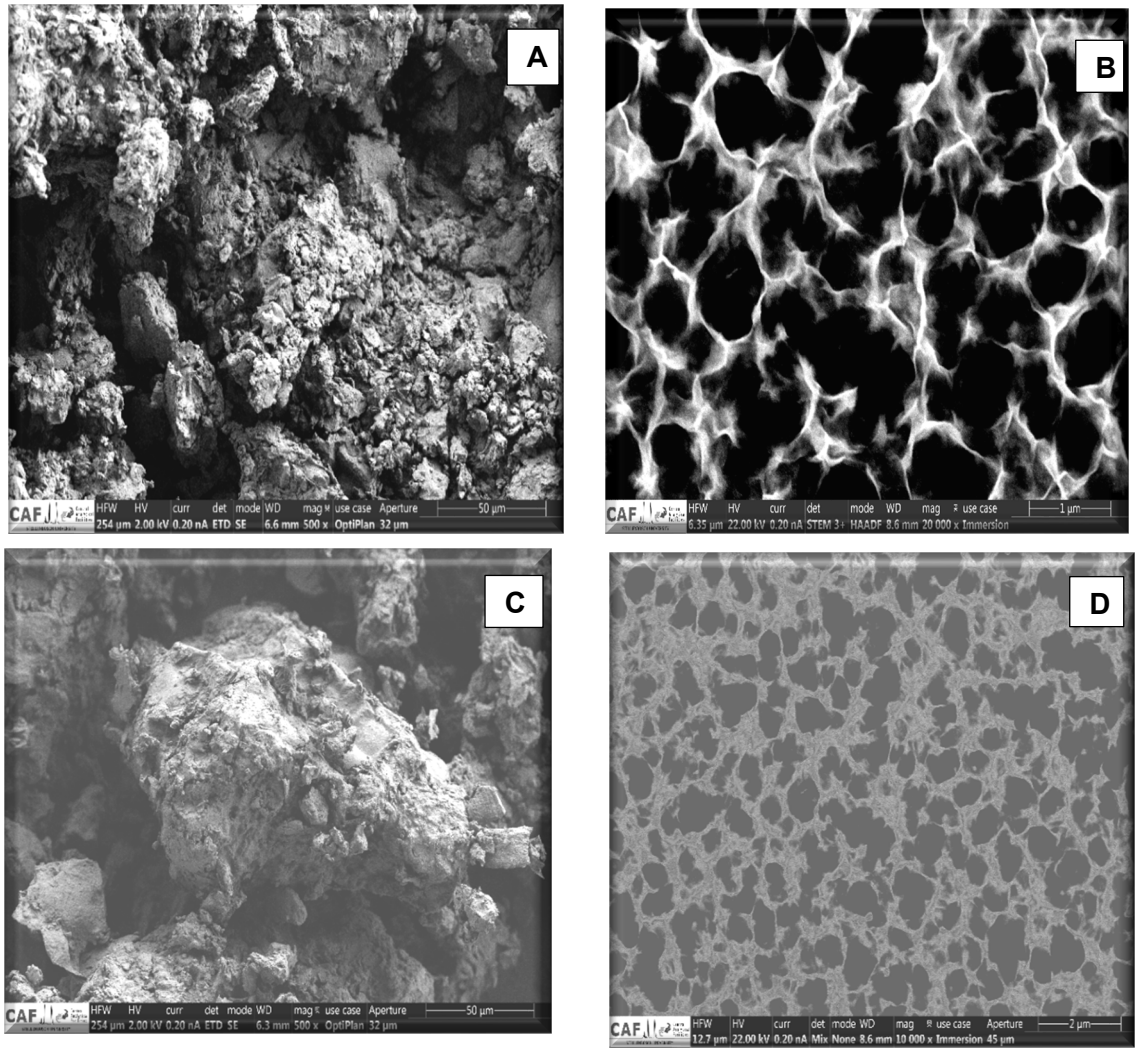

Figure 3.8 SEM and STEM microstructures of SDMOLPs prepared through freeze-drying method using PEG4000 and PEG6000, respectively. (A) SEM and (B) STEM micrographs of MOLP-PEG4000 dispersions. (C) SEM and (D) STEM micrographs of MOLP-PEG600. Note: SEM magnification: 1000 and STEM magnification 20 000.

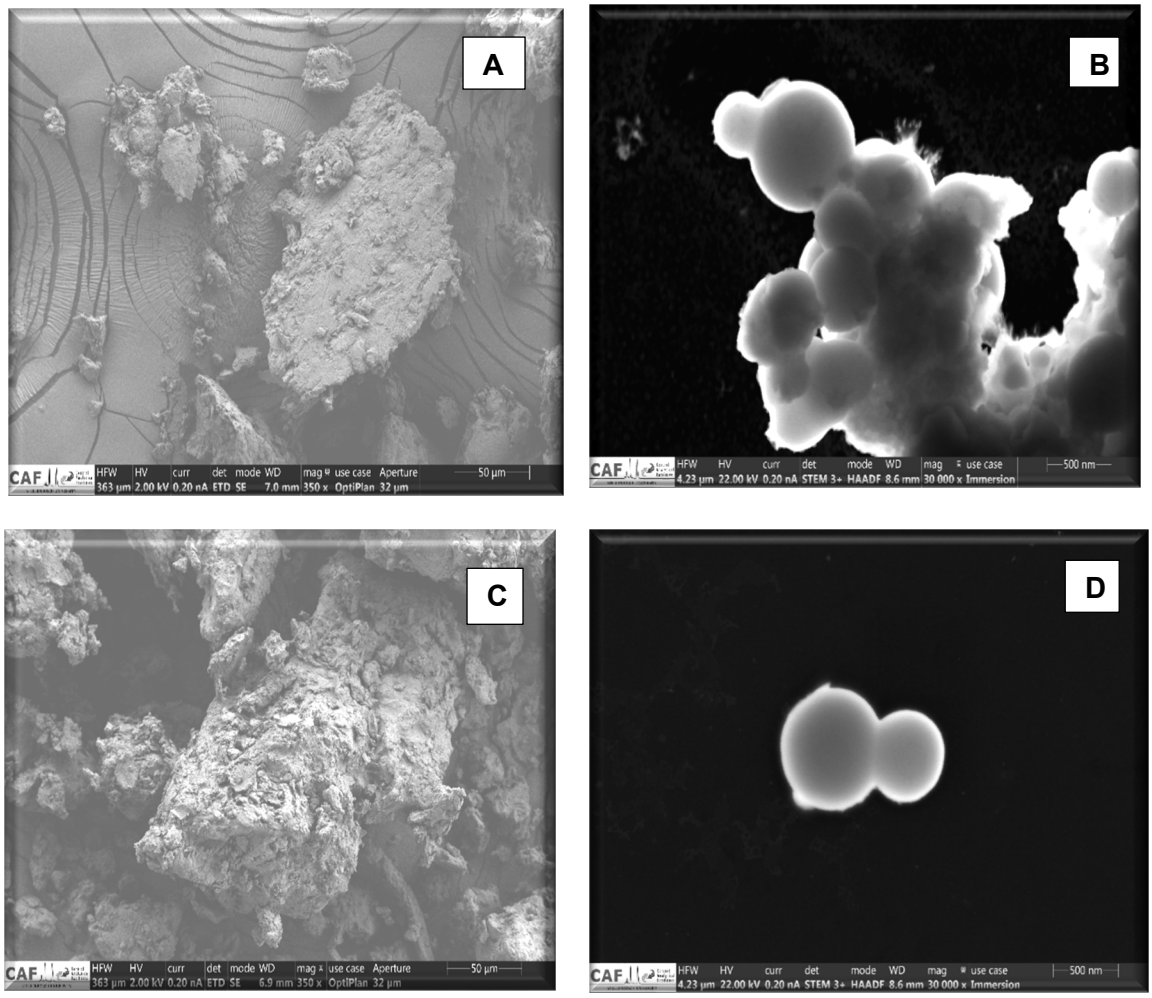

Figure 3.9 SEM and STEM microstructures of MOLP solid dispersions prepared through solvent evaporation method using PEG4000 and PEG6000, respectively. (A) SEM and (B) STEM micrographs of MOLP-PEG4000 dispersions. (C) SEM and (D) STEM micrographs of MOLP-PEG6000. MOLP-PEG600. Note: SEM magnification: 1000 and STEM magnification: 20 000.

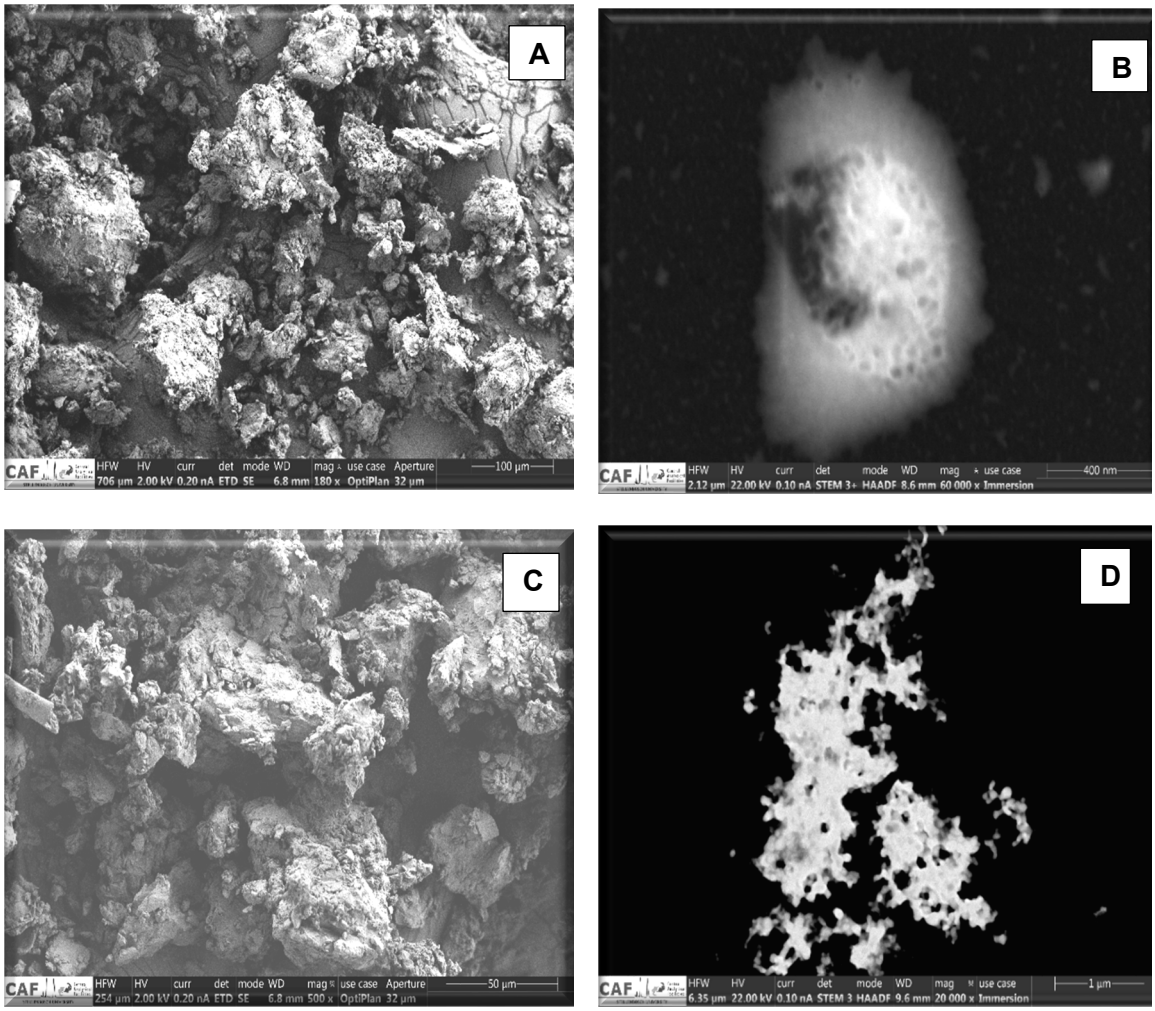

Figure 3.10 SEM and STEM microstructures of MOLP solid dispersions prepared through microwave method using PEG4000 and PEG6000, respectively. (A) SEM and (B) STEM micrographs of MOLP-PEG4000 dispersions. (C) SEM and (D) STEM micrographs of MOLP-PEG6000. MOLP-PEG600. Note: SEM magnification: 1000 and STEM magnification: 20 000.

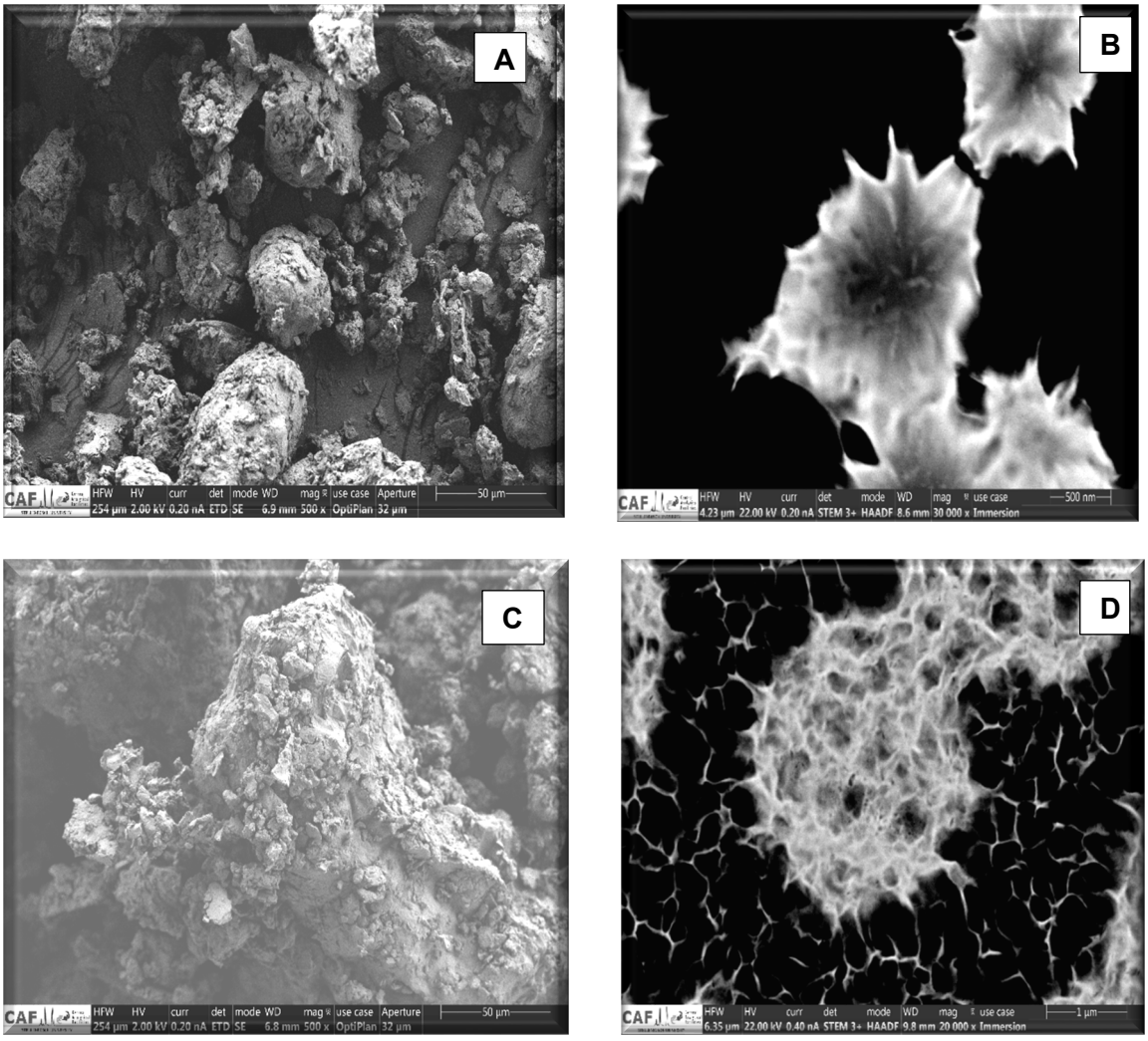

Figure 3.11 SEM and STEM microstructures of MOLP solid dispersions prepared through melting method using PEG4000 and PEG6000, respectively. (A) SEM and (B) STEM micrographs of MOLP-PEG4000 dispersions. (C) SEM and (D) STEM micrographs of MOLP-PEG6000. MOLP-PEG600. Note: SEM magnification: 1000 and STEM magnification: 20 000.

The micrograph of solid-dispersed MOLP prepared by the melting method also revealed crystalline and uniform particles. This finding concurs with the physical appearance observations of the aforementioned solid dispersions, which were highly crystalline and glassy. In the case of solid dispersions, the STEM microstructures are shown in Figures 3.7-3.11 (Bs and Ds). The structural analysis of solid-dispersed complexes by STEM revealed that the samples were clustered globular/ spherical morphologies. However, the surfaces of samples varied significantly in size and shape. The STEM microstructures of solid dispersion prepared through the freeze-drying method (both PEG4000 and

PEG60000) (Figure 3.8B and D) were observed to be porous. This concurs with the SEM structures. The big intra pores were attributed to the characteristic freeze-drying (lyophilisation) process. During freeze-drying, ice sublimation causes significant changes in the shape and volume of the food products. Similar results were observed for freeze-dried chitosan hydrogels, revealing a highly porous surface morphology (Falsafi *et al.*, 2020). The micrographs of solid dispersions prepared by a solvent evaporation method (Figure 3.9B and D) were more globular, spherical-shaped, clustered, and cloud-like globes. The structural changes may be due to the dehydration and volatilisation of raw materials (Dahlberg, 2010; Singh *et al.*, 2017). Singh *et al.* (2017) reported similar morphology (spherical shape) for Elmisartan solid dispersion prepared with poloxamer 188 and modified locust bean gum carriers. Micro pores were also observed on the STEM microstructure of microwave solid dispersions (Figure 10B and D). The pores could be attributed to the effect of microwave heating which causes an increase in internal pressure due to high vapour pressure inside the cells and thus cell rupture which thereafter results in porosity. Minute differences were observed on the SDMOLP structures (micro and macrospores), thus indicating the existence of intermolecular interactions, perhaps through hydrogen bonding or van der Waal interactions between MOLP and PEGs as confirmed from our PXRD and FTIR studies. These findings further demonstrated that MOLP was thoroughly mixed in carriers with little loss of crystallinity. The STEM analysis for solid dispersions showed transformations in the surface morphology as compared to pure MOLP. The STEM images correspond to the SEM images as well as the SEM-EDX images.

3.14 Elemental composition of *Moringa oleifera* leaf powder and solid dispersions determined by SEM-Energy Dispersive X-ray Spectroscopy (EDX)

Moringa oleifera leaf powder

Scanning electron Micrographs-Energy-dispersive X-ray spectroscopy (SEM-EDX) is a characterization technique that provides an elemental composition of various constituent elements in a material. SEM-EDX technique determines the distribution of various elements on the surfaces. Table 3.6 illustrates the elemental composition of MOLP and SDMOLPs. The EDX spectrum of MOLP is shown in Figure 3.12. The results of mineral composition of MOLP as tableted in Table 3.6 exhibited a high concentration of Ca (40.56%), K (8.29%), Mg (16.02%), P (13.71%), S (21.32%), Fe (6.09%), Cu (4.93%) and Zn (0.40%). The elemental analysis of *Moringa oleifera* revealed that it is high in biologically significant elements with therapeutic properties, suggesting that it could supplement macro and microelements in the body. The results obtained in this study are similar to those obtained

Table 3.6 EDX elemental (weight %) composition of MOLP and solid dispersions[1,2]

Sample	Ca	Mg	P	Fe	Cu	S	Zn	Na
Pure MOLP	40.56 ± 6.14[a]	16.02 ± 7.69[a]	13.71 ± 1.13[a]	6.09 ± 4.78[a]	4.93 ± 0.11[a]	21.32 ± 2.38[a]	0.40 ± 0.08[a]	3.47 ± 2.70[a]
PEG4000								
Freeze-drying	45.84 ± 3.88[a]	6.69 ± 9.17[b]	2.22 ± 0.66[b]	1.56 ± 2.20[b]	6.70 ± 7.83[a]	36.98 ± 23.0[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Solvent evaporation	46.87 ± 1.07[a]	8.92 ± 2.08[c]	13.52 ± 3.41[a]	3.75 ± 3.87[b]	7.29 ± 1.79[a]	17.63 ± 0.49[a]	1.48 ± 2.08[a]	0.56 ± 0.78[a]
Melting	65.05 ± 4.54[c]	11.53 ± 10.3[c]	3.79 ± 3.41[b]	0.15 ± 0.21[b]	5.33 ± 3.67[a]	13.65 ± 0.21[d]	0.03 ± 0.04[a]	0.48 ± 0.67[a]
Microwave	55.96 ± 2.69[d]	6.04 ± 3.54[b,c]	7.20 ± 2.44[c]	2.13 ± 3.01[b]	2.97 ± 4.20[a]	22.22 ± 0.51[a]	3.26 ± 4.60[ab]	0.28 ± 0.39[a]
PEG6000								
Freeze-drying	64.83 ± 9.16[b]	4.24 ± 3.61[b]	1.73 ± 1.05[b]	3.20 ± 4.53[b]	5.65 ± 7.02[a]	18.64 ± 4.74[a]	0.34 ± 0.47[a]	1.40 ± 1.97[a]
Solvent evaporation	38.28 ± 1.51[a]	4.26 ± 6.02[b]	2.77 ± 3.91[b]	3.88 ± 0.19[b]	27.05 ± 25.0[b]	18.33 ± 0.35[a]	0.66± 0.93[a]	0.67 ± 0.95[a]
Melting	60.42 ± 5.06[c]	17.97 ± 1.48[a]	11.16 ± 8.51[a]	1.45 ± 1.89[b]	2.33 ± 0.82[a]	6.00 ± 1.07[c]	0.00 ± 0.00[a]	0.68 ± 0.95[a]
Microwave	38.31 ± 5.44[a]	10.02 ± 3.32[c]	6.61 ± 0.42[c]	2.53 ± 2.76[b]	7.00 ± 3.76[a]	24.14 ± 5.58[a]	8.49 ± 4.72[b]	2.93 ± 3.94[a]

[1]Values are expressed as mean ± standard deviation. Means in a column not sharing the same letter are significantly different ($p < 0.05$).
[2]MOLP: *Moringa oleifera* leaf powder. Ca: calcium; Mg: magnesium; S: sulphur; P: phosphorus; Fe: iron; Zn: zinc; Na: sodium

by Raju *et al.* (2019) using the same technique, which exhibited 76.70% Ca, 2.53% Cu, 15.64% Zn, and 5.03% Mg. However, slight variation in the mineral composition may be explained by the difference in soil, weather, and maturity of the plant leaves, since the macronutrients of the plants might vary according to biotic and abiotic conditions of the environment as well as the maturity of the leaves (Melo *et al.*, 2013). MOLP mineral results also complement the previous studies that MOLP is a source of macro and microelements. The minerals found *in Moringa oleifera* leaves play a curative and preventive role in combating human disease (Jideani & Diedericks, 2014; Murdiana *et al*., 2018; Kim & Kim, 2019). For example, calcium as a macro element is a multifunctional nutrient essential to the body's metabolism, and calcium deficiency leads to osteoporosis. Therefore, *Moringa oleifera* leaf powder is known as a natural remedy for osteoporosis. Furthermore, magnesium intake is good for diabetes (Valdez-Solana *et al.*, 2015). The elemental composition of the MOLP revealed low contents of zinc. The finding is in agreement with several studies that reported low zinc content and no zinc (Bello *et al.*, 2017). Some elements, including titanium, silicon iron, and chlorine were detected in small concentrations, as evidenced by the smaller EDX peaks and low intensity. Compared to the daily requirement of elementals, Moringa leaves are a good dietary source for calcium, sulphur, phosphorous, and magnesium, and one micro-element: copper.

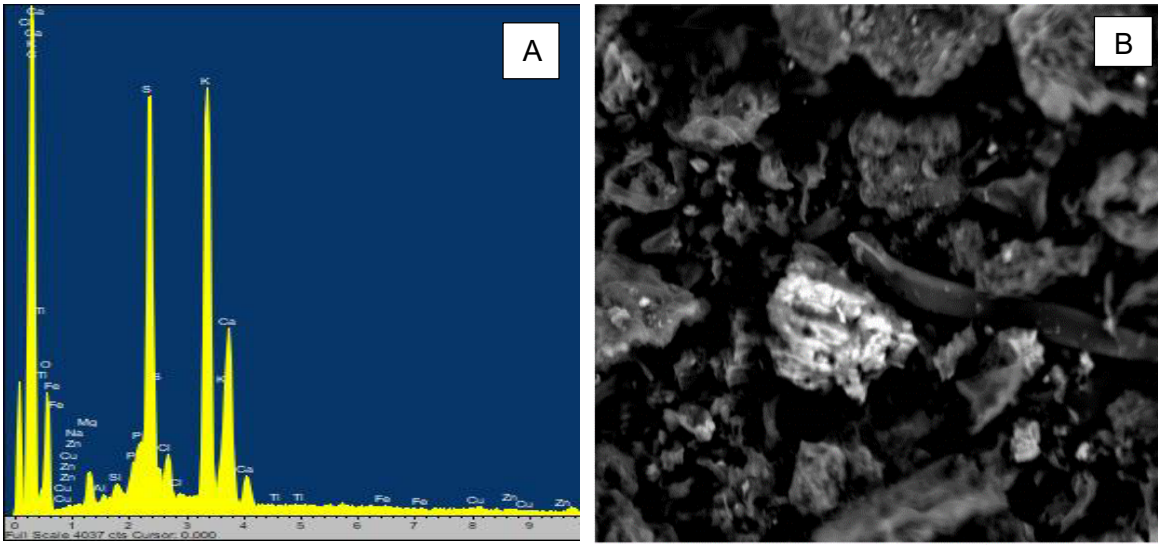

Figure 3.12 EDX spectrum of pure *Moringa oleifera* leaf powder and its SEM site of interest micrograph 100 µm. Note: Accelerating voltage: 15.0 kV; magnification: 100

Given the recommended dietary allowance (RDA) for minerals: calcium (1000 mg/day); phosphorus (800 mg/day); magnesium (400 mg/day), and iron (8 mg/day), MOLP could cover RDA and contribute substantially for improving human diet (Oulai *et al.* 2014). The corresponding SEM micrograph of the point of interest for MOLP is illustrated in Figure 3.12b. The micrograph appears to have a non-homogeneous and rough surface with presumably pored interspaces. Moreover, the micrograph shows the leaf cells with various crystals, such as protein and fibre, pigment, and other diverse substances (Huang *et al.*, 2020). The surface morphology of *Moringa oleifera* powder showed that the powder was an assemblage of fine particles which did not have a regular, fixed size and shape. The particles were granular, spherical, and long strands of various dimensions and all of them contained a large number of steps surface with edges and pores. This proved the heterogeneity of MOLP. Similar observations were reported by Araujo *et al* (2013).

Freeze-drying

The freeze-drying method in Figure 3.13 revealed almost all the elements reported in the MOLP EDX spectrum except for zinc and sodium in FD4000 solid dispersion. The EDX spectra of freeze-dried solid dispersions (both FD6000 and FD4000) exhibited prominent peaks of calcium, phosphorus, magnesium, sulphur, and small peaks of zinc, sodium, and iron in the accelerating voltage of 0.2-3.8 keV. It can be deduced that the stronger the elemental peak intensity the higher the elemental composition, and the smaller the peak the lower the concentration of the element in that sample. The elemental composition of FD6000 solid dispersion, shown in Table 3.8, revealed a high concentration of calcium (64.83%) followed by sulphur (18.64%), copper (5.65%), magnesium (4.24%), iron (3.20%), phosphorus (1.73%), sodium (1.40%) and zinc (0.34%). Whereas FD4000 solid dispersion showed the highest concentration of calcium (45.84%), sulphur (36.98%), copper (6.70%), magnesium (6.69%), phosphorus (2.22%), iron (1.56%), and the lowest ones being zinc and sodium. Solid dispersion (FD6000) had significantly higher ($p < 0.05$) calcium than FD4000 and MOLP. However, there was no significant ($p > 0.05$) difference in zinc, copper, iron, sodium, and sulphur between FD6000 and MOLP. Given the recommended dietary allowance (RDA) for minerals: calcium (1000 mg/day); phosphorus (800 mg/day); magnesium (400 mg/day) and iron (8 mg/day), FD6000 and FD4000 both could cover RDA and contribute substantially for improving human diet (Oulai *et al.,* 2014). Significant

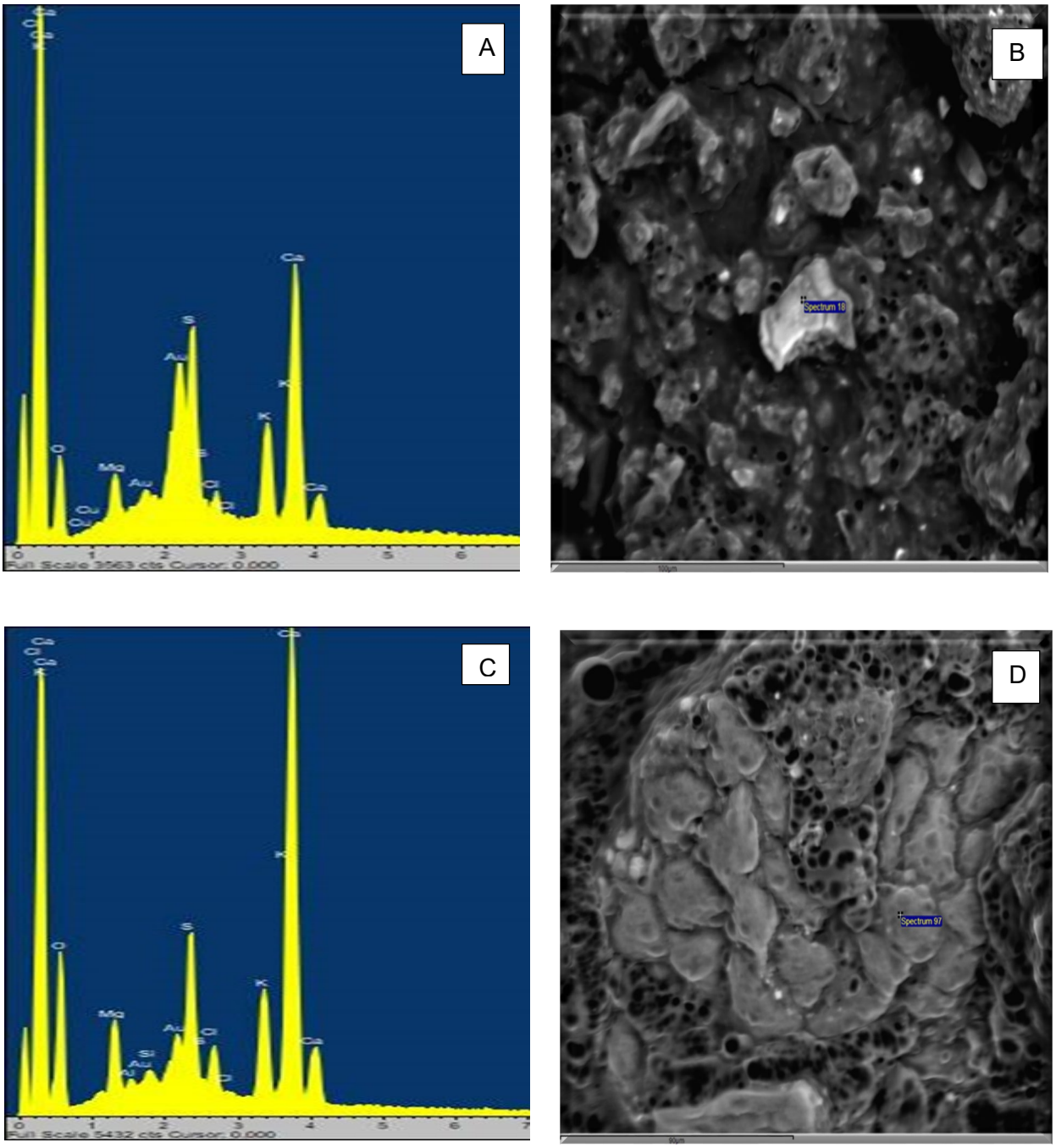

Figure 3.13 EDX spectra and SEM micrographs (site of interest) of MOLP solid dispersions prepared through freeze-drying method using PEG4000 and PEG6000, respectively. (A) EDX spectrum and (B) its SEM site of interest micrograph of MOLP-PEG4000 dispersions. (C) EDX and its SEM site of interest micrograph of MOLP-PEG6000 dispersions. Note: Accelerating voltage: 15.0 kV; 100 µm, magnification: 100

difference existed in macro-and micro-elements between FD4000 and MOLP. Variations in elemental composition among the freeze-dried solid dispersions may be attributed to different polyethylene glycol carriers used. The results obtained in this study are comparable to the ones reported by Raju *et al.* (2019) for MOLP and cassava leaf powder by Kolawole *et al.* (2017). As it can be seen in Figures 3.5b and d, the micrographs of freeze-dried solid dispersions (PEG6000 and PEG4000) exhibit variation in pore size and there is no clear pore arrangement. The macro-pores in the solid dispersions were larger than the ones observed in the pure MOLP structure. However, the solid dispersion by the PEG4000 carrier showed even larger macro-pores than the PEG 6000 solid dispersions. The large pores are thought to be due to the method of preparation which involved a lyophilisation process. Freeze-drying is carried out in two stages; the product is first frozen and then the ice is removed by sublimation directly from the solid to the vapour phase. During freeze-drying, ice sublimation causes significant changes in the shape and volume of the food products. Depending on the process conditions, pores or gaps with different characteristics are created by the ice crystals which sublimated. Similar observations were recorded by Palzer *et al.* (2012) and Fazaeli *et al.* (2012) for freeze-dried food products. The bulk density decreased (as evidenced in this study), and porosity increased when decreasing the applied pressure during freeze-drying, thus resulting in carbonaceous surfaces. Moreover, the pores were observed to be inter pores, intra pores, and organic matter pores. The micrographs show crystals that are attributed to diverse substances such as protein, among others.

Melting

The EDX spectrographs with corresponding SEM micrographs of solid dispersions (M4000 and M6000) prepared by the melting method are illustrated in Figure 3.14. Typical strong peaks of calcium elements were observed in spectrographs of both M4000 and M6000 solid dispersions. The elemental composition in Table 3.8 shows that M4000 had high concentration of calcium (65.05%) followed by sulphur (13.65%), magnesium (11.53%), copper (5.35%), phosphorus (3.79%), iron (0.15%), sodium (0.48%) and 0.03% zinc. M6000 also displayed high calcium content of 60.42% followed by 17.97% magnesium, phosphorus (11.16%), sulphur (6.00%), copper (2.33%), iron (1.45%), sodium (0.68%) and zinc (0.00%). These solid dispersions are sources of calcium and other macro elements. Calcium is the major constituent of bone and teeth and is needed for the regulation of nerve and muscle function. The calcium in the two solid dispersion was significantly ($p < 0.05$) higher than in MOLP. There was no significant ($p > 0.05$) in Mg, P, Cu, Zn, and Na concentrations between M6000 and MOLP. Slight differences in elemental composition

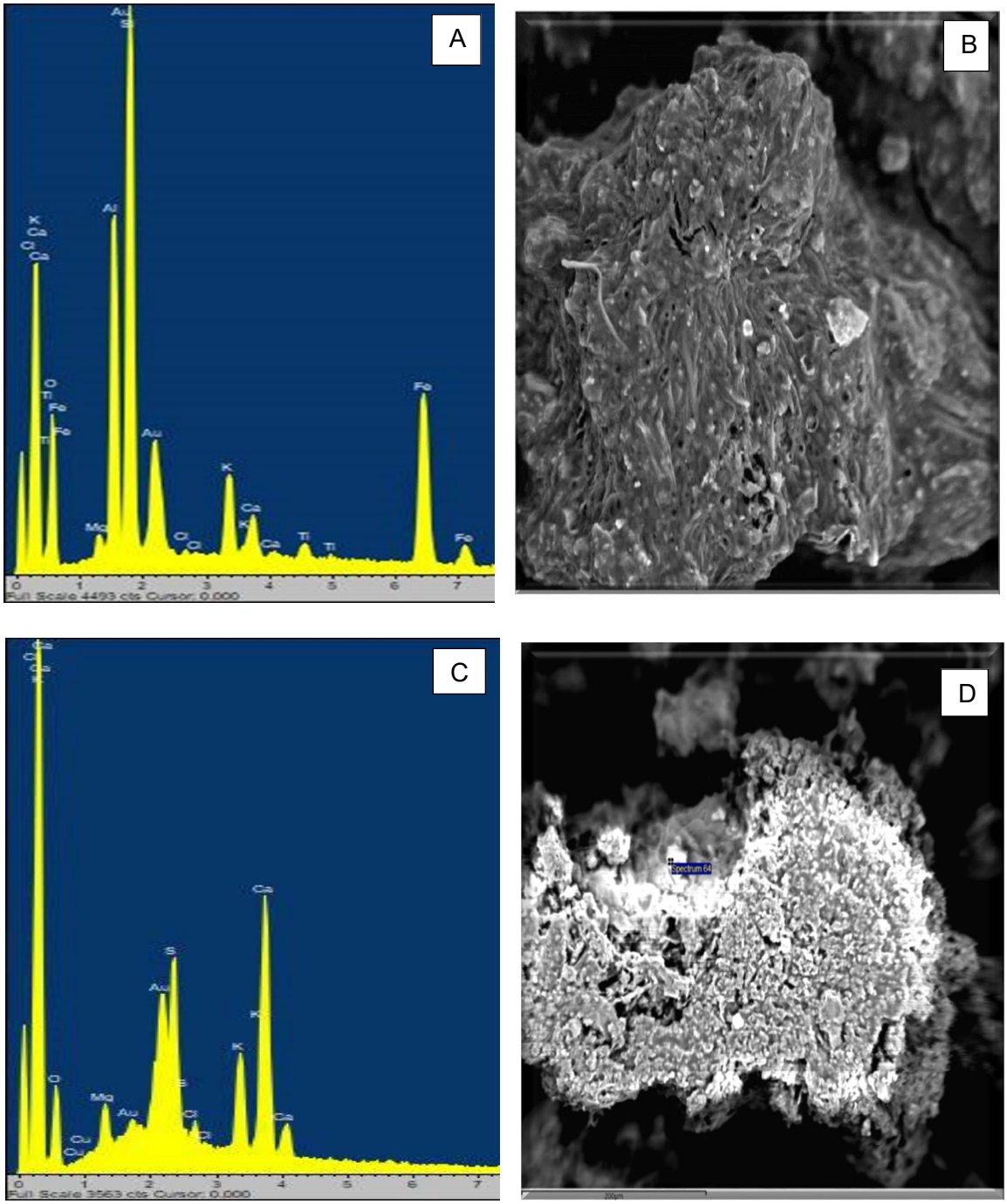

Figure 3.14 EDX spectra and SEM micrographs (site of interest) of MOLP solid dispersions prepared through melting method using PEG4000 and PEG6000, respectively. (A) EDX spectrum and (B) its SEM site of interest micrograph of MOLP-PEG4000 dispersions. (C) EDX and its SEM site of interest micrograph of MOLP-PEG6000 dispersions. Note: Accelerating voltage: 15.0 kV; 100 µm, magnification: 100

between M6000 and M4000 may be due to varying carriers (PEG6000 and PEG4000) that have slightly different physical and chemical properties. The SEM site of interest micrographs for the melting method, as shown in Figures 3.14b and d, revealed morphologies that are stone-like/shaped and lightly porous and smooth surfaces compared to the pure MOLP. Some scattered particles were also observed. Moreover, the surfaces were uneven. Small and few pores were observed. It was further noted that the structure of MOLP is completely different from the melting method-based solid dispersions. This indicates that a kind of new structure was formed in solid dispersions. These findings showed that MOLP was thoroughly mixed in the carriers with loss of crystallinity. Moreover, there was no significant difference between the PEG6000 and PEG4000 structures. The solid dispersion morphologies appeared uniform and homogeneously mixed mass with wrinkled surfaces.

