Effects of formulation on the stability of green *Cyclopia subternata* extract during spray-drying and storage

by

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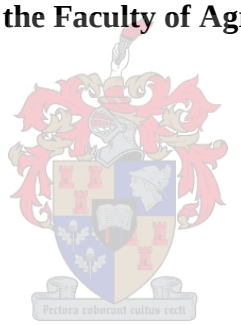

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Declaration

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Abstract

Green *Cyclopia subternata* (honeybush) hot water extracts (GCSE) have potential as value-added functional food ingredients based on their anti-obesity and anti-diabetic properties. They are thus suitable for inclusion in reduced-kilojoule instant iced tea powder formulations. The aims of this study were to determine the effect of spray-drying, with and without carriers, on the stability of GCSE and the effect of product formulation and storage at different conditions on iced tea powder mixtures containing GCSE.

GCSE was spray-dried without (control) and with added carriers, i.e. corn syrup solids (CS) and inulin (IN) at four treatment levels (0, 25, 50 and 75%). IN was selected for the final iced tea powder formulation due to its prebiotic properties, but solubility limited practical inclusion of IN in iced tea powder to levels of 25% (IN25). Six formulations (T1 = spray-dried GCSE; T2 = IN25; T3 = IN25 + sugar; T4 = IN25 + xylitol + stevia; T5 = T3 + citric acid + ascorbic acid; T6 = T4 + citric acid + ascorbic acid) were subjected to a six month shelf-life stability trial at ambient (25 °C/55% relative humidity (RH)) and accelerated (40 °C/75% RH) conditions.

Physicochemical properties of the spray-dried and iced tea powders were characterised in terms of phenolic retention, moisture content (MC), water activity (a_w), moisture sorption isotherms (MSI) and objective colour measurements. Isothermal microcalorimetry (IMC) was used to determine if the ingredients of mixtures interacted with one another and to assess the effect of raised RH conditions (25°C/55% RH and 40°C/75% RH) on the stability of the iced tea powders. Differential thermal analysis (DTA) was applied to measure the phase transition temperatures of the powders, X-ray powder diffraction (XRPD) measurements confirmed the crystalline or amorphous nature of the powders, and contact angle measurements indicated wettability of the powder.

Spray-drying produced fine, light brown, amorphous and free-flowing powders. The MC and a_w of the powders fell within the range of the monolayer moisture values calculated using the BET model from MSI data. IN and CS produced powders with similar characteristics and were compatible with GCSE, with the exception of mixtures containing 75% CS. Heating conditions during spray-drying had a negligible effect on the bioactive phenolic content and the free radical scavenging capacity of the extract. Therefore spray-drying was considered to be a suitable method of producing dried honeybush extracts.

XRPD and DTA showed no significant phase transition for the iced tea powders during storage. IMC detected no incompatibilities between ingredients in the mixtures. Physicochemical

characteristics of the powders remained stable and adequate phenolic retention was achieved at ambient temperature (25 °C). However, when the powders were stored at 40 °C, the presence of the acids caused drastic degradation of phenolic compounds and physicochemical changes resulting in prominent colour changes. IMC at 55% RH showed that amorphous powders (spray-dried extract and stevia) deliquesced. At 75% RH mixtures containing xylitol underwent deliquescence, while those with sugar remained stable. An iced tea powder containing GCSE should therefore be stored at ambient temperature in moisture impermeable packaging to ensure adequate stability.

Uittreksel

Groen *Cyclopia subternata* warm water ekstrakte (GCSE) het potensiaal as waarde-toegevoegde funksionele voedsel bestandele gebaseer op hul anti-vetsug en anti-diabetiese eienskappe. Sulke ekstrakte is dus geskik om in ‘n verminderde-kilojoule kits-ystee poeier formulasie gebruik te word. Die doelstellings van hierdie studie was om die effek van sproeidroging, met en sonder draers, op die stabiliteit van GCSE te bepaal, asook die effek van produk formulasie en opberging by verskillende toestande op ystee-mengsels wat GCSE bevat te bestudeer.

GCSE is gesproeidroog sonder (kontrole) en met draers, d.i. mieliestroop-vastestowwe (MS) en inulin (IN) teen vier behandelingsvlakke (0, 25, 50 en 75%). IN is gekies vir die finale poeier ystee formulasie weens sy pre-biotiese eienskappe maar oplosbaarheid het die byvoeging van IN in ystee-poeier beperk tot 25% (IN25). Ses formulasies (T1 = gesproeidroogde GCSE; T2 = IN25; T3 = IN25 + suiker; T4 = IN25 + xylitol + stevia; T5 = T3 + sitroensuur + askorbiensuur; T6 = T4 + sitroensuur + askorbiensuur) is onderwerp aan ‘n ses maande rakleeftyd studie by normale (25 °C/55% relatiewe humiditeit (RH)) en versnelde (40 °C/75%RH) opbergings toestande.

Die fisies-chemiese eienskappe van die gesproeidroogde en ystee-poeiers is gekarakteriseer in terme van fenoliese behoud, voginhoud (VI), water aktiwiteit (a_w), vog-sorpsie-isoterme (VSI) en objektiewe kleur metings. Isotermiese mikrokalorimetrie (IMK) is gebruik om te bereken of daar interaksie is tussen die bestandele van die mengsels, en om die uitwerking van opberging RH toestande (25 °C/55% RH en 40 °C/75% RH) op die stabiliteit van ystee-poeiers te bepaal. Differensiële termiese analiese (DTA) is gebruik om die fase oorgangs temperature van die poeiers te bepaal, X-straal poeier diffraksie (XRPD) meetings het die kristal- of amorfe-karaktereienskappe van die poeiers bevestig, en kontak-hoek metings het die benatingspotesiaal van die poeier aangedui.

Sproeidroging het fyn, ligte-bruin, amorfe en vry-bewegende poeiers geproduseer. Die VI en a_w van die poeiers was in die omvang van die monolaag voginhoud waardes, soos bereken met die BET model van die VSI data. IN en MS het poeiers met soortgelyke eienskappe opgelewer en het nie interaksie met GCSE getoon nie, behalwe in geval van mengsels met 75% MS. Die verhittingskondisies gedurende sproeidroging het ‘n minimale effek op die bioaktiewe phenoliese-inhoud en die vryradikaal blussings vermoë van die ektrak gehad. Daarom is sproeidroging aanvaar as ‘n effektiewe manier om gedroogde heuningbostee ekstrakte te vervaardig.

XRPD en DTA het geen verskille in die fase oorgangs van ystee-poeiers gedurende opberging getoon nie, terwyl IMK het geen interaksie tussen bestandele van die mengsels getoon het nie. Die

fisies-chemiese eienskappe van poeiers was stabiel en aanvaarbaare behoud van fenoliese verbindings is behaal by kamertemperatuur (25 °C). Opberging by 40 °C het egter getoon dat die sure drastiese afbreking van die fenoliese verbindings asook fisies-chemiese veranderinge veroorsaak. Die fisies-chemieses veranderings het kleur veranderings veroorsaak. IMK teen 55% RH het bewys dat amorfepoeiers (gesproei-droogde ekstrak en stevia) het vloiebaar geword. By 75% RH het die mengsels met xylitol ook vloeibaar geword, terwyl die met suiker stabiel gebly het. ‘n Ystee-poeier met GCSE moet dus by kamertemperatuur en in vog-ondeurlaatbare verpakking opgeberg word om stabiel te bly.

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"For the joy of the Lord is your strength" Nehemiah 8 vs.10

Notes

This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the research objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and reference format used are in accordance with the requirements of the International Journal of Food Science and Technology. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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Chapter 1

General introduction

Sugar sweetened beverages (SSB) have been highlighted as one of the leading causes of obesity and the metabolic disorders which are associated with this condition (Ludwig *et al*., 2001; Bray *et al*., 2004; Ebbeling *et al*., 2006; Malik *et al*., 2006; Bleich *et al*., 2009). Surveys in the US have revealed that from 1977 to 2001 the energy intake from soft drinks and fruit juices increased by 135%. Over the same period of time the prevalence of adult obesity doubled (Bleich *et al*., 2009). At present South Africa is following a similar trend. In a recent study by Ronquest-Ross *et al*., (2015) it was revealed that between 1994 and 2012 the consumption of soft drinks increased by 68.9%. It is not surprising that South Africa is also the most obese population in sub-Saharan Africa. The biggest downside of SSB is that they contribute high amounts of kilojoules to the diet while providing limited nutrients for the total energy intake (Malik *et al*., 2006).