Solvent evaporation

Figure 3.15 represents the EDX spectra, and SEM micrographs of solid dispersions (SE6000 and SE4000) prepared through solvent evaporation using PEG6000 and PEG4000 hydrophilic carriers. The spectra of solid dispersions showed the presence of calcium, sulphur, potassium, phosphorus, magnesium, copper, zinc, iron, and sodium. Prominent and strong peaks of calcium, sulphur, magnesium were observed in both spectra. This corresponds to their high elemental concentration. Likewise, small elemental peaks of iron and sodium confirm that the elements were detected in smaller concentrations. Almost all the elements found in pure MOLP were detected in the solid dispersions. The mineral content of solid dispersions is given in Table 3.8. Calcium content was relatively high in both solid dispersions. SE6000 solid dispersion exhibited the highest concentration of Ca (38.28%), Cu (27.05%) and S (18.33%), followed by lowest Mg (4.26%), Fe (3.88%), P (2.77%), Na (0.67%) and Zn (0.66%) concentrations. While SE4000 displayed high concentration of Ca (46.87%), S (17.63%), P (13.52%), Mg (8.92%), Cu (7.29%), Fe (3.75%), Na (2.93%) and the lowest Zn concentration (1.48%). SE6000 and SE4000 both showed the highest Ca, S, Cu, and Mg concentrations and lowest microelements contents. According to the recommended dietary allowance (RDA) for minerals: calcium (1000 mg/day); phosphorus (800 mg/day); magnesium (400 mg/day) and iron (8 mg/day), solid dispersions could cover RDA and contribute substantially for improving human diet (FND, 2005). It's worth underlining that calcium and phosphorus are associated with the growth and maintenance of bones, teeth, and muscles. Sodium less than 5% has been recommended for the prevention of high blood pressure (Selladurai *et al.*, 2018). The calcium in SE4000 (46.87%) was significantly ($p < 0.05$) higher than MOLP

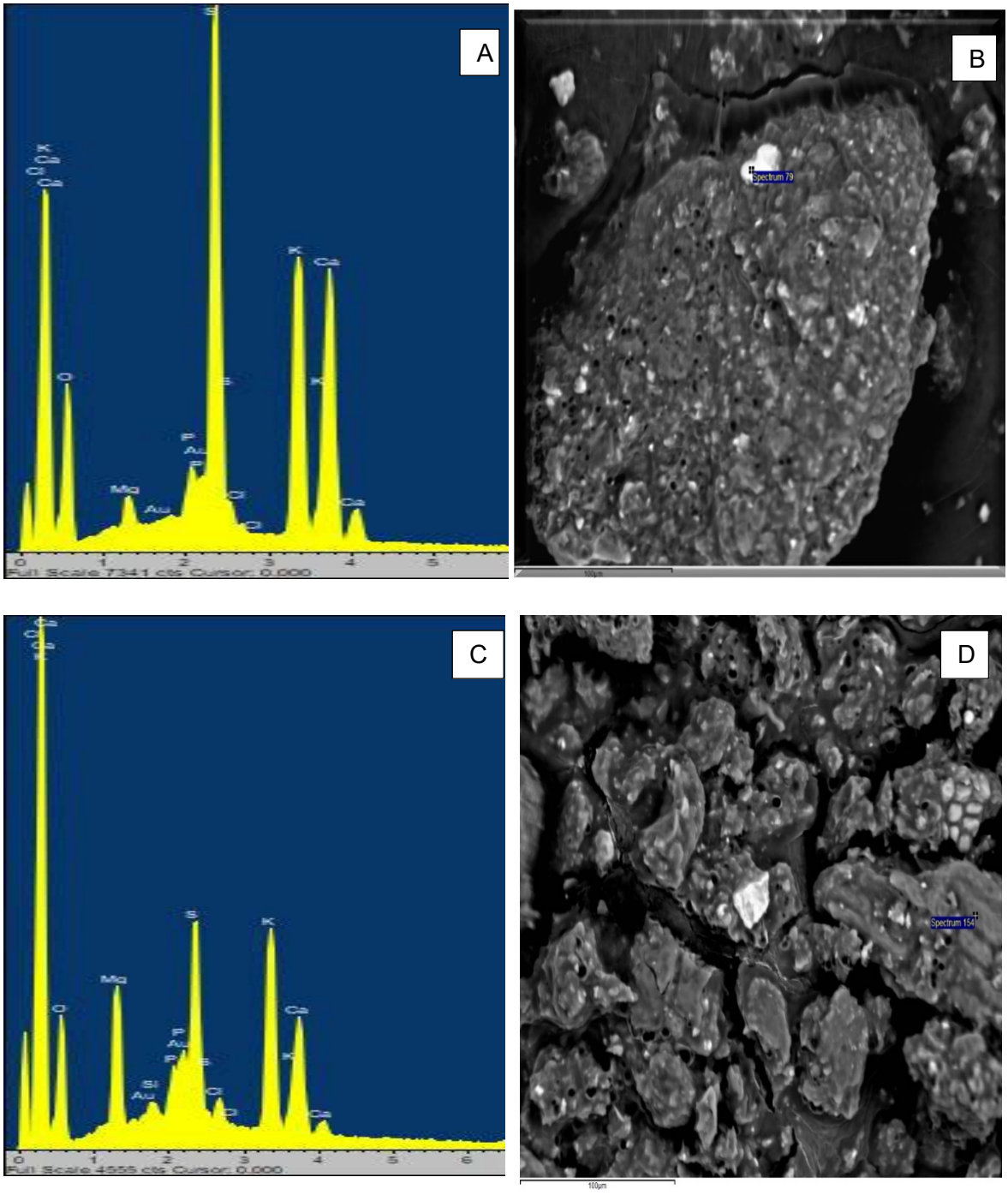

Figure 3.15 EDX spectra and SEM micrographs (site of interest) of MOLP solid dispersions prepared through the solvent evaporation method using PEG4000 and PEG6000, respectively. (A) EDX spectrum and (B) its SEM site of interest micrograph of MOLP-PEG4000 dispersions. (C) EDX and its SEM site of interest micrograph of MOLP-PEG6000 dispersions. Note: Accelerating voltage: 15.0 kV; 100 µm, magnification: 100

(40.56%) and SE6000 (38.28%), While SE6000 calcium was comparatively lower than MOLP. Moreover, no significant difference existed in copper, iron, sulphur, and sodium between SE4000 and MOLP. More so, there was no significant ($p > 0.05$) difference in iron, sulphur and zinc between SE6000 and MOLP. The macro minerals and trace minerals obtained in this study are high compared to cassava leaves (Kolawole *et al.*, 2017) and other domesticated leafy vegetables (Aletor *et al.*, 2002; Arasaretnam *et al.*, 2018; Punchay *et al.*, 2020). The differences in elemental composition between SE6000 and SE4000 solid dispersion may be attributed to different carriers used as well as varying nutritional content i.e., different ash content. The SEM point of interest micrographs for EDX (Figure 3.7b and d) revealed large crystal mass, stone-shaped morphologies with slightly rough surfaces, few and small spherical pores. Furthermore, the SEM structures appeared uniform and homogeneously mixed mass, unlike the pure MOLP structure. This confirms the success of solid dispersion complexes. The small pores observed on surfaces of the solid dispersions were also observed on the MOLP micrograph. Moreover, the small crystal fragments were confirmed from the PXRD analysis. It was discovered that the diverse conditions of solid dispersion preparation had a substantial impact on the surface morphology of various solid products.

Microwave

Figure 3.16 shows EDX spectrographs and SEM micrographs of solid dispersions (MWI6000 and MWI4000). The EDX spectra reveal a strong signal in the calcium, sulphur, potassium, and magnesium (0.5-4 keV) regions. Calcium, potassium, and sulphur had high accumulation in both solid dispersions. The mineral composition of the solid dispersions is tableted in Table 3.8. MWI6000 exhibited the presence of Ca (38.31%), S (24.14%), Mg (10.02%), Zn (8.49%), Cu (7.00%), P (6.61%), Na (2.93%) and Fe (2.53%). SE4000 also showed high Ca (55.96%), S (22.22%), P (7.20%), and Mg (6.04%) concentrations followed by low Zn (3.26%), Cu (2.97%), Fe (2.13%) and Na (0.28%). The solid dispersions analysed in this study contained relatively high amounts of calcium, comparable with the value reported for MOLP (40.56%). Moreover, calcium and other macro minerals values are extremely higher when compared with leafy vegetables, as reported by Arasaretnam *et al.* (2018). Low values for sodium obtained in this study are recommended for the

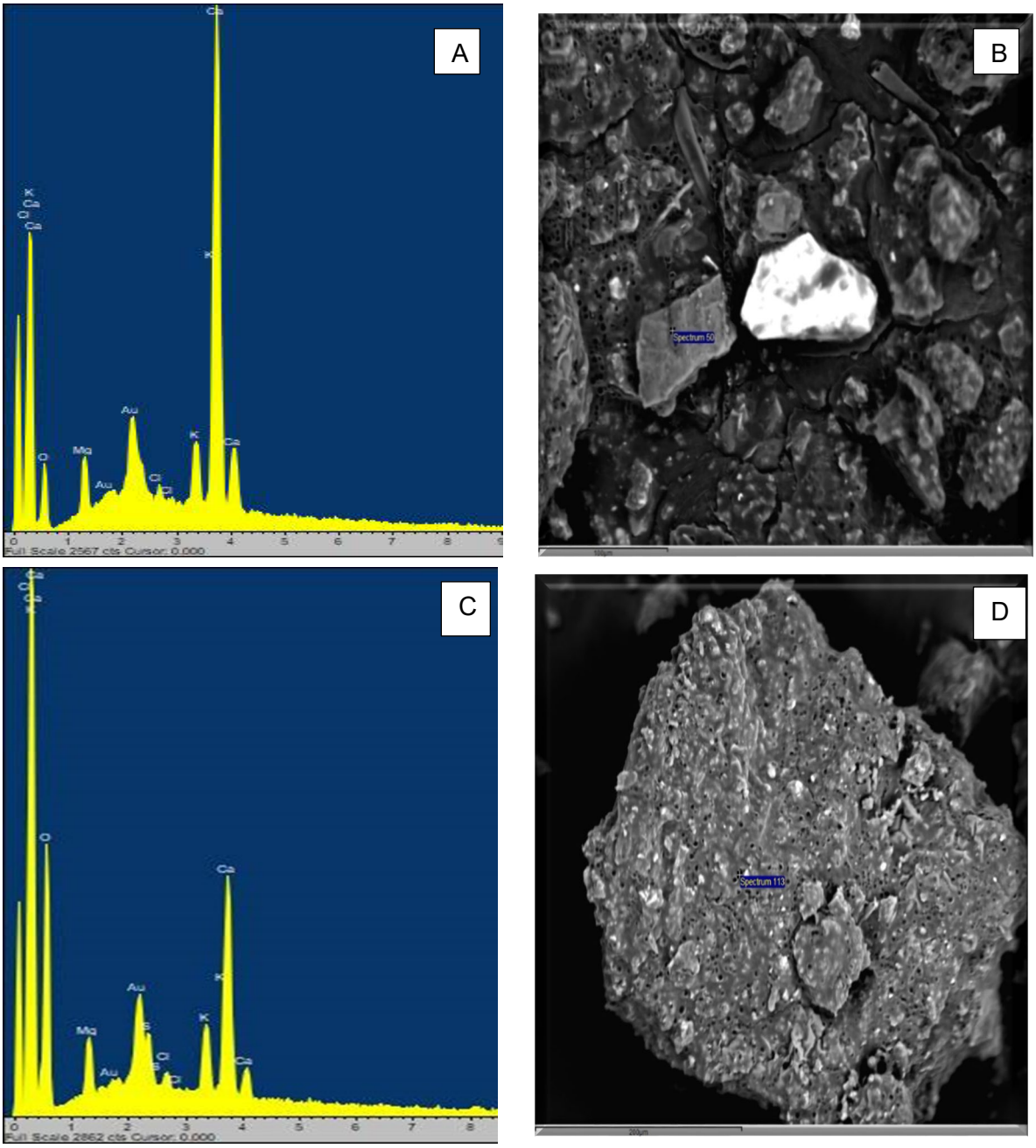

Figure 3.16 EDX spectra and SEM micrographs (site of interest) of MOLP solid-dispersions prepared through the microwave irradiation method using PEG4000 and PEG6000, respectively. (A) EDX spectrum and (B) its SEM site of interest micrograph of MOLP-PEG4000 dispersions. (C) EDX and its SEM site of interest micrograph of MOLP-PEG6000 dispersions. Note: Accelerating voltage: 15.0 kV; 100 µm, magnification: 100.

prevention of high blood pressure. In terms of SEM micrograph, solid dispersions (Figure

3.26b and d) appear to have smaller pores than pure MOLP, almost the same as the pure MOLP micrograph. However, solid dispersion with PEG6000 reveals crystal, stone-like shapes similar to the structure of pure PEG6000 (Figure 3.9), which shows crystal rock-like rectangular shapes. This is demonstrated by the PXRD data, which indicate significant crystallinity. Moreover, less rough surfaces are also seen on the surface of the solid dispersions compared to pure MOLP. There is no considerable variation in the pore size of the solid dispersion. The pores were classified as inter pores and intra pores.

3.15 Conclusion

This study demonstrated the feasibility of using PEG4000 and PEG6000 as hydrophilic carriers in the development of solid dispersions of MOLP. Solid-dispersed MOLPs were successfully prepared through melting, freeze-drying, solvent evaporation, and microwave irradiation methods as indicated by the improved aqueous solubility and WAC in solid-dispersed MOLP. This might be attributed to improved wetting in the presence of PEG4000 and PEG6000 or association between the functional groups of carriers and MOLP. Furthermore, biochemical information on nutritional and mineral compositions suggests that SDMOLPs are good sources of macro and micronutrients and that they will contribute greatly towards meeting nutritional requirements for normal growth while providing adequate protection against diseases arising from malnutrition. The solid-dispersed MOLP will be useful as functional ingredients in food, food, and beverage fortificant and nutraceutical formulations.

3.16 References

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CHAPTER FOUR
CRYSTALLINITY, THERMAL AND COMPATIBILITY PROPERTIES OF SOLID-DISPERSED *MORINGA OLEIFERA* LEAF POWDER

Abstract

Moringa oleifera leaf powder (MOLP) has been identified as the most important functional ingredient owing to its rich nutritional profile and healthy effects. The functional properties of this ingredient can be enhanced through the use of solid dispersion technology. This study aimed to investigate the effects of polyethylene glycols (PEGs) 4000 and 6000 as hydrophilic carriers and solid dispersion techniques (freeze-drying, melting, solvent evaporation, and microwave irradiation) on the crystallinity and thermal stability of solid-dispersed *Moringa oleifera* leaf powders (SDMOLPs). SDMOLPs were dully characterised using powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FTIR). The PXRD results revealed that the solid dispersions were partially amorphous with strong diffraction peaks at 2θ values of 19° and 23°. The calorimetric and thermogravimetric curves demonstrated that PEGs conferred more stability on the dispersions. The FTIR results revealed the existence of strong intermolecular hydrogen bond interactions between MOLP and PEG functional groups. MOLP solid dispersions may be useful in functional foods and beverages and nutraceutical formulations.

4.1 Introduction

Solid dispersion technology is a solubility enhancement technology in which poorly water-soluble ingredients are mixed in an inert matrix with a hydrophilic carrier resulting in solid dispersions with improved solubility, dissolution, bioavailability, and stability (Zhu *et al.*, 2012; Lan *et al.*, 2019). Moreover, off flavours from food ingredients like pea protein isolate have been reported to be mitigated with this technique (Lan *et al.*, 2019a). The mechanism for increasing the solubility and dissolution rate of the ingredient is the included reduction of particle size to molecular size ratio. Furthermore, the ingredient is converted from crystalline to amorphous form, a high-energy state that is extremely soluble. (Zawar & Bari, 2013). However, the physical state of the solid dispersion depends on the physicochemical properties of the carrier and ingredient, the ingredient-carrier interactions, and the preparation methods. Polyethylene glycols (PEGs) with molecular weights of 1500−20,000 are commonly used to manufacture solid dispersions. The specific benefit of PEGs in preparing SD is that they are miscible/soluble in numerous used organic solvents. The melting points of PEGs are under 65°C [e.g., the melting ranges of PEG4000 and PEG6000

are 50−60 and 55−63°C, respectively] Zawar & Bari (2013). Lower melting points of PEGs are helpful in the manufacturing of solid dispersions by different methods. Furthermore, they are also able to improve the wettability of poorly soluble compounds. PEG4000 and PEG6000 are partially crystalline. Zhu *et al.* (2012) reported that, for dispersions prepared with a semi-crystalline polymeric carrier, the ingredient could be amorphous, crystalline, or semi-crystalline. The phase behaviour can be very complex. Thus, the identification of the physical state of the ingredient in solid dispersion is important. It is equally important to understand the crystallisation behaviour of both the carrier and the ingredient since it will affect the microstructure of the solid dispersion, and subsequently influence the dissolution behaviour and solubility. Several researchers have investigated the effects of solid dispersion on properties of food ingredient powders, such as beta-carotene (Ishimoto *et al.*, 2019), pea protein isolate (Lan *et al.*, 2019), *Rebaudioside D* (a natural sweetener from plant Stevia), *Kaempferia parviflora* extract (Weerapol *et al.*, 2017). Up to now, no systematic study has been reported about solid dispersion technology on the MOLP. However, Huang *et al.* (2020) studied the influence of superfine grinding on the structural and thermal properties of MOLP. The findings indicated that superfine grinding and particle size influenced the physicochemical, thermal, and structural properties of MOLP. More so, some previous studies have investigated the effect of PEG molecular weights on the dissolution rate and physicochemical characteristics of the solid-dispersed ingredients (Bolourchian *et al.*, 2013). Key factors that affect the properties of solid dispersions include glass transition temperature (T_g), molecular mobility, miscibility, and crystallinity (Janssens & Van den Mooter, 2009; Ma & Williams, 2019). These factors must be evaluated to ensure a stable solid dispersion. Only a few characterisation methods have been reported on the evaluation of solid dispersions. These include; powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), among others (Zhao *et al.*, 2014; Delgado *et al.*, 2016; Alshehri *et al.*, 2017; Recharla *et al.*, 2017; Hassouna *et al.*, 2019; Oliete *et al.*, 2019). FTIR is used to detect the vibration in the energy distribution of interaction between ingredients and carriers, and it is also applied to follow changes in bonding between functional groups (Pang *et al.*, 2015; Raj and Kumar, 2018; Sharma, 2019). Thermo-analytical techniques (DSC and TGA) are employed to characterise thermal stability and thermal properties (da Silva *et al.*, 2019). Furthermore, DSC and X-ray diffraction are other advanced analytical techniques that are used to determine the amorphisation or crystallisation of ingredients in polymers based on changes in thermal behaviour and crystallinity of ingredients (Enose *et al.*, 2014). Although a recent study reported on the thermal and crystallinity characterisation of MOLP (Huang *et al.*, 2020), this is the first study reporting the use of MOLP to form solid dispersions with

polyethylene glycol, as stated in Chapter 3. Here, a further investigation of the effect of polyethylene glycols (PEG4000 and PEG6000) on crystallinity, thermal, and interaction properties of the MOLP-PEG solid dispersion is described.

4.2 Materials and Methods

4.2.1 Source of materials and equipment

Moringa oleifera leaf powder (MOLP) was procured from Supa Nutri PTY Ltd in Cape Town, South Africa from which the solid-dispersed MOLPs (SDMOLPs) were produced as detailed earlier (Chapter 3).

The major equipment utilised in this study includes Differential Scanning Calorimetric (Perkin Elmer, DSC 6000, United Kingdom), TGA (Perkin Elmer, TGA 4000), and FTIR (Perkin Elmer ATR-FTIR), sourced from Textile Department, CPUT. Powder X-ray Diffraction (PXRD) (D2 Phaser, Bruker AXS, D2 Phaser A26-X1-A2BOB2A, D 76181 Karlsruhe, Germany) was sourced from the Chemistry department, Cape Peninsula University of Technology. Figure 4.1 outlines the analyses that were carried out in this chapter.

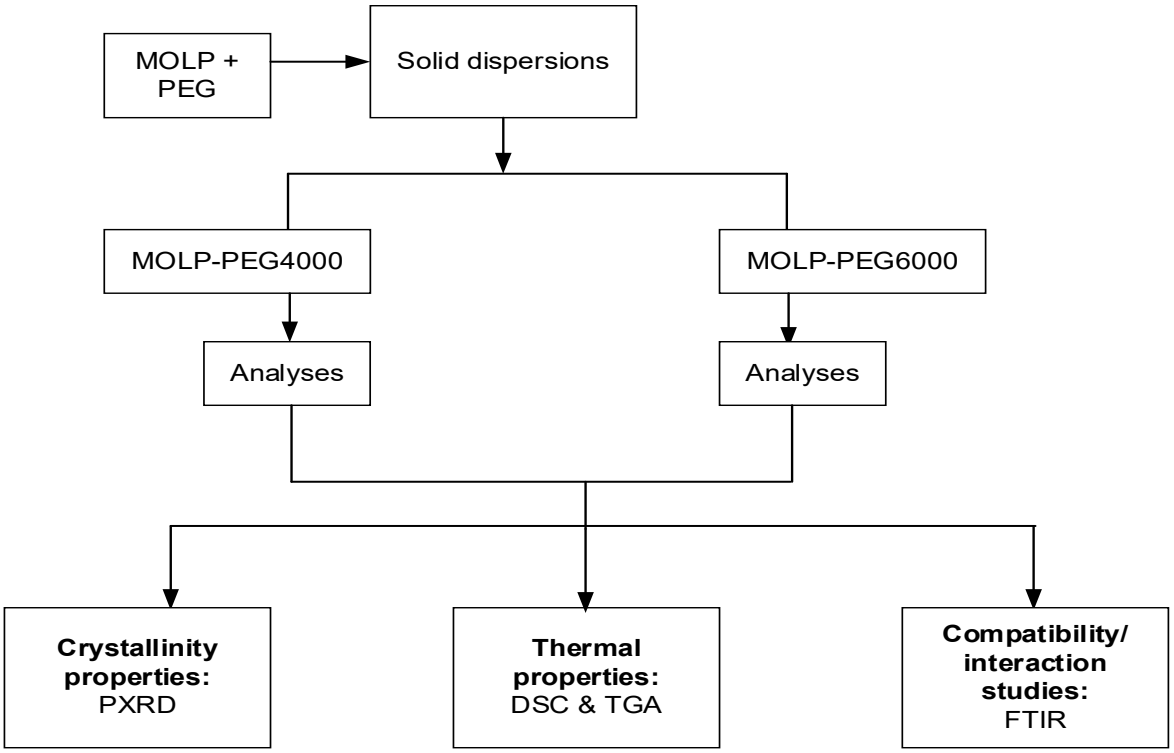


Figure 4.1 Chapter four outline.

4.3 Characterisation of MOLP and Solid Dispersions

4.3.1 Powder X-ray Diffraction (PXRD) analysis

Solid-state properties such as the amorphous and crystalline nature of the MOLP, PEG, and SDMOLP formulations were assessed by PXRD diffraction utilising an X-ray diffractometer, equipped with a CuKa radiation source (1.5406 Å) with the generator current and voltage of 10 mA and 30 kV, respectively. The samples mounted on plate holders were measured for diffraction patterns ambient conditions by scanning in the (2θ) range of 5–50^{o} using a 0.02^{o} (2θ) step size and a time constant of 2 s per step (Khatri et al., 2018; Fitriani et al., 2018).

4.3.2 Determination of thermal properties of MOLP and SDMOLP complexes using DSC

The changes in the physical properties of MOLP and solid dispersion complexes as a function of temperature and time were determined using Differential Scanning Calorimetry (DSC) (Perkin Elmer, DSC 6000, United Kingdom) under nitrogen flow with a rate of 20 ml/min. The adsorption of heat by the complexes at a temperature range of 25-425^{o}C at a rate of 10^{o}C/ min was assessed. The samples (3-4 mg) were accurately weighed into DSC aluminium pans and sealed. A chromel-alumel thermocouple was used to measure the sample, which was placed directly above a constantan disc. An empty pan was used as a reference. The temperature difference across the sample and the reference chromel wafers gave a measurement of heat flow. Pure Indium standard was used for calibration and the experiments were conducted in duplicate. The thermal behaviour of pure MOLP and SDMOLP complexes was observed as peaks. The glass transition temperature (T_g), onset, peak, and end of each peak temperature as well as the enthalpy (ΔH_m) of samples were obtained accordingly using Pyris software.

4.3.3 Determination of thermal stability of MOLP and SDMOLP complexes using thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed using the Thermogravimetric Analyser (Perkin Elmer, TGA 4000) apparatus outfitted with an autosampler to analyse the decomposition stages and thermal stability of MOLP and solid dispersion samples. The samples were accurately weighed (3.0 mg) into the aluminium pans of the TGA analyser and empty pans were used as references. The experiments were carried out in the heating range of 25-600^{o}C with an accelerated heating rate of 10^{o}C/min under a nitrogen atmosphere at a flow rate of 20 mL/min. The TGA results were graphically represented and evaluated according to the weight loss as the function of temperature.

4.3.4 Fourier transform infrared (FTIR) spectroscopy analysis

Fourier transform infrared spectroscopy analysis or compatibility study was carried out

using the FTIR Spectrometer (Perkin Elmer ATR-FTIR, Spectrum Two Spectrophotometer). This technique was also used to identify functional groups present in the samples. MOLP and solid-dispersed MOLPs samples were sampled using an attenuated total reflectance (ATR) sampling technique then analysed in the beam of the FTIR spectrophotometer. The spectra were analysed in the resolution interval of 400-4000 cm^{-1}.

4.4 Data Analysis

Multivariate analysis of variance (MANOVA) was employed to determine the significant differences ($p < 0.05$) in attributes among samples. Duncan's multiple range test was used to separate means where a significant difference existed (IBM SPSS version 26, 2020).

4.5 Results and Discussions

4.5.1 MOLP and SDMOLPs crystallinity determined by Powder X-ray diffraction

X-ray diffraction was used to identify the degree of crystallinity achieved in pure MOLP and SDMOLPs. The X-ray diffraction patterns of pure MOLP, PEGs (PEG6000 and PEG4000), and SDMOLP samples are presented in Figures 4.2 to 4.4, respectively. The X-ray diffraction spectrum of MOLP exhibited four low intensity, distinguishable diffraction peaks at 2 Theta (2θ) values of 15.22°, 21.81°, 22.92°, and 22.94°, reflecting both its amorphous and crystalline domains. In general, crystalline materials show a series of sharp peaks, while amorphous products produce a broad background pattern (Fernandes *et al.*, 2014). The diffraction patterns of MOLP were exceedingly small, implying that the crystallite size (part of crystalline cellulose and hemicellulose) is small (Huang *et al.*, 2020). Furthermore, due to high protein present in the composition of the MOLP (28-35%), the X-ray diffraction patterns are attributed to the predominance of the amorphous nature of native proteins, thus, corresponding to the α-helix and β-sheet structures in proteins (Ferreira *et al.*, 2021) surrounding the other components that have a more amorphous nature. Zhao *et al.* (2015) also reported that the diffraction peak angles of α-helix and β-sheet structures from proteins in X-ray patterns were at 2θ around 10° and 20°, respectively, which were similar to 2θ values of 15.22° and 21.81° obtained in this study. Furthermore, the high background base and wider pattern found in the MOLP diffractogram may be related to its amorphous character, which might be attributable to its lipids because it contains polyunsaturated fatty acids (PUFAS). MOLP is a heterogeneous and complex matrix with several bioorganic substances, including proteins, carbohydrates, ash, and minerals, as substantiated in Chapter 3. Therefore, a few unassigned diffraction peaks observed could be due to these bioorganic compounds in the leaf. This finding concurs with the reports made by Huang *et al.* (2020) [small diffraction peak intensity at 2θ of 19.6° and 22.4°] for MOLP. Fernandes & Sellappan, (2019) also obtained a similar pattern for *Strobilanthes* species leaves.

Fernandes *et al.* (2016) found a similar conformation with *S. Brasillensis* dried extract. Furthermore, Araújo *et al.* (2010) reported comparable results for *Moringa oleifera* seed powder.

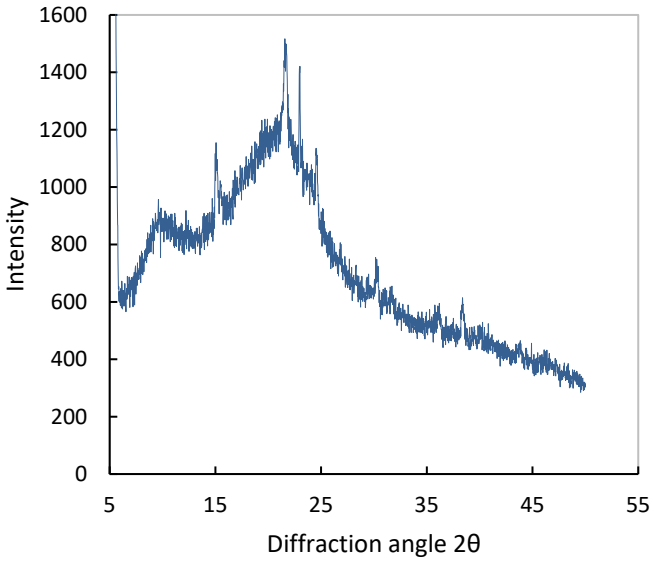


Figure 4.2 Powder X-ray diffraction pattern of pure MOLP

The PXRD spectra of PEG4000 and PEG6000 are shown in Figure 4.3. Although the XRD of PEGs showed similarities in diffraction patterns, a slight difference in the value of 2θ diffraction angles was observed. PEG4000 exhibited two strong and distinct diffraction peaks at 2θ values of 19.35°, 23.57°, and some small peaks at 2θ= 15.47, 26.97, 27.72, 36.99 and 40.84°. The strong peaks suggest that the carrier is semi-crystalline or partially amorphous in structure. These results are in agreement with previous studies for PEG4000 (19.35° and 23.5°) reported by Vasa *et al.* (2014) and Obaidat *et al.* (2017). PEG6000 diffractogram exhibited two major, highly intense and defined diffraction peaks at 2θ values of 19.29°, 23.65° and a few minor peaks at 2θ = 15.37°, 22.32°, 26.16°, 27.38°, 28.85° and 36.59°, which are consistent with the data in previous reports [19.12° and 23.23°] (Jayaramudu *et al.*, 2016; Obaidat *et al.,* 2017) suggesting that PEG6000 is also semi-crystalline or partially amorphous. Two characteristic diffraction peaks observed in the diffractograms of PEGs could be ascribed to the PEG lattice plane of (1 2 0) and (1 3 2), respectively (Kou *et al.*, 2019). Moreover, both PEG4000 and PEG6000 PXRD patterns were in good agreement with the reported literature of the international centre of Diffraction Data Card (JCPDS-19-0629). This study confirmed that PEG4000 and PEG6000 are semi-crystalline and may be beneficial as solid dispersion carriers due to their ability to create amorphous dispersions with increased solubility.

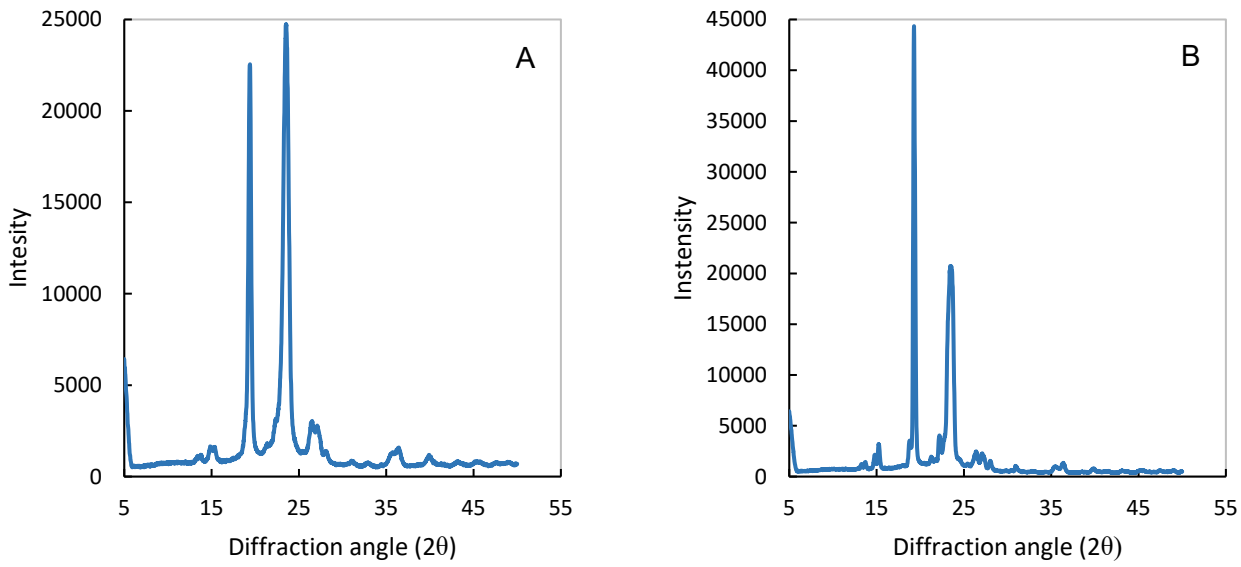


Figure 4.3 Powder X-ray diffraction pattern of (a) PEG4000 and (b) PEG6000

PXRD patterns of solid dispersions

Powder X-ray diffractograms of SDMOLP samples (Figure 4.4) exhibited strong and highly intense characteristic diffraction peaks appearing at 2θ values of 19.35-19.49^{o} and 23.55-23.72^{o}, and a few tiny, halo characteristic peaks at 2θ value of 15.29, 26.69, 36.69, 40.23 and 45.77^{o} were observed. The presence of distinct peaks indicated that SDMOLP samples were present as semi-crystalline to amorphous material. Solid dispersions showed diffraction patterns that corresponded to the carriers (PEG4000 and PEG6000) while peaks of pure MOLP were absent. The disappearance of the characteristic peaks of MOLP (from crystalline cellulose) suggested that MOLP was completely dispersed in PEG carriers. This proves the conversion of the crystalline form of MOLP into an amorphous form in solid dispersion. Thus, only the characteristic peaks of the carriers were observed in the MOLP-PEG solid dispersions, which also suggested the absence of any physical interaction between MOLP and PEGs (Alshehri *et al.*, 2020). Moreover, a decrease in peak intensity and the presence of the slightly high base of the solid dispersion diffractograms noticed compared to pure PEGs corresponds to amorphisation, thus, suggesting the presence of intermolecular interactions between MOLP-PEG via hydrogen bonding and van der Waal interactions. Weak physical bonds formed by non-covalent interactions such as hydrogen bonds, ionic bonds, van der Waals, dipole-dipole interactions, and acid-base interactions are common interactions that occur between components in solid dispersions (Krstić *et al.*, 2020). The formation of these bonds between an ingredient and a carrier component may prevent self-association between ingredient molecules, thus, leading to changes in the crystallization kinetics. These results, therefore, suggest that a phase transition of MOLP

from the crystalline form (crystalline diffractions) to the or amorphous or semi-crystalline form occurred during the preparation of the solid dispersion. This proves the success of the solid dispersions. Thus, the results of XRD support the findings of the DSC study.

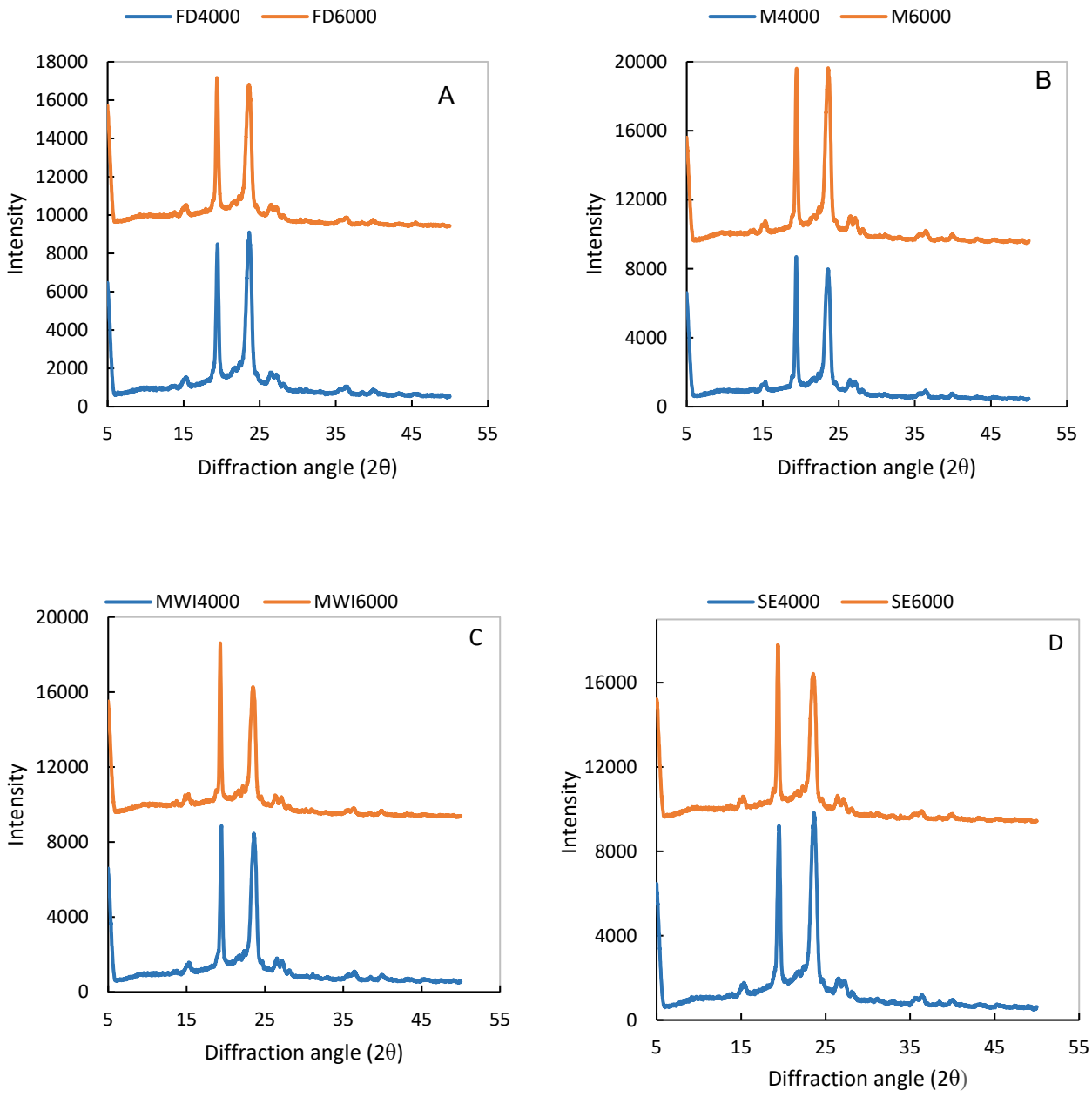


Figure 4.4 Powder X-ray diffraction patterns of the solid dispersions of MOLP using PEG as a carrier prepared through (a) freeze-drying method (FD4000 and FD6000), (b) melting method (M4000 and M6000), (c) solvent evaporation (SE4000 and SE6000), and (d) microwave irradiation method (MWI4000 and MWI6000)

Moreover, the major peaks slightly shifted towards a higher diffraction angle, whilst the small characteristic diffraction peaks slightly shifted towards lower diffraction angles, suggesting that intermolecular interactions occurred in solid-state between the two entities (MOLP and PEG). This small interaction was also confirmed in the FTIR study through band shift and intensity reduction appearance because of Van der Waal interactions and hydrogen bonding between N-H, O-H, or C=O functional groups of MOLP and –O- or O-H groups of PEGs. Weak physical bonds formed by non-covalent interactions such as hydrogen bonds, ionic bonds, van der Waals, dipole-dipole interactions, and acid-base interactions are common interactions that occur between components in solid dispersions (Krstić *et al.*, 2020; Tran & Tran, 2020). The formation of these bonds between an ingredient and a carrier component may prevent self-association between ingredient molecules, thus, leading to changes in the crystallisation kinetics. Moreover, the reduction in peak intensity and the presence of the high base in solid dispersion diffractograms corresponds to the amorphous nature, indicating MOLP was present as amorphous material inside the PEG matrix. Additionally, the slightly broadened peaks in solid dispersions indicated that MOLP in solid dispersion was molecularly dispersed in carriers, resulting in improved solubility of solid dispersions compared to pure MOLP (discussed in Chapter 3). However, no diffraction peaks other than those that could be assigned to PEGs were detected in the solid dispersions, indicating no chemical interaction between the two components. Rakesh *et al.* (2008) reported similar results for furosemide and PEG6000 solid dispersions, where the solid dispersions diffraction patterns (semi-crystalline) showed more resemblance to PEG, suggesting no chemical interactions. Alshehri *et al.* (2020) also obtained similar results for Luteolin and PEG4000 solid dispersions prepared by microwave irradiation, melting, and solvent evaporation methods. Douroumis *et al.* (2010) reported similar findings. Moreover, the XRD results are similar to the curcumin-PEG solid dispersion reported by Muthu *et al.* (2019). There was no significant difference in PXRD patterns among solid dispersions, suggesting that the type of method of solid dispersion did not significantly affect the crystallinity of the solid dispersions. However, a significant difference existed in diffraction patterns between MOLP and SDMOLP samples. All the solid dispersions possessed semi-crystallinity/amorphicity characteristics compared to MOLP. This can be due to the successful formation of solid dispersion between MOLP and PEG.

4.5.2 Thermal properties of MOLP and SDMOLPs

Diffraction scanning calorimetric (DSC) is a commonly used thermal analysis method due to its capacity to provide detailed information about physical properties. The DSC thermograms of MOLP, carriers and solid dispersions are displayed in Figures 4.5.

Moreover, the thermal properties are presented in Table 4.1. The thermogram of MOLP (Figure 4.5a) presented five broad and not well-defined endothermic peaks at a heating rate of 10^{o}C min^{-1}. The peaks were at 80^{o}C, 162^{o}C, 210^{o}C, 253^{o}C and 312.56^{o}C temperatures, respectively. The broad characteristic peaks confirmed that MOLP possessed both crystalline and amorphous characteristics, as indicated in the PXRD study. The first endothermic peak and its melting peak were observed at approximately 80^{o}C with an onset of 44^{o}C and end set of 89^{o}C, and amount of endothermic heat (ΔH) fusion of 129.30 J/g, attributed to the evaporated water from the matrix, lattice, surface, and pores of MOLP sample. The high enthalpy indicates that more energy was expended in the disruption of noncovalent bonds in MOLP. (Jeyakumar *et al.*, 2020) reported similar results with an enthalpy of 177.4 J/g at 71.34^{o}C for MOLP antioxidants.