As the incidence of metabolic syndrome take on global proportions there is increasing consciousness amongst consumers of the link between diet and health (Baboota *et al*., 2013; Braithwaite *et al*., 2014; Anon., 2014; Anon., 2015a). For nine consecutive years a rising awareness of the detriments of carbonated beverages has led to a decrease in sales in the US (Anon., 2015b). Parallel to this has been an increased demand for products which are associated with the prevention of nutrition-related diseases, as well as the health and well-being of the consumer (Siró *et al*., 2008; Ozen *et al*., 2012). This has led to the emergence of new categories of food products, namely functional foods and nutraceuticals (Arvanitoyannis &Van Houwelingen-Koukaliaroglou, 2005).

Functional beverages have been identified as the fastest growing functional food category due to the fact that they are convenient and flexible in meeting consumer needs and provide many opportunities to incorporate desirable nutrients and bioactive compounds (Corbo *et al.*, 2014). Ready-to-drink iced tea falls into this category due to its well-documented health benefits which are associated with a high phenolic content (Bender, 2014). Green and herbal teas in particular are gaining traction in the on-going search for new sources of bioactive-enriched foods (Bender, 2014).

Cyclopia species (commonly known as honeybush) are indigenous to the fynbos biome in South Africa and have long been consumed as herbal tea by local populations (Joubert *et al.*, 2008a). Research into honeybush as a commercial entity emerged during a time when consumers were becoming more health conscious and aware of the benefits of natural substances, particularly antioxidants (Joubert *et al.*, 2011). Green or "unfermented" (unoxidised) honeybush has been identified as a sustainable source of bioactive phenolic compounds (Joubert *et al.*, 2008b; De Beer *et al.*, 2012; Schulze *et al*., 2014; Beelders *et al.*, 2015). The xanthone mangiferin, a potent antioxidant, in particular is sought after due to its well-documented health benefits which include anti-diabetic, anti-mutagenic, anti-carcinogenic, anti-inflammatory and hypolipidaemic properties, amongst others (Vyas *et al.*, 2012). Aqueous extracts of *C. subternata*, which contains mangiferin and other bioactive phenolic compounds such as α-glucosidase inhibiting benzophenones (Beelders *et al.*, 2015), have been patented for their anti-diabetic properties (Mose Larsen *et al*., 2008). Dudhia *et al*. (2013) demonstrated anti-obesity properties for aqueous *C. subternata* extract. These findings present opportunities for creating value-added products which can be used in the nutraceutical industry, including applications such as instant iced tea (Joubert *et al.*, 2011).

The production of natural extracts for the nutraceutical industry presents a number of challenges. In their unprocessed form natural extracts are often sticky, highly susceptible to degradation and practically difficult to work with (Sansone *et al*., 2011). Furthermore the bioactive content of plant material, including *C. subternata* plant material, exhibits large variation which makes the production of standardised extracts difficult (Bott *et al*., 2010; De Beer *et al*., 2012). In order to address the first challenge various drying and encapsulation technologies have been developed over the years (Desai & Park 2005; Murugesan & Orsat, 2011; Fang & Bhandari, 2010). Of these, spray-drying remains the oldest and most widely used technique for the drying and microencapsulation of natural extracts (Gharsallaoui *et al*., 2007). Carriers are frequently used during spray-drying to protect heat-sensitive compounds and improve the physical properties of the resulting powder (Desai & Park, 2005; Munin and Edwards-Lévy, 2011).

There are a number of factors which affect the stability of powders. Understanding the interplay of these factors assists in selecting the correct packaging, establishing adequate shelf-life periods, as well as determining optimal storage conditions (Gabas *et al*., 2007). Most importantly it is essential to ensure consistent quality and the delivery of bioactive compounds within the final product (Bott *et al.*, 2010).

The presence of free water, as well as conditions which result in moisture uptake, leads to degradation of powders such as caking and agglomeration (Al-Muhtaseb *et al*., 2002; Ortiz *et al*.,

2008; Mauer & Taylor, 2010). During storage of powders state transformations can occur (Bott *et al.*, 2010). Spray-dried powders are often in an amorphous (glassy) or metastable state, and if not stored correctly, could change to a crystalline state (Müller *et al.*, 2015). The stability of the extract and carrier during storage is dependent on their glass transition temperatures, susceptibility to crystallisation and the presence of moisture in the system (Sansone *et al.*, 2011). When the physical state of powdered natural extracts is compromised it could result in degradation of important bioactive compounds. Phenolic retention of natural extracts is typically assessed using high performance liquid chromatography (HPLC), total antioxidant and total polyphenol assays (Ersus & Yurdagel, 2007; Da Silva *et al.*, 2011; Cortés-Rojas & Oliveira, 2012; Couto *et al.* 2012; Nunes *et al.*, 2015).

A critical factor to consider in the development of functional beverages is the effect of other ingredients on the stability of bioactive compounds (Corbo *et al.*, 2014). Interactions with other ingredients could lead to reactions which cause precipitate formation, oxidation, insolubility or degradation which would compromise the functionality of bioactive compounds (Chadha & Bhandari, 2014; Corbo *et al.*, 2014). The addition of other ingredients could also affect the relative humidity at which deliquescence occurs, a reaction which causes caking and irreversible degradation of powders (Kwok *et al.*, 2010; Stoklosa *et al.*, 2012). Many of these degradation reactions are reflected in changes in the colour and visual appearance of the product which compromises consumer acceptance (Hetrick *et al.*, 2013; Cortés-Rojas *et al.*, 2014).

At present small volumes of spray-dried honeybush extract are produced in industry, mainly for use as a food ingredient. Along with growth in this sector there is a drive to increase the amount of processed vs. unprocessed plant material for export in order to increase economic returns to South Africa (Anon, 2013). However, there is no literature on the effects of this process on the stability of the phenolic compounds or the physicochemical properties of *Cyclopia* spp. powder. Furthermore, there has been no research performed on the stability of powdered extracts of *Cyclopia* spp. during storage or when in contact with other food ingredients used in product formulations.

The overall objective of this study was to create a healthy honeybush iced tea which is able to deliver the bioactive compounds from green *C. subternata* extract to consumers in a convenient and stable form. In order to achieve this, the effects of spray-drying and the addition of two carriers on the physicochemical stability and phenolic content of the extract were assessed. The powder extract with the most suitable powder characteristics was then used to develop an instant iced tea formulation with reduced sucrose content. The stability of the extract when in contact with different ingredients used in iced tea was tested during a six month period at ambient and accelerated storage conditions.

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Chapter 2

Literature review

2.1 Introduction

This literature review aims to give an overview of the developments in the functional food industry, highlighting efforts to address the global metabolic syndrome epidemic. Within this sphere the development of honeybush tea, as a potential nutraceutical ingredient, will be discussed with the view of using it to produce dry instant iced tea powder. To this end the challenges associated with the production of spray-dried natural extracts will be discussed. Particular emphasis is placed on the stability of bioactive compounds throughout processing, storage and when combined with other ingredients used in product applications such as instant iced tea. Methods used for the physicochemical characterisation of powders will be outlined as a means of determining if suitable product characteristics and quality standards have been met.

2.2 The global burden of metabolic syndrome

Metabolic syndrome (MS) is a modern day epidemic which has reached global proportions and is continuously increasing at an alarming rate. MS has been broadly defined as the cluster of metabolic complications resulting from obesity and has been linked to a number of other co-morbidities of great public health concern (Beilby, 2004; Malik *et al*., 2006). These include hypertension, cardiovascular disease, diabetes, various forms of cancer as well as depression, all of which are leading causes of death in the developed world (Beilby, 2004, Malik *et al*., 2006). Furthermore the decrease in productivity and quality of life associated with these ailments are linked to increased medical, psychological and social costs (Malik *et al*., 2006).

During the past three decades obesity levels have doubled, affecting developed and developing countries alike. According to World Health Organisation statistics, 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese (Anon, 2015a). With these climbing levels of obesity it is no surprise that in 2014 the International Diabetes Federation (IDF) reported that 387 million people have diabetes and by 2035 this will rise to 592 million (Anon, 2014). It is also important to note that the number of people with type 2 diabetes is increasing in every country and 77% of people with diabetes live in low- and middle-income countries. Alarmingly almost half of people with diabetes remain undiagnosed and in 2014 diabetes caused 4.9 million deaths. To put this

in perspective every seven seconds a person dies from complications associated with diabetes. This inevitably has a devastating effect on global healthcare systems (Anon, 2014).