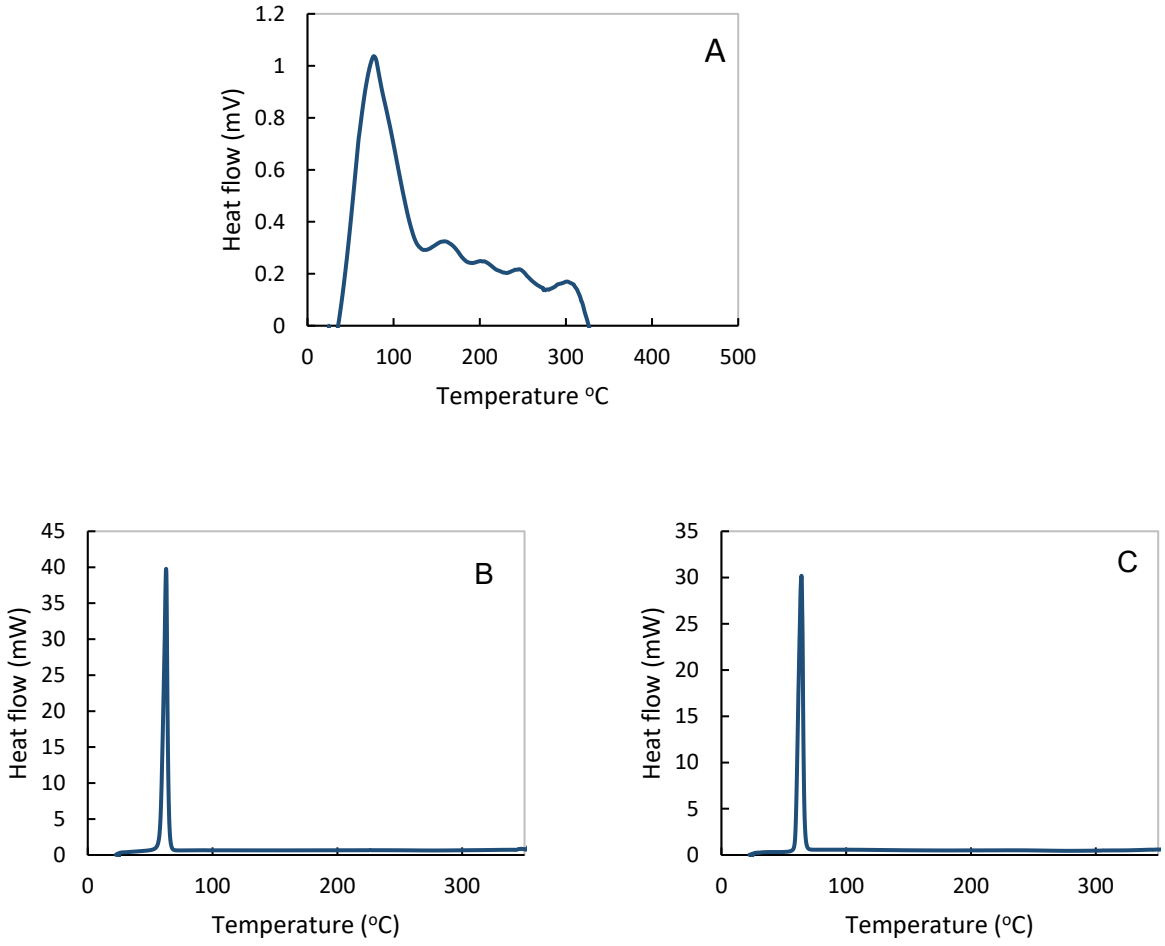


Figure 4.5 DSC thermograms of (a) MOLP (b) PEG4000 (b) and (c) PEG6000

The second peak was observed at approximately 159^{o}C, showing an onset at 128.23^{o}C and end set at 179.97^{o}C with Tg of 141.97^{o}C and fusion enthalpy 5.0 J/g. Huang *et al.* (2020*)* reported similar fusion enthalpy (2.52-3.93 J/g) for MOLP. Low ∆H indicates less energy expended for disruption of noncovalent bonds, which could be due to high temperatures. The third and fourth peaks were observed at 210^{o}C and 253^{o}C temperatures, respectively, depicting the melting of tannic acid (218^{o}C). The fifth small endothermic peak appeared at approximately 312.56^{o}C, having an onset at 347.48^{o}C and an end set at 345.79^{o}C and fusion enthalpy ∆H of 23.84 J/g and glass temperature of 277.72^{o}C. These endothermic peaks are attributable to the evaporation of bonded water from the MOLP, which is incorporated in different structures. Furthermore, the breaking down or decomposition of organic matter and protein molecules is attributed to the presence of the other peaks. In addition, the decomposition (above 100^{o}C) may be associated with a wide variety of secondary metabolites, principally phenolics, present in the dried MOLP (Hugo *et al.*, 2013). The second, third, fourth, and fifth endothermic peaks might also be associated with the melting of fats, saponins (150^{o}C), polysaccharides, de-methoxylation, dihydroxylation, and decarboxylation. All the obtained results confirmed the heterogeneity of *Moringa oleifera* leaves. The leaves are high in minerals, protein, vitamins, phenolics, and phytochemicals. Similar DSC thermograms were reported for *Moringa oleifera* gum with several peaks of fusion, green tea extract (Dzulhi *et al.*, 2018), and green tea and curcumin (Pandit *et al.*, 2019). Four similar endothermic peaks for the *Moringa oleifera* leaf powder antioxidant were reported by (Jeyakumar *et al.*, 2020)

In the case of PEG4000 and PEG6000 (Figure 4.5), the DSC thermogram of PEG4000 exhibited a sharp endothermic peak at 60^{o} C [with enthalpy (∆H) fusion of 179.70 J/g], corresponding to its melting point. Similar results were reported by (Zawar and Bari, 2013). PEG6000 also exhibited a clear, single, sharp, and narrow endothermic peak at 63.10^{o}C with a corresponding enthalpy (∆H) fusion of 193.4 J/g. The observed endothermic peak was associated with the melting point of the carrier, indicating the semi-crystallinity. The fusion temperature of PEG6000 has been reported as 62-65^{o}C (El Maghraby & Elsergany, 2014). Biswal *et al.* (2008) reported a similar melting temperature (61.9^{o}C) but slightly lower ∆H of 188.6 J/g than obtained in this study. Therefore, these results were in agreement with previous similar studies. PEG4000 and 6000 are indeed semi-crystalline carriers; this was also confirmed in the PXRD study.

DSC thermograms of solid dispersions

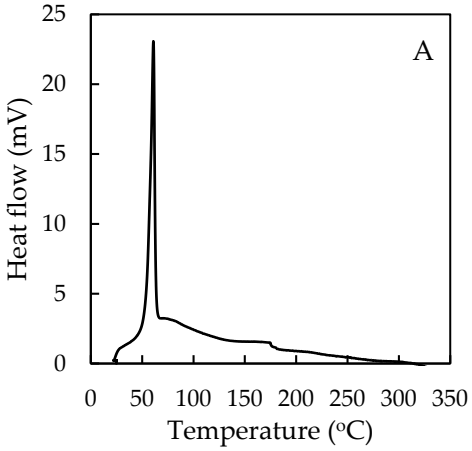
Solid dispersion thermograms in Figure 4.6 exhibited characteristic and single endothermic peaks with melting temperatures ranging from 57.76-61.76^{o}C and heat enthalpy (∆H) ranging from 75.22-108.44 J/g. Sharp with slightly broadened base endothermic peaks were

obtained for all the solid dispersions, thus, proving their semi-crystallinity to amorphicity. The characteristic endothermic peak corresponding to the carriers melting point (60-63^{o}C) was broadened and shifted toward lower temperature, with reduced intensity, in all the solid dispersion. This could be attributed to the uniform distribution of MOLP in the crust of the polymer, resulting in the complete miscibility of the molten MOLP in PEG carriers. The lower intensity of peaks and disappearance of MOLP endothermic peaks in solid dispersions compared to PEG and MOLP, and reduction of fusion enthalpy (ΔH) could be explained by dissolving of MOLP in melted carriers during the DSC measurement (Febriyenti *et al.*, 2019). The complete disappearance of the MOLP melting peaks observed in solid dispersions suggests the molecular dispersion of MOLP in PEG carriers or absence of crystalline nature of MOLP, as discussed in the PXRD study. This indicates conversion of MOLP from crystalline to partially amorphous form in solid dispersion. The reduction of fusion enthalpy (ΔH) and melting point in DSC spectra of solid dispersions indicated the lower crystallinity of MOLP in the solid dispersions. The melting temperature of PEG gradually shifted to the lower temperature (from 63^{o}C to 58^{o}C) in solid dispersions, thus, losing its sharp and distinctive appearance. This suggests that intermolecular interactions between MOLP and PEGs occurred (Patil & Gaikwad, 2011) thus, leading to the amorphisation of MOLP in solid dispersions. Consequently, the existence of strong hydrogen bonds between the ingredient (MOLP) and polymer decreased and/or lowered the overall free energy of mixing. Thus, promoting miscibility between the ingredient and polymer. This corresponds with the improved solubility of solid dispersion obtained in Chapter 3. Furthermore, hydrogen bonding is thought to be important to increase the physical stability of solid dispersions over time (Khodaverdi *et al.*, 2012). Different methods of solid dispersion preparation did not have a significant effect on the melting temperature of SDMOLPs. However, significant differences in ΔH existed, which suggested that solid dispersions prepared by microwave irradiation and melting methods had a slightly higher degree of crystallinity than those prepared by freeze-drying and solvent evaporation methods. Febriyenti *et al.* (2019) also reported similar DSC findings for their gliclazide and PEG6000 solid dispersions, where the endothermic peaks resembled that of PEG and showed complete absence of the pure ingredient endotherm peaks. da Silva *et al.* (2019) reported similar thermal profiles in their atorvastatin calcium (ATV).

Table 4.1 Thermal properties of MOLP and solid dispersions at the heating rate of 10°C min^{-1}

Sample	Peak	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	ΔH (J/g)	T_g (°C)
Pure MOLP	1	44.14	58.79	76.78	111.35	37.27
	2	128.23	159.35	179.97	5.0176	141.97
	3	185.86	210	230	1.54	180.69
	4	230	253.7	274	3.69	238.56
	5	347.48	312.56	345.79	23.84	277.72
Pure PEG4000	1	59.62	60.21	61.38	179	22.89
Pure PEG6000	1	59.62	63.10	64.68	193.74	60.11
PEG4000						
Freeze-drying	1	53.35	57.78	60.80	94.45	64.01
Melting	1	56.24	59.10	61.40	102.21	62.85
Solvent evaporation	1	53.32	60.19	62.22	75.22	54.95
Microwave irradiation	1	55.10	59.48	61.29	108.44	52.84
PEG6000						
Freeze-drying	1	56.48	60.88	62.50	90.29	64.86
Melting	1	57.43	60.93	62.84	104.07	64.44
Solvent evaporation	1	55.76	60.96	63.05	95.59	50.34
Microwave irradiation	1	58.00	61.76	63.67	94.77	56.95

[1]MOLP: *Moringa oleifera* leaf powder. PEG: Polyethylene glycol. T_{onset}: onset temperature. T_p: Peak temperature. T_{endset}: end set temperature. Tg: ΔH: enthalpy


A
Heat flow (mV)
Temperature (°C)
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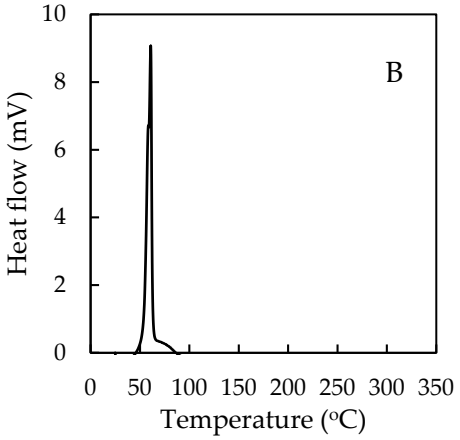
5
10
15
20
25
0 50 100 150 200 250 300 350


B
Heat flow (mV)
Temperature (°C)
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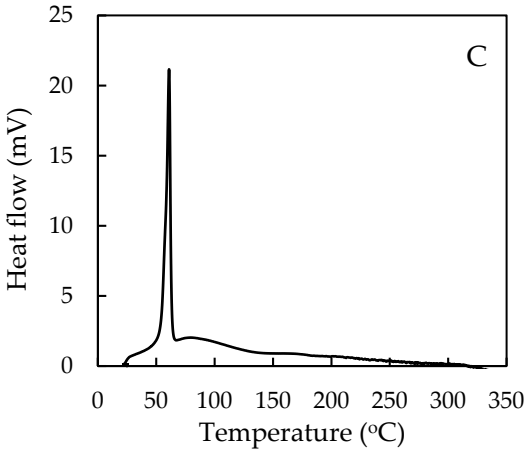
0 50 100 150 200 250 300 350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Heat flow (mV)
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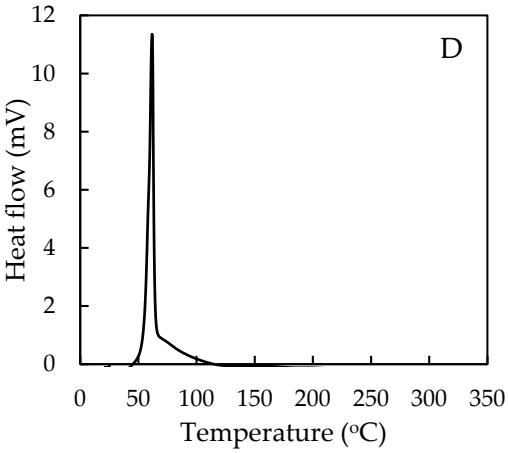
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Heat flow (mV)
Temperature (°C)
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12
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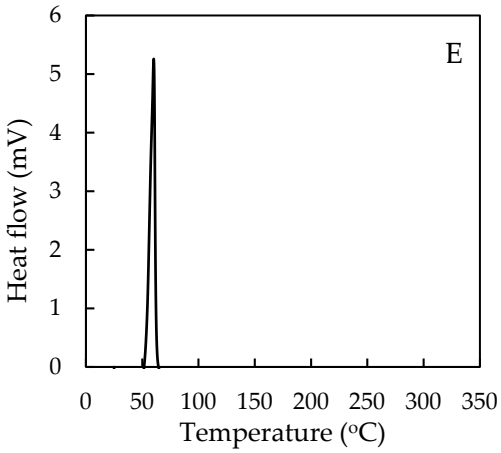
E
Heat flow (mV)
Temperature (°C)
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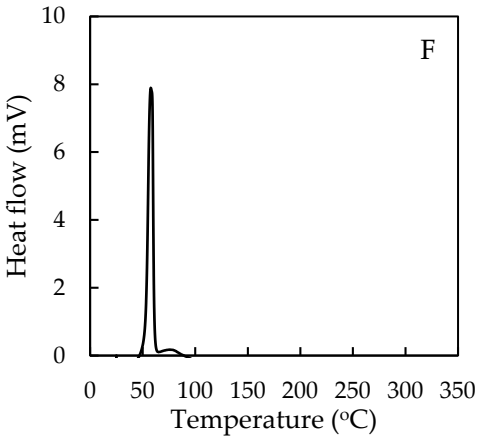
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Heat flow (mV)
Temperature (°C)
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6
8
10
0 50 100 150 200 250 300 350

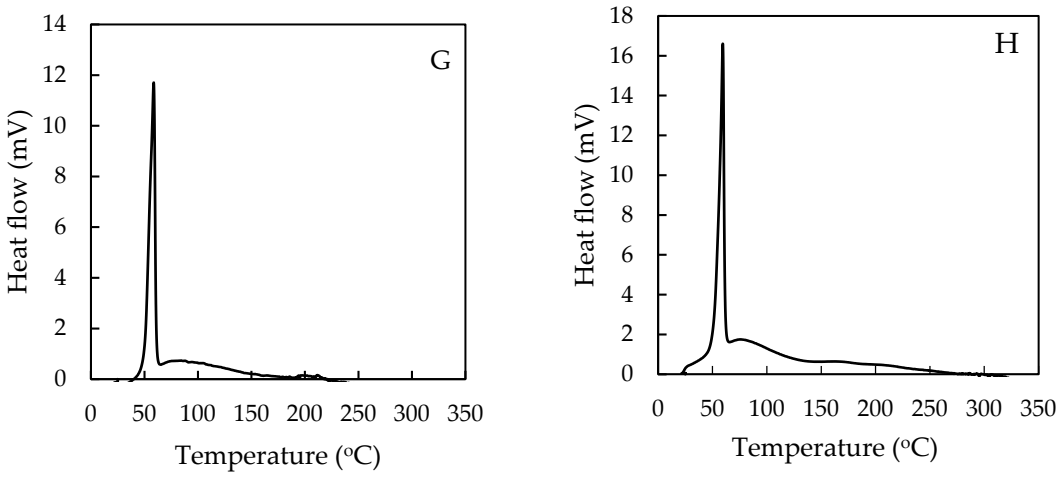

Figure 4.6 DSC thermograms of solid-dispersed MOLP through freeze-drying with PEG6000 (a), PEG4000 (b), (c) and microwave (d) using PEG6000; and (e) Solvent evaporation, (f) Freeze-drying, (g) Melting and (h) microwave prepared using PEG4000

4.5.3 Thermogravimetric properties of MOLP and SDMOLPs

Thermogravimetric analysis (TGA) was used to characterise the decomposition and thermal stability of the samples. As the temperature increased, the weight of the sample decreased indicating the continuous decomposition of the sample. TGA thermograms of pure MOLP, carriers (PEG6000 and PEG4000), and solid dispersion samples are displayed in Figures 4.7 to 4.9. Moreover, Table 4.2 presents the values of mass loss (%) as a function of temperature. MOLP thermogram (Figure 4.7a) exhibited several stages of the decomposition process. The mass loss of MOLP can be divided into three stages. In the first stage, MOLP slightly lost 4.96% weight after the heating commenced up to 151.07°C, associated with moisture loss or water desorption (dehydration of adsorbed and surface water from the MOLP). The amount of water lost from MOLP is similar to the 8.44% moisture content found in Chapter 3 and literature (Valdez-Solana *et al.*, 2015; Ali *et al.*, 2017; Mikore and Mulugeta, 2017). In the second stage, a significant major weight loss of 44.16% was observed over a temperature range of 151.07-371.86°C. This stage was related to the decomposition of organic matter, probably the protein components (amino acids which have a melting point of 200-300°C), fat, and carbohydrate (265°C) present in Moringa leaves. Moreover, this stage might also occur due to the decomposition of fatty acids, for example, oleic acid, which has a boiling point of 360°C (Araújo *et al.*, 2010).

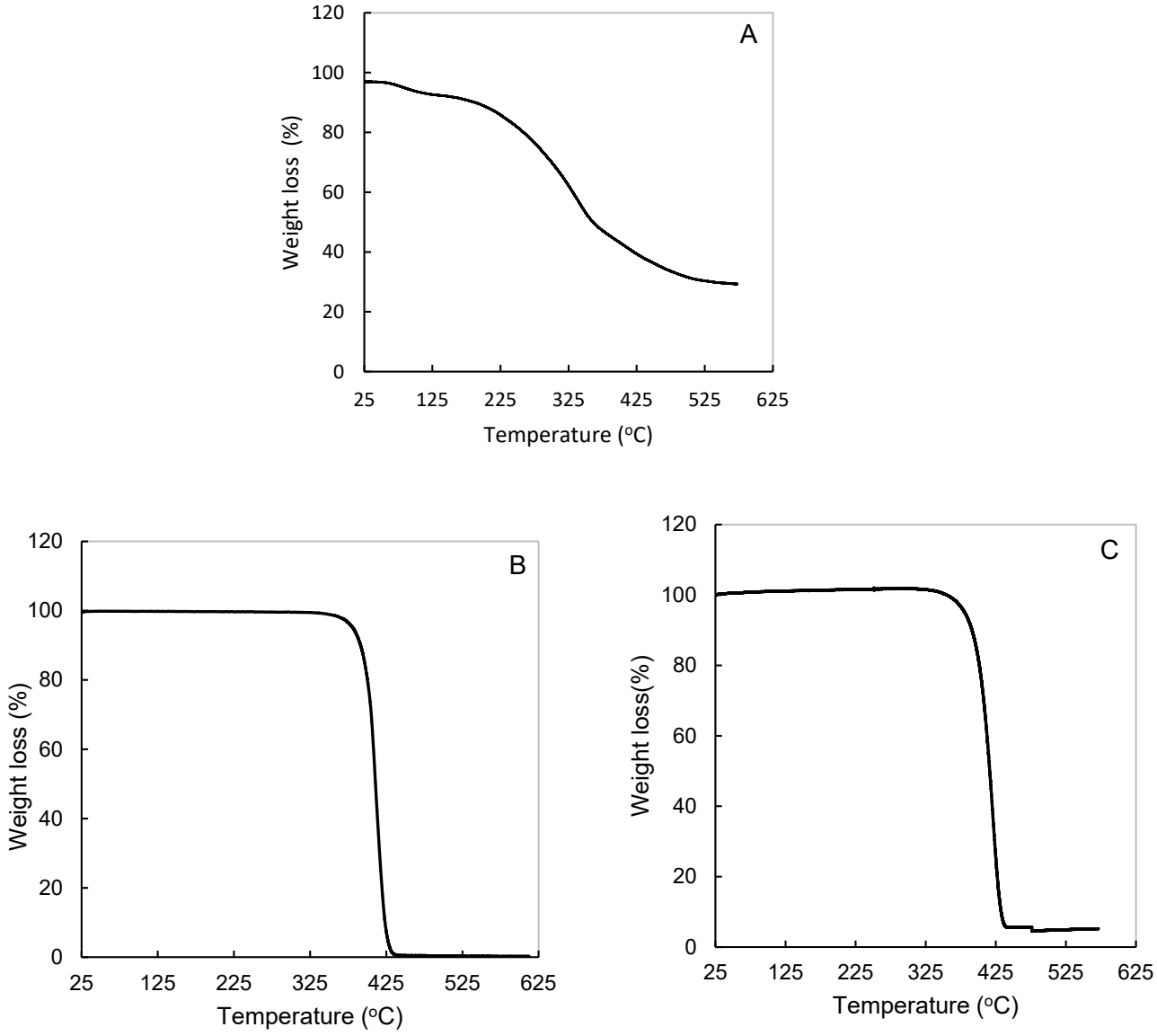

Figure 4.7 Thermogravimetric curves of (A) MOLP, (B) PEG4000, and (C) PEG6000 at a heating rate of 10°C· min $^{-1}$

Since MOLP is a good source of phenolic compounds and phytochemicals, the second stage may further denote the decomposition of remaining phytochemical compounds such as kaempferol (276-278°C), quercetin (316°C), gallic acid (26°C), and tannic acid (above 200°C), thus indicating the presence of antioxidant components in *Moringa oleifera* leaves (Jeyakumar *et al.,* 2020). In addition, 200-450°C is associated with the degradation of cellulose and hemicellulose (Malucelli *et al.*, 2018). Similar results were reported for *Moringa oleifera* leaf antioxidants by Jeyakumar *et al.* (2020). The final stage of degradation with 17.91% weight

Table 4.2 Thermogravimetric data showing decomposition behaviour of MOLP, PEG6000, and solid dispersions as a function of temperature at a heating rate of 10°C·min $^{-1}$

Method	Treatment	∆Mass loss (%)	Temperature range (°C)
-	Pure MOLP	4.96	32.21-151.07
		44.16	151.07-371.86
		24.91	392.16-531.96
	Pure PEG4000	98.22	356.4-483.84
-	Pure PEG6000	96.0	330.78-451.32
Freeze-drying	PEG4000	1.93	25.06-130.87
		22.19	130.87-369.82
		59.88	369.82-569.78
	PEG6000	1.93	25.06-130.87
		22.19	130.87-369.82
		55.02	369.82-569.78
Melting	PEG4000	1.83	32.12-131.35
		19.49	131.35-371.74
		60.69	371.74-572.01
	PEG6000	1.27	24.97-111.48
		18.30	111.48-371.74
		54.38	371.74-572.01
Solvent evaporation	PEG4000	2.10	32.13-130.27
		22.27	130.27-369.72
		57.66	369.27-569.86
	PEG6000	2.10	32.13-169.82
		22.27	169.82-389.85
		57.66	389.85-489.99
Microwave	PEG4000	2.26	31.97-130.34
		22.93	130.34-369.77
		57.18	369.77-569.55
	PEG6000	2.26	31.97-130.34
		22.93	130.34-369.77
		57.18	369.77-569.55

[1]MOLP: *Moringa oleifera* leaf powder. PEG: Polyethylene. $\Delta m/\% = \%m_{end} - \%m_{initial}$ represents the percentage of mass loss at each stage

loss appeared after 390°C with the decomposition of particles in the range of 371.86°C to 571.76°C, which probably include fatty acids, ash, or mineral content, and inorganic oxides. MOLP is high in minerals (11.82%) as obtained in Chapter 3, thus the last stage can be attributed to the mineral decomposition and residues mass which is usually carried out at high temperatures (500°C). Moreover, lignin degradation happens between 450-800°C (Malucelli *et al.*, 2018). In this temperature range, oxygen-containing functional groups such as lactones or esters (-COO-) and carboxyls (COOH carboxylic acid) decompose. It has been reported that functional groups including hydroxyl, carboxyl groups, and esters were decomposed at 326.85°C and 499.85-723.85°C. (Li *et al.*, 2014). MOLP exhibited poor thermal stability, which corroborates with the calorimetric (DSC) curve data discussed in section 4.4.2. More so, Araújo *et al.* (2010) and Bello *et al.* (2017) observed similar behaviour during the thermal assessment of *Moringa oleifera* seed powder. Another similar behaviour was described by Ram *et al.* (2014) for *Carica papaya* leaves with 6.23% mass loss in the first stage and 28.00% and 17.46% in the second and third stages, respectively. *Dicksonia sellowiana* extract also showed three stages of degradation with mass losses of 7.19, 64.73, and 27.81%, respectively, as reported by Malucelli *et al.* (2018).

PEG4000 (Figure 4.7b) exhibited a single thermal decomposition of 98.22% in the temperature range of 356.4-483.84°C. This thermal behaviour for PEG4000 is similar to those (99.16%) reported by Liu *et al.* (2016) and Kou *et al.* (2019). Meanwhile, the PEG6000 thermograph (Figure 4.7c) also exhibited a uniform thermal degradation behaviour and greater stability. A single mass loss stage was observed between 330.78°C and 451.32°C and peaked at 391.01°C with 96.0% weight loss. This corresponds to the decomposition of the materials or PEG chains, carbonaceous material originating from the decomposition of the high molecular weight polymer (PEG6000). These results concur with the previously reported weight loss (98.79%) of PEG6000 (Regdon & Korteby, 2018). Jayaramudu *et al.* (2016) also reported a similar weight loss of 95.97% for PEG6000 in the same temperature range. Both PEG4000 and PEG6000 undergo a substantial weight loss at ~400°C, corresponding to the pyrolysis of PEG functional groups. Based on these findings, it can be concluded that all the PEG samples were thermally stable.

Thermogravimetric curves of solid dispersions

Figures 4.8 and 4.9 show the thermogravimetric curves of solid-dispersed MOLP obtained by freeze-drying, melting, solvent evaporation, and microwave irradiation methods of solid dispersion. Moreover, Table 4.2 presents the values of mass loss as the function of temperature. All solid dispersions' TGA degradation profiles exhibited a continuous weight loss

with three stages as the MOLP. These TGA curves confirmed the sample heterogeneity, which is the same as that of MOLP. In contrast, the solid dispersions showed greater thermal stability than pure MOLP. It is observed that solid dispersions are stable up to 300^{o}C, whereas MOLP is stable up to 150^{o}C. Thus, it can be inferred that the complexation of MOLP onto PEG has significantly increased the thermal stability of SDMOLPs.

Solid dispersions prepared by the freeze-drying method (PEG4000 and PEG6000) in Figure 4.8a revealed three stages of thermal degradation occurring at 130.87^{o}C, 369.82^{o}C, and 569.78^{o}C. The first stage occurs from 25.06-130.87^{o}C with a mass loss of 1.93%. In the second stage, a weight loss of 22.19% was observed in the temperature range of 170.33–389.80^{o}C. The third stage was observed in the temperature range of 389.80- 469.91^{o}C with greater mass loss of 59.88% (MOLP-PEG4000) and 55.02% (MOLP-PEG6000). The two solid dispersions from PEG4000 and PEG6000 carriers showed similar results for stages 1 and 2 but significantly differed in terms of stage 3 weight loss. This may be attributed to strong ingredient-polymer interactions which occur through hydrogen bonding, elaborated in the FTIR compatibility study. However, improved thermal stability was observed in both MOLP-PEG4000 and MOLP-PEG6000 dispersions.

The melting method solid dispersions (PEG6000 and PEG4000) in Figure 4.8b also revealed three mass loss stages. M4000 solid dispersions (prepared by MOLP-PEG4000) showed three stages of mass losses 1.83%, 19.49% and 60.69% were observed in the temperature ranges of 32.12-131.35^{o}C, 131.35-371.74^{o}C and 371.74-572.01^{o}C, respectively. While in the M6000 solid dispersion (prepared by MOLP-PEG6000), the first weight loss of 1.27% occurred from 24.97 to 111.48^{o}C, the second stage occurred in the temperature range of 111.48-371.74^{o}C with 18.30% mass loss, and the last major mass loss of 54.78% can be observed in the temperature range of 371.74-572.01^{o}C.

The solid dispersions prepared through the solvent evaporation method (PEG4000 and PEG6000) in Figure 4.9 exhibited their first mass loss (2.10%) between 32.13 and 130.27^{o}C. In the second stage, 22.66% mass loss is observed in the temperature range of 130.27-369.72^{o}C and the final stage had the greatest mass loss of 57.57% which is shown in the temperature range 369.72-569.86^{o}C. There was no significant difference in weight loss between the MOLP-PEG4000 and MOLP-PEG6000 solid dispersions. Furthermore, solid dispersions prepared by the microwave method (PEG4000 and PEG6000) (Figure 4.9) also indicated three stages of weight loss, where the first mass loss of 2.26% is occurring from 31.97-130.34^{o}C. The second mass loss 22.93%, occurs in the temperature range 130.34-369.77^{o}C, and in the last stage, 57.18% of major mass loss is observed in the temperature range of 369.77-569.55^{o}C. Solid dispersions prepared by microwave did not significantly differ in weight loss and thermal stability.

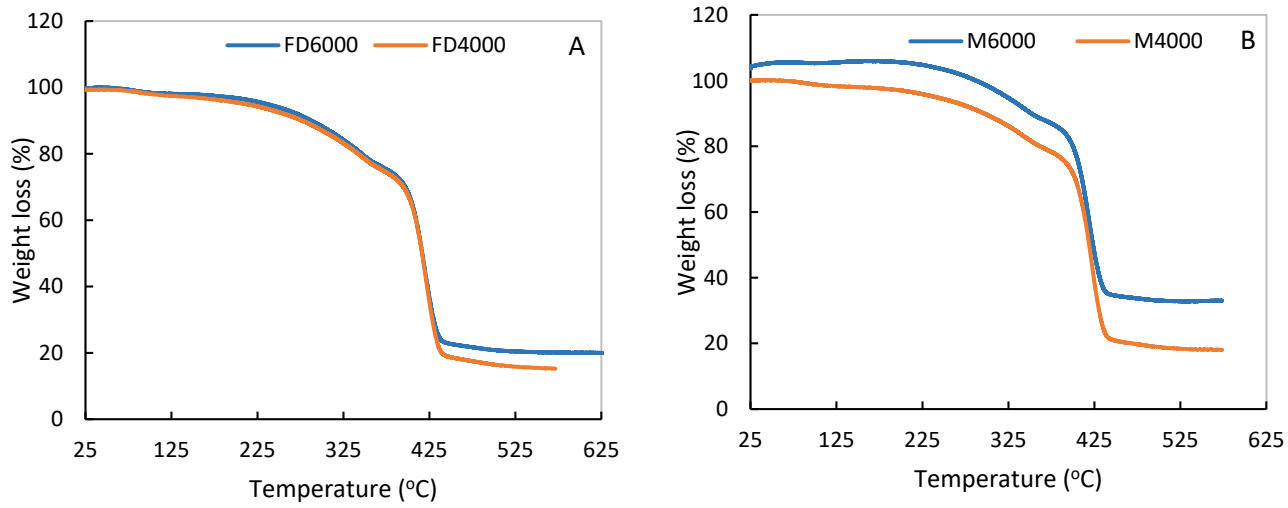

Figure 4.8 Thermogravimetric curves of MOLP solid dispersions prepared through (a) freeze-drying method (FD4000 and FD6000), and (b) melting method (M4000 and M6000)

Thermogravimetric curves and thermal behaviour of all the solid dispersions were divided into three consecutive stages. Whereby, i) the first stage of mass loss (1.27-2.26%) was attributed to water desorption and loss of loosely bound water due to sample dehydration (Malucelli *et al.*, 2018; Raj & Kumar, 2018). The amount of water loss from the solid dispersions determined by this technique was never reported before, as this is a new study of MOLP-PEG solid dispersions. However, the water loss in this region is similar to the values of moisture content obtained in Chapter 3 section 3.5 for the solid dispersion samples (2.65-4.03%). Moreover, the weight loss in this region was found to be lower ($p > 0.05$) than that of pure MOLP (4.96%). ii) In the second stage, weight loss (18.30-22.93%) in the temperature range of 130-371°C was observed in the solid dispersions. This stage occurs due to the decomposition of organic matter, probably protein components present in Moringa leaves which have a decomposition temperature of 185-280°C (Araújo *et al.*, 2010). However, the mass loss observed in this stage is significantly lower ($p > 0.05$) than 44.16% in MOLP, this evidences that pure MOLP has higher protein (~30.0%) than the solid dispersions (12.54-14.23% protein content), as established in Chapter 3.

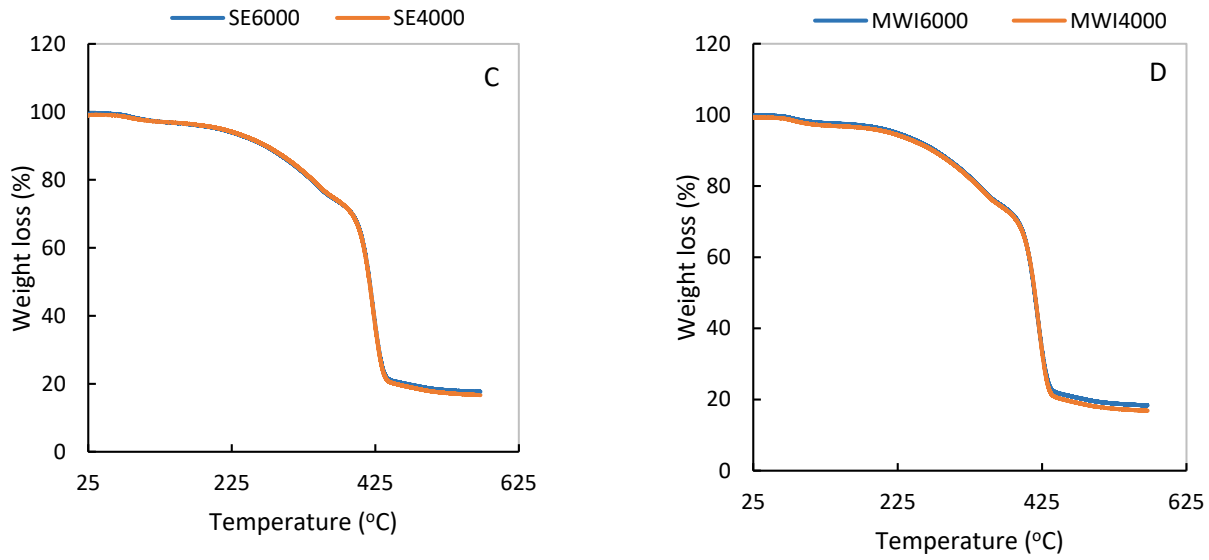

Figure 4.9 Thermogravimetric curves of solid-dispersed MOLPs prepared through (a) solvent evaporation method (SE4000 and FD6000), and (b) microwave irradiation method (MWI4000 and MWI6000)

The results further indicate the presence of a well-defined interaction between MOLP and PEG. Strong ingredient-polymer interactions may have occurred through hydrogen bonding via the N-H amine functional group of MOLP and the O-H hydroxyl group of PEGs, and via the C=O ester carbonyl group of MOLP (which is found in the primary amide region of Moringa protein) and the O-H of PEG, as confirmed from the PXRD and FTIR compatibility studies. Consequently, the physical stability is improved. The good physical or thermal stability in solid dispersions was attributed to the strong hydrogen bonds between MOLP and PEG which might reduce the molecular mobility and retard recrystallisation during storage. Moreover, it could be seen that the TGA thermograms of all the solid dispersions shifted to the left side. This proves the possible interaction between the MOLP and PEG in solid-state, thus high thermal stability in solid dispersion than MOLP was observed. According to Li *et al.* (2014), the improvement of physical stability might be influenced by ingredient/carrier ratio and ingredient-carrier interactions. iii) In the third stage, solid dispersions had 54.38-60.69% weight loss in the temperature range of 369-572°C, attributed to the organic phase desorption. This stage occurs with the decomposition of the greater part of the solid dispersions, which probably include fatty acids from MOLP such as oleic acid which has a boiling point of 360°C amino acid (371°C). Moreover, solid dispersions had mineral contents. Therefore, this stage may also be attributed to the decomposition of mineral residues, which may be associated with the ash content usually obtained at high temperatures (500°C) in the furnace oven (Kou *et al.*, 2019). According

to Li *et al.* (2014), oxygen-containing functional groups such as hydroxyl (O-H), esters (-COO-), and carboxyl (COOH) groups decompose at below 326.85^{o}C, 499.85-723.85^{o}C, respectively. Therefore, this stage can also be associated with the decomposition of these functional groups present in the essential amino acid (shown in Figure 4.11) and phenolic compounds of MOLP. Additionally, both PEG4000 and PEG6000 undergo a substantial weight loss at ~400^{o}C, corresponding to the pyrolysis of PEG functional groups; this stage could also be due to the pyrolysis of functional groups (-O- and O-H) in solid dispersions. Furthermore, it is worth mentioning that the third stage of mass loss for solid dispersions was significantly ($p < 0.05$) higher than the pure MOLP (17.91%), the addition of the PEG increased the residual weight of the solid dispersions. It was observed that the solid dispersions exhibited good thermal stability than the MOLP. This suggests that the PEG interfered in all solid dispersions, contributing to greater stability of the MOLP. These findings corroborate with the calorimetric thermograms discussed in this study, which is extra indicative of the physical characteristics of the solid dispersions obtained by the four techniques. Similar results were reported for Atorvastatin calcium and PEG6000 solid dispersions, however, unlike in this, they showed six stages of weight loss (da Silva *et al.*, 2019).

4.5.4 Fourier Transform Infrared (FTIR) Spectra and Functional groups of MOLP and SDMOLPs

FTIR was performed to further investigate the possibility of interactions between MOLP and PEGs (PEG4000 and PEG600) in the solid-state, this technique was equally employed to characterise the functional groups present in the samples. Figures 4.10 to 4.15 and Table 4.3 show the infrared (IR) spectra of pure MOLP and SDMOLPs. MOLP spectrum contained many characteristic bands connected with main *Moringa oleifera* leaf components. The MOLP spectrum (Figure 4.10) exhibited a broad peak centred at 3295.4 cm^{-1}, attributed to the stretching vibration band of O-H. This band is associated with polyphenol structures such as flavonoids, tannins, glycoside derivatives, and fibre. This functional group also appears predominantly in the protein and fatty acid structures in *Moringa oleifera* leaves (Araújo *et al.*, 2010; Huang *et al.*, 2020). Similar results (3290 and 3309 cm^{-1}) were reported for MOLP by Huang *et* al. (2020). Moreover, since MOLP is a source of protein, the region at
3295.4 cm^{-1} can also be associated with the N-H functional group, evidenced in Figure 4.11. Other sharp characteristic peaks at 2921.2, 2850.6, 1990, 1640.9, 1553.6, 1420.6, 1316.6, 1246, 1058.9, 530.95, and 468.6 cm^{-1} were observed. The peaks at 2921.2 cm^{-1} and 2850.6 cm^{-1} were attributed to C-H's asymmetric and symmetric stretching vibrations in aliphatic chains, respectively. These methyl groups are usually present in fatty acids. Exact results were reported by Araújo *et al.* (2010) for the *Moringa oleifera* seed powder, which revealed the

methyl groups at 2923 cm^{-1} and 2852 cm^{-1}, respectively. Moreover, Bello *et al*. (2017) also observed similar functional groups at 2917 and 2849 cm^{-1} in the FTIR spectrum of raw MOLP. The characteristic peak observed at 1640.9 cm^{-1} was associated with the primary amide (amide I) of the protein group in MOLP, corresponding to the C=O carbonyl asymmetric. The maximum amide I band centred at 1640.9 cm−1 indicates that MOLP contains proteins with pronounced α-helix and β-sheet secondary structures. The C=O vibrations have the predominant role in this band, followed by C-N. There is also some in-plane N-H bending contribution to amide (Gowri *et al.*, 2015; Tran *et al.*, 2020). This region also indicates the C=O carbonyl stretching of the ester carbonyl functional group of the triglycerides of fatty acids, this carbonyl group is present in the fatty acids, and protein structure of the *Moringa oleifera* leaves. Moreover, the signals at 1640.9 cm^{-1} also correspond to C=O bonds of ether, ester, and phenol found in MOLP. These results are in good agreement with the values reported by Bello *et al.* (2017) for the *Moringa oleifera* leaf powder (1636 cm^{-1}). More so, the characteristic peak around 1553.6 cm^{-1} marked the secondary amide (amide II) band, showing the N-H group of the protein in *Moringa oleifera* leaves, corresponding to C=O and N-H bending vibration (Huang *et al.*, 2020). The amide III bands were relatively weak and appeared at about 1420.6 and 1316 cm^{-1}. It was associated with N-H plane bending coupled with C-N stretching and C–H and N-H deformation vibrations (Tran *et al.*, 2020). These bands also were representatives of C=O asymmetric stretching as well as the deformation band of aliphatic methylene group CH_2.