These increases in health care costs could have a crushing effect on resource-poor countries which now have to cope with the double burden of chronic and infectious diseases (Malik *et al*., 2006). South Africa is no exception to this rule. Diabetes is a huge problem country wide and is currently the number one cause of death in the Western Cape (Anon, 2013a).

The issue of obesity is a complex one and can be related to extrinsic factors such as an increase in sedentariness and lack of exercise, as well as an increase in the availability of cheap and high calorie foods. Intrinsic factors include genetic, epigenetic and developmental factors (Baboota *et al*., 2013). Over the years a number of anti-obesity medications have been developed. However, the negative side-effects of these agents have been found to far outweigh the effects of losing weight (Baboota *et al*., 2013). Interventions related to diet and physical activity remains the best treatment for obese and overweight patients.

2.2.1 The beverage industry and obesity

Numerous scientific studies have shown a positive association between the consumption of sugar sweetened beverages (SSB) and an increase in weight gain and obesity in both adults and children (Ludwig *et al*., 2001; Bray *et al*., 2004; Bleich *et al*., 2009; Ebbeling *et al*., 2006; Malik *et al*., 2006). Beverages which fall into this category include drinks which contain cane sugar, high fructose corn syrup (HFCS) or fruit juice concentrates as these all have the same metabolic effects (Brownell *et al*., 2009).

The biggest concern about SSB is that they contribute high amounts of sugar to the diet (Fig. 2.1) with very little nutrient compensation for the total energy intake. A single can of carbonated SSB contains in the range of 7-10 teaspoons of sugar (16.3 kJ/g) which contributes on average 626.9 kJ in energy (Malik *et al*., 2006). If these kilojoules were added to the average western diet without reducing intake from other sources, 1 can of soda per day could lead to a weight gain of 6.75 kg in a year (Malik *et al*., 2006).

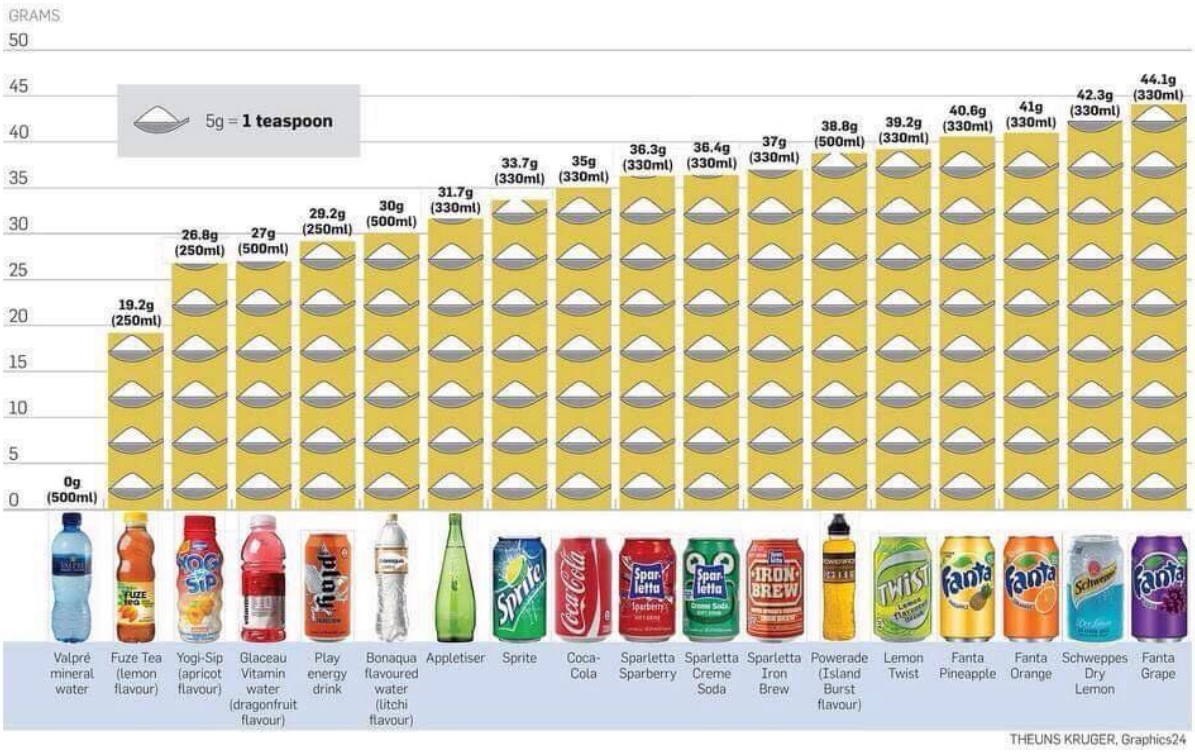


Figure 2.1 Amount of sugar consumed in a single serving of popular sugar sweetened beverages in South Africa.

Another concern with these beverages is the fact that they lack nourishment and therefore have low satiety. Many people mistake hunger as being a signal that the body requires more energy when in fact it is a signal that the body requires more micronutrients such as minerals and phytonutrients. This is due to the fact that 95% of our body's activities are reliant on over 4000 enzymes which require minerals to function. Improvement of diet includes both energy intake restrictions and identifying bioactive functional food ingredients that could modulate pathways and gene/protein expressions in a beneficial way (Baboota *et al*., 2013).

2.3 Functional foods and nutraceuticals

The concept of using foods to improve health is certainly not a new one. The use of health-promoting foods has been the practice of many ancient cultures. Nowadays society is returning to this way of thinking and looking for natural solutions to chronic diseases. Therefore foods are no longer expected to simply satisfy hunger and to provide the necessary nutrients, they are also associated with the prevention of nutrition-related diseases and the health and well-being of the consumer (Siró *et al*., 2008). Over the past decade there has been an increase in the demand for foods and beverages that improve or benefit health (Ozen *et al*., 2012). This surge in demand has been linked to an increase in consumer awareness, rising health-care costs and a desire to improve the quality of life of the aging population (Siró *et al*., 2008, Sun-Waterhouse, 2011). These consumer demands as well as an increased awareness of the inherent benefits of certain foods have led to the emergence of new categories of products namely functional foods and nutraceuticals. These developments have created a need to accurately define these terms as well as set up appropriate legislation to ensure that health claims made about these products are not misleading.

2.3.1 Defining nutraceuticals and functional foods

The term functional food was first used in Japan in the 1980s to define processed foods products which had been fortified with bioactive compounds that possess health benefits in addition to providing nutrients (Siró *et al*., 2008). To date there is no global definition of functional foods. They can be broadly defined as "foods similar in appearance to conventional foods that are consumed as part of a normal diet and have demonstrated physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions" (Clydesdale, 1997). Functional foods were originally considered to be products fortified with vitamins and minerals such as vitamin C, vitamin E, folic acid, calcium, iron and zinc. In recent years micronutrients such as omega-3 fatty acids, phytosterols and soluble fibres have also gained attention for their ability to promote health and prevent disease (Siró *et al*., 2008). Functional foods must be proven to be effective when consumed at levels found in normal diets and as part of a normal food pattern (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005).

The term nutraceutical was first used in 1989 by the US Foundation for Innovation in Medicine and was described as "any substance that is a food or a part of a food that provides medical or health benefits, including the prevention and treatment of disease" (Corbo *et al*., 2014). This definition has been refined to "natural, bioactive chemical compounds that are characterised by health-promoting, disease-preventing and medicinal properties" (Arvanitoyannis & Van

Houwelingen-Koukaliaroglou, 2005). It is important to differentiate that nutraceuticals are derived from food stuffs but are often concentrated and administered in the form of dietary supplements. They can also be added to foods and supplements to produce bioactive-enriched foods (BEFs). Pathway 27 is an EU funded project which aims to provide guidelines for the production of BEFs by small to medium sized enterprises as well as submitting convincing health claim dossiers to the European Food Safety Authority (EFSA) for approval (Anon, 2013b).

Dietary supplements have been defined as "a food, not in its conventional form, providing a component to supplement the diet by increasing the total dietary intake of that component". This category includes a variety of food components such as vitamins, minerals, herbs, plant-derived substances, amino acids and extracts of these substances. Supplements are consumed in forms such as tablets, capsules, powders, etc. It is important to note that they are not considered to be conventional foods, nor the sole item of a meal or diet. Furthermore they are neither replacements for conventional diets nor drugs (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005).