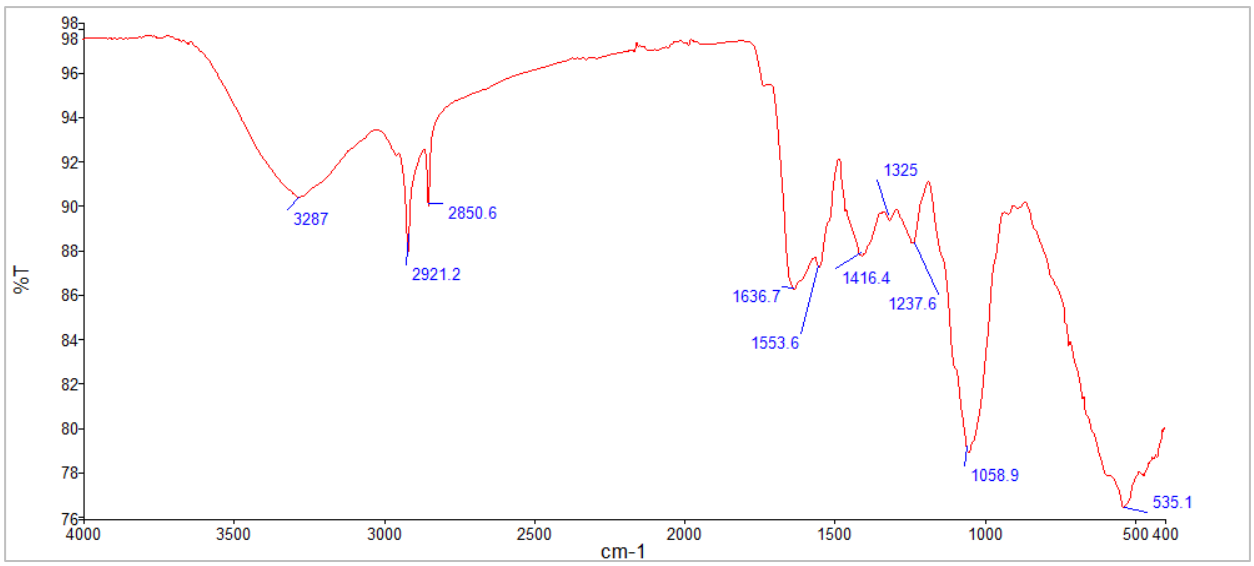

Figure 4.10 FTIR spectrum of MOLP

The band observed at 1246 cm^{-1} corresponds to the C-O stretching, which is common in several organic compounds. Due to the high protein content in *Moringa oleifera* leaves there

might also be a contribution in this from the N-H stretching of the amides group. The intense characteristic peak observed at 1058.9 cm^{-1} corresponds to C-O-C bonds of ether, phenol, and ester or related compounds. This band is characteristic of carbohydrates. Tran *et al.* (2020) also reported that the absorption of infrared energy in the 1200 cm^{-1} to the 900 cm^{-1} region is due to carbohydrate components. Furthermore, stretching vibrations located at around 473-703 cm^{-1} represent C-H, C=C, and N-H (Matinise *et al.*, 2017). The peak at 651-535 cm^{-1}, attributed to C-N. Moreover, the absorption band at 651 cm^{-1} is related to C-C-N bend amines and N-C=O bend amide (Girma *et al.*, 2018). The presence of this bend confirms the protein structure in the Moringa leaves. Thus, the components of *Moringa oleifera* leaf powder could be aliphatic or aromatic.

Table 4.3 Functional groups from the FTIR spectra of MOLP, PEG, and solid dispersions[1]

Method	Carrier	Wavenumber (cm^{-1})
Pure MOLP		3287, 2921.2, 2850.6, 1990, 1640.9, 1553.6, 1420.6, 1316.6, 1246, 1058.9, 530.95, 468.6
Freeze-drying	PEG6000	3380, 2883.59, 1466.43, 1359.72, 1341.67, 1279.27, 1241.01, 1146.89 1100.39, 1059.90, 961.28, 841.58, 528.43
	PEG4000	3315, 2893, 1628, 1467, 1342, 1280, 1241, 1104, 962, 842, 526
Melting	PEG6000	3361, 2892, 1634, 1467, 1342, 1280, 1241, 1101, 962, 842,531
	PEG4000	3289, 2891, 1634, 1467, 1342, 1241, 1104, 960, 840, 529, 427
Solvent evaporation	PEG6000	3424, 2888, 1634, 1468, 1342, 1280, 1242, 1105, 960, 842, 530
	PEG4000	3288, 2892, 1649, 1467, 1342, 1280, 1242, 1104, 960, 842, 531
Microwave	PEG6000	3351, 2891, 1652, 1467, 1467, 1342, 1280, 1149, 1103, 962, 842, 526
	PEG4000	3341, 2891, 1628, 1467, 1342, 1280, 1241, 1104, 961, 842, 532

[1]SD: solid dispersion; MOLP: *Moringa oleifera* leaf powder; PEG4000 and PEG6000: Polyethylene glycol

It may therefore be inferred that aliphatic or aromatic alcohols, phenols, amine, ketones, esters, and some nitrogen-containing compounds are the constituents of the leaf of *Moringa oleifera*. The FTIR results obtained in this study for MOLP were per those reported in the literature (Huang *et al.*, 2020). FTIR spectra of PEG4000 and PEG6000 (not reported) indicated important vibrations, including the broadband at 3425 cm-1 due to O-H stretching, aliphatic C-H stretching at 2875 cm-1, and C-O at 1100 cm^{-1} and 960 cm^{-1}, and O-H primary or secondary, OH in-plane bending or stretching at 1341 cm^{-1}. These results are in good agreement with the previously recorded spectrum or values for the polymer (Shameli *et al.*, 2012; El Maghraby & Elsergany, 2014).

FTIR spectra and functional groups of solid dispersions

Melting method

Solid dispersions prepared by the melting method (Figure 4.12) resulted in clear band shifts and increased peak intensity. The appearance of broadband at 3413 cm^{-1} and 3416 cm^{-1} in MOLP-PEG4000 and MOLP-PEG6000 solid dispersions belongs to stretching vibration of a phenolic hydroxyl group (O-H) which represents hydrogen bonding between MOLP and carriers. The hydrogen bonding occurs between the amide (NH_2) functional group of MOLP and the ether group (-O-) of the PEG polymer, depicted in Figure 4.10. Another hydrogen bonding interaction occurs between NH_2 (MOLP) and O-H (PEG6000). Hydrogen bonds are essential in solid dispersions because they can reduce molecular mobility, depress the driving force of crystallization, and thus enhance the stability and solubility of the solid dispersion compared to pure MOLP. Kumavat *et al.* (2013) reported similar results (intermolecular hydrogen bonding) for Curcumin and PEGs (PEG4000 and PEG6000) solid dispersions. Guedes *et al.* (2011) also reported the same findings for Imidazolidinedione and PEG000 solid dispersions. In pure MOLP (3287 cm-1), the O-H peak position was greatly shifted to 3413 cm^{-1} and 3416 cm^{-1} in MOLP-PEG4000 and MOLP-PEG6000 solid dispersions, respectively. This indicated that there was an intermolecular interaction between MOLP and PEG after the preparation of solid dispersions, which may be from Van der Waal interactions, hydrogen bonding (through O-H and N-H groups) (Yu *et al.*, 2020). The intermolecular hydrogen bonds between MOLP and PEG facilitated the stability of the solid dispersions during storage through reduced moisture content from 8.44% (pure MOLP) to 3.07-3.65% (MOLP-PEG6000 and MOLP-PEG4000 solid dispersions), as determined in Chapter 3. Moreover, when comparing the solid dispersions, the redshift of the O-H spectral peak in MOLP-PEG6000 solid dispersion was greater than MOLP-PEG4000. This may be due to the increase of the PEG molecular weight (El Maghraby & Elsergany, 2014). The spectral peak observed at 2992 cm^{-1} (C-H) in

pure MOLP disappeared in the spectral analysis of solid dispersions, suggesting that binding occurred between MOLP-PEG. Whereas the peak at 2853 cm^{-1} in pure MOLP attributed to the symmetric C-H group shifted to 2892 cm-1 (higher wavenumber), indicating hydrogen bonding between ether (-O-) group in PEG and methyl (C-H) group in MOLP, as shown in Figure 4.11. The spectral peaks at 1641, 1541, 1237, 1064, 668 cm^{-1}, assigned to C=O and N-H, primary amide group, secondary amine group, symmetric bending of C-O, C-H bending, and C-N, respectively, all shifted to higher wavenumber. In addition to peak shifts of the primary and secondary amide groups, the peak intensity of these peaks was reduced. These results prove that MOLP and PEG interacted with one another via hydrogen bonds through their N-H and O-H functional groups. Significant changes in the position of characteristic peaks of the MOLP when mixed with PEG carriers indicated compatibility between the two components.

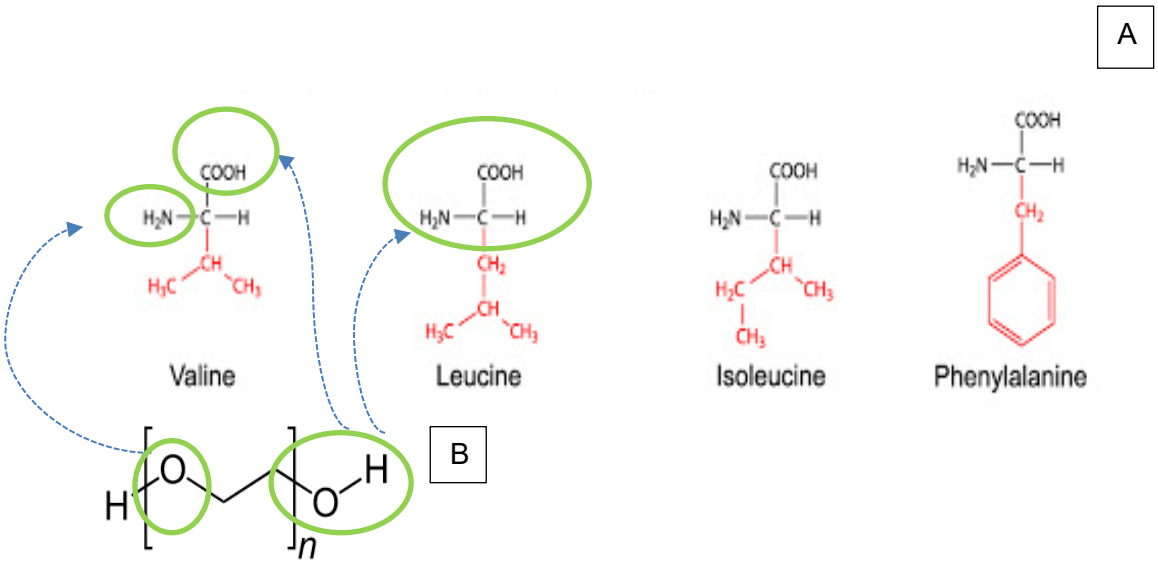

Figure 4.11 Chemical structures of some of the essential amino acids present in (a) MOLP and (b) general chemical structure of polyethylene glycol showing potential positions of hydrogen bonding between the components

As shown in Figure 4.13, the FTIR spectra of MOLP-PEG4000 and MOLP-PEG6000 solid dispersions prepared by the freeze-drying method indicated broadband at 3309 cm^{-1} and 3388 cm^{-1} and, respectively. The broad peak corresponds to an O–H stretch vibration, which suggests the presence of hydroxyl groups in solid dispersions. Those groups are attributed both to hydroxylated molecules of the MOLP (e.g., polyphenols) and to the moisture content of the solid dispersion samples. In addition to broadening the O-H region peaks in solid dispersion, changes in peak shape, intensity, and position have occurred in the solid

dispersions' FTIR spectra.

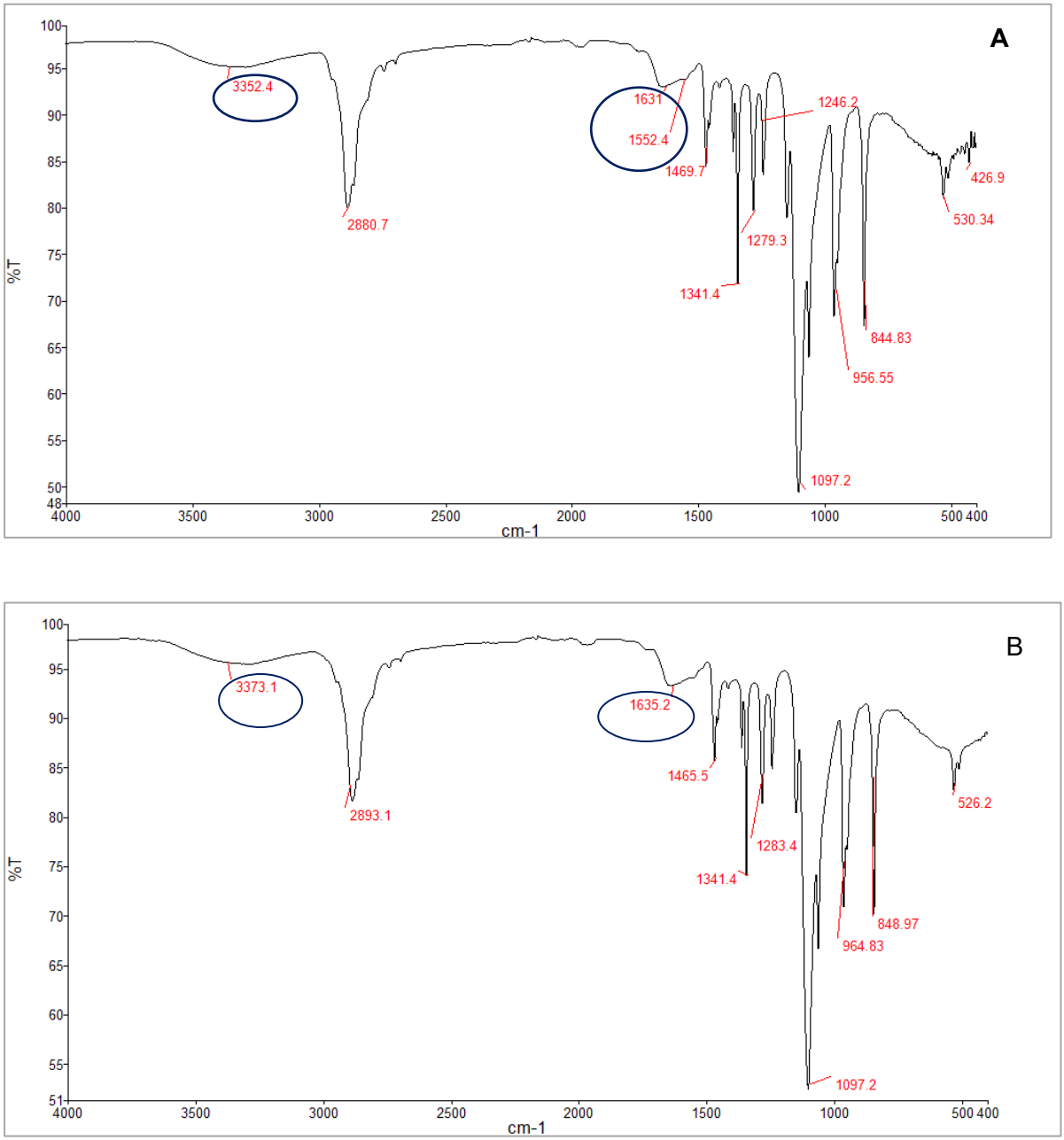

Figure 4.12 FTIR spectra of solid-dispersed MOLP samples prepared through melting method using (a) PEG4000 and (b) PEG6000.

These significant changes in the position of characteristic peaks of the MOLP when mixed with PEG carriers indicated compatibility between the two components. Both peaks for the solid dispersion (MOLP-PEG4000 and MOLP-PEG6000) samples shifted towards a higher wavelength, and peak intensity got reduced compared to MOLP. This, therefore, suggests the

presence of physical interactions (intermolecular interactions) between MOLP and PEG carriers (Yu *et al.*, 2020), which might have happened through hydrogen bonding via O-H of PEG and N-H of MOLP functional groups as substantiated in Chapter 3 (proximate analysis). The hydrogen bonding also occurs between the amide group of MOLP and the ether group (-O-) of the PEG polymer, depicted in Figure 4.11. Hydrogen bonding between an ingredient and carrier is important in solid dispersions, as it promotes greater stability and increases solubility.

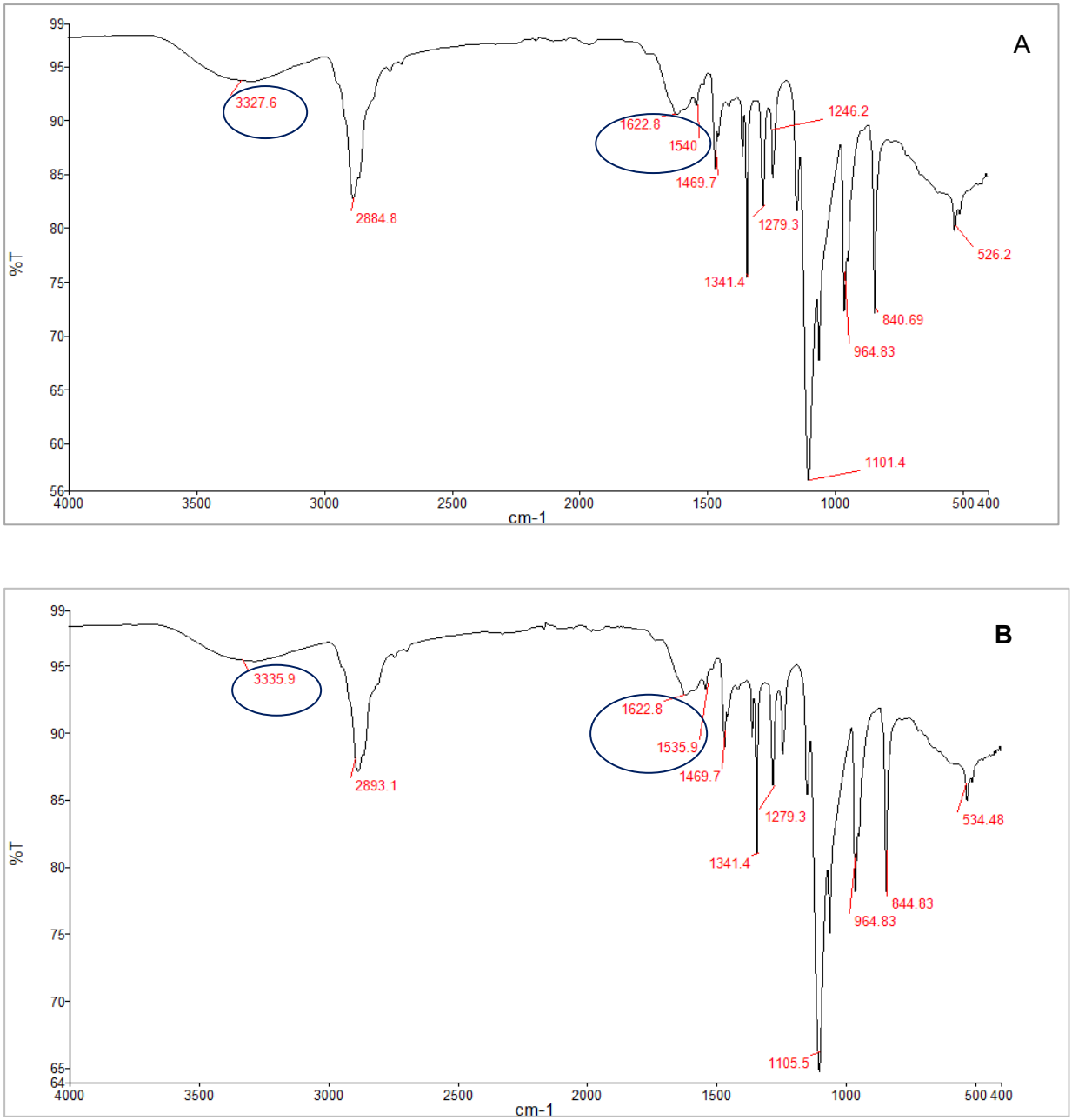

Figure 4.13 FTIR spectra of solid-dispersed MOLP complexes prepared through freeze-drying method using (a) PEG4000 and (b) PEG6000

Kumavat *et al.* (2013) reported similar results (intermolecular hydrogen bonding) for Curcumin and PEGs (PEG4000 and PEG6000) solid dispersions, suggesting successful interaction between the components. The characteristic peaks for the aliphatic C-H asymmetric group at 2922 cm^{-1} in the MOLP spectrum completely disappeared in all solid dispersions. While the symmetric C-H at 2853 cm^{-1} in MOLP greatly shifted to 2883.59 cm^{-1} and 2893 cm^{-1} in MOLP-PEG4000 and MOLP-PEG6000 solid dispersions, respectively, indicating hydrogen bonding between ether (-O-) group in PEG and C-H methyl group in MOLP. These changes also suggest that the coordination bond formed between *Moringa oleifera* particles and the hydroxyl groups of PEG (Jayaramudu *et al.*, 2016). The peak disappearances and shifts indicate chemical and intermolecular interactions between the pure MOLP and the carrier. The characteristic peaks at 1640.9 cm^{-1} and 1548 cm^{-1} in MOLP, attributed to the primary (C=O, N-H, C-N) and secondary (N-H) amides, respectively, greatly shifted towards lower wavenumbers 1622.8 cm^{-1} and 1538.8 cm^{-1}, in solid dispersions. This proves a successful interaction between MOLP (N-H) and PEG (O-H) functional groups via hydrogen bonding. The C-N stretching at 651 cm^{-1} in the pure MOLP spectrum also shifted to a lower wavenumber 529 cm^{-1}. The downward shift is caused by the lengthening of the N-H bond, which occurs in hydrogen bond formation. This can also be due to the relatively reduced protein content in solid dispersions, MOLP had 28.44% while 12.43% and 12.70% were obtained for the solid dispersions. Similar results were reported by Febriyenti *et al*. (2019) for gliclazide and PEG6000 solid dispersions.

The FTIR spectra of MOLP-PEG4000 and MOLP-PEG6000 dispersions prepared using the solvent evaporation method are displayed in Figure 4.14. The functional groups of MOLP investigated were N-H and O-H (3287 cm^{-1}), C=O (1770–720 cm^{-1}), and aromatic groups (900–675 cm^{-1}). The spectra of the solid dispersions showed a clear reduction on the O-H and N-H bands of MOLP. In addition, the O-H/ N-H absorption peak in MOLP at 3287 cm^{-1} shifted to a higher wavenumber 3398 cm^{-1} and 3424 cm^{-1} in MOLP-PEG4000 and MOLP-PEG6000 solid dispersions, respectively. This indicated the intermolecular interaction between MOLP and PEG carriers, which may form a hydrogen bond through the hydroxyl group of PEG and amide group of MOLP (Yu *et al.*, 2020). Another hydrogen bonding in this region occurred between the amide group of MOLP and the ether group (-O-) of the PEG polymer, displayed in Figure 4.11. A large upward shift of the O-H vibration in solid dispersions indicates that the hydrogen bond strength dramatically decreased, suggesting that the mass of the water and amino acid molecules was reduced. This, therefore, is in good agreement with the results obtained in Chapter 3 since a decrease in moisture (from 8.44% MOLP to 2.65-2.74% solid

dispersions) and protein contents (28.44% to 15%) was observed. The disappearance of the MOLP asymmetric C-H peak at 2922 cm^{-1} in solid dispersions was observed.

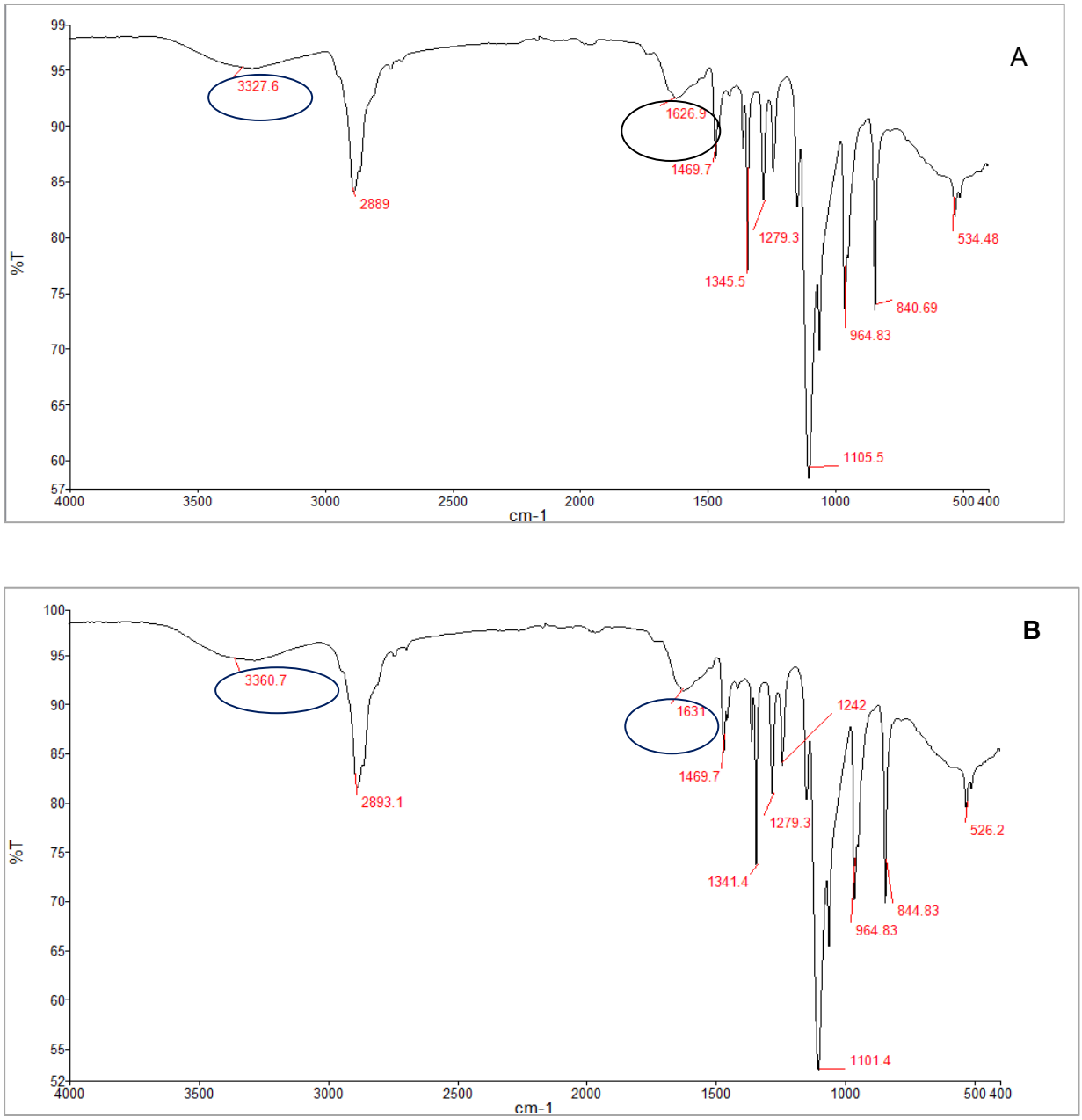

Figure 4.14 FTIR spectra of solid-dispersed MOLP complexes prepared through solvent evaporation method using (a) PEG4000and (b) PEG6000

This may be interpreted as the consequence of the hydrogen bonds between the final O-H group (PEG) and the C-H of MOLP. The MOLP spectral peak at 2853 cm^{-1} associated with symmetric C-H shifted towards a higher wavenumber in solid dispersions, 2884.8 and 2893.1 cm^{-1} (MOLP-PEG4000 and MOLP-PEG6000, respectively), indicating hydrogen bonding

between ether group in PEG and methyl group in MOLP. Moreover, a characteristic peak C=O and N-H peak at 1636 cm^{-1} in MOLP attributed to the primary amide (amide I) shifted towards a lower wavenumber. Thus, indicating a blue shift predominantly due to strong hydrogen bonding between the final O-H group of the PEG and the C=O group of MOLP or between the N-H group of MOLP and the O-H group of PEG. The significant changes in the position of characteristic peaks of the MOLP when mixed with PEG carriers indicated compatibility between the two components. Strong intermolecular interactions via hydrogen bonding are important in solid dispersions. They may help avoid the thermal degradation of ingredients and improve solid dispersions' miscibility and physical stability. Thus, in chapter 3, we observed an improved solubility of solid-dispersed MOLPs. While the region for aromatics (900-530 cm^{-1}) of MOLP displayed a marked increase in intensity, suggesting the presence of van der Waals-type interactions in both solid dispersions. The presence of hydrogen bonds and hydrophobic interactions between MOLP and the hydrophilic carrier in the solid dispersions is the primary cause of the rise in their solubility and dissolution rate. These results are similar to the ones reported by Kumavat *et al.* (2013) for curcumin and PEGs (PEG4000 and PEG6000) solid dispersions. Febriyenti *et al*. (2019) also reported similar findings for the gliclazide and PEG6000 solid dispersion, where physical interactions between the components were caused by hydrogen bonds between the amine group and –O- group.

Solid dispersions prepared by the microwave irradiation method (Figure 4.14) also resulted in a shift and displacement in some spectral bands of MOLP (O-H or N-H and C=O) in solid dispersions. Moreover, disappearances of peaks in the solid dispersions were also observed. The band at 3280 cm^{-1}, attributed to the O-H group in the MOLP spectrum greatly shifted to a higher wavenumber at 3581 cm^{-1} and 3400 cm^{-1} in MOLP-PEG4000 and MOLP-PEG6000 solid dispersions, respectively. This evidenced the physical and intermolecular interaction between MOLP and carriers through hydrogen bonding. The hydrogen bonding occurred between the amide (N-H) group of MOLP and the ether (-O-) group of the PEG polymer, depicted in Figure 4.10. Another hydrogen bonding occurred between the N-H group and the O-H group. These significant changes in the position of characteristic peaks of the MOLP when mixed with PEG carriers indicated compatibility between the two components. Similar results (intermolecular hydrogen bonding) were reported by Kumavat *et al.* (2013) for Curcumin and PEGs (PEG4000 and PEG6000) and Febriyenti *et al.* (2019) for gliclazide and PEG6000 solid dispersions, suggesting successful interaction between the two components. The disappearance of the characteristic asymmetric C-H peak at 2921.2 cm^{-1} in MOLP was observed in both solid dispersions. Whereas the spectral band observed at 2853 cm^{-1} associated with the symmetric C-H group in MOLP shifted towards higher wavenumber,

2883.8 cm^{-1} and 2892 cm^{-1} in MOLP-PEG4000 and MOLP-PEG-6000 solid dispersions, respectively, indicating hydrogen bonding between the -O- ether group of PEG and C-H methyl group of MOLP.

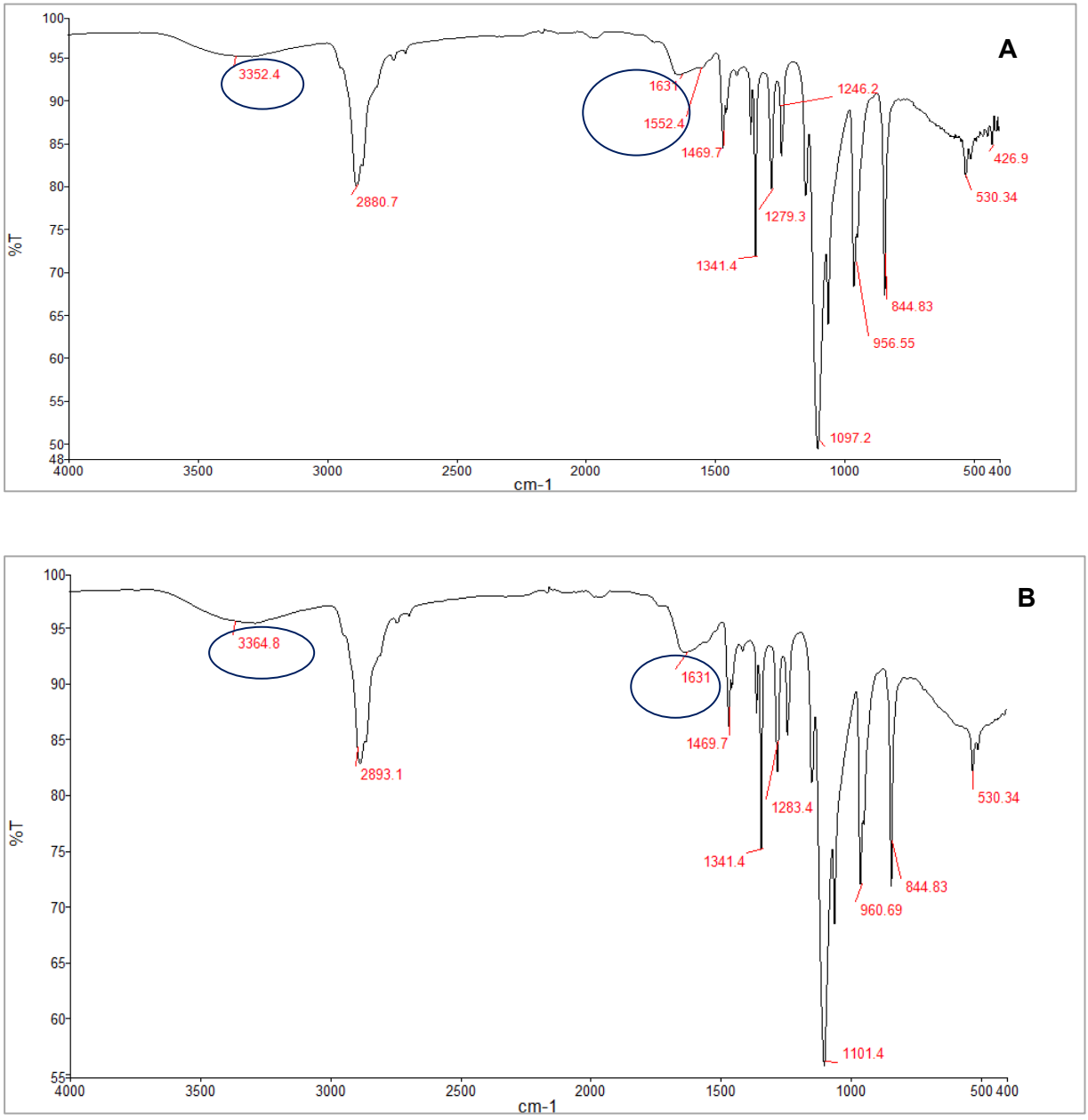

Figure 4.15 FTIR spectra of solid-dispersed MOLP prepared through microwave method using (a) PEG4000 (A) and (b) PEG6000

The intensity of these peaks also increased. Moreover, the primary amide group (N-H, C=O) peak at 1640.9 cm^{-1} in MOLP had a downward peak shift in solid dispersion (1631 cm^{-1}), proving strong hydrogen bonding between MOLP and PEG that occurred between N-H and

O-H functional groups. Thus, a decrease in protein content from 28.53% (MOLP) to 12.52-12.90% in MOLP-PEG4000 and MOLP-PEG6000 solid dispersion prepared by microwave irradiation method. More so, the spectral peak at 1553.6 cm^{-1} associated with secondary amide N-H groups in MOLP decreased to 1552.4 cm^{-1} and 1469.7 cm^{-1} in solid dispersions (MOLP-PEG4000 and MOLP-PEG6000, respectively). The significant changes in the FTIR spectra solid dispersions confirmed the effect of the PEG carrier on the MOLP, thus proving compatibility between the two components. Moreover, the shift in peak values or positions may be due to intra and intermolecular bonds between functional groups present in MOLP and PEG. Changes in the environment of the chemical bonds affect the intensity and position of the peaks. These changes prove the physical interaction between MOLP and PEGs in the solid-state and are mainly caused by hydrogen bonds between the components. The formation of hydrogen bonds in solid dispersions is essential for improved stability and solubility. However, no new peaks were formed in solid dispersions, suggesting that no new chemical bonds were created. Febriyenti *et al.* (2019) reported similar results for gliclazide and PEG6000 solid dispersions.

4.6 Conclusion

The novel solid dispersions of MOLP successfully prepared by freeze-drying, melting, solvent evaporation, and microwave irradiation methods using PEGs molecular weights 6000 and 4000 hydrophilic carriers were more thermally stable than the pure MOLP; this suggests that the PEGs conferred greater stability to the solid dispersions. Moreover, the solid dispersions were found to be partially amorphous. The FTIR spectra of the solid dispersions revealed the formation of MOLP-PEG intermolecular interactions via hydrogen bonding between hydroxyl (O-H) or amine (N-H) functional groups on MOLP with O-H and –O- ether functional groups of PEGs. These interactions were observed in all the solid dispersions and further confirmed by the PXRD study. Moreover, the significant changes in the position of characteristic peaks of the MOLP when mixed with PEG carriers indicated compatibility between the ingredient and the carrier. Interactions between ingredients and polymers play an important role in the solid dispersion process and influencing the product properties. Strong intermolecular interactions may help avoid the thermal degradation of ingredients and improve solid dispersion's miscibility and physical stability. Furthermore, the study has provided a better understanding of the role of PEG in the improved functionalities of MOLP via solid dispersion-based processing. Therefore, it is hoped that the novel solid dispersions of MOLP with improved solubility and good thermal stability would be a promising technology to broaden the application of MOLP in foods and beverages.

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CHAPTER FIVE
FLOW AND DISSOLUTION PROPERTIES OF SOLID-DISPERSED *MORINGA OLEIFERA* LEAF POWDER

Abstract

Moringa oleifera leaf powder (MOLP) has been identified as the most prominent ingredient due to its beneficial effects (nutritional and medicinal). However, its low solubility severely limits its utilisation in the food and beverage industries. Therefore, there is a need to enhance the dissolution rate of MOLP. The purpose of the present study was to investigate the effect of polyethylene glycol (PEG) molecular weights 4000 and 6000 as solid dispersion hydrophilic carriers on the *in vitro* dissolution behaviour of the solid-dispersed *Moringa oleifera* leaf powder (SDMOLP). Solid dispersions of MOLP-PEG (4000 and 6000) prepared through melting, freeze-drying, solvent evaporation, and microwave methods were evaluated in the solid-state for flow properties using Hausner's ratio, Carr's index, and angle of repose. The solid dispersions were further characterised in the liquid state for their dissolution behaviour using a Vankel's dissolution apparatus [United States Pharmacopeia (USP) basket method] and analysed with a UV-vis spectrophotometer. The results demonstrated moderate to excellent flow properties for the SDMOLP with the Hausner's ratio ranging from 1.29-1.34, Carr's index from 22.36-25.54%, and angle of repose from 27.16-28.29^{o}, while pure MOLP exhibited poor flowability. Moreover, the MOLP solid dispersions showed significantly improved ($p < 0.05$) than pure MOLP in 60 minutes. In comparison to MOLP, the solid dispersion generated by the melting method displayed a three-fold increase in dissolution. As a result, the solid dispersion approach and PEG carriers have the potential to increase MOLP flow characteristics and dissolution rate. SDMOLPs with improved dissolution may be useful in the beverage and pharmaceutical industries. However, the use of markers is recommended in future dissolution studies of MOLP since it is a heterogeneous plant.

5.1 Introduction

The use of herbal products in the food industry and the management of various ailments has gained popularity in recent years. With the advancement in herbal product technology, plant-based products have received substantial attention from the functional foods segment, dietary supplements, and the medical community and consumers. *Moringa oleifera* leaf powder is one of the highly nutritious parts of the *Moringa oleifera* plant that is mostly used to combat malnutrition and cure diseases. Nonetheless, like any herbal leaves, *Moringa oleifera* leaf powder (MOLP) has been reported to possess poor flowability, low

aqueous solubility, and dissolution rate (Ali *et al.*, 2018, 2019). MOLP's poor solubility may be due to its high protein content, vitamins, minerals, and various phenolic compounds. To address these challenges and enhance the effectiveness of herbal products/plants, several novel systems have been employed in recent times. These include solid dispersion technology, among others. Therefore, preparing solid dispersions of poorly soluble ingredients with hydrophilic carriers is a promising technique for improving the dissolution characteristics of the ingredients/compounds (Ghareeb *et al.*, 2009; Daravath *et al.*, 2017; Younis, 2017; Ma & Williams, 2019). There have been few published studies on the dissolution of solid-dispersed herbal products. These include *Kaempferia parviflora* extract (Weerapol *et al.*, 2017), *Selgnella deadline* extract (Chen *et al.*, 2020). Ali *et al.* (2018) studied the flow and dissolution properties of pure MOLP and MOLP treated with excipients and the findings showed that MOLP has poor flow and dissolution properties. However, no previous studies have been reported on the flow and dissolution properties of solid-dispersed MOLP. As a result, it is necessary to introduce and implement new techniques that will increase the application of MOLP in the food industry. Recently, characterising the flowability of food powders has gained great importance in the food industry to prevent production stoppages caused by powdered foods' unpredicted behavior and prevent poor product quality (Doğan *et al.*, 2019). Since the texture and mouthfeel of the end product are affected by the different granularity of the powders, the knowledge about the food powder properties is also significant for both commercial and domestic users. The flowability of powder is defined as the ease with which a powder can flow under a specific set of conditions (Intipunya & Bhandari, 2010). The flowability of the food powder cannot be explained by the physical appearance or shape of the particle. Various techniques such as the angle of repose, Hausner’s ratio, and Carr’s index have been employed (Ali *et al.*, 2019). The literature data on flow behaviour mostly analysed the milk and pharmaceutical powders. There is limited information on the flow properties of herbal or food powders. The essential knowledge regarding the powders' bulk, flowability, and processability characteristics is required to get a reliable prediction of the flow performance.

Dissolution is the amount of substance that goes into solution per unit time under standardised conditions of liquid interface and temperature (de Sousa *et al.*, 2011; Mbamalu, 2015; Byrn *et al.*, 2017). At present, dissolution testing is a crucial tool employed for quality control of conventional drug formulations. It is also used to optimise formulations and desired release specifications for drug products (Mbamalu, 2015). As widely accepted as this test is for conventional drug and active ingredient (API) formulations, it is new for herbal products. Since herbal products have gained great popularity in recent years and may diversify in the food, dietary supplements, nutraceuticals, and pharmaceutical

industries, perhaps serving as new formulations or improved versions of previous formulations, there is a need to implement new approaches that will improve their quality profiles. Dissolution studies of these materials, carried out following relevant guidelines, may provide the desired development. Currently, few herbal products classified as dietary supplements, curcuminoids, turmeric, and soy isoflavanoids, have monographs with dissolution specifications in the USP (Mbamalu, 2015). Because of their importance, dissolution tests are often used to control the quality of synthetic drugs, but their use for evaluating natural products has not become widespread (Taglioli *et al.*, 2001; Mbamalu, 2015). The biopharmaceutical characterisation of herbal products (*in vivo / in vitro*) can be very challenging due to their complex composition and high metabolites (Costa *et al.*, 2011; de Sousa *et al.*, 2011). Given the above, the dissolution test on *Moringa oleifera* leaf powder is essential because the population extensively consumes it, and the lack of this kind of study involves herbal products and nutraceuticals. Therefore, in this study, the solid dispersions of MOLP developed in Chapter 3 were characterised for flow and dissolution properties.

5.2 Materials and Methods

5.2.1 Sources of materials and equipment

Moringa oleifera leaf powder (MOLP) was procured from Supa Nutri PTY Ltd in Cape Town, South Africa. The polyethylene glycol polymers were purchased from Spellbound Company, South Africa. Chemicals used in this study were of analytical grade. The dissolution apparatus (VanKel VK 700, USA) was sourced from the School of Pharmacy at the University of Western Cape. UV-vis spectrophotometer (Perkin Elmer Lamba 25) was obtained from the Department of Food Science and Technology, CPUT. Figure 5.1 shows Chapter 5 outline.

5.3 Determination of physical and flow properties of MOLP and SDMOLPs

5.3.1 Bulk density and tapped density

The bulk density (ρBulk) was determined by weighing 30 g of SDMOLP effervescent granules into a 100 ml graduated measuring cylinder without compacting. The granules were carefully levelled without compacting and the apparent unsettled volume was read and recorded. The bulk density was calculated using equation 5.1.

$$\rho\text{Bulk}\ (g/mL) = \frac{Weight\ of\ SDMOLP\ granules\ (g)}{Volume\ of\ SDMOLP\ (mL)} \qquad \textit{Equation 5.1}$$

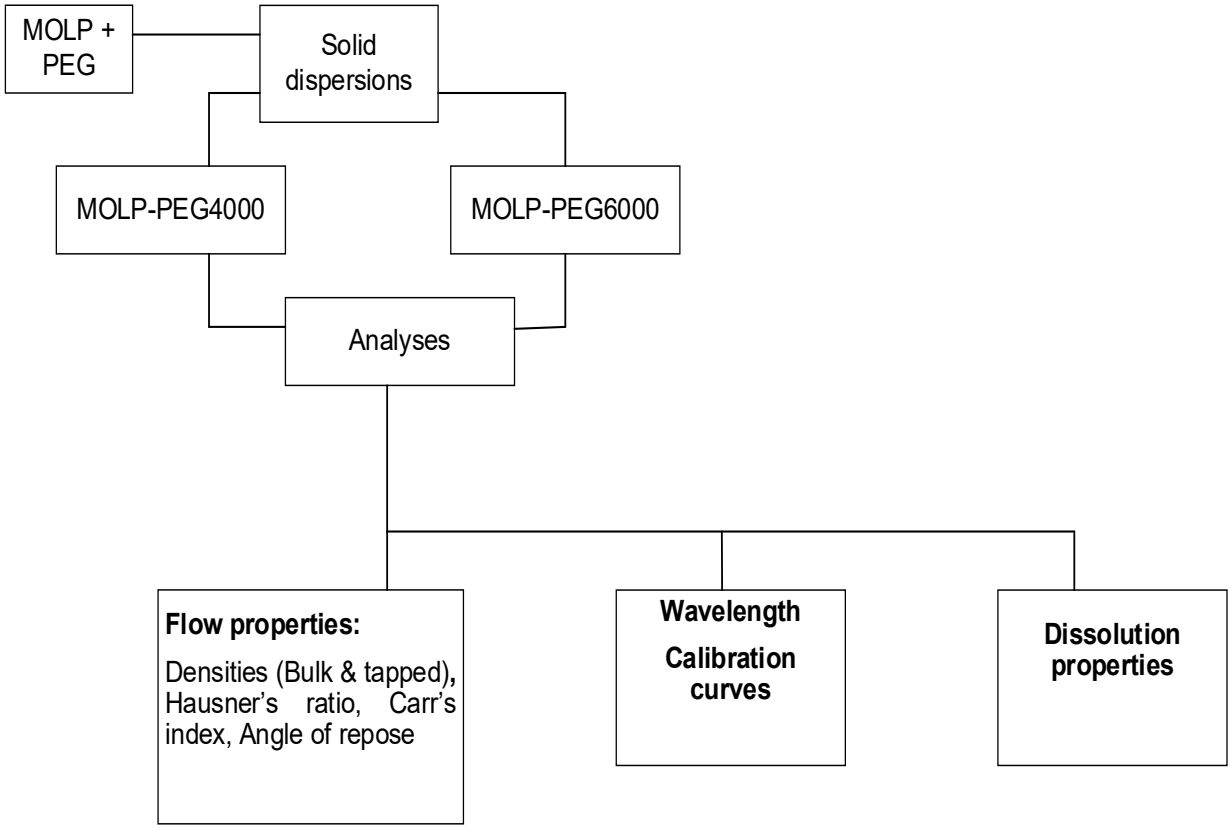

Figure 5.1 Chapter 5 outline

5.3.2 Tapped Density

For tapped density determination, the same measuring cylinder containing the SDMOLP or MOLP (as weighed in section 5.3.1 was tapped 50 times at the height of 2.5 cm at 2-second intervals until no further change was noticed in the volume. The tapped density was calculated in g/mL using equation 5.2.

$$\rho\text{Tapped (g/mL)} = \frac{Weight\ of\ SDMOLP\ (g)}{Volume\ of\ tapped\ SDMOLP(mL)} \qquad \textit{Equation 5.2}$$

5.3.3 Hausner's ratio

Hauser's ratio was determined and calculated as the tapped density divided by the bulk density. Hausner's ratio is related to interparticle friction and can be used to predict powder flow properties. Low Hausner's ratio (< 1.25) indicates better and good flow properties than higher ones. Hausner's ratios, from 1.25 to 1.6, depict moderate flowing properties (Pandey *et al.*, 2013). While Hausner's ratios with more than 1.6 show poor flow properties and

cohesive powders (Ali et al., 2018; Hiola & Tungadi, 2018). Hausner's ratio was therefore calculated using equation 5.3.

$$\text{Hausner's ratio} = \frac{\rho Tapped}{\rho Bulk} \qquad \textit{Equation 5.3}$$

Where, ρTapped = tapped density, ρBulk = bulk density

5.3.4 Measurement of Carr's index

Compressibility (Carr's) index is known as percent compressibility, indirectly related to the flow rate, cohesiveness, and particle size. Compressibility is the ability of a powder to decrease in volume under pressure. It is a simple, fast, and accurate method of predicting powder flow characteristics. Carr's compressibility index of the powder samples was obtained from density determinations (Ali et al., 2018, 2019). Carr's index for each formulation was calculated using equation 5.4.

$$\text{Carr's index (\%)} = \frac{\rho\text{Tapped} - \rho\text{Bulk}}{\rho\text{Tapped}} \times 100 \qquad \textit{Equation 5.4}$$

Where ρTapped = tapped density, ρBulk = bulk density

5.3.5 Angle of Response

The flowability of solid dispersions was evaluated by measuring the angle of repose and the flow rate through a funnel. A fixed funnel method was used (Ali et al., 2018; Aslani & Fattahi, 2013; Hiola & Tungadi, 2018; Kumavat et al., 2013; Yeo et al., 2020). Specifically, a fixed funnel (100 mm) with an orifice inner diameter of 10 mm and specially designed to evaluate the flowability of powders or granules were used. The funnel was fixed above a flat horizontal surface at an appropriate height (2 cm). The orifice of the funnel was closed, and 10 g of the sample (pure MOLP, PEG, solid dispersions) was poured into the funnel. Then, the orifice of the funnel was opened, allowing the sample to pass through. The radius of the base of the conical pile was measured. The angle of repose (θ) was calculated using equation 5.5.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right) \qquad \textit{Equation 5.5}$$

Where, θ = angle of repose (°), *h* = fixed height (cm), and *r* = radius of the formed powder base (cm).

5.4 Determination of maximum wavelength

The identification of *Moringa oleifera* leaf powder (MOLP) and the solid dispersions was done using an ultraviolet-visible (UV-vis) spectrophotometric method reported by Shousha *et al.* (2019) with modifications. Sample equivalent to 100 mg was accurately weighed and taken in a 100 mL volumetric flask and filled with distilled water up to the mark. The flask was closed, and the solution was vigorously stirred on a vortex for 5 minutes until the MOLP or SDMOLPs was dissolved. The solution was then transferred into 50 mL centrifuge tubes and centrifuged (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc, USA) for 5 min at 12 000 rpm. The supernatant was then filtered through a 0.45µm filtration membrane. The prepared stock solution (1 M) was transferred into a beaker and further diluted with distilled water (1 ml of stock solution was diluted up to 10 ml with distilled water). Using distilled water as the reference, the diluted solution was finally scanned for maximum absorbance and wavelength (λmax) using the UV-vis spectrophotometer in the range of 200-400 nm. The characteristic peaks were detected, and the peak values of the UV-Vis were recorded.

5.5 Preparation of Standard Calibration Curve

Standard stock solutions of MOLP and SDMOLP were prepared by dissolving 100 mg sample in 100 mL volumetric flask and filled with distilled water up to mark. The stock solution was subsequently diluted with distilled water to get desired concentration range: 200, 300, 400, 500, 600, 700, 800, 900 µg/mL. The absorbance of the dilutions was measured at 286 nm and 382 nm, respectively using a UV-vis spectrophotometer against a blank of distilled water. A calibration graph was plotted by taking absorbances on Y-axis and concentration (µg/ml) on X-axis. The data were fitted to a linear regression equation: $y = mx + c$, R^2 (R^2 is a statistical measure of how close the data are to the fitted regression line), the concentration of the compound released was calculated using the linear equation derived from the slope of the curve.

5.6 Determination of Dissolution Properties of Solid-dispersed MOLP

The MOLP and SDMOLPs were evaluated using a dissolution apparatus for the in vitro dissolution tests (VanKel VK 700, USA). The dissolution medium of 900 mL distilled water was used. Samples equivalent to 200 mg pure MOLP and solid-dispersed MOLPs were accurately weighed and filled into size 0 capsules. The capsules were weighed before and after filling to determine the uniformity of weight.

The United States Pharmacopeia (USP) basket apparatus method described by Mbamalu (2015) with modifications was employed to determine the dissolution of the capsules. The dissolution medium of 900 mL distilled water maintained at 37 ± 0.5°C was used. One capsule containing the appropriate MOLP or SDMOLPs material was placed in

each dissolution vessel basket, and vessel contents were stirred at 100 rpm. At predetermined time intervals (10, 20, 30, 40, 50, and 60 minutes), 5 ml of sample was withdrawn from each vessel through a 0.45 µm nylon membrane syringe filter. The samples were filtered during withdrawal to prevent the continuation of dissolution and replaced with an equal volume of the same pre-warmed medium to maintain constant volume and temperature. The filtered withdrawn samples were analysed using UV-vis spectroscopy at 286 nm and 382 nm, respectively. The percentage release was calculated using an equation obtained from the calibration curve (equations 5.6-5.8). Each sample was performed in triplicate.

$$Y = mx + C \qquad \textit{Equation 5.6}$$

Where Y= Absorbance, m= gradient, x= concentration,

Amount of compound (mg) = Conc. × volume of the dissolution medium × DF/1000

Equation 5.7

Where Conc. = concentration; DF= dilution factor

$$\text{Release percentage (\%)} = \frac{Amount\ of\ compound}{Sample\ size} \times 100 \qquad \textit{Equation 5.8}$$

5.7 Data Analysis

Multivariate analysis of variance (MANOVA) was used to determine the significant differences ($p \leq 0.05$) in attributes among samples. Duncan's multiple range test was used to separate means where a significant difference existed (IBM SPSS version 26, 2020). Principal Component Analysis (PCA) was used to extract the components that explained the variability in the flow properties of pure MOLP and solid dispersions (Unscrambler ver 10.4). The Smoothing Savitsky-Golay method was used.

5.8 Results and Discussion

5.8.1 Physical properties of *Moringa oleifera* leaf powder and solid-dispersed *Moringa oleifera* leaf powder

Densities

The bulk and tapped densities of pure MOLP and solid dispersions are presented in Table 5.1. The pure MOLP showed a bulk density and tapped density of 0.38 g/mL and 0.50 g/mL, respectively. The relatively low bulk density of MOLP may be attributed to its fluffy structure and cohesiveness. Usually, cohesive powders have low bulk density and high compressibility (Carr's index) as compared to non-cohesive powders. These findings are in

good agreement with previous studies conducted by Ali *et al*. (2018, 2019), where the bulk density and tapped density of pure MOLP were 0.33 g/mL and 0.58 g/mL, respectively. The bulk density of MOLP was comparable to the one of cassava flour (*Chila* and *Mweru*) 0.40 and 0.47 g/mL, respectively, while their tapped densities were 0.62 and 0.67 g/mL, respectively. Usually, lower protein and lipid contents are characterised by lower bulk densities, as exhibited in *Chila* (1.21% protein and 0.15% lipid). However, that is not the same with MOLP, which has more than 28% protein content. Obatolu *et al.* (2000) showed that low bulk density is an advantage because high bulk limits the caloric and nutrient intake per feed per child to satisfy their energy and nutrient requirements. More so, instant products require reduced bulk densities.

Table 5.1 Bulk and tapped densities of pure *Moringa oleifera* leaf powder and solid-dispersed *Moringa oleifera* leaf powder[1]

Method	Carrier	Bulk density (g/ml)	Tapped density (g/ml)
Pure MOLP	-	0.34 ± 0.0^{a}	0.50 ± 0.0^{a}
Freeze-drying	PEG4000	0.48 ± 0.0^{b}	0.64 ± 0.0^{b}
	PEG6000	0.48 ± 0.0^{b}	0.64 ± 0.0^{b}
Solvent evaporation	PEG4000	0.57 ± 0.0^{c}	0.76 ± 0.0^{c}
	PEG6000	0.58 ± 0.0^{c}	0.76 ± 0.0^{c}
Melting	PEG4000	$0.59 \pm 0.0^{c,d}$	0.76 ± 0.0^{c}
	PEG6000	$0.59 \pm 0.0^{c,d}$	0.76 ± 0.0^{c}
Microwave irradiation	PEG4000	0.61 ± 0.0^{d}	0.79 ± 0.0^{d}
	PEG6000	0.61 ± 0.0^{d}	0.79 ± 0.0^{d}

[1]Values are mean $\pm$ standard deviation of three replicates. Means within a column followed by different superscripts are significantly ($p < 0.05$) different. MOLP: *Moringa oleifera* leaf powder. PEG: Polyethylene glycol.

On the other hand, bulk density and tapped bulk density ($p < 0.05$) of SDMOLP samples were observed in the range of 0.48–0.61 and 0.64–0.79 g/mL, respectively. The highest bulk density was recorded in microwave solid dispersions (0.61 g/mL) for both PEG6000 and PEG4000 and lowest in freeze-dried solid dispersions (0.48 g/mL). These results concur with those reported by Kumavat *et al.* (2013) for the solid dispersed curcumin

(PEG4000 and PEG6000) prepared by the melting method (041-0.46 and 0.55-0.62, bulk and tapped densities, respectively). The low bulk densities in freeze-dried solid dispersions may be attributed to their high porosity structure, as found in Chapter 3 (SEM and STEM study) section 3.16.1. As reported by previous researchers, the bulk density is reversely associated with porosity [1- (bulk density/granule density) × 100] (Mirhosseini & Amid, 2013). This study observed that the food powder with lower bulk density has a higher porosity and vice versa. Moreover, due to the sublimation of the ice, regarding the remaining solid, residual moisture content affects the properties of the powder, such as bulk density during freeze-drying. Caliskan & Dirim (2016) reported similar findings for the freeze-dried sumac extract powders (0.267–0.282 g/mL) their bulk densities were significantly lower than spray dried sumac extract powders (0.369– 0.508 g/ml). In a similar study, Mirhosseini & Amid (2013), found that the bulk density of freeze-dried Grewia (0.140 g/mL) was lower than that of oven-dried (0.160 g/mL). The bulk and tapped densities of the SDMOLPs were significantly ($p < 0.05$) higher than pure MOLP. The increase in the densities of SDMOLPs may be due to the carrier acting as an excipient. Caliskan & Dirim (2016) reported similar findings that bulk and tapped densities show a significant ($p < 0.05$) increase with an increase in carrier agent concentration. Bulk density and porosity depend on particle size, inter-particle voids of powders, properties of food powders and carrier agents, drying methods. Particles with higher moisture amounts tend to have a higher bulking weight due to the presence of water which is denser than dried solids (Tkacz *et al.*, 2020). However, in this study, no correlation was found between moisture content and bulk density. Instead, the opposite was observed, solid dispersions with lower moisture contents had higher bulk densities than MOLP. This study also observed that drying methods had a larger impact on bulk density than carrier agents. Thus, powders in terms of decreasing bulk density can be ordered as follows: microwave irradiation > melting > solvent evaporation > melting solid dispersions (0.61, 0.59, 0.58 and 0.48 g/mL, respectively). This behaviour can be explained by the crystal structure of the SDMOLPs obtained in microwave irradiation, melting, and solvent evaporation methods and by the reduction of interstitial air content (less porous) between the particles and the reduction of the volume occupied. However, the low bulk density in freeze-dried solid dispersions can be explained through its high porosity, as evidenced by the SEM study in Chapter 3. Since the density and volume are inversely proportional to each other, the higher density results in lower packaging volume. This phenomenon is prominent from the industrial point of view.

High bulk density is critical and economically positive in food packaging and transportation since products require smaller packaging, and transport and storage costs are lower. Moreover, bulk density is desirable as it offers a greater packaging advantage as

greater quantity may be packed within a constant unit volume (Chisenga *et al.*, 2019). In contrast, lower bulk density is associated with air entrapment in voids, thereby facilitating oxidation and less stability. The tapped density, also known as packed density, increased significantly ($p < 0.05$) in SDMOLPs than MOLP. The tapped density is the maximum packing density of powder or blend of powders achieved under the influence of well, externally applied forces (Ali *et al.*, 2017, 2018; Sarfraz *et al.*, 2019). The bulk density of the solid-dispersed MOLP could be used in determining their handling requirement because it is the function of mass and volume. The bulk density values can find use in packaging, handling, and processing requirements. Bulk density is a very complex product property for food powders and is of great importance for economic and functional reasons. High bulk density is desirable for reducing shipping and packaging costs. Contrarily, low bulk density, as seen in agglomerated or fluffy products, influences other powder properties such as flowability and instant characteristics.

5.8.2 Flow Properties of MOLP and SDMOLPs

The flowability and cohesiveness properties of MOLP and SDMOLPs are detailed in Table 5.2. MOLP had a Carr's index, Hausner's ratio, and angle of repose of 31.29%, 1.46, and 30.0^{o}, respectively. SDMOLPs had Carr's indexes, Hausner's ratios, and angles of repose ranging from 22.36-25.62%, 1.29-1.33, and 27.16-28.61^{o}, respectively. The classification of powder flowability based on Carr index is very good (> 15), good (15–20), fair (20–35), poor (30–35), and very poor (36-45) (Ali *et al.*, 2018; Hiola & Tungadi, 2018). The powder flowability based on Hausner's ratio is classified as good (< 1.2), moderate (1.2–1.4), and low (> 1.4) (Caliskan & Dirim, 2016). Pure MOLP exhibited fair to poor flowability. The higher Hausner's ratio that MOLP meant that the powder was more cohesive and less able to flow freely. These results are in agreement with those previously found by Ali et al. (2018). However, their Carr's index (42.98%) and Hausner's ratio (1.75) were extremely high, thus suggesting extremely poor flowability. Similar results were also reported by Okoye *et al.* (2013) where the Carr's index (30.10%), Hausner's ratio (1.42), and angle of repose (42%) were the same except for their angle of repose that was higher than the one obtained in this study which depicted extremely poor flowability properties. The variation in the flow properties of MOLP could be explained by differences in particle size and moisture content. This was also evidenced by PCA in section 5.4.2 which illustrated a positive correlation between moisture content and flow properties.

Table 5.2 Flow properties of pure MOLP and solid-dispersed MOLP[1]

Samples	Carrier	Hausner's ratio	Carr's index (%)	Angle of repose (º)
Melting	PEG4000	1.29 ± 0.034[a]	22.36 ± 2.095[a]	28.07±0.315[a]
	PEG6000	1.29 ± 0.034[a]	22.36 ± 2.095[a]	28.61 ± 0.510[a]
Microwave irradiation	PEG4000	1.29 ± 0.030[a]	22.94 ± 1.830[a]	28.18 ± 0.185[a]
	PEG6000	1.29 ± 0.030[a]	23.12 ± 1.518[a]	28.50 ± 0.510[a]
Solvent evaporation	PEG6000	1.29 ± 0.034[a]	22.36 ± 2.095[a]	28.07 ± 0.000[a]
	PEG4000	1.33 ± 0.035[a,b]	25.31 ± 1.994[b]	28.29 ± 0.375[a]
Freeze-drying	PEG4000	1.34 ± 0.084[b]	25.54 ± 0.468[b]	27.16 ±0.595[a]
	PEG6000	1.34 ± 0.008[b]	25.54 ± 0.477[b]	27.16 ± 0.610[a]
Pure MOLP	-	1.46 ± 0.025[c]	31.30 ± 1.176[c]	30.00 ± 1.109[b]

[1]Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly (p> 0.05) different. MOLP: Moringa oleifera leaf powder. PEG: Polyethylene glycol.

The poor flowability of MOLP could be attributed to its high cohesion strength (high Hausner's ratio), moisture content (6.10%), and fluffy texture. The particle size is one of the most important parameters that affect flowability. The powder cohesiveness increases when the particle size decreases because a decrease in the particle size heightens the contact surface area and results in greater van der Waals attractions among the particles (Caliskan & Dirim, 2016). The fine MOLP is cohesive since its average particle size was < 200 µm. Therefore, the higher rate of Carr's index and cohesion index (Hausner's ratio) of MOLP makes the addition of excipients or carriers essential.

SDMOLPs, on the other hand, exhibited acceptable flowability properties and low cohesiveness, with moderate Carr's indexes (22.36-25.56%), Hausner's ratios (1.22-1.33), and excellent angles of repose (27.16-28.61°). The excellent flowability of the solid dispersion was evidenced with the angle of repose within the range of 27-28.61°, which was < 30°. The flow properties of solid dispersions were significantly ($p < 0.05$) lower than those

of MOLP. There was no significant ($p > 0.05$) difference in Carr's index, Hausner's ratio, and angle of repose among the solid dispersions prepared by melting, solvent evaporation, and microwave irradiation methods (both PEG6000 and PEG4000). However, solid dispersions prepared by the freeze-drying method significantly differed ($p < 0.05$) from all the samples. This variation could be attributed to the moisture content of the freeze-dried dispersions (4.0%), which was higher than that of other solid dispersions (ranged from 1.87-3.16%), and its high porosity structure as evidenced by SEM in Chapter 3 section 3.16.1. However, even though the solid dispersions varied, their Hausner's ratios were still below 1.4, indicating moderate and passable flowability properties, and were significantly ($p < 0.05$) different from those of MOLP. The difference could be because pure MOLP is cohesive, as evidenced by the 1.42 Hausner's ratio. High Hausner's ratio means high cohesiveness (Ali *et al.*, 2018). Thus, implying a lesser free-flowing capacity (poor flowability). It was further observed that solid dispersions resulted in better flow properties ($p < 0.05$) than pure MOLP in Carr's index and angle repose. The increased flowability in SDMOLPs may be due to their low moisture content. Fitzpatrick *et al.* (2004) and Caliskan & Dirim (2016) reported that an increase in moisture content of powders had been found to increase the cohesion between powder particles, resulting in lower flowability. Our study concurs with previous studies, and the PCA demonstrated that the flow properties, moisture content, fat content were robustly connected, indicating a positive or strong correlation. Previous studies showed that the cohesiveness of powders is not only dependent on the moisture content or particle size but also the fat content, and the distribution of the fat located on the surface or in the inner part of the particle can be important (Kim *et al.*, 2005; Doğan *et al.*, 2019). This is also true, based on our findings in Chapter 3 section 3.14, MOLP had a significantly high-fat content than solid dispersions, which further explains its poor flowability compared to SDMOLPs. It was evident that the excipients (PEG4000 and PEG6000) and solid dispersion methods contributed to the acceptable flow characteristics for SDMOLPs. This study, therefore, proved that the solid dispersion technique does not only improve solubility and dissolution, as previously reported, but it improves the flow properties of the ingredients as well. In addition, the texture, morphology also played a significant role in the flowability differences between SDMOLPs and MOLP. SDMOLPs were denser and more spherical than pure MOLP (fluffy texture), thus making them easy to flow. There are no previous studies on the solid dispersions of MOLP. However, comparable results were reported by Caliskan & Dirim (2016) for the freeze-dried Sumac extract (with maltodextrin as a carrier). Their Carr's indexes ranged from 19.42% to 25.02%, and Hausner's ratios (1.241-1.333) indicated good flowability. Furthermore, Kumavat *et al.* (2013) reported similar results for the solid-dispersed curcumin, with Carr's indexes (18.18-

30.90%), Hausner's ratio (1.22-1.35), and angle of repose (22.42-28.47^{o}) which indicated good flowability since their angles of repose were < 33^{o}.

5.8.3 Identified Principal Components on flow properties of SDMOLPs and MOLP

The principal component analysis (PCA) was applied to evaluate the variability among the samples. Figure 5.2 shows the score and loading plots of principal components. The first principal component explains 68% of the variability in the data and the second principal component explains 14%. The two principal components (PC1 and PC2) well described the relationship among variables in the model, explaining 82% of the total variance. MOLPs represent the untreated samples, while SDMOLPs represent samples treated with PEGs (4000 and 6000) or with different methods (freeze-drying, solvent evaporation, melting, and microwave irradiation). The graphs show a separation between MOLP and SDMOLP samples along the projected plane. MOLP is at the left, and SDMOLPs is at the right; this suggests a negative correlation. Therefore, the PCA biplots demonstrate that SDMOLPs are significantly ($p < 0.05$) different from pure MOLP, indicating effective treatment. On a loading plot, variables on the same axis correlate positively, while those on the opposite side show inverse correlation. By this measure, flow properties (Hauser's ratio, angle of repose, Carr's index), moisture content, and macronutrients (protein and fat) were strongly correlated. The score plots among various PEG4000 and PEG6000 solid dispersions grades suggest similarity among specific grades that overlap. Based on the closeness in the spatial positions on the score plots, SDMOLP (PEG4000) grades are more similar to SDMOLP (PEG6000). The proximity of these grades on the score plot implies that their flow properties, nutritional composition, and densities are similar. On the other hand, all solid dispersions (PEG4000 and PEG6000) correlated inversely with MOLP, suggesting they differed in flow properties, densities, and nutritional components.

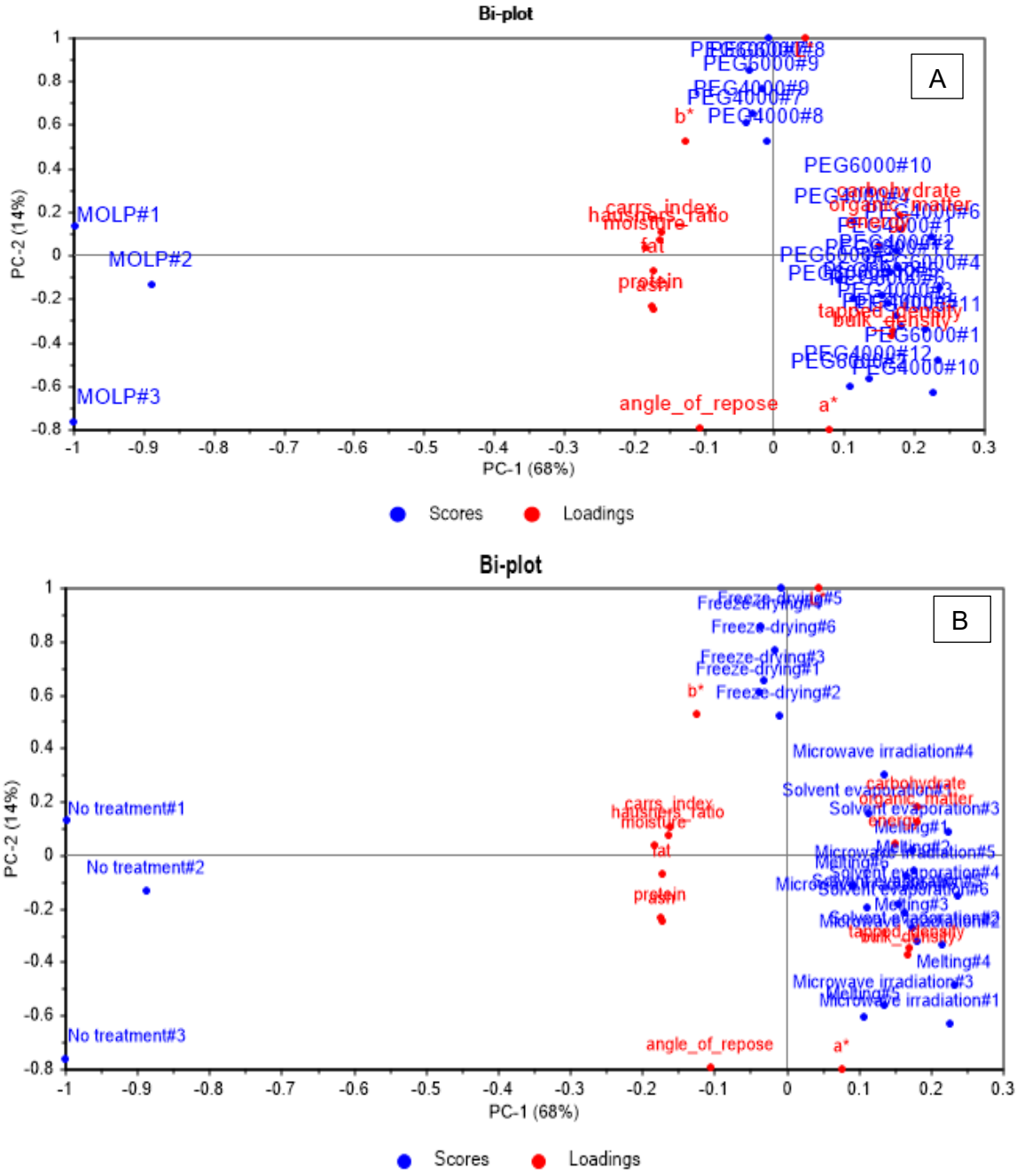

Figure 5.2 PCA biplots of the component loading vectors and principal components core vectors arranged by a) PEG treatment and b) method of preparation

5.8.4 Maximum absorbance of MOLP and solid-dispersed MOLP

The scanned wavelengths of MOLP and SDMOLP spectra are shown in Figure 5.3. All the samples exhibited multiple peaks, with the highest sharp peaks at 382 nm and the lowest sharp peaks at 286.06 nm when scanned between 200 and 400 nm wavelength. In distilled water, the UV absorption spectrum of pure MOLP demonstrated maximum absorption at 382.76 nm and 286.87 nm, respectively, with absorption values of 0.94854 and 0.68175.

The maximum wavelengths of the solid MOLP dispersions were similar to those of the pure MOLP, ranging from 382.76 nm to 382.87 nm and 286.06 nm to 288.06 nm, with absorbances ranging from 0.92969 to 0.9335 and 0.61538 to 0.62253, respectively. The maximum absorbances of MOLP and SDMOLPs were not significantly different. Most of the absorbance occurred in the UV region, which suggests the presence of phenolic and flavonoid compounds in *Moringa oleifera* leaves. The samples had maximum absorbance at 286 nm and 382 nm; this suggests that at 382 nm, there was a higher concentration of phytochemicals at 286 nm. Higher absorbance intensity implies a higher concentration of phytochemicals. This study concurs with previous studies that reported that flavonoids have characteristic absorption spectra in the UV region due to their chemical structure, with two peaks of absorption, one occurring between 240 and 285 nm and another between 300 and 400 nm (Costa *et al.*, 2011). Similar results were reported by Vicente *et al.* (2019) where the maximum absorbance for MOLP was observed at 270 nm and 360 nm. In addition, another similar study by Dhivya (2017) reported that absorption maxima bands in the ranges 230-285 nm (band 1) and 300-430 nm (band 2) are characteristics for phenolic compounds and flavonoids, respectively. Moringa leaves are the source of essential amino acids and phytochemicals such as phenolics, flavonoids, and antioxidants, and these secondary metabolites contribute to their medicinal value.

The scanned maximum wavelengths for MOLP and SDMOLPs also correspond with the results reported for absorbance profiles of *Moringa oleifera* leaf protein (280 nm, 320 nm, and 340 nm) and *Moringa oleifera* seed protein (300 nm and 320 nm) by Adewale *et al*. (2015). The results confirmed that MOLP is high in protein (28%). All the maximum wavelengths obtained for MOLP and solid-dispersed MOLP revealed the presence of protein and other protein molecules. Previous studies showed that various protein molecules were detected at wavelengths between 300 nm and 960 nm (Adewale *et al.*, 2015). The findings in this study were also comparable to other plant proteins such as Dika nut, soybean, African bean (brown), groundnut, and Bambara groundnut, which showed maximum absorbance at 280-312 nm and 274-300 nm, respectively, due to the presence of tryptophan and tyrosine amino acids (Okoronkwo *et al*., 2017).

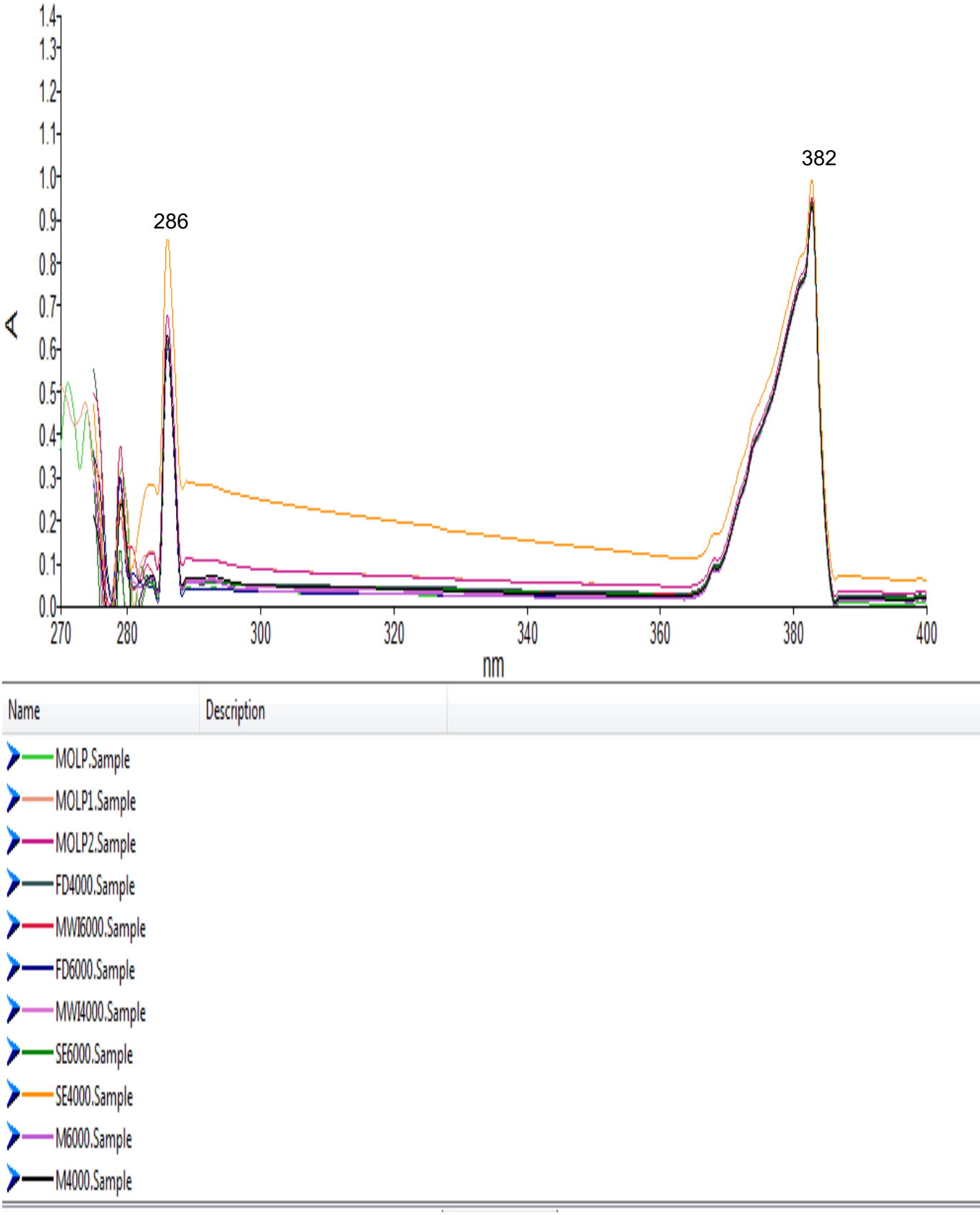

Figure 5.3 UV-visible spectra of MOLP and solid-dispersed MOLP λmax values in distilled water.

5.8.5 Calibration Curves of MOLP and SDMOLP

To determine linearity, various concentrations of the samples were set up, and calibration curves analysed. The R-squared measures the proportion of variation in the dependent variable that can be attributed to the independent variable. The calibration curves of MOLP and SDMOLP examined at 286 nm, and 382 nm wavelengths are shown in Figures 5.4-5.8. The calibration curves for the MOLP and SDMOLP samples showed a linear correlation, i.e., R^2 ranged from 0.9481-0.9934, suggesting that the samples had a strong linear association (between absorbance and concentration). Practically, R^2 values 0.95-0.99 are considered very high and fall under the accepted range because a 5% or less variability error is acceptable. According to Analytical Methods Committee (2001); Prichard & Barwick (2003), a perfect positive linear association is whereby the points are exactly on the trend line with $R^2 = 0.99$.

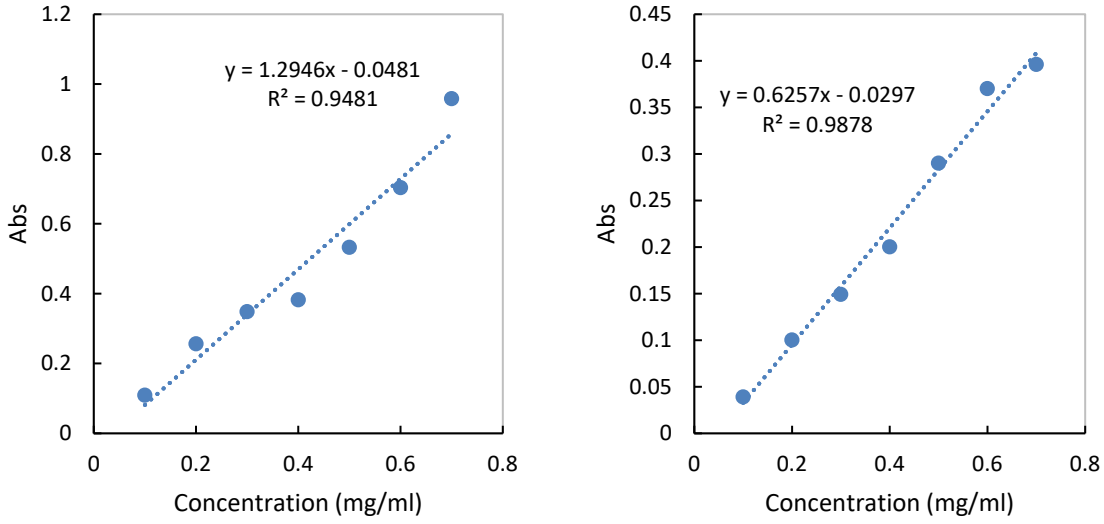

Figure 5.4 Calibration curves of MOLP a) 286 nm and b) 382 nm

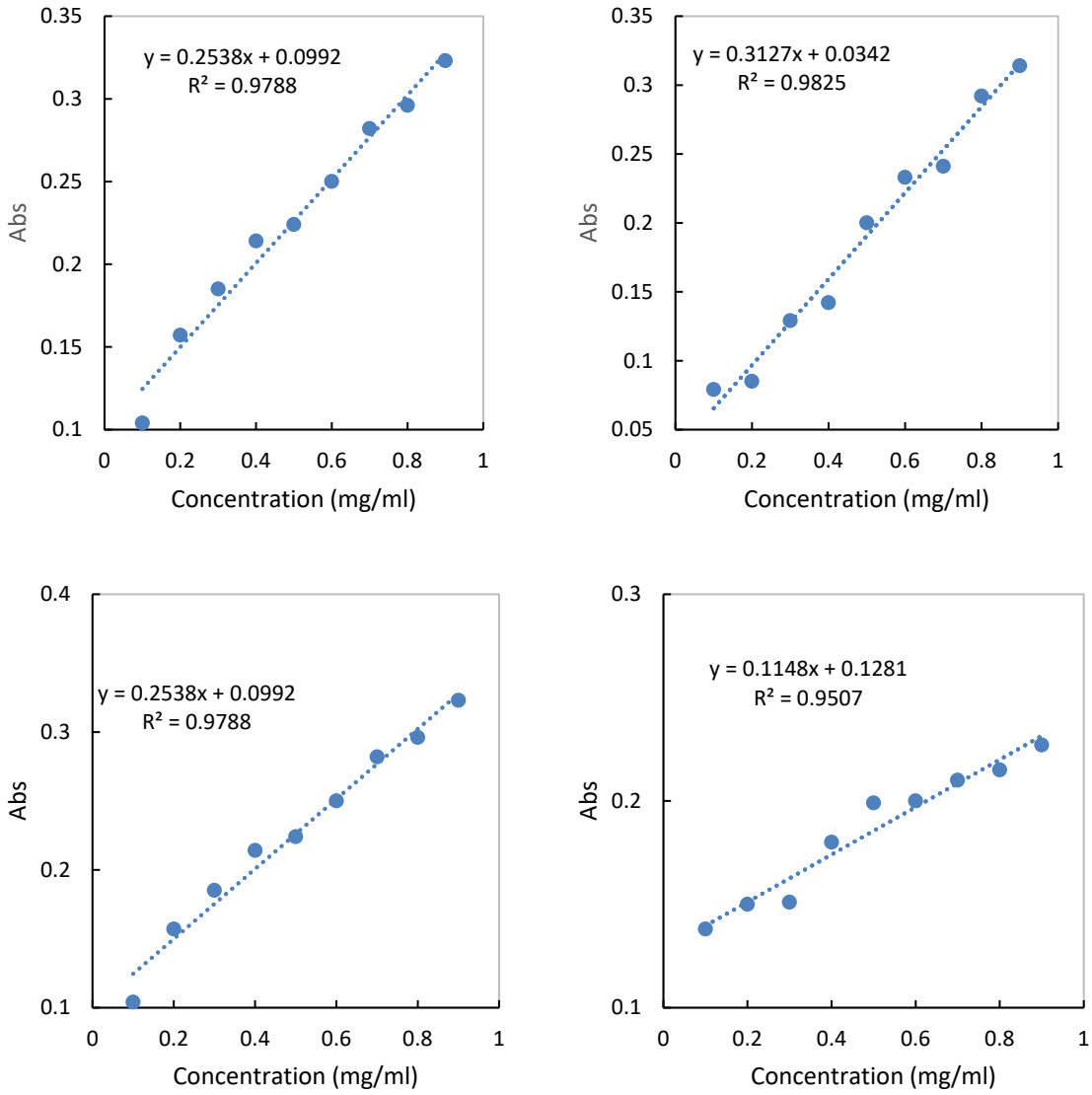

Figure 5.5 Calibration curves of solid-dispersed MOLP prepared through freeze-drying method a) 286 nm peg4000 and 286 nm peg6000, 382 nm c) PEG4000 and d) PEG6000

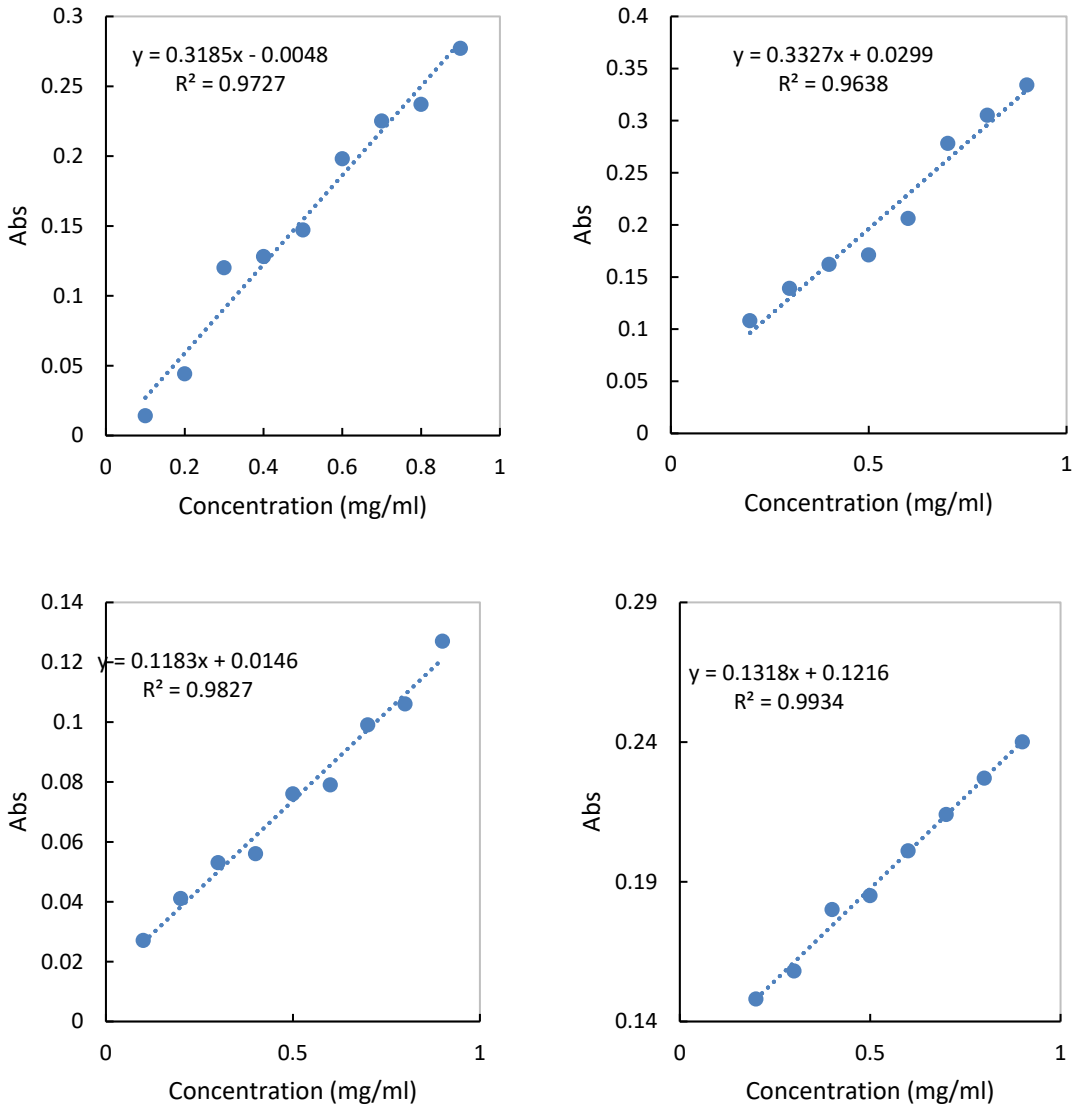

Figure 5.6 Calibration curves of solid-dispersed MOLP prepared by melting method 286 nm a) PEG4000 and b) PEG6000, 382 nm c) PEG4000 and d) PEG6000

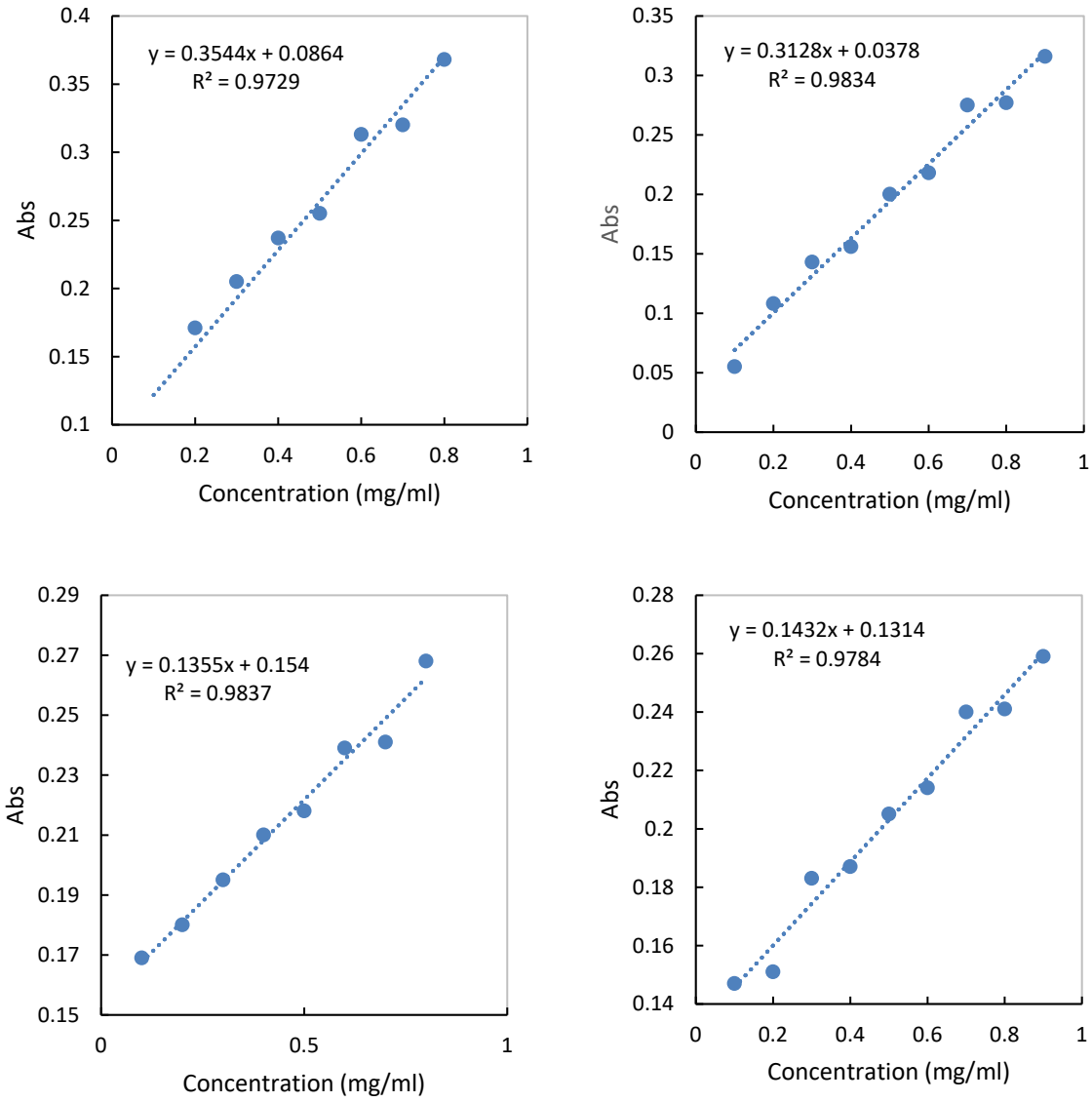

Figure 5.7 Calibration curve of solid-dispersed MOLP prepared by solvent evaporation method 286 nm a) PEG4000 and b) PEG6000, 382 nm c) PEG4000 and d) PEG6000

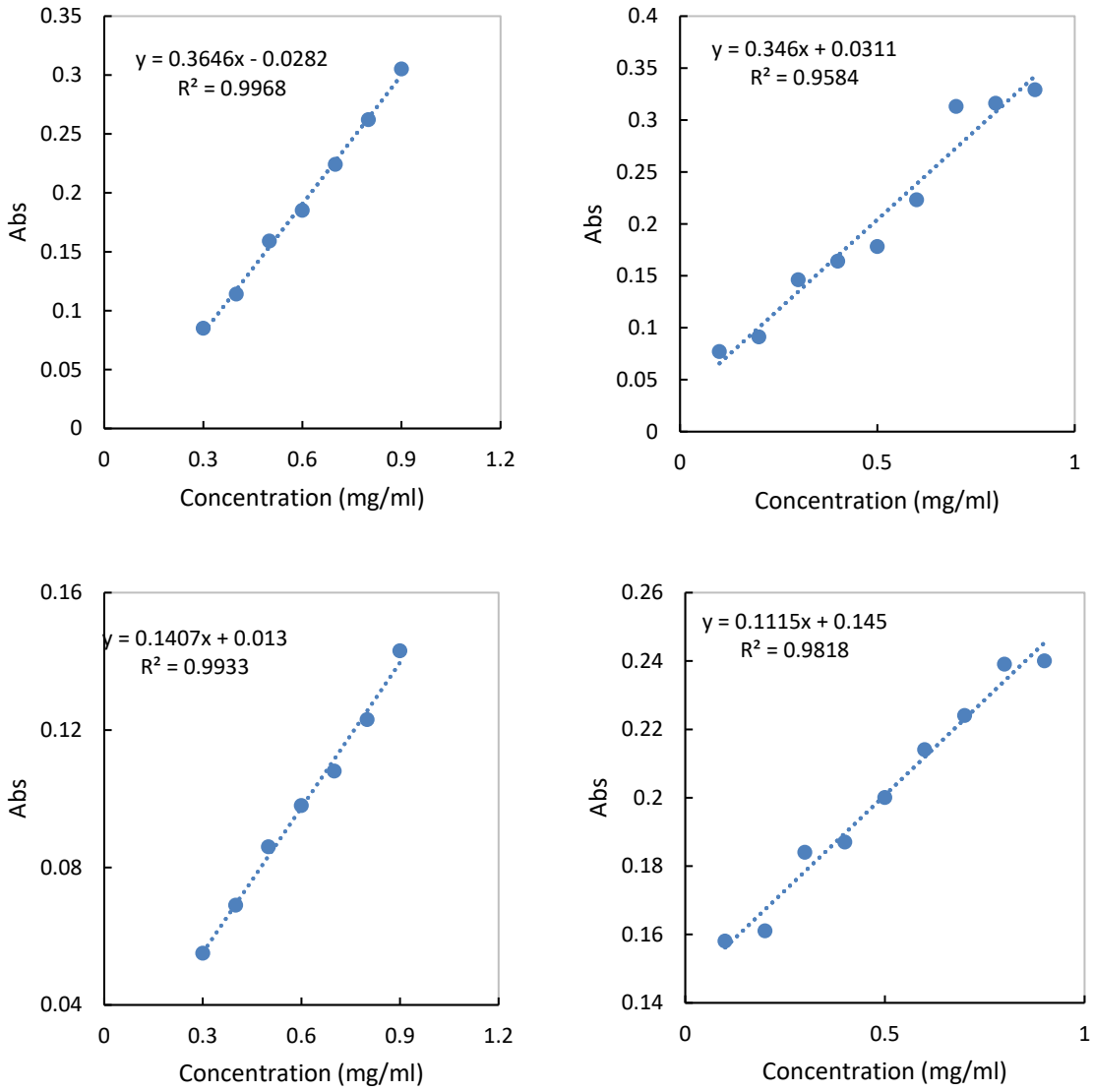

Figure 5.8 Calibration curves of SDMOLP prepared by microwave method a) 286 nm (PEG4000) and b) 286 nm (PEG6000), 382 nm c) PEG4000, and d) PEG6000

5.8.6 *In vitro* Dissolution Studies

In-vitro dissolution of SDMOLP and MOLP was studied using distilled water as dissolution medium and analysed at 286 nm and 382 nm wavelengths using the UV-vis spectrophotometer. The dissolution profiles of SDMOLP and pure MOLP are shown in Figures 5.9-5.12. An enhanced dissolution rate of SDMOLP was attained in all cases. Pure MOLP showed a significantly ($p < 0.05$) low dissolution rate (0.76% and 2.62% at 286 nm and 382 nm, respectively) within the total dissolution time (one hour). The release of SDMOLP (at 286 nm) ranged from 2.77% to 3.10% at the end of one hour. More so, the SDMOLP (at 382 nm) highest release ranging from 3.07% to 9.42%. The dissolution rate of

the prepared solid dispersions significantly exceeded the plain MOLP at both 286 nm and 382 nm. Whereas the SDMOLP samples had a dissolution rate of 9.42%, 8.34%, 6.92%, 6.79%, 6.13%, 5.50%, 5.06%, and 3.70% for melting (PEG4000), microwave irradiation (PEG4000), melting (PEG6000), freeze-dried (PEG4000), freeze-dried (PEG6000), solvent evaporation (PEG4000), microwave irradiation (PEG6000), and solvent evaporation (PEG6000) at 382 nm, respectively after 60 min. The significant difference between the dissolution rates of samples analysed at 382 nm and 286 nm is attributed to the high concentration of compounds at 382 nm. This was confirmed in the maximum absorbance (λ max) results in section 5.3.1, where 382 nm demonstrated a big and narrower peak than 286 nm (Figure 5.3). However, the significant increase in the dissolution rate of SDMOLPs could be because of increased wettability conferred by the carrier properties. The pure MOLP exhibited the slowest dissolution rate because of its hydrophobicity that caused the powder to perhaps float on the surface of the dissolution medium and prevented its surface from contacting the medium. In all cases, the dissolution rates of MOLP solid dispersions were remarkably enhanced compared to their corresponding pure MOLP.

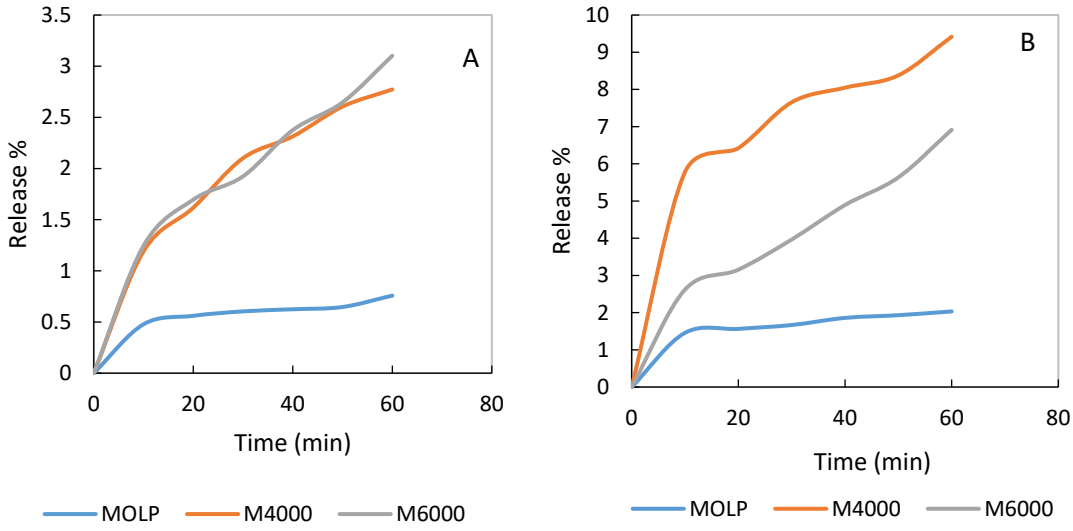

Figure 5.9 Dissolution profiles of SDMOLP prepared by melting method (PEG4000 and PEG6000) a) at 286 nm and b) 382 nm

The highest dissolution was achieved from systems prepared with PEG4000, whereas the least dissolution was obtained from systems prepared with PEG6000. This might be due to the low molecular weight of the polymer, which increases the solubility of the MOLP compared to the high molecular weight polymer. This result is in good agreement with those observed by Kumavat *et al.* (2013) for the solid-dispersed Curcumin, where the dissolution

rate of PEG4000 solid dispersions was higher compared to the PEG6000's. Furthermore, the melting method and PEGs (4000 and 6000) based solid dispersions systems displayed superior dissolution rates compared to other prepared systems (freeze-dried, microwave irradiation, and solvent evaporation).

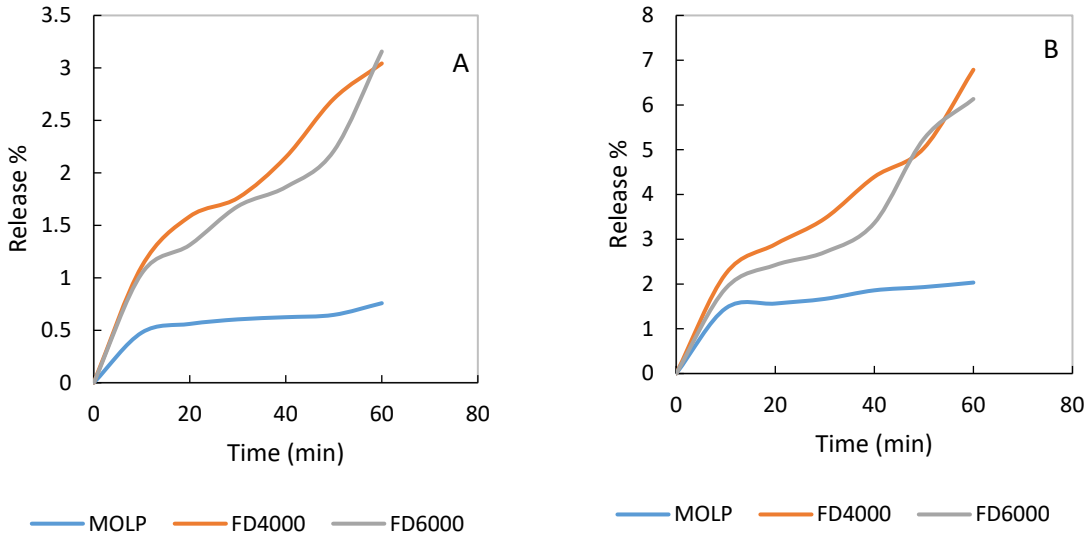

Figure 5.10 Dissolution profiles of Freeze-dried solid dispersions (PEG4000 and PEG6000) a) at 286 nm and b) 382 nm

However, compared to the melting formulations based on the PEG, dispersions with PEG4000 showed a higher release than those with PEG6000. The improved dissolution rate of SDMOLP within the prepared systems can be attributed to several factors, including the incorporation of strongly hydrophilic polymers to improve the ingredient's wettability (Nayak, 2015; Alshehri *et al.*, 2020; Jha *et al.*, 2020) an increase in solubility, the solubilizing effect of the carrier (Kumavat *et al.*, 2013), and the formation of high energy partial amorphous state as confirmed by DSC, FTIR. Moreover, the improved dissolution profile of SDMOLPs was also anticipated, since the solubility result, as shown in section 3.15.2, indicated that SDMOLP prepared with hydrophilic carriers was more water-soluble than pure MOLP with solubility values almost twice that of pure MOLP. Methods of preparing solid dispersions and types of hydrophilic carriers can also affect dissolution rates. There was a noticeable difference between dissolution rates among solid dispersions made using different methods and carriers. Overall, a higher dissolution rate was achieved with all two PEG types compared with pure MOLP. PEG's nature greatly contributed to improving the dissolution rate. Moreover, they can form physically stable, soluble complexes with MOLP via intermolecular hydrogen bonding. MOLP contains one hydroxyl group which can form

hydrogen bonds with ether functional group (-O-) in PEG molecules (Fouad *et al.*, 2021). Hydrogen bond formation due to these functional groups in both polymers respectively was observed in the FTIR study (Chapter 4, section 4.5.3). Therefore, an enhanced dissolution rate of SDMOLPs results from the interaction of MOLP with PEG by hydrogen bonds, thus converting MOLP to its amorphous form in solid dispersions.

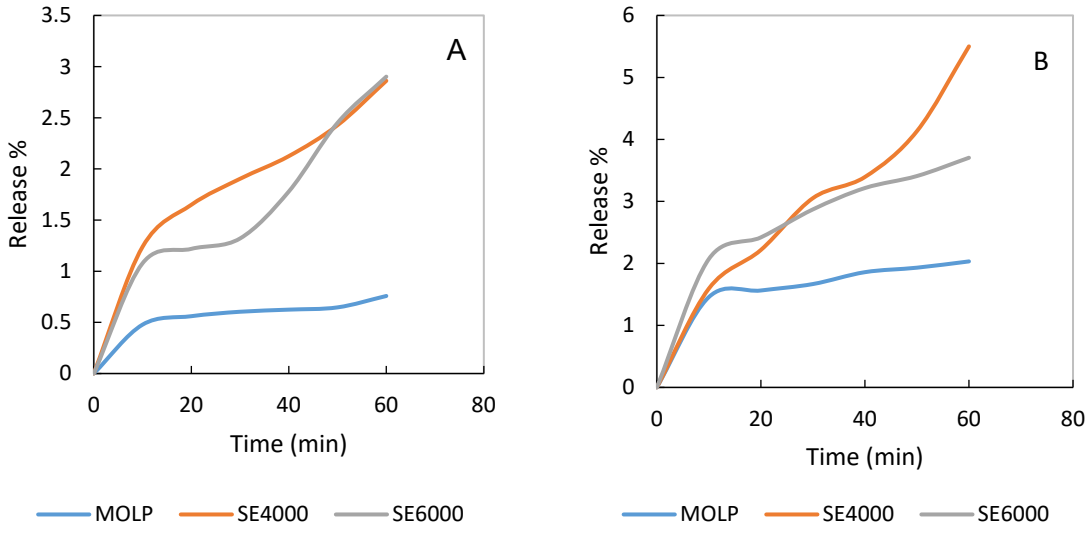

Figure 5.11 Dissolution profiles of SDMOLP solvent evaporation method (PEG4000 and PEG6000) a) at 286 nm and b) 382 nm

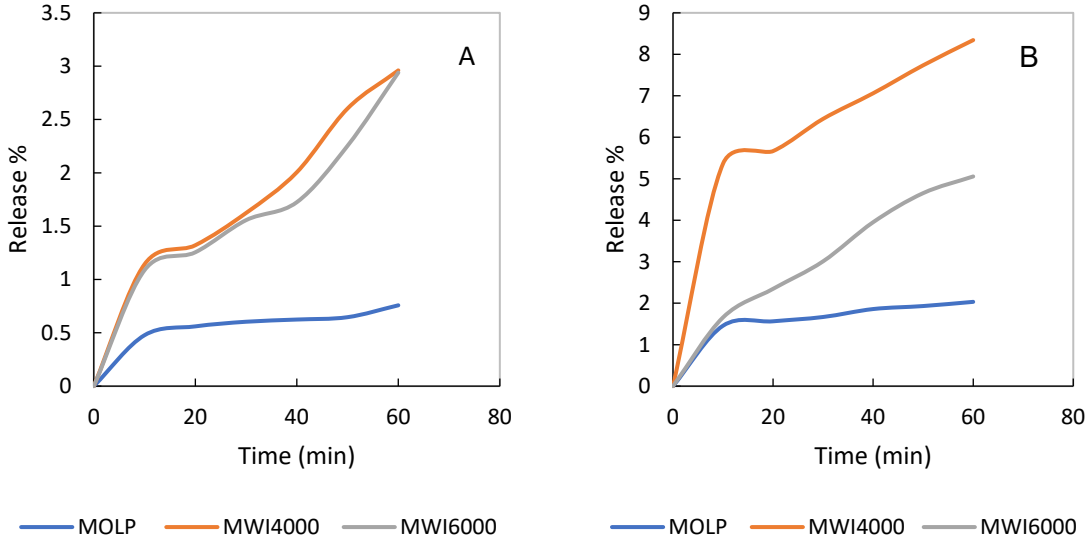

Figure 5.12 Dissolution profiles of SDMOLP prepared by Microwave irradiation method (PEG4000 and PEG6000) a) at 286 nm and b) 382 nm

A study by de Sousa *et al.* (2011) demonstrated a similar occurrence in the dissolution profile of *Senna sp*; the release was less than 10% from capsules containing the extract in 60 min even though they used markers. Similar results were also reported by Kumavat *et al.* (2013) for curcumin (> 10%), and solid-dispersed curcumin (35-60%), a significant increase in dissolution rate was observed in curcumin solid dispersions. These results also coincide with those obtained by Yu *et al.* (2020) for curcumin and curcumin solid dispersions. Archer *et al.* (2020) observed similar results for *Cassia sieberiana* extract. In addition, Jha *et al.* (2020) reported comparable results for solid-dispersed Naringenin (51%) and pure Naringenin (22%) for two hours. These studies demonstrated that the dissolution rate was better for solid-dispersed herbal products than raw ingredients. Even though the dissolution behaviour of SDMOLP was improved, no markers were used in this study. Thus, it is important to note that the objective of this study was just to compare the dissolution behaviour of SDMOLP and MOLP samples to see if the SDMOLPs would demonstrate an improved dissolution behaviour compared to pure MOLP. As a result, it does not meet pharmaceutical standards for the dissolution rate of herbal products. However, since the solid dispersion technique and PEGs have been shown to increase MOLP dissolution rate, marker compounds such as flavonoids, antioxidants, catechin, and/or quercetin can be used to develop a novel method for standardizing SDMOLP *in vitro* dissolution. Finally, it should be noted that standardisation of vegetable raw materials is one of the most significant issues facing the herbal product market (Costa *et al.*, 2011). New technologies are being studied and explored to modernise the preparation of traditional herbs as part of pharmaceutical goods, with the ultimate goal of maximising the opportunities and overcoming the obstacles inherent in herbal ingredients.

5.9 Conclusion

The overall objective of the chapter was to test the hypothesis that solid dispersion techniques and hydrophilic carriers (PEG4000 and PEG6000) could improve the release profile of the solid-dispersed MOLP. The novel solid dispersions of MOLP successfully prepared by the solvent evaporation, freeze-drying, melting, and microwave irradiation methods were characterised for their flow and dissolution properties. Given the flow properties, all the solid dispersions had improved flowability with excellent angles of repose. The flow properties of the solid dispersions were significantly differentiated by the methods of preparation and not by the carrier (PEG) type. Furthermore, the *in vitro* dissolution behaviour of the solid dispersions was also significantly improved. The remarkable enhancement in the dissolution rate may be attributed to the molecular dispersion of MOLP within PEG owing to the specific interaction between the molecules. However, SDMOLPs

prepared by the melting method and PEG4000 and PEG6000 demonstrated the highest dissolution rate and good flow properties among the solid-dispersed MOLPs. In conclusion, the results can be useful in developing innovative and high-quality products such as functional foods or beverages, herbal medicines, and nutraceuticals.

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CHAPTER SIX
FLOW BEHAVIOUR AND CONSUMER ACCEPTABILITY PROFILE OF *MORINGA OLEIFERA* EFFERVESCENT BEVERAGE GRANULE

Abstract

In recent years, attention has been focused on functional foods and beverages due to heightened health awareness. *Moringa oleifera* leaf powder (MOLP), rich in various phenolic compounds, vitamins, and minerals, can be developed into a functional beverage in the form of effervescent granules. The objective of this study was to design, formulate and physicochemically evaluate the effervescent beverage granules of solid-dispersed MOLP (SDMOLP). SDMOLP Effervescent beverage granules with varying acid concentrations 10% [formulation 1], 15% [formulation 2], and 20% [formulation 3] (citric and tartaric acids at 1:1) respectively, were developed using the wet granulation technique and optimised using a quadratic response design. The resulting effervescent granules were evaluated for physical and flow properties such as bulk density and tapped density, Hausner's ratio, Carr's index and angle of repose, mean particle size, pH, moisture content, and solubility time. In addition, the consumer acceptability of 65 g of effervescent granules in 1000 mL water was assessed for appearance, colour, aroma, taste, texture, and overall acceptability using a 5-point hedonic test scale, ranging from 1 (dislike very much) to 5 (like very much), conducted with 53 consumer panellists. Bulk and tapped densities ranged from 0.40 to 0.44 g/mL and 0.46 to 0.51 g/mL, respectively. The three formulations of effervescent granules exhibited excellent flow properties. The pH and moisture content of effervescent granules differed significantly ($p < 0.05$). Mean hedonic scores of sensory attributes ranged from neither like nor dislike to like moderately for appearance (3.19-3.92), colour (3.23-4.04), aroma (4.00-4.13), taste (3.08-3.77), texture (3.04-3.66) and overall acceptability (3.40-4.00). Formulations 2 and 3 were the most acceptable in terms of aroma, taste, texture, and overall acceptability. This study has shown the feasibility of using SDMOLP as a functional ingredient in developing easy-to-drink effervescent beverage granules.

6.1 Introduction

Effervescent granules are granular forms combining active ingredients and an effervescent base made up of sodium bicarbonate, citric acid, and tartaric acid. When these acids and bases react with water, carbon dioxide gas is released, resulting in effervescence (Aslani and Jahangiri, 2013a; Pandey *et al.*, 2013; Jassim *et al.*, 2018b; Murdiana *et al.*, 2018; Patel and Siddaiah, 2018; Giyatmi and Lingga, 2019). The liberation of carbon dioxide gas will

enhance the dissolution of the ingredient and mask the bitter taste of the herbal ingredients (Kuswardhani *et al.*, 2020). Effervescent granules can increase aesthetics and pleasure for consumers and mask the slightly bitter taste of active ingredients from *Moringa oleifera*, antioxidants, and other various phenolic compounds. Moreso, the carbon dioxide gas, the fizzing, makes effervescent products appeal to the people's senses. According to Giyatmi & Lingga (2019), effervescent granules can flavour up to 680 grams of water. Suggesting that a consumer can turn any liquid into a functional beverage without purchasing a new bottle every time. Thus, reducing the usage of plastic and other petroleum-based products. Another benefit is that some active ingredients are sensitive to moisture or pH, but an effervescent product is manufactured under extremely low-moisture conditions and packaged so that moisture cannot penetrate until the product is used. In most cases, effervescent granules are prepared from a combination of citric and tartaric acids rather than a single acid since using either acid alone presents difficulties. When tartaric acid is the only acid present, the resultant granules readily crumble, while citric acid alone produces a sticky, difficult-to-granulate mixture (Palanisamy *et al.*, 2011; Aslani & Jahangiri, 2013; Shamsi, 2017; Bharat, 2018; Hiola & Tungadi, 2018; Jassim *et al.*, 2018; Kuswardhani *et al.*, 2020). Meanwhile, citric acid combined with tartaric acid can enhance the interactions or bonds between particles in effervescent granules; therefore, a well-balanced acid-base formulation is critical. Effervescent technology is a proven dietary supplement delivery system with several advantages in the beverage market. This technology allows for creating pleasant-tasting beverages while masking the bitter taste of antioxidants, vitamins, minerals, and herbal extracts or ingredients, such as *Moringa oleifera* (Kuswardhani *et al.*, 2020). In addition, effervescent technology can improve dissolution and bioavailability (Manthena & Artham, 2014; Hiola & Tungadi, 2018).

Numerous *Moringa oleifera* leaf powder (MOLP) based products have been on the market in the form of herbs, teas, capsules and fortified foods and beverages. MOLP herbal teas/beverages are high in antioxidants, carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins, and terpenoids, among other natural bioactive chemicals (Chandrasekara & Shahidi, 2018; Etti & Sani, 2020). Herbal traditional beverages in the form of effervescent are still uncommon, with dietary supplements and vitamins being the sole exceptions. People still think of MOLP as a bitter-tasting herbal ingredient or medicine with a foul odour, even though it can help maintain and improve health when used daily. As a result, a new paradigm must be established: MOLP drink consumption is not always synonymous with bitter, odorous, and impractical flavors. It can, however, be made practical in the form of granules that dissolve quickly in water, such as effervescent granule MOLP.

Effervescent granule preparations are influenced by solubility, taste, texture, effervescent quality (Kuswardhani *et al.*, 2020); there has, however, been no information on how to manufacture effervescent MOLP granules with a proportion of sodium bicarbonate and acids until now (tartaric and citric). Okoye *et al.* (2013) studied the micrometric properties of MOLP granules. However, no effervescent study was reported on the MOLP granules. More so, Murdiana *et al.* (2018) produced the first *Moringa oleifera* leaf extract-based effervescent tablets (anti-anaemic dosage) bitter even after adding flavours, perhaps due to high antioxidants and phenolic content in the extract. However, nothing has been reported on the effervescent of MOLP or solid-dispersed MOLP. More study on the effervescent granules of MOLP easy-to-drink beverages is required.

In Chapter 3, solid-dispersed MOLP samples were produced through solid dispersion techniques such as freeze-drying, melting, solvent evaporation, and microwave irradiation using polyethylene glycols (PEG4000 and PEG6000) as hydrophilic carriers. Chapters 3, 4, and 5 determined these dispersions' functional, nutritional, mineral, structural, thermal, and dissolution properties. Solid dispersion prepared by melting method and a PEG6000 carrier was chosen as the candidate for product development due to its improved solubility and dissolution rate. The objectives of this chapter were to establish the flow behaviour and consumer acceptability of MOLP effervescent beverage granules.

6.2 Materials and Methods

6.2.1 Sources of materials and equipment

Citric acid, tartaric acid, and sodium bicarbonate were purchased from Spellbound company, Cape Town. Ethanol was purchased from Kemix Co, maltodextrin was procured from Tongaat Hulett, Cape Town, and flavours were a gift from Chemtrade, Cape Town and Ingreto, Johannesburg. Sucrose was procured from Spar supermarket. All chemicals used were of analytical grade. Sieves (MINOR, Endicott Ltd., England), air oven, a Hunterlab Colorflex Spectrophotometer, moisture analyzer (Denver instrument IR-30), and pH meter (GLP pH-Meter, Lasec) were provided by the Department of Food Science and Technology, Cape Peninsula University of Technology, South Africa. Figure 6.1 displays the Chapter 6 outline.

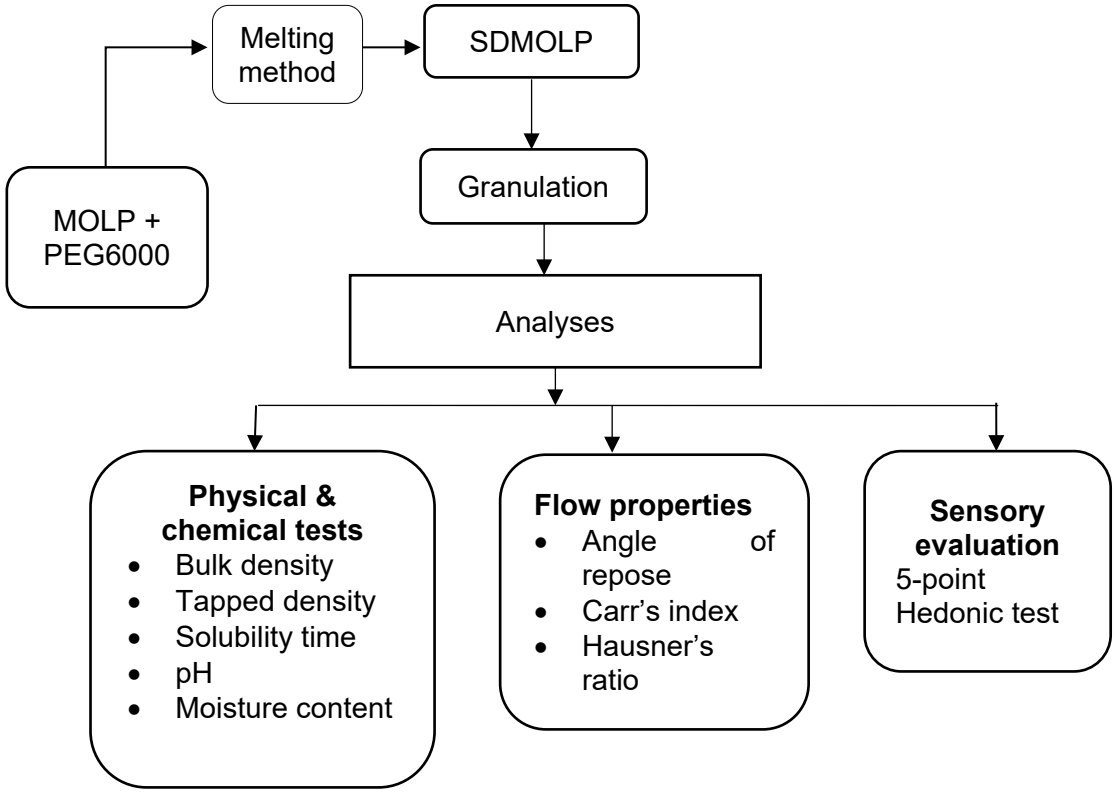

Figure 6.1 Chapter six outline

6.2.2 Effect of acids on the functional properties of solid dispersed *Moringa oleifera* leaf effervescent granules

A preliminary study on the effect of acid ratios and sodium bicarbonate on the functional properties of solid-dispersed *Moringa oleifera* leaf powder effervescent granules was conducted to get the standard pH (results not reported). The results indicated that 25% of sodium bicarbonate and 20% of acid ratio would give a pH of 7. However, this pH was out of range because the standard pH for effervescent granules must be neutral (6 - 6.5). As a result, a comparative design experiment was carried out in which the sodium bicarbonate was kept constant while the acid ratios were varied in order to obtain the optimal acid ratio for maximising the pH of the beverage granules. Three formulations with varying acid concentrations (10%, 15%, and 20%, respectively) and constant sodium bicarbonate (25%) were employed.

6.2.3 Production of *Moringa oleifera* leaf powder effervescent granules

The process (Figure 6.1) of making effervescent granules in this study was done using a wet granulation method described by Giyatmi & Lingga (2019) with slight modification.

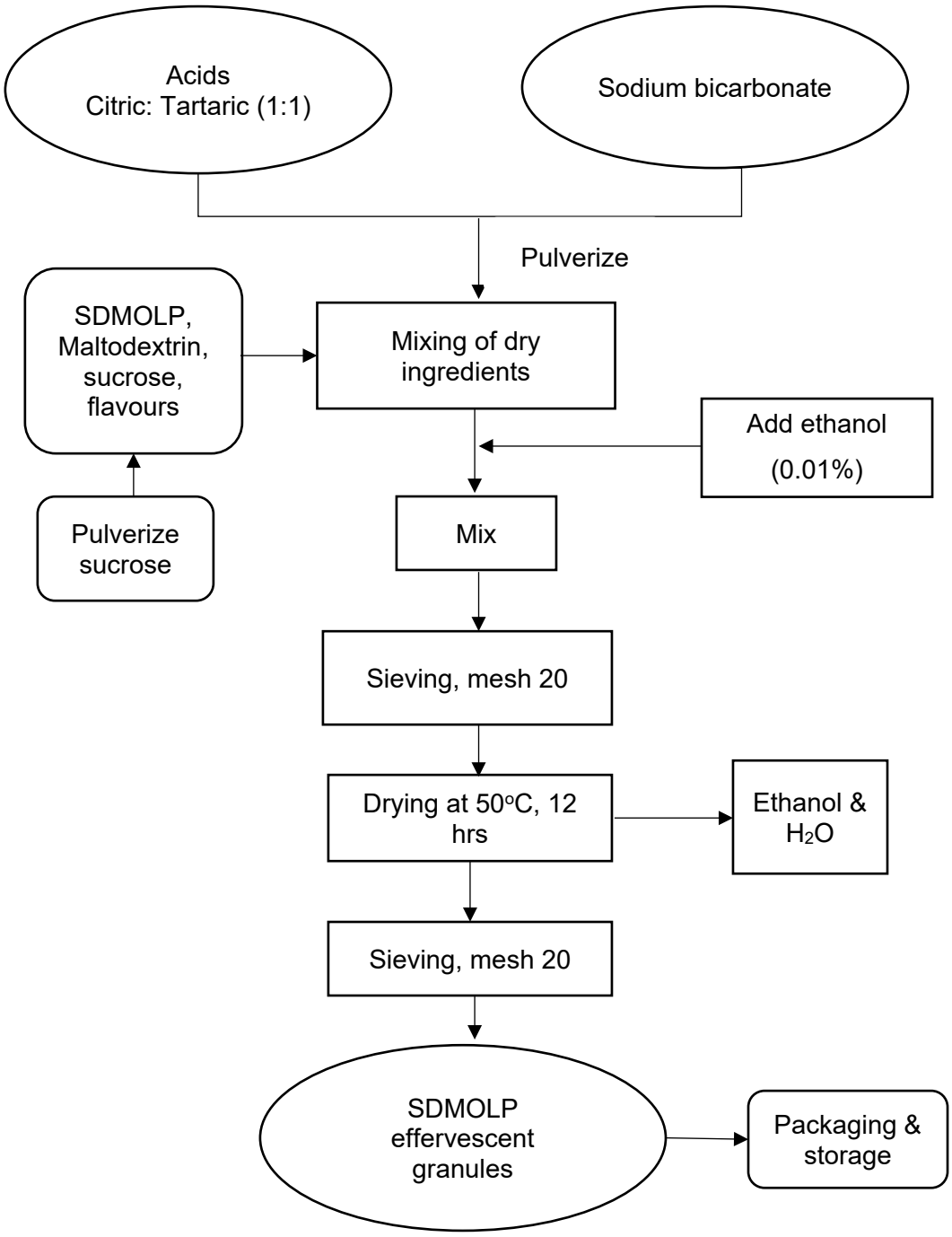

Figure 6.2 The process of making SDMOLP effervescent granules using a wet granulation technique.

Solid-dispersed *Moringa oleifera* leaf powder (SDMOLP), citric and tartaric acids (1:1) ratio (10%, 15%, and 20% acids, respectively), sodium bicarbonate(25%), maltodextrin, and sucrose were accurately weighed (according to Table 6.1) and mixed to form a homogeneous mixture of dry ingredients. The mixture was put into a stainless-steel basin

container for the wet granulation process by adding an ethanol binder solution (absolute ethanol 99%), 50 mL. Orange, strawberry, and soda powder flavours were added into the moist mixture, then granulated using a 20-mesh sieve. The obtained granules were dried using an air oven at 50°C for 12 hours. The dried granules were sieved again with a 40-mesh sieve to form a granule mass and then packed in airtight containers (sealable glass jars). Physicochemical properties (moisture content, densities, solubility time and pH) and consumer acceptability of the effervescent granules were evaluated.

Table 6.1 Formulations of solid-dispersed MOLP effervescent beverage granules[1]

	Formulations		
Ingredients (g)	**F1**	**F2**	**F3**
SDMOLP	100	100	100
Sodium bicarnonate	25	25	25
Tartaric acid	5	7.5	10
Citric acid	5	7.5	10
Sucrose	10	10	10
Maltodextrin	5	5	5
Flavours: orange, strawberry, banana, cream soda	5	5	5

SDMOLP: solid-dispersed *Moringa oleifera* leaf powder. F1: 10% acid, F2:15 % acid and F3: 20% acid

6.2.4 Physicochemical measurement of SDMOLP effervescent granules

6.2.4.1 Bulk density

The bulk density was determined by weighing 30 g of SDMOLP effervescent granules into a 100 ml graduated measuring cylinder without compacting. The granules were carefully levelled without compacting and the unsettled apparent volume was read and recorded. The bulk density was calculated using equation 6.1.

$$\rho\text{Bulk}\ (g/mL) = \frac{\text{Weight of SDMOLP granules (g)}}{\text{Volume of SDMOLP (mL)}} \qquad \textit{Equation 6.1}$$

6.2.4.2 Tapped density

For tapped density determination, the same measuring cylinder containing the SDMOLP effervescent granules as weighed in section 6.1.1 was tapped 50 times at the height of 2.5 cm at 2-second intervals until no further changes in the volume were noted. The tapped density was calculated in g/mL using equation 6.2.

$$\rho\text{Tapped}\ (g/mL) = \frac{\text{Weight of SDMOLP (g)}}{\text{Volume of tapped SDMOLP(mL)}} \qquad \textit{Equation 6.2}$$

Where: ρTapped = tapped density

6.2.4.3 Carr's index

Compressibility (Carr's) index is related to the flow rate, cohesiveness, and particle size in an indirect way. Compressibility refers to a powder's capacity to shrink in volume under pressure. It's a simple, quick, and accurate way to forecast powder flow characteristics (Ali e*t al*., 2018, 2019). Density measurements (equation 6.3) were used to determine Carr's index for the granules.

$$\text{Carr's index (\%)} = \frac{\rho\text{Tapped} - \rho\text{Bulk}}{\rho\text{Tapped}} \times 100 \qquad \textit{Equation 6.3}$$

Where, ρTapped = tapped density, ρBulk = bulk density

6.2.4.4 Hausner's ratio

Hauser's ratio was determined and calculated as the tapped density divided by the bulk density. Hausner's ratio is related to interparticle friction and can be used to predict powder flow properties. Low Hausner's ratio (< 1.25) indicates better and good flow properties than higher ones. From 1.25 to 1.6, Hausner's ratios depict moderate flowing properties (Pandey *et al.*, 2013). While values of more than 1.6 show poor flow properties and cohesive powders (Ali *et al*., 2018; Hiola & Tungadi, 2018). Hausner's ratio was calculated using equation 6.4.

$$\text{Hausner's ratio} = \frac{\rho Tapped}{\rho Bulk} \qquad \textit{Equation 6.4}$$

Where, ρTapped = tapped density, ρBulk = bulk density

6.2.4.5 Angle of repose

The flowability of solid dispersions was further evaluated by measuring the angle of repose and the flow rate through a funnel. For measurement of the angle of response, a fixed funnel

method was used (Ali *et al*., 2018; Aslani & Fattahi, 2013; Hiola & Tungadi, 2018; Kumavat *et al*., 2013; Yeo et al., 2020). Specifically, a fixed funnel (100 mm) with an orifice inner diameter of 10 mm and specially designed to evaluate the flowability of powders or granules were used. The funnel was fixed above a flat horizontal surface at an appropriate height (2 cm). The orifice of the funnel was closed, and 10 g of MOLP or solid-dispersed MOLP was poured into the funnel. Then, the orifice of the funnel was opened, allowing the sample to pass through. The radius of the base of the conical pile was measured. The angle of repose (θ) was calculated using equation 6.5.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right) \qquad \textit{Equation 6.5}$$

Where, θ = angle of repose (º), h= fixed height (cm), and r = radius of the formed powder base (cm).

6.2.4.6 Colour measurements of SDMOLP effervescent granules

The colour of the SDMOLP effervescent granule samples was measured using a Hunterlab Colorflex Spectrophotometer 45°/0° standard, set at standard observer 10° and D65 and standardized with white tile (L* = 93.41, a* =−1.18, b* = 0.75) and black tile. Moringa samples were placed in the glass sample holder and reflectance was measured for L*, a*, b*, and LCh colour scales. L* is the lightness with 100 as maximum, indicating an ideal reflecting diffuser and the minimum is zero, which is black. The a* and b* axes have no specific numerical limits; negative a* is green, positive is red. Negative b* is blue, positive b* is yellow (Aidoo et al. 2010). Chroma (C) is the quality that distinguishes a pure hue from a grey shade and describes hue saturation or purity; its axis extends from the values (lightness) axis toward the pure hue (Murevanhema & Jideani, 2015). Colour differences among the solid-dispersed MOLP effervescent granules were estimated using the colour difference ΔE given as in equation 6.6:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^{*2} + \Delta b^{*2}} \qquad \textit{Equation 6.6}$$

where L = lightness, a = green-red scale, b* = blue-yellow scale.*

6.3 Solubility time

The effervescent granules (5 g) were thoroughly weighed and dispersed in a 250 mL glass beaker with 200 mL distilled water. The solubility of dispersion time was measured as the time it took for all granules to be homogeneously disseminated in the distilled water. For

effervescent granules, the solubility time requirement is 5 minutes (Giyatmi and Lingga, 2019; Grajang and Wahyuningsih, 2019).

6.4 pH test of the SDMOLP effervescent beverage granules

The effervescent solution was prepared by weighing 5g of mass of effervescent granules and dissolving them in 200 mL of water. To calibrate the pH meter, the pH 4.0 and pH 7.0 buffer solutions were used, respectively. After calibration, the electric pH meter was immersed in an effervescent solution that no longer had gas bubbles. The pH of the sample was recorded. A good pH for the effervescent solution is between 5.6-7 (Grajang & Wahyuningsih, 2019).

6.5 Moisture content

Moisture content evaluation was carried out by weighing 2 g of the effervescent granules into a foil dish and placing them on the moisture content analyser (Moisture analyser IR-30). The instrument was run for 15 minutes with an annealing temperature of 105^{o}C. After 15 minutes, the moisture content was automatically displayed on the instrument in percentage (%).

6.6 Sensory evaluation

A consumer tasting panel of 53 was invited among staff and students of Cape Peninsula University of Technology to determine the consumer acceptability of SDMOLP effervescent beverages. The sensory evaluation was carried out in the sensory laboratory at 25^{o}C. Panellists received written and prior verbal instruction regarding the evaluation procedures. Panellists were presented with three coded digit samples in clear cups on a white tray of 9.6 x 6.6 cm; water was provided to the panellists to rinse their palate between the tasting of each sample. Before starting the evaluations, the panellists were asked to sign an approved informed consent form regarding ethical protocol (Appendix A) of research involving human subjects (Ethic approval attached in Appendix B). Panellists were instructed to rate the samples for appearance, colour, aroma, taste, texture, and overall acceptability using a five-point hedonic scale. The five-point hedonic scale was used to rate each sample as (1) dislike extremely, (2) dislike moderately, (3) neither like nor dislike, (4) like moderately, (5) like extremely.

6.7 Data Analysis

Where the data follows a normal distribution, the data were analysed using multivariate analysis of variance (MANOVA) to determine the significant differences ($p < 0.05$) in

attributes among samples. Duncan's multiple range test was used to separate means where a significant difference existed (IBM SPSS version 26, 2020). Where the data violates normality conditions, the non-parametric independent-Sample Kruskal Wallis Test was used to establish differences between treatments.

6.8 Results and Discussion

6.8.1 Physical properties of effervescent granules

Bulk and tapped densities

Determination of granule and powder density in the food industry can contribute to quality control. The bulk and tapped densities of MOLP effervescent granules are shown in Figure 6.3. The bulk density ranged between 0.40-0.44 g/ml. The lowest bulk density was recorded for solid-dispersed MOLP effervescent granules (SDMOLPG10) (10% acids) and the highest for both SDMOLPG15 (15% acids) and SDMOLP20 (20% acids). The tapped densities were in the range of 0.47-0.50 g/ml. The results obtained are comparable with 0.43-0.53 g/mL for *Moringa oleifera* leaf powder granules (Okoye *et al.*, 2013). Shamsi (2017) also reported similar results (0.50-0.57 g/ml) for the effervescent granules. In comparison, Pandey *et al.* (2013) reported slightly higher bulk and tapped densities for herbal effervescent granules of *Martynia Annua* extract (0.55 and 071 g/ml, respectively). Structural differences, raw materials, and moisture content could be the reason for the differences observed. Jassim *et al.* (2018) also reported higher bulk densities (0.60-0.81 g/ml) and tapped densities (0.75-1.00 g/ml) for Lornoxicam sachet effervescent granules. The tapping density of granules or powder (or a blend of powders) is the maximum packing density attained under the influence of well-defined, externally applied forces (Chisenga *et al.*, 2019). The packed densities were higher than the bulk densities. This variation could be due to the surface properties of the granules. The bulk density can find use in packaging, handling, and processing requirements. Instant products require reduced bulk densities (Chisenga *et al.*, 2019). Therefore, the bulk densities reported in this research suggest that the granules possessed the least porosity (best packability) in comparison to MOLP. This is advantageous since the granules will take up less space in the package, saving money. Effervescent granules should therefore be placed in smaller containers in terms of final product packaging.

The concentration of acids significantly affected the bulk and tapped densities of SDMOLP effervescent granules (Figure 6.4). High acid concentration resulted in high bulk density and tapped density and vice versa. This could be attributed to the densities of the acids. Tartaric acid has a greater density than citric acid, so that granules containing tartaric acid will have greater density.

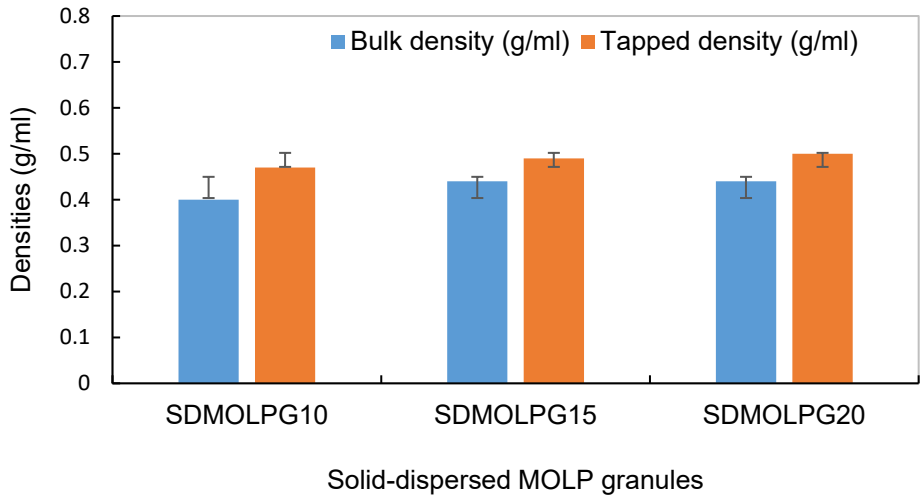

Figure 6.3 Bulk and tapped densities of MOLP effervescent beverage granules

Huge molecular weights indicate large densities, which will flow more freely due to gravity (Okoye *et al.*, 2013). Citric acid has a specific density of 1.665 mg/mL, but tartaric acid has a specific density of 1.7598 mg/mL, according to Mohrle (1980). Hence the bulk and tapped densities of SDMOLP effervescent granules with an acid concentration of 15% and 20% were greater than effervescent granules with 10% acids (SDMOLPG10). Bulk density and tapped density are critical factors in determining the size of containers or packaging required for transporting, handling, and storing raw materials and finished goods. Moreover, these densities are essential for determining the flow properties of the powder and granulated products. It follows that the higher the bulk density, the denser the packaging material required. According to Okoye *et al.* (2013) and Chisenga *et al.* (2019), the increase in bulk density is beneficial because it provides a better packaging advantage by allowing more products to be packed into a given unit volume. Due to their reduced moisture content, granules produced by the wet granulation process have a lesser tendency for adhesion. Moreover, their larger particle sizes led to a decrease in the particle contact area. Thus, friction between particles decreased, resulting in increased flowability, as discussed in section 6.3.2.

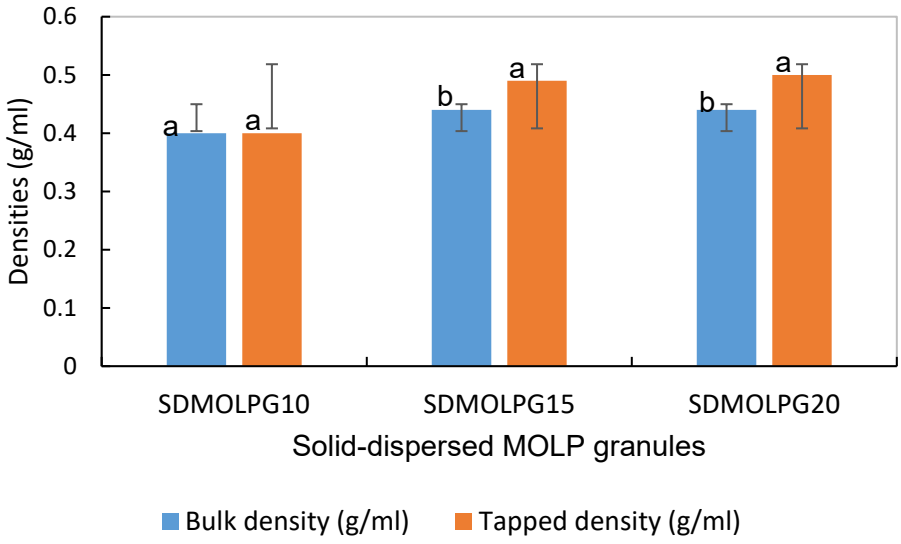

Figure 6.4 Effect of acids on the bulk density and tapped density of SDMOLP effervescent granules

6.8.2 Flow Properties- Hausner's ratio, Carr's index, and angle of repose

The flowability of SDMOLP effervescent granules is tabulated in Table 6.2. Carr's Index of the granules was in the range of 11.21-14.86%. The compressibility index (Carr's index) is a measurement of a granule's or powder's proclivity to be compressed (Maysarah *et al.*, 2020). It is calculated using the bulk and tapped densities. In theory, less compressible materials are more flowable; therefore, this suggests that the three formulations of SDMOLP effervescent granules obtained in this study possessed good flow properties since their Carr's index values were less than 15% (Pandey *et al.*, 2013; Bharat, 2018; Diyya and Thomas, 2018).

Hausner's ratios were in the range of 1.13-1.18. Lower Hausner's ratios (1.25) imply better flow qualities than higher ones, with 1.25 to 1.5 indicating moderate flow and greater than 1.5 indicating poor flow (Okoye *et al.*, 2013; Pandey *et al.*, 2013; Saifullah *et al.*, 2016). The observed Hausner's ratio for the SDMOLP granules was ranged from 1.13 to1.18, indicating good flow properties. Moreover, granules with a Hausner ratio below 1.2 are said to have good compressibility, so the granules of the three formulas possess this property. While there was no significant difference in Carr's index, Hausner's ratio and angle of repose among the SDMOLP effervescent granules ranged from 30.28 to 30.86°. The angle of repose 25-30° shows excellent flow properties, 31-35° shows good flow properties, 36-40° shows fair flow properties, and 41-45° shows passable or poor flow properties (Patel, 2015; Hiola and Tungadi, 2018). The angle of repose observed was 30.28-30.86° which indicates excellent to good flow properties. As a result, all three formulations displayed good

flow properties, possibly due to the preparation method. In general, enlarging particle size can also improve flow characteristics. (Okoye *et al.*, 2013). Differences in citric acid and tartaric acid (1:1) concentrations can affect the effervescent granular flow characteristic. The greater the flow characteristic, the higher the acid content, as was also discovered by Grajang & Wahyuningsih (2019). The flowability obtained in this study is comparable with 18-32^{o} angle of repose, 1.12-2 Hausner's ratio, Carr's index reported by Alaayedi & Mohamed (2019) for Ibuprofen effervescent granules. Similar results for an effervescent granule of corn milk, supplemented with probiotics lactobacillus strain *Shirota* (Hiola & Tungadi, 2018), lornoxicam sachet effervescent granules (Jassim *et al.*, 2018) were reported. However, red ginger extract effervescent granules had poor flow properties (40-42^{o}), as Giyatmi & Lingga (2019) reported. The poor flowability, low tensile strength, and high caking strength of pure herbs and leaves are not desirable because they create difficulties in storage and handling (Ali *et al.*, 2018).

Table 6.2 Physical and flow properties of SDMOLP effervescent granules[1]

Formulation	ρbulk (g/ml)	ρtapped (g/ml)	Carr's index (%)	Hausner's ratio	Angle of repose (^{o})
SDMOLPG10	0.40 ± 0.0^{a}	0.47 ± 0.0^{a}	14.86 ± 2.0^{a}	1.18 ± 0.0^{a}	30.86 ± 0.0^{a}
SDMOLPG15	0.44 ± 0.0^{b}	0.49 ± 0.0^{a}	11.21 ± 1.1^{a}	1.13 ± 0.0^{a}	30.46 ± 1.0^{a}
SDMOLP20	0.44 ± 0.0^{b}	0.50 ± 0.0^{a}	11.97 ± 2.4^{a}	1.13 ± 0.0^{a}	30.28 ± 0.0^{a}

[1]ρbulk: bulk density; ρtapped: tapped density. SDMOLPG: Solid-dispersed MOLP effervescent granules with (10% acids), (15% acids) and (20% acids)
[2]Values are the mean $\pm$ standard deviation of three replicates. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different. N=3

The effect of acid concentration on the flow properties of MOLP effervescent granules is displayed in Figure 6.5. The angle of repose test was conducted to assess indirect granular flow properties, as it is affected by cohesion bonds between particles. Cohesive bonds among particles illustrate friction between particles. If the angle of repose formed by the granules is less than 31^{o}, they have excellent flow properties. A small angle of repose will produce a good flow and better illustrate the cohesion and friction bonds between particles. The results showed that SDMOLPG20 (20% acids) with a combination of effervescent acids concentrations greater than SDMOLPG10 (10% acids) and SDMOLP15 (15% acids) possessed a better angle of repose. This was most likely due to rising amounts of effervescent acids, which resulted in stronger ionic bonds and weaker van der Waals bonds,

resulting in an effervescent product with decreased cohesivity, friction and superior flow qualities (Maysarah *et al.*, 2020). Furthermore, the particle shape of the granule-forming substance also affects the flowability of granules. Compounds with a crystalline form will have a better flow rate than amorphous powders. SDMOLPG20 contained greater citric acid compared to SDMOLPG10 and SDMOLPG15, so that the SDMOLPG20 effervescent granule had a better flow property. Nevertheless, all three formulations met the pH requirements of effervescent granules.

6.8.3 Colour Characteristics of SDMOLP Effervescent Granules

The colour attributes of the effervescent granules measured were lightness (L*), greenness (-a*), redness (+a*), blueness (-b*), yellowness (+b*), hue, and chroma as tabulated in Table 6.3. Lightness is the luminous intensity of colour measured on a scale of 0 to 100, with 0 indicating black and 100 indicating white. Hue is how the colour of an object is perceived and chroma is the vividness or dullness of colour; it indicates the richness or saturation of a colour (Murevanhema & Jideani, 2015). On the colour scale, a* describes redness or greenness, with positive a* denoting redness, negative a* denoting greenness, and 0 denoting neutrality.

Table 6.2 shows that the lightness (L*) of the granules ranged from 30.15 to 31.21. The SDMOLP effervescent granules before and after drying are displayed in Figure 6.7. There was no significant difference in lightness among the samples, suggesting that different acid concentrations did not affect the granules' color lightness. In contrast, Kuswardhani *et al.* (2020) found the quantity of sodium bicarbonate and tartaric acid used in producing effervescent tablets of temulawak (*Curcuma zanthorrhiza*) had a considerable impact on the product's lightness. Possibly, this variation may arise from the fact that only acid proportion was altered in this study, while their study employed various sodium bicarbonate and acid concentrations. Compared to the lightness of the MOLP and SDMOLP (51.2 and 53.10), determined in Chapter 3, the lightness of the granules significantly decreased, and the colour became dark green-brownish. It can be attributed to the addition of flavours with different colours, such as strawberry flavour, red. Furthermore, temperatures below 60^{o}C can cause less browning as the deterioration of chlorophyll causes the changes. The air oven-drying temperature-time has a significant effect ($p < 0.05$) on the lightness of the products. Therefore, the degradation may be due to the longer time rather than the action of polyphenol oxidase. Murdiana *et al.* (2018) reported similar observations for the *effervescent tablets of Moringa oleifera leaf powder extract*. In addition, this study obtained lightness (L*) comparable to those reported by Ali *et al.* (2014) for oven-dried *Moringa oleifera* leaves at 50^{o}C (36.52 and 37.69).

The greenness of the granules changed to reddish (a*) ranging from 4.05 to 6.87, depicted in Figure 6.5. Significant variations in greenness were observed between solid-dispersed MOLP effervescent granules with 10% acids (SDMOLPG10) and SDMOLPG15 (15% acids). The natural green colour of the leaves, which is attributed to a mixture of chlorophylls directly related to magnesium, suggests that magnesium molecules were converted to pyropheophytin and pheophytin during oven drying of the granules (Ali *et al.*, 2014). Therefore, at higher temperatures (+60^{o}C), greenness is reduced, due to chlorophyll degradation, which subsequently reveals the yellow carotenoid pigments. In this study, the effervescent granules were oven-dried at 50^{o}C for 5 hours; as stated before, temperatures below 60^{o}C can also cause less browning due to chlorophyll deterioration; a similar observation was reported by Raja *et al.* (2019) for the papaya leaf powder. Additionally, it is also possible that the colour of the effervescent granules was affected by the various flavours, which came in different colours, including strawberry (red), orange and banana (off-white), and cream soda (green). The yellowness (+b*) of the granules ranged from 16.60 to 23.27, with no significant difference between them. The yellowness for all formulas increased, compared to pure MOLP (11.45-12.87) dried at 50^{o}C. The increase in b* of the granules showed that granules became more yellow, perhaps due to the yellow carotenoid pigments. That was also related to brightening the colour and loss of chlorophylls. As a result of the loss of green pigments, other pigments, such as carotenoids, might become more visible. Chloroplasts (primarily in leaves) contain both carotenoids and chlorophyll. Visually, the dark green colour of the granules seemed dull green-yellow due to the degradation of chlorophyll, shown in Figure 6.6.

Table 6.3 Colour attributes of SDMOLP effervescent granules[1]

Formulation	L*	a*	b*	Chroma	Hue (^{o})
SDMOLPG10	30.15 $\pm$1.2^{a}	6.87 $\pm$ 1.1^{b}	17.77 $\pm$ 3.2^{a}	19.11 $\pm$ 2.9^{a}	68.40 $\pm$ 5.8^{a}
SDMOLP15	31.21 $\pm$ 0.0^{a}	4.05 $\pm$ 1.0^{a}	23.27 $\pm$ 4.22^{a}	23.62 $\pm$ 4.3^{a}	80.22 $\pm$ 0.7^{b}
SDMOLP20	30.71 $\pm$ 1.6^{a}	6.25 $\pm$1.2^{a,b}	16.60 $\pm$ 3.9^{a}	17.74 $\pm$ 4.0^{a}	69.26 $\pm$ 1.5^{a}

[1]SDMOLPG10,15 and 20: Solid-dispersed *Moringa oleifera* leaf powder effervescent granules with 10% acid, (15% acid), and 20% acid, respectively. Values are the mean $\pm$ standard deviation of three replicates. Means within a column followed by the same superscript are not significantly [p > 0.05] different. N=3.

A similar increase in the level of the yellow colour was observed by Sledz & Witrowa-Rajchert (2012) in herbs (basil, mint, lovage, parsley, and rocket leaves) dried with oven.

Colour saturation, or chroma (C), is one of the most important factors determining how the chromatic parameters (a* and b*) contribute to the overall perception of colour. Chroma describes the vividness or dullness of color, such as how close the colour is to either grey or the pure hue. Colours in the centre are grey (dull) and become more saturated (vivid) as they move toward the perimeter. Chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] describes the colour richness, the colour strength. All three formulations of the effervescent granules had chroma ranging from 17.74 to 23.62, with no significant difference between them. The relatively low saturation suggests that the granules were dull in colour or less chromatic, or less saturated. Similar results were reported for green beans (*Phaselus vulgarus L.*) [20.63 and 21.46] by Aydogan & Turhan (2015).

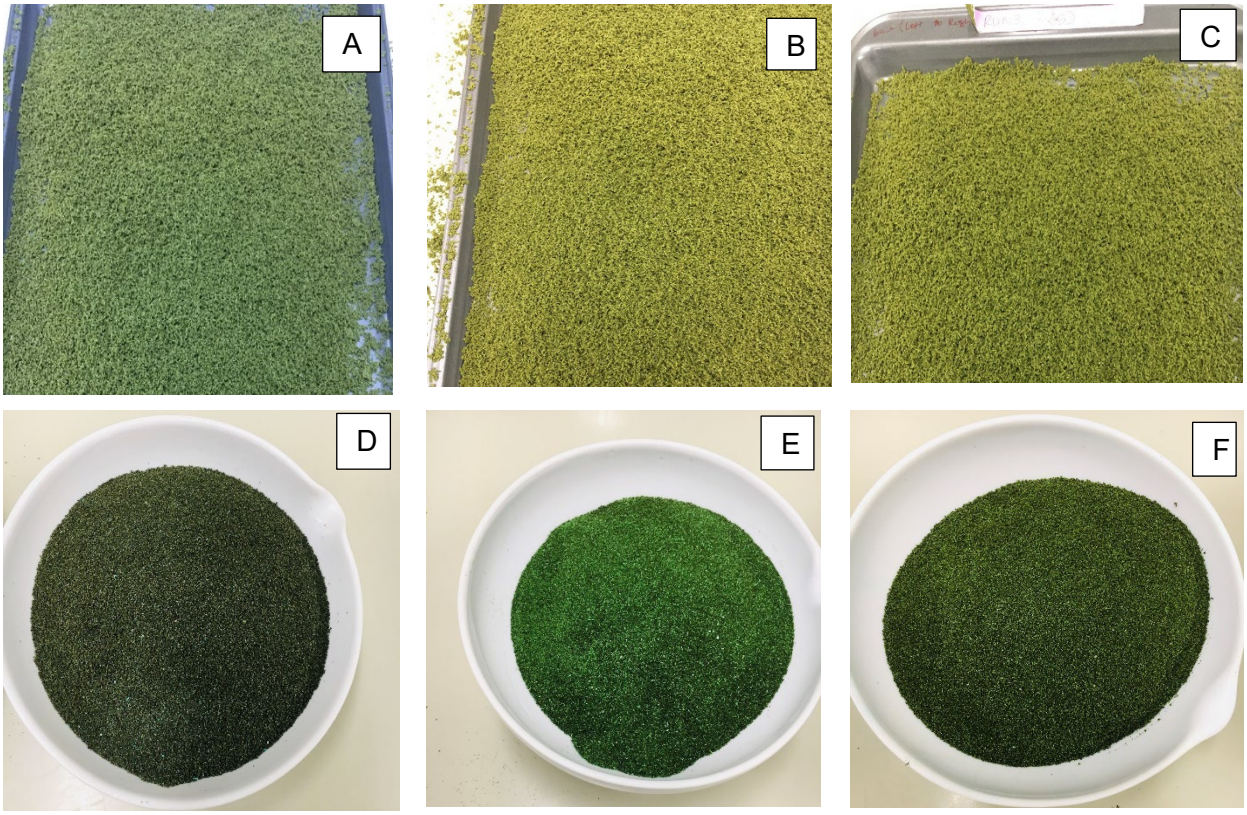

Figure 6.5 Pictorials of solid-dispersed *Moringa oleifera* leaf powder effervescent granules before drying [(a) SDMOLPG10, (b) SDMOLPG15, (c) SDMOLPG20], and after drying (d) SDMOLPG10, (e) SDMOLP15, and (c) SDMOLP20

Sledz & Witrowa-Rajchert (2012) also reported similar results for the herbs, parsley (18.40), mint (14.32) basil (29.0). The low chroma of the effervescent granules could also be attributed to a loss of chlorophylls and/or an increase in pheophytins and other chlorophyll derivatives during drying (Montesano *et al.*, 2016).

Hue, the actual colour of an object, is usually expressed as a ratio such as a/b. Moreover, the visual impression is determined by a and b- values [$h^o = \tan^{-1}(b^*/a^*)$]. Hue angles of effervescent granules ranged from 68.40 to 80.22^o. Hue angles of granules with 10% acid and 20% acid significantly ($p < 0.05$) differed from the granules with 15% acid. Granules with 15% acid were greener and yellower than 10% and 20% acid, respectively. However, all the hue angles were within the 90^o range, indicating apparent yellowish-red. According to Downham & Collins (2000), a hue-angle of 0/360^o represents pure redness while a hue-angle of 180^o represents pure greenness. A hue-angle of 90^o represents pure yellowness, while a hue-angle of 270^o represents pure blueness. Therefore, the hue angles of the granules in this study were closer to the yellow. Granule yellowing may be due to colour loss during drying. In an effervescent mix, colour changes occur after wet granulation. This means that the hue was changed from green to yellowish-red, and this can be attributed to heat exposure during drying (50^{o}C) and colours from flavours (red colour from the strawberry flavour and off white from banana flavour). Giyatmi & Lingga (2019) also stated that processes and raw materials impact colour.

Colour differences among the SDMOLP effervescent beverage granules

A colour difference (ΔE) of 1 is known as the threshold at which a trained observer would notice the difference between two colours (Sharma, 2005). This difference is referred to as a just-noticeable difference (JND). Moreover, the difference can be perceivable but still deemed acceptable. An ΔE between 4 and 8 is acceptable. However, ΔE above 8 is perceivable and unacceptable and likely to be rejected by consumers (Sharma, 2005). The effervescent granules showed acceptable colour differences with ΔE ranging between 1.44 and 7.04 because the values are below 8. The colour difference between granules with 10% acid and granules with 15% acid was 6.27, while that of granules with 15% and 20% acid was 7.04. The colour difference between granules with 10% acid and 20% acid was 1.44. The differences may be attributed to variations in acid concentrations.

6.8.4 Chemical Properties of SDMOLP Effervescent Granules

6.8.4.1 Moisture content

The moisture content of the three formulations of SDMOLP effervescent beverage granules ranged from 1.82 to 3.43%, as shown in Table 6.4. SDMOLPG10 (10% acids) had a significantly lower moisture content than SDMOLPG15 (2.45%) and SDMOLPG20 (3.43%). All granules have low moisture content, indicating their ability to retain effervescent quality as well as free flow. Moreover, the low moisture suggests that the granules will have a prolonged shelf life due to low microbial activity. SDMOLPG15 and SDMOLPG20

(containing 15% and 20% acid, respectively) showed comparatively more moisture content than SDMOLPG10, which contains 10% acid. The low moisture content in SDMOLPG10 may be associated with its low concentration of acids and the high moisture content in SDMOLPG15 and SDMOLPG20 may be due to their high acid concentrations (15% and 20%, respectively). The higher the concentration of acids, the higher the moisture content. This can be explained through the hygroscopicity of acids. Both tartaric and citric acids are hygroscopic and may absorb water under ambient conditions (Peng *et al.*, 2001). In comparison to citric acid and tartaric acid, sodium bicarbonate is less capable of absorbing moisture during storage. As a result, the lower the moisture content of effervescent granules, the more sodium bicarbonate is utilised in the manufacturing process. Sodium bicarbonate is non-hygroscopic and has less than 1% moisture content at room temperature (Grajang & Wahyuningsih, 2019). However, all the SDMOLP effervescent granules met the effervescent moisture content requirement, stipulating that standard granules must have a moisture content of ≤5% (Manthena & Artham, 2014; Giyatmi & Lingga, 2019; Kuswardhani *et al.*, 2020). The different acid concentrations affected the moisture content of SDMOLP effervescent granules. Similar results (3.40, 3.62, and 3.50%) were recorded by Grajang & Wahyuningsih. (2019) for *Sechium edule* extract effervescent granules. Giyatmi & Lingga (2019) reported a significantly lower moisture content of 0.41-0.47% for the red ginger extract effervescent granules. The differences in the moisture content of the effervescent granules may be attributed to different environmental conditions such as relative humidity. It is stated that effervescent formulations should be prepared at 20-25^{o}C with 25% relative humidity (Grajang & Wahyuningsih, 2019). Effervescent granules easily absorb moisture from the air. Therefore, a controlled environment is imperative for controlling active ingredient flow characteristics and dissolution rates since changes in moisture parameters affect these properties.

6.8.4.2 pH of solid-dispersed MOLP effervescent beverages

The pH of the SDMOLP effervescent beverage granules after reconstitution ranged from 6.04 (SDMOLPG20, 20% acid) to 6.65 (SDMOLPG10, 10% acid) as tabulated in Table 6.4. The difference in the acid concentrations significantly impacted the pH ($p < 0.05$). The three formulations of effervescent granules significantly ($p < 0.05$) differed in pH. The pH of the SDMOLPG20 (20% acid) was lower than those of SDMOLPG10 (10% acid) and SDMOLPG15 (15% acid). Increased acid concentration caused a significant decline in pH. Increasing the acid percentage increases the concentration of unreacted ions [H30+] in the solution (Grajang & Wahyuningsih, 2019). Moreover, decreasing the acid proportions in formulations 1 (10% acid) and 2 (15%) increased their pH due to increased alkalinity. In

short, a higher acid concentration will result in lower pH of SDMOLP effervescent beverage granules, while a lower acid concentration will result in higher pH. The pH of effervescent beverages is good if it is between 6-7 or close to neutral (Grajang & Wahyuningsih, 2019). The three SDMOLP effervescent beverage granules produced in this study met the requirements because their pH values ranged between 6.04 and 6.65. Therefore, effervescent beverages were safe for consumption. pH is one of the key parameters influencing consumer acceptance of effervescent preparations. Preparations with high acidic pH irritate the stomach or gastrointestinal tract, while preparations with high alkaline pH are bitter and unpleasant (Grajang & Wahyuningsih, 2019). Similar results were reported by Palanisamy *et al.* (2011); Aslani & Jahangiri (2013); Patel (2015); Hiola & Tungadi (2018); Patel & Siddaiah (2018); Giyatmi & Lingga (2019) for effervescent granules.

Table 6.4 Chemical properties of SDMOLP effervescent granules

Formulation	Moisture content (%)	pH	Solubility time (min)
SDMOLP10	1.82 ± 0.1^{a}	6.65 ± 0.6^{a}	4.0
SDMOLP15	$2.45 \pm 0.6^{a,b}$	6.44 ± 0.0^{b}	3.48
SDMOLP20	3.43 ± 0.0^{b}	6.04 ± 0.0^{c}	3.21

[1]SDMOLPG10, 15 and 20: *Moringa oleifera* leaf powder effervescent granules with 10% acid, 15% acid and 20% acid, respectively.
[2]Values are the mean $\pm$ standard deviation of three replicates. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different.

6.8.5 Solubility Time

The solubility times of effervescent granules are measured to determine how long it takes to dissolve in water. Effervescent solubility ends when all the effervescent solid components dissolve into the solution, and no further gas bubbles are produced. According to Kuswardhani *et al.* (2020), the relatively short time needed for the effervescent component to dissolve indicates highly soluble. The soluble time of SDMOLP effervescent granules with variations in the proportion of acids can be seen in Table 6.4. The solubility times for SDMOLPG10, SDMOLPG15, and SDMOLP20 were 4.0, 3.48, and 3.21 min, respectively. Among the three formations, SDMOLP20 showed the least solubility time, which may be attributed to its high acid content. SDMOLP10 and SDMOLP15 had fairly long dissolution times of 4.0 min and 3.48 min, respectively. The results showed that the proportion of acids in the manufacture of effervescent granules of SDMOLP significantly affected the solubility time of granules. Based on the observations, citric acid has a high solubility in water and is

particularly hygroscopic; therefore, the higher the percentage of citric acid added, the faster the solubility. According to Giyatmi & Lingga (2019) and Kuswardhani *et al.* (2020), good effervescent granules or powder have a dissolving time of fewer than 5 minutes, suggesting that the preparation meets the requirements of dissolution time. These findings are similar to those reported by Shamsi (2017) for effervescent granules from an Unani powder, Aslani & Jahangiri (2013) ranitidine effervescent tablets, Diyya & Thomas (2018) Metronidazole effervescent granules, Kuswardhani *et al.* (2020) *Curcuma zanthorrhiza*, and Giyatmi & Lingga (2019) effervescent of red ginger extract.

6.8.6 Sensory Evaluation and Consumer Acceptability

The organoleptic and sensory evaluations, including appearance, colour, aroma, taste, texture, and overall acceptability, were carried out to determine the panellists' acceptance. Furthermore, the demography of the 53 consumer panellists, tabulated in Table 6.5, shows 60.38% female, 39.62% male, 77.36% students (75.47% local students, 24.53% international students), 22.64% staff, 66.04% <20-29 age group, 24.53% 30-39 age group, and 9.43% 40 and above age group. Panellists' responses significantly ($p \leq 0.05$) differed in the evaluation of the beverage. However, no significant ($p > 0.05$) differences were found in the likeness of the effervescent beverages. The results obtained from the 5-point hedonic scale evaluation are illustrated in Table 6.7 and Figure 6.6. Table 6.9 shows the results of Kruskal-Wallis sum of ranks of consumer acceptance. The pictorial representation of three SDMOLP effervescent beverages is displayed in Figure 6.7. The Independent-Sample Kruskal Wallis Test (Table 6.7) revealed that the null hypothesis that MOLP effervescence beverages have the same appearance distribution and color was rejected, inferring that effervescent beverages differed in appearance. The colour (Figure 6.7) of the beverages ranged from 3.23 to 4.04 (neither like nor dislike to like moderately) with significant differences among the samples. Colour assessment is used in organoleptic testing because it plays a significant role in the level of product acceptance visually. Evaluation results showed that the percentage of panellists who neither liked nor disliked to liked moderately from the colour parameter was 64.60-80.80%. Several panellists commented that the beverages' appearance and colour did not appeal to them, perhaps due to MOLP's dark green colour (high chlorophyll) and the variety of flavours used. In the future, it will be important to improve the colour properties by adding dyes such as light green or yellow to enhance the granule color's brightness. Box and whisker plots for appearance and colour of the beverages in Figure 6.8 revealed that the scores for the beverages were spread over the whole scale range. Moreover, the difference between the lower quartile (Q1, lower edge of the box) and upper quartile (Q3, upper edge of the box) was between 1 and 2 points on

the scale for all beverages. Meanwhile, in terms of aroma, 80-83% of panellists moderately liked the aroma of the beverages.

Table 6.28 Demography of SDMOLP effervescent beverage granules[1]

Item	Frequency (%)
Age	
20-29	35 (66.04)
30-39	13 (24.53)
40 & above	5 (9.43)
Race	
Black	47 (88.68)
White	0
Coloured	6 (11.32)
Occupation	
Staff	12 (22.64)
Student	41 (77.36)
Gender	
Male	21 (39.62)
Female	32 (60.38)
International	
Yes	13 (24.53)
No	38 (75.47)

*Numbers are frequency and percentage in brackets.

Table 6.6 Mean sensory attributes of the solid-dispersed MOLP effervescent beverage[1,2]

Beverages	Appearance	Colour	Aroma	Taste	Texture	Overall acceptability
SDMOLPEB10	3.19 ± 0.2^{a}	3.23 ± 0.1^{a}	4.00 ± 0.2^{a}	3.08 ± 0.2^{a}	3.04 ± 0.2^{a}	3.40 ± 0.2^{a}
SDMOLPEB15	3.92 ± 0.2^{bc}	4.04 ± 0.2^{bc}	4.13 ± 0.2^{bc}	3.81 ± 0.2^{b}	3.66 ± 0.2^{bc}	3.92± 0.2^{bc}
SDMOLPEB20	3.60 ± 0.2^{c}	3.53 ± 0.2^{c}	4.00 ± 0.2^{c}	3.77 ± 0.2^{c}	3.58 ± 0.2^{c}	4.00 ± 0.2^{c}

[1]SDMOLPEB10,15 and 20: Solid-dispersed *Moringa oleifera* leaf powder effervescent beverage with 10%, 15% and 20% acids, respectively.
[2]Values are the mean ± standard deviation of three replicates. Means within a column followed by the same superscript are not significantly [p > 0.05] different.

Table 6.30 Kruskal-Wallis Test ranks describing solid-dispersed MOLP effervescent beverage sensory attributes[1,2]

Beverage	Appearance	Colour	Aroma	Taste	Texture	Overall acceptability
SDMOLPEB10	63.49 ± 0.00^{a}	64.65 ± 0.00^{a}	77.16 ± 0.34^{a}	58.35 ± 0.00^{a}	63.27 ± 0.00^{a}	60.90 ± 0.00^{a}
SDMOLPBE15	95.26 ± 0.00^{b}	98.54 ± 0.00^{b}	87.07 ± 0.34^{a}	92.20 ± 0.00^{b}	89.73 ± 0.00^{b}	88.92 ± 0.00^{b}
SDMOLPBE20	81.25 ± 0.00^{c}	76.81 ± 0.00^{c}	75.77 ± 0.34^{a}	89.45 ± 0.00^{c}	87.00 ± 0.00^{c}	90.18 ± 0.00^{c}

[1]SDMOLPEB10,15 and 20: Solid-dispersed *Moringa oleifera* leaf powder effervescent beverage with 10%, 15% and 20% acids, respectively.
[2]Values are the mean ± standard deviation of three replicates. Means within a column followed by the same superscript are not significantly [p = 0.05] different

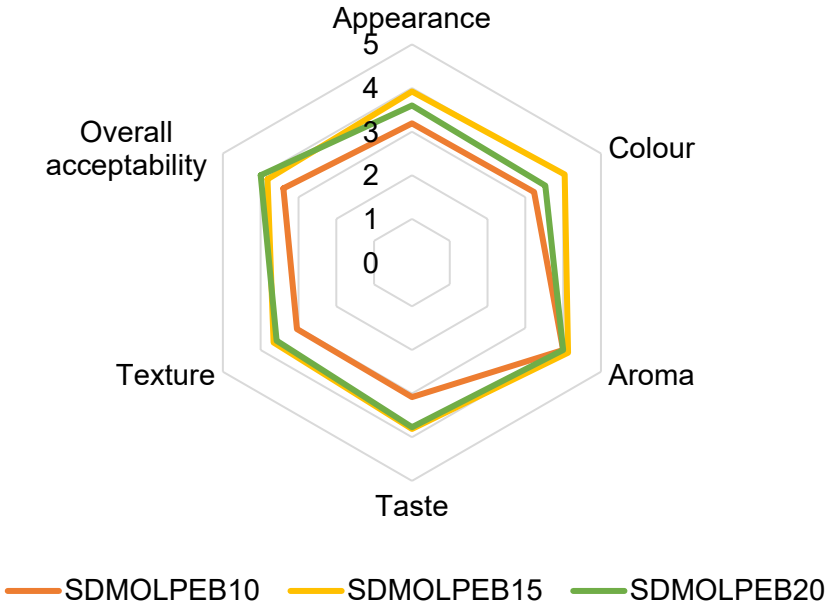

Figure 6.6 Mean sensory scores of SDMOLP effervescent beverage granules (1= Dislike very much, 2= Dislike moderately, 3= Neither like nor dislike, 4= Like moderately, 5= Like very much). SDMOLPEB10%, SDMOLP15% and SDMOLPEB20%

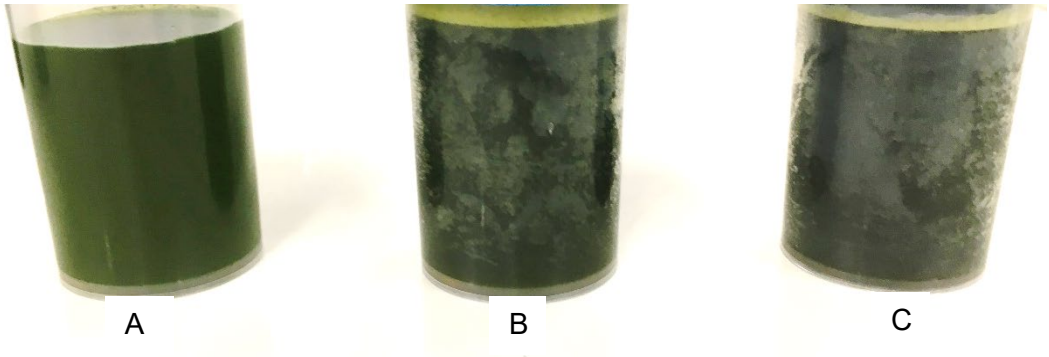

Figure 6.7 SDMOLP effervescent beverages (a) SDMOLPEB10 (10% acid), (b) SDMOLPEB15 (15% acid), and (c) SDMOLPEB20 with 20% acid

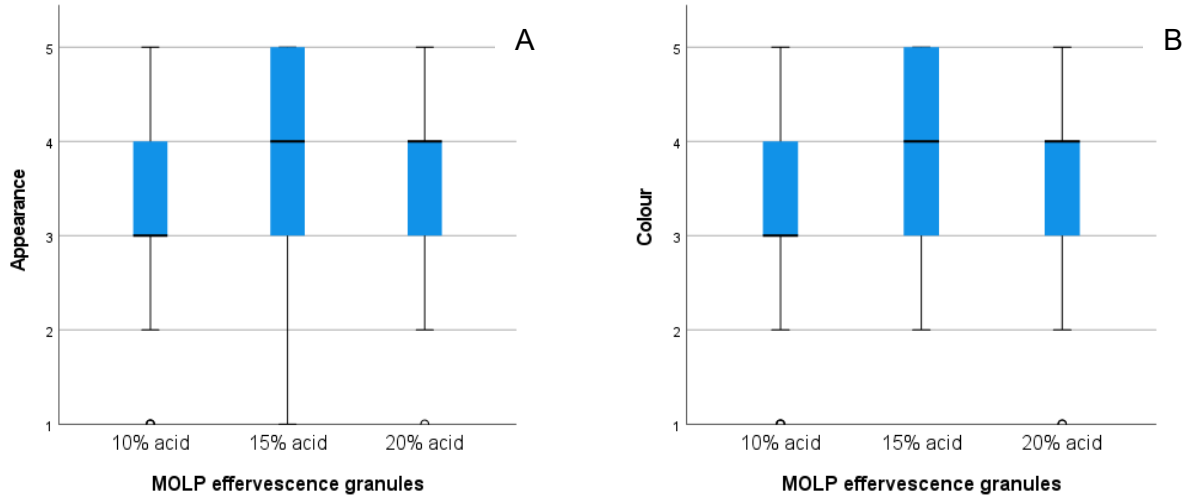

Figure 6.8 Box-and-whisker plots of descriptive statics for consumers' liking scores of MOLP effervescent beverages: (A) appearance and (B) colour. The lower and upper edges of a box represent the (Q1) and third (Q3) quartile, while the line across within the box show the median and the mean, respectively. The whiskers represent the lower and upper extremes of data.

The aroma of the effervescent beverages was liked moderately (4.00-4.13), with SDMOLPEB15 (4.13) being the highly scored beverage. Different concentrations of acids in effervescent beverages did not affect the aroma of effervescent beverages. The Independent-Sample Kruskal Wallis Test proved this by accepting a null hypothesis that aroma distribution is the same across categories of MOLP effervescence granules. Similarly, Giyatmi & Lingga (2019) observed no significant difference in aroma between the effervescent red ginger extracts (with varying acid concentrations). The aroma attribute was the most scored hedonic sensory attribute during the sensory evaluation, suggesting that the panellists liked the scent or flavour of the beverages. The flavours used were orange, cream soda, and strawberry. Positive comments from panellists such as "nice developed aroma," "Moringa not potent," "nice flavour," "smells nice," "I like the aroma," I like the aroma, I would rate it 8/10," and "subtle flavour" were noticed. However, negative comments such as "I don't like the colour or appearance," "I enjoyed the aroma, not sure about the texture and colour" were indicated. Box and whisker plots for the aroma of the beverages in Figure 6.9 revealed that the scores for the beverages were not spread over the whole scale range. Moreover, the difference between the lower quartile (Q1, lower edge of the

box) and upper quartile (Q3, upper edge of the box) was between 1 and 2 points on the beverage scale. This suggests that there was no wide variability among consumers' scores.

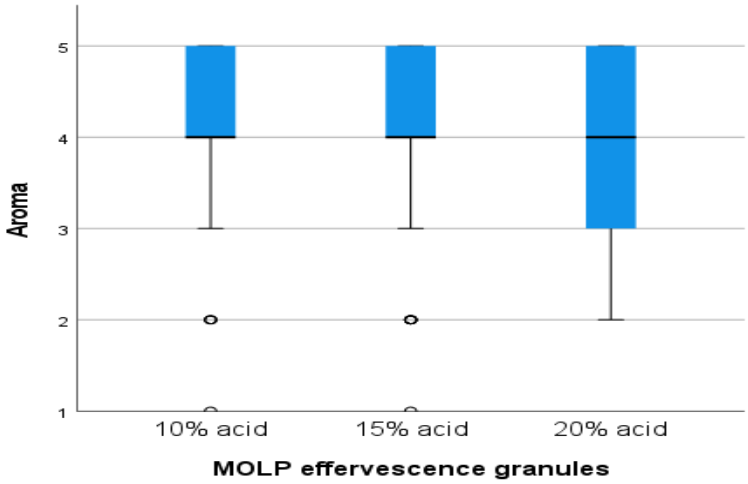

Figure 6.9 Box-and-whisker plots of descriptive statics for consumers' liking scores of MOLP effervescent beverages: Aroma. The lower and upper edges of a box represent the (Q1) and third (Q3) quartile, while the line across within the box shows the median and the mean, respectively. The whiskers represent the lower and upper extremes of data.

Taste is a very important factor in determining the consumer's final decision to accept or reject a food product. Taste is judged by chemical stimulation responses given by the tongue and the trigeminal neurons of the nose and mouth in the mouth of the taster (Hiola & Tungadi, 2018). Taste sensitivity or perception can be affected by several factors, including the substances being tested and the panellist and temperature conditions. The taste scores of the effervescent beverage granules ranged from 3.08-3.81, indicating that the beverages were neither liked nor disliked (SDMOLPEB10) or liked moderately (SDMOLPEB15 and SDMOLP20). Significant ($p < 0.05$) differences existed in taste among the samples with comments like "bitter taste," "tastes moderately well," "I can taste the strong Moringa taste, "needs more sugar" were indicated. Majority of the bad comments were from the age group <20-29. However, good comments were also observed including "no plant or herb after taste, sweetness 4-6/10", "good aroma and taste but not an appealing colour", "the taste is quite nice but improve sweetness," "tastes delicious" and "I can buy it." The bitter taste can be associated with high mineral content, phenolic compounds, protein, vitamins, and antioxidants present in *Moringa oleifera* leaf powder. The results (bitterness) obtained in this study concur with those Murdiana *et al.* (2018) reported for *Moringa oleifera* lam. Extract-based effervescent tablets showed that the effervescent tablets were even

bitter after using lemon and strawberry flavors. Their bitterness was attributed to the bitter taste of the natural extract in which phenolic content is likely the 'culprit' for the bitter taste. In the future, masking agents can be used; cyclodextrin, for instance, is a bitter masking agent widely used in food and pharmaceutical products. The Independent-Sample Kruskal Wallis Test revealed that the null hypotheses that MOLP effervescence beverages have the same distribution of taste and texture were rejected. Box and whisker plots for taste and texture of the beverages in Figure 6.10 exhibited that the scores for the beverages were not spread over the whole scale range. Moreover, the difference between the lower quartile (Q1,lower edge of the box) and upper quartile (Q3, upper edge of the box) was only 1 point on the scale for beverages. This suggests that there was no wide variability among consumers' scores.

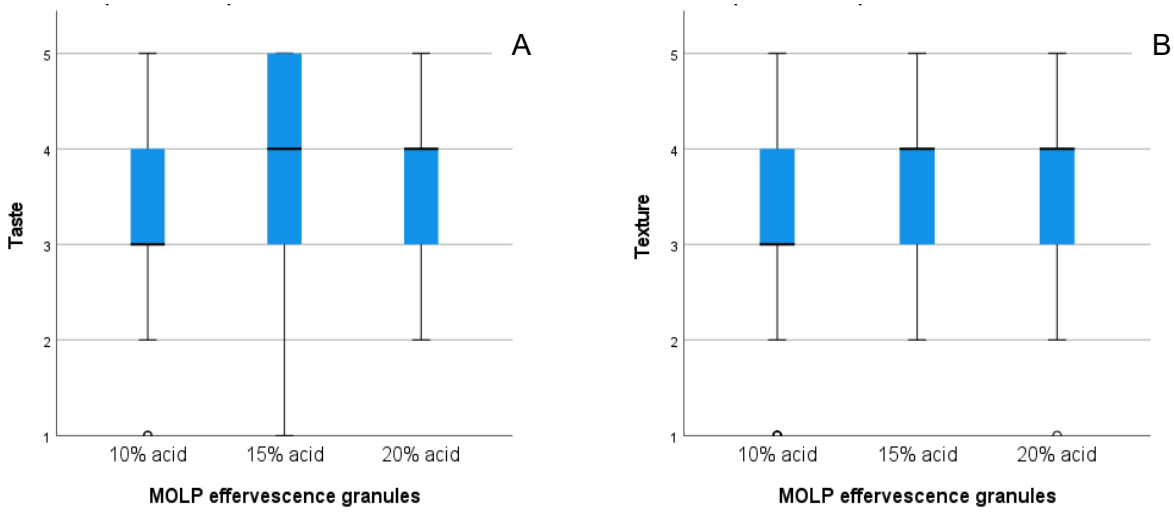

Figure 6.10 Box-and-whisker plots of descriptive statics for consumers' liking scores of MOLP effervescent beverages: (A) Taste and (B) texture. The lower and upper edges of a box represent the (Q1) and third (Q3) quartile, while the line across within the box show the median and the mean, respectively. The whiskers represent the lower and upper extremes of data.

The texture of the effervescent beverages was also neither liked nor disliked or moderately liked, with scores ranging from 3.04 to 3.66, with SDMOLPEB10 being the lowest-rated beverage, while SDMOLPEB15 and SDMOLP20 beverages were liked moderately. Comments like " nice taste and aroma but small particles or sediments are not

appealing.” The lower scores could be attributed to the sedimentation of particles in the beverages, which affected the texture and appearance of beverages. The particles noticed in the beverages could be due to the texture of Moringa leaf powder, which should be made finer; leaves do not completely dissolve in water. In addition, MOLP is a heterogeneous mixture, high in vitamins, protein, and minerals, thus, making it less soluble in water. Furthermore, particles in the beverage could be explained through the particle size of the granules (>850 microns)—the bigger the granule or particle size, the lower the dissolvability. Therefore, further decreasing the granule size or sifting the beverage can be recommended. The overall acceptability of the effervescent beverages ranged from 3.40-4.00 (neither like nor dislike to like moderately. SDMOLPEB20 (4.00) beverage had the highest overall acceptability, followed by SDMOLPEB15 and SDMOLPEB10 (3.92 and 3.40, respectively). It is observed that SDMOLPEB15 and SDMOLPEB20 beverages were the most accepted ones, exceeding the acceptances of the beverages based on SDMOLPEB10. This fact can be corroborated in Figure 6.8, where the greater scores are attributed to SDMOLPEB15 and SDMOLPEB20 beverages, which in turn significantly differed ($p < 0.05$) from the SDMOLPEB10 beverage. There was no significant difference between SDMOLPEB15 and SDMOLPEB20 samples. However, SDMOLPEB10 and SDMOLPEB15 or SDMOLPEB10 and SDMOLPEB20 beverages significantly varied. Different acid concentrations could explain the variability in taste and overall acceptability among the two samples (SDMOLPEB10 and SDMOLPEB20). Based on all sensory attributes, SDMOLPEB15 and SDMOLPEB20 beverages had the best overall acceptability, with positive comments like “I like the fizziness, the aroma is gentle and nice, I can buy it, doesn’t taste like Moringa, nice aroma,” as displayed in Figure 6.12 (word cloud). Negative comments like “the colour are too dark, add more sugar, smells and tastes nice but particles are not appealing.” The negative sentiments were welcomed to improve the beverages to be commercially acceptable. Moreover, these comments displayed each panelist's different options; therefore, effervescent beverage granules from all acid ratios could be accepted if subsequently improved. For more effervescence or fizziness, the acid and base ratio need to be improved. The hypothesis of producing an acceptable beverage was tested and accepted; however, the taste and texture of the beverages could be improved in future work. The beverages could be filtered, carbonated, and blended with other beverages to boost the taste and nutritional content to suit the young generation. International students are most familiar with herbal beverages in comparison to South African citizens. The Independent-Sample Kruskal Wallis Test revealed that the null hypotheses that MOLP effervescence beverages have the same distribution of overall acceptability was rejected.

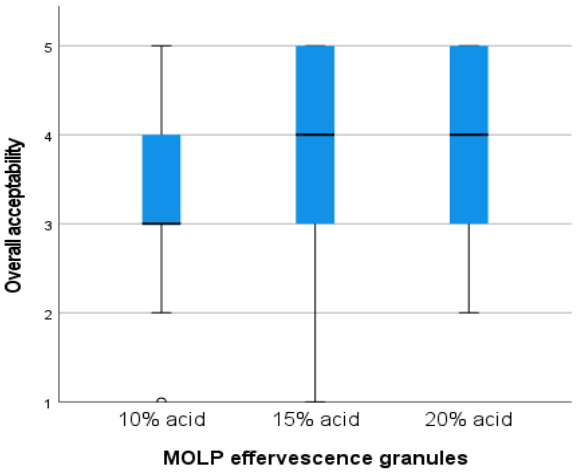

Figure 6.11 Box-and-whisker plots of descriptive statics for consumers' liking scores of MOLP effervescent beverages: Overall acceptability. The lower and upper edges of a box represent the (Q1) and third (Q3) quartile, while the line across within the box shows the median and the mean, respectively. The whiskers represent the lower and upper extremes of data.

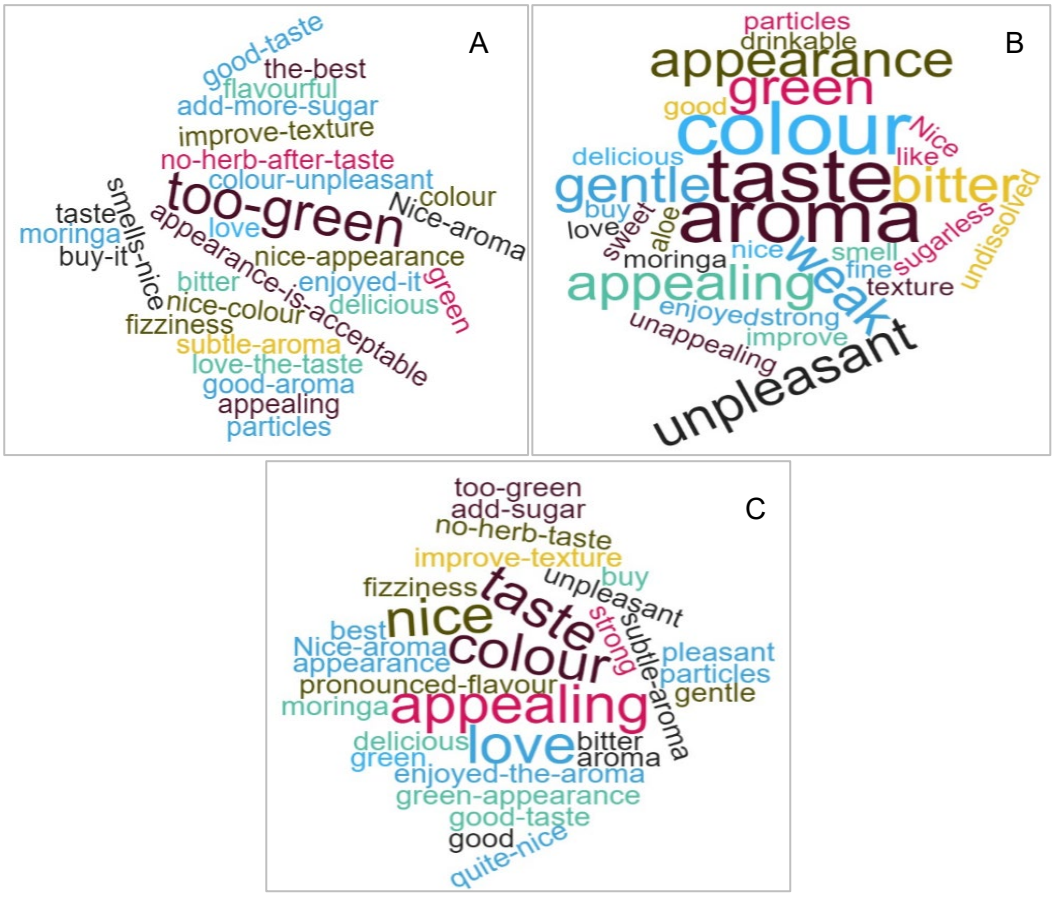

Figure 6.12 Word cloud based on the comments from panellists on effervescent beverages (a) SDMOLPEB10, (b) SDMOLPEB15, and (c) SDMOLPEB20

6.9 Conclusion

This study successfully formulated novel SDMOLP into three formulations of effervescent granules which differed in acid concentration. Solid-dispersed MOLP effervescent beverages contained 10%, 15%, and 20% acid, respectively. The SDMOLP effervescent granule formulations exhibited excellent flow characteristics, uniform particle size distribution, and quality requirements. In all three formulations, the solubility time was less than 5 minutes, and the pH ranged between 6.04 and 6.65 (neutral pH). The acid proportions significantly affected the moisture content and pH of the beverage granules. The three beverages were acceptable to the panellists with sensory scores ranging from 3.05 to 4.13, neither liked nor disliked, and liked moderately. In addition, beverages with 15% acid and 20% acid were the most acceptable in terms of colour, taste, aroma, texture, and overall acceptability.

6.10 References

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CHAPTER SEVEN

GENERAL SUMMARY AND CONCLUSIONS

The physicochemical, nutritional, and dissolution properties of the solid dispersed *Moringa oleifera* leaf powder were investigated in this study. This study aimed to develop solid-dispersed MOLP using polyethylene glycol carriers with the view of improving its solubility and dissolution and presenting it as an easy-to-drink effervescent beverage for the food industry. This study identified the following objectives:

1 Establishing solid dispersions of MOLP using a suitable hydrophilic carrier (PEG) and solid dispersion techniques such as freeze-drying, melting, solvent evaporation, and microwave irradiation method.

2 Determining the physicochemical, nutritional, mineral, functional, and structural properties of the solid -dispersed MOLP.

3 Determining the thermal, compatibility, and crystallinity properties of solid-dispersed MOLP.

4 Establishing the flow and dissolution properties of solid-dispersed MOLP.

5 Production of effervescent beverage granules using wet granulation method and establishing their physical, chemical, and flow properties.

6 Establishing the sensory properties of the beverage and consumer acceptance.

The first objective was successfully achieved through the use of hydrophilic carriers such as PEGs (PEG4000 and PEG6000) and solid dispersion techniques, including melting, freeze-drying, solvent evaporation, and microwave irradiation. The hypothesis that new solid-dispersed MOLP will be produced as a food ingredient using PEGs was accepted.

The second objective was achieved by characterising the new solid-dispersed MOLP complexes. Characterisation of nutritional composition was done through the AOAC methods and mineral composition through Energy Dispersive X-Ray (EDX) methods. Functional properties evaluated were solubility, water absorption capacity, and structural properties characterised using scanning electron microscopy and transform electron microscopy. The nutritional composition showed that the SDMOLP samples were high in protein, fat, carbohydrate, energy, and low moisture content. Low moisture content is desirable in food powders; it retards microbial activity, thereby increasing the shelf life. High mineral composition in SDMOLPs and pure MOLP (calcium, potassium, magnesium,

sulphur) was observed, suggesting that the same nutrients found in pure MOLP can also be found in SDMOLP. Furthermore, SDMOLPs had significantly improved solubility from 25% (pure MOP) to 65% (SDMOLP) and water absorption capacity (from 350 to 560%). Moreover, the increased solubility of the dispersions can be attributed to the increased surface area of the samples as well as the hydrogen bonding interaction between MOLP and PEGs. Thus, the hypothesis that new solid-dispersed MOLP will have improved solubility was accepted.

The third objective was achieved by characterising the thermal, compatibility, and crystallinity properties of the SDMOLPs. Diffraction scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were used to characterise thermal properties. Compatibility and crystallinity properties were determined using Fourier transform infrared spectroscopy (FTIR) and powder x-ray diffraction (PXRD). The DSC thermographs showed that SDMOLPs had a semi-crystalline structure, as indicated by the single sharp endothermic peaks with melting temperatures in the 59-61^{o}C range. While the TGA results confirmed that SDMOLPs are more thermally stable at high temperatures than pure MOLP, this is due to the carriers used, which are thermally stable at high temperatures. The thermal stability of solid dispersions is closely related to the shelf life. Thermal instability results in shortened shelf life, while thermal stability prolongs the shelf life of products. Furthermore, the PXRD study evidenced that SDMOLPs were partially amorphous or semi-crystalline with diffraction angles at 2 theta values of 15, 19, 23^{o}. The FTIR results of MOLP confirmed that it is made up of carbon, hydrogen, nitrogen, and oxygen molecules which make up the gross structure of essential amino acids. Furthermore, those results indicated that MOLP is made up of essential amino acids which also contribute to its high protein content (28%). The characteristic peaks for the aliphatic C-H group at 2922 cm^{-1} in the MOLP spectrum completely disappeared in solid dispersions. In addition, a significant shift in peaks was observed in FTIR spectra of SDMOLPs compared to pure MOLP. Peak shifts in the hydroxyl (O-H) group, primary and secondary amide groups (N-H), and carboxylic acid (COOH) group were attributed to the intermolecular interactions which occurred through hydrogen bonding between MOLP and PEG molecules. The fact that the solid-dispersed MOLPs showed a chemical interaction (hydrogen bond) and still retained PEG's hydrophilic nature may be the reason why these complexes became highly soluble. Therefore, this intermolecular interaction played a vital role in the solubility enhancement of SDMOLPs in comparison to pure MOLP. The hypothesis that SDMOLP will have different structures and thermal stability compared to MOLP was accepted.

The fourth objective was achieved by establishing the flow properties and dissolution behaviour of solid-dispersed MOLP. Flow properties were determined through

Carr's index, Hausner's ratio, and angle of repose. While dissolution behaviour was carried out using the USP basket method. Pure MOLP had poor flowability. In contrast, the SDMOLPs had excellent to moderate flowability. This suggests that pure MOLP was high in cohesiveness, and SDMOLPs were low, and they can freely flow without vibration during processing or packaging. In addition, the dissolution behaviour of SDMOLP was also significantly improved in comparison to MOLP. This suggests that the hydrophilic carriers used in the study (PEG4000 and PEG6000) reduced the particle size, increased the surface area, improved wettability, and solubility, and consequently improved dissolution rate. The differences observed in the dissolution of each solid dispersion MOLP may be due to differences in their release from the enclosing material; factors that affect the dissolution rate ingredients include the solid dispersion method and the type of hydrophilic carrier. It was observed that solid dispersions prepared by PEG4000 carriers had markedly enhanced dissolution than PEG6000. This is due to the low molecular weight of PEG4000. In addition, solid dispersions of MOLP by melting method with PEG4000 or PEG6000 had the highest dissolution rate than those prepared with solvent evaporation, freeze-drying, and microwave irradiation. Thus, it can be said that the melting method is a good method for the preparation of MOLP solid dispersions. The hypothesis that solid-dispersed MOLP will have improved flow and dissolution properties was accepted. However, improvements such as markers (flavonoids, antioxidants, catechins, quercetins, alkaloids, etc.) for the dissolution characterisation are recommended since MOLP is an herbal plant with a complex composition.

The fifth objective was achieved by producing effervescent granules using the solid-dispersed MOLP. The effervescent granules were successfully produced using the wet granulation method and analysed for physical, chemical, flow properties. Consumer acceptability was conducted using a 5-point Hedonic scale test (ranging from 1 dislike very much to 5 like very like). The sensory attributes evaluated were colour, appearance, flavour, taste, texture, and overall acceptability. Effervescent granules had bulk and tapped densities suitable for instant product packaging. The granules exhibited low moisture contents, advantageous for prolonged shelf life and low microbial activity. Also, the pH ranged from 6.05-6.65, indicating good pH because it is close to neutral. The consumer acceptability exhibited that the effervescent granule beverages ranged from 3 to 5 for all the sensory attributes; this suggests that beverages were neither liked nor disliked to liked moderately in terms of sensory score. This proves that the beverage granules have the potential in the functional food market. The taste and texture could be improved by adding sweeteners and flavourings/masking agents to remove the bitter taste of MOLP that is perhaps caused by the high mineral content, antioxidants, and phenolic compounds. The

texture could be improved by further reducing the particle size and increasing the effervescent concentration. Therefore, the hypothesis that the production of effervescent granules using solid-dispersed MOLP will be possible was accepted.

The following conclusions can be drawn from this study:

1. Polyethylene glycols (PEG4000 and PEG6000) can be used as hydrophilic carriers in the MOLP solid dispersion process.
2. Solid dispersion technology has the potential to improve the solubility of MOLP. This study provided a better understanding of the enhanced solubility mechanism of solid dispersion processing, which can facilitate further improvement and application of solid dispersion MOLP in food and beverages.
3. PEGs can enhance the flow and dissolution behaviour of SDMOLP. However, the use of markers is recommended in future studies.
4. The nutritional, mineral, and functional properties of SDMOLP make them suitable for use in the food industry as functional foods or ingredients, nutraceutical formulations.
5. Effervescent beverages can be produced as easy-to-drink beverages. However, an improvement on the acid: base ratio and texture of the product is required. Future works can be directed towards the use of powerful bitter masking in the formulation, such as chitosan-cyclodextrin, which was previously reported to attenuate the bitterness of natural extract significantly.

In the future, the following could be of interest:

a) Use a particle size analyzer to obtain more accurate sizes, as that the manual method may miss some particles.
b) Chemical markers can be employed during the dissolution study of MOLP. Component compounds in the plant can be used as references to perform dissolution tests on herbal products. In such a case, they can be referred to as marker compounds because they can characterise or identify the product. Thus, when herbal products are tested for dissolution, the dissolution of these compounds is assessed. For most herbal products, chemical marker compounds that can serve as standards or references are not readily available due to their complex nature; for or instance, MOLP is a good source of protein, minerals, vitamins, antioxidants, and various phenolic compounds. Antioxidants and other phenolic compounds (flavonoids, quercetin, kaempferol, alkaloids, catechin) can be employed as markers for MOLP and SDMOLPs.
c) In this study, a capsule of MOLP or SDMOLPs was immersed in a 900 ml dissolution medium for the test. This method can be modified by perhaps decreasing the volume of

the dissolution medium to 450 ml and immersing 2 or 3 capsules containing each plant material, so as to improve the sensitivity capacity of the method by increasing the concentration of the marker thereby enhancing detection capacity which can then result in an improved dissolution rate.

d) Digestibility assessment of the Moringa product (protein and Carbohydrate) should assessed using in vitro stimulated gastrointestinal models to ensure that the enhanced dissolution and other properties do not alter the digestion and release of the nutrients.
e) Bio-accessibility assessment of the minerals and vitamins must be assessed using in vitro stimulated gastrointestinal models to ensure that the enhanced dissolution and other properties do not alter the digestion and release of the nutrients.

Appendix A: Approved ethical clearance

Cape Peninsula University of Technology

Statement of Permission

Data/Sample collection permission is not required for this study.

Reference no.	214308561/06/2020
Surname & name	Tafu, N.
Student Number	214308561
Degree	Master of Food Science and Technology
Title	Physicochemical, nutritional and dissolution properties of soliddispersed *Moringa oleifera* leaf powder effervescent beverage granules.
Supervisor(s)	PROF VICTORIA ADAORA JIDEANI
FRC Signature	
Date	2020 July 02

Cape Peninsula University of Technology

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Ethics Approval Letter **Reference no:** 214308561/06/2020

Office of the Chairperson Research Ethics Committee	Faculty of Applied Sciences

On 29 June 2020, the Faculty Research Ethics Committee of the Faculty of Applied Sciences granted ethics approval to Tafu, N. for research activities related to a project to be undertaken for a degree (Master of Food Science and Technology) at the Cape Peninsula University of Technology.

Title of project:	Physicochemical, nutritional and dissolution properties of solid-dispersed *Moringa oleifera* leaf powder effervescent beverage granules.

Comments (Add any further comments deemed necessary, e.g. permission required)

1. Human subjects are included in the proposed study.
2. This permission is granted for the duration of the study.
3. Research activities are restricted to those detailed in the research proposal.
4. The research team must comply with conditions outlined in AppSci/ASFREC/2015/1.1 v1, CODE OF ETHICS, ETHICAL VALUES AND GUIDELINES FOR RESEARCHERS.

Signed: Chairperson: Research Ethics Committee	29/06/2020 Date

Appendix B: Informed consent form signed by volunteers prior tasting

Department of Food Science and Technology

P. O. Box 1906
Bellville 7535

Cape Peninsula University of Technology

INFORMED CONSENT FOR *MORINGA OLEIFERA* RESEARCH

Hello

We are scientists from Cape Peninsula University of Technology. We are conducting a research to find new food use for Africa's indigenous crop. No value will be added to any produce except the consumers endorse it. Hence, we are approaching you to be part of this study. We realize you need to make an informed decision whether or not to be part of this study, hence we have provided below further details with regards to the research to assist in your decision process.

Title of Research Project:
Physicochemical, nutritional and dissolution properties of solid-dispersed *Moringa Oleifera* leaf powder effervescent beverage granules

Investigator:

N. Noxolo Tafu Tel: 073576 4994
Victoria Jideani Tel: 021 9538749

Purpose of the Research:
Moringa oleifera is an indigenous plant grown in many countries in semi-arid, subtropical and tropical regions of the world. The crop is reported to have originated in India, and now is distributed around the world. Moringa leaves are good source of protein, vitamins, minerals, phytochemicals, essential amino acids, unsaturated fatty acids, antioxidants, and various phenolic compounds, among others. *Moringa oleifera* tree is resistant to drought and it is less demanding on climate and soil nutrients. That is due to the formation of large tuberous roots, and thus are mostly important for famine foods. *Moringa oleifera* in South Africa is known as makgonatsohle in Sepedi, which means the ability to do everything; it is also known as the miracle tree worldwide. Moringa is an important multipurpose crop. The leaves of Moringa are considered a complete food owing to their nutritional quality and have a chemical composition which is comparable to that of soybean. Despite its high nutritional value, much of its use in South Africa is limited at household level. The objective of the study is to evaluate the physicochemical, nutritional and dissolution properties of solid-dispersed *Moringa oleifera* leaf powder while presenting it as an effervescent beverage (easy to drink).

Description of the Research:
This is an invitation to participate in the sensory study. The procedure to be adopted in the study as well as the terminologies on the score form will be explained to the panelists prior to tasting sessions. You will receive three 40 ml non-alcoholic beverage samples processed differently. You will not be bound to finish the 40 ml serving size of the beverage. The

samples contain water, moringa oleifera leaf powder, sugar (sweeteners), stabilizer, citric and tartaric acid, sodium bicarbonate, binders, dextrin, and flavorings. During the production of these samples good manufacturing practices (GMP) and standard operating procedures (SOPs) were followed to ensure the samples are safe for consumption. You will be required to test them and rate your preference (on a simple questionnaire) for each based on appearance, colour, taste, aroma, mouthful, smoothness and overall acceptability. Each tasting session will last for 15 - 30 minutes depending on individual.

Potential Harm, Injuries, Discomforts or Inconvenience:
Moringa oleifera leaves are a source of food and health for thousands of Africans; its consumption does not pose any hazard to human health. Therefore, there is no known harm associated with tasting *M. oleifera* leaf products in this study.

Potential Benefits:
You will not benefit directly from participating in this study.

Confidentiality:
Confidentiality will be respected and no information that discloses the identity of the participant will be released or published.

Participation:
Participation in this research is voluntary. If you choose not to participate in this study you may withdraw at any time.

Contact
If you have any questions about this study, please contact:

N. Noxolo Tafu	Tel:	073 576 4994
Victoria Jideani	Tel:	021 9538749

Consent:
By signing this form, I agree that:

1. The study was explained to me and all my questions answered.
2. I have the right to participate and the right to stop at any time.
3. I have been told that my personal information will be kept confidential
4. There is no likely harm from tasting *Moringa Oleifera* effervescent beverage granules.

I hereby consent to participate in this study:

.....	.....
Name of Participant	**Signature & Date**
.....	.....
Name of Researcher	**Signature & Date**

Appendix C: Score card used to rate the beverages

SCORE CARD- HEDONIC RATING SCALE
***Moringa Oleifera* leaf powder effervescent beverage granules**

Instruction:
You are provided with three samples of *Moringa* leaf beverages. Please take a sip of water before you start tasting and in between tasting the different samples. Please rate each sample on its own merit based on the given attributes. Do not compare the samples

Name of product: …… **Code: …….**

	Dislike very much (1)	**Dislike moderately (2)**	**Neither Like nor Dislike (3)**	**Like moderately (4)**	**Like very much (5)**
Appearance					
Colour					
Aroma					
Taste					
Texture					
Overall acceptability					

Comments:

.....

.....

We would like to obtain information about you. Kindly complete this brief questionnaire appropriately:

1. What is your Gender: Female ☐ Male ☐

2. What is your race? Black ☐ Coloured ☐ White ☐ Indian ☐ Other ☐

3. Are you a student or staff? Student ☐ Staff ☐

4. If you are a student, are you an international student? Yes ☐ No ☐

5. What is your age group?
☐ Less than 20-29
☐ 30-39
☐ 40 & above

Thanks for assisting us!!!!

Appendix D: Research outputs presented at national and international conferences

1. Poster presentation: 6th International ISEKI-FOOD Conference

Nontsikelelo Noxolo[1] Tafu and Victoria A. Jideani[1]. Physical and consumer acceptability profile of *Moringa oleifera* leaf powder effervescent beverage granules. 6th International ISEKI-Food Conference. 23-25 June 2021. (Poster presentation & Award participation).

2. Oral presentation: SaaFost Congress 2021 conference

Nontsikelelo Noxolo Tafu and Victoria A. Jideani. Solubility and Dissolution of Food Ingredients: Solid Dispersion Technology. SaaFost's 24th Biennial International Virtual Congress. 20-22 September 2021. (Oral presentation).

3. Poster presentation: CPUT Postgrad 2021 conference

Nontsikelelo Noxolo Tafu and Victoria A. Jideani. Solubility and Flow Properties of Solid-dispersed *Moringa oleifera* Leaf Powder. Cape Peninsula University of Technology Post Graduate 2021 Conference. 30 November 2021. (Poster presentation).

Appendix E: Manuscript for publication in peer-reviewed journals (MDPI: Processes special issue journal)

Nontsikelelo Noxolo Tafu[1] and Victoria A. Jideani[1,*]. Characterization of Novel Solid Dispersions of *Moringa oleifera* Leaf Powder Using Thermo-Analytical Techniques. *Processes,* **5.**

[Processes] Manuscript ID: processes-1467500 - Accepted for Publication

Processes Editorial Office <processes@mdpi.com>
to me, Victoria, Processes, Isabella

Dear Ms. TAFU,

Congratulations on the acceptance of your manuscript, and thank you for your interest in submitting your work to Processes:

Manuscript ID: processes-1467500
Type of manuscript: Article
Title: Characterization of Novel Solid Dispersions of Moringa oleifera Leaf Powder Using Thermo-Analytical Techniques
Authors: Nontsikelelo Noxolo Tafu *, Victoria A. Jideani *
Received: 1 November 2021
E-mails: tafunoxolo@gmail.com, JideaniV@cput.ac.za
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Properties and Processing Process of Flour Products
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