2.3.2 Regulation of health food claims

The sale of nutraceutical products and functional foods is very lucrative and development in this area continues to grow rapidly (Bigliardi & Galati, 2013). In 2011 the global sales of nutraceuticals was estimated at US $ 142 billion and was predicted to grow as much as US $ 207 billion by 2016 (Muratoglu, 2013). With an increased demand for health-promoting food products, there is a growing need for efficient regulation of the manufacturing and labelling of these products. The health-related claims that are made on packaging and advertising have a direct impact on the dietary choices that consumers make as well as their education regarding diet-related diseases (Williams, 2005). Therefore existing legislation is being updated worldwide to ensure that consumers are properly informed about the use of these foods (Reis, 2011).

Japan, the USA and Europe are considered to be the leading consumers of functional foods and nutraceuticals (Bigliardi & Galati, 2013). Of these Japan remains the only country where functional foods are recognised as a distinct product category by regulatory authorities (Brown and Chan, 2009). These products are distinguished from normal food products with the "FOSHU" symbol (Food for Specified Health Uses) which was introduced by the Japanese Ministry of Health in 1991 (Siró *et al*., 2008). In order to qualify for the FOSHU symbol, manufacturers must supply regulatory bodies with scientific evidence to substantiate claims. This includes all publications or internal reports on the effectiveness of the product and/or its ingredients including *in vitro* metabolic and biochemical

studies, *in vivo* studies and randomised control studies on the Japanese population (Yamanda *et al*., 2008).

In America claims that may be made on food products and dietary supplements fall into three categories (Hasler, 2008). Firstly *health claims* describe the relationship between a food substance (a food, food component, or dietary supplement ingredient), and reduced risk of a disease or health-related condition. Secondly *nutrient content claims* are regulated by The Nutrition Labeling and Education Act of 1990 (NLEA). They allow for label claims that specify the level of a nutrient in a food product as long as they have been authorised by the FDA and are made in accordance with FDA's authorising regulations. Lastly *structure/function claims* describe the role of a nutrient in the normal structure or function of the human body as well as the means by which a nutrient acts to maintain such a structure or function for example 'antioxidants maintain cell integrity'. These claims are regulated by The Dietary Supplement Health and Education Act of 1994 (DSHEA) (Anon, 2013c).

In Europe all nutrition and health claims made on food products are regulated by the regulation EC No. 1924/2006 which came into effect in December 2006. According to this regulation a list of authorised claims has to be published for all members of state and nutrient profiles have to be established for all foods with health claims. Manufacturers may only make 'function claims' and 'reduction of disease risk claims' (Siró *et al*., 2008). Furthermore the EFSA is responsible for verifying the scientific evidence available to support claims (Reis, 2011).

Keeping in step with global trends, regulations in South Africa continue to undergo rigorous restructuring and revision. In March 2012 the Department of Health (DOH) instituted new regulations relating to the labelling and advertising of foodstuffs, No. R146 of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (commonly known in industry as R146). This first phase aims to address simpler aspects of food labelling such as ingredient lists and nutrient contents. The second phase was introduced in May 2014 as draft regulations relating to the labelling and advertising of foodstuffs (R.429). These regulations are far more stringent and are currently under discussion (Sunley, 2014).

New regulations present many challenges to the food industry to provide accurate and reliable information about their products. These challenges are further complicated by the global export of many products and the differences in jurisdiction between those countries (Reis, 2011). However, these developments are aimed at protecting the consumer and providing products which are safe and effective.

2.4 Functional beverages: Providing health and convenience

Functional beverages are increasingly used to deliver nutraceuticals to consumers. They have been identified as the fastest growing functional foods category due to the fact that they are convenient and flexible in meeting consumer needs, easy to transport and store and provide many opportunities to incorporate desirable nutrients and bioactive compounds (Corbo *et al.*, 2014). There has been much interest in functional beverages from the perspective of research and innovation as can be seen from the vast number of papers published on this topic (as reviewed by Corbo *et al*., 2014).

2.4.1 Product development of functional beverages

There are two major challenges associated with the development of functional beverages. The first is to remove ingredients which are deemed as unhealthy. In the beverage industry, high levels of sugar are the biggest concern. The second is to incorporate bioactive ingredients which are able to contribute added benefits to the consumer.

2.4.1.1 Alternative sweeteners

Non-caloric artificial sweeteners (NAS) have been developed over the past century as a means of providing sweetness in products without the harmful side effects of consuming too many kilojoules. Over the years they have gained much popularity owing to their low cost and their supposed health benefits. However the safety of NAS is being questioned and a recent article published in Nature revealed that NAS induces glucose intolerance by altering gut microbiota (Suez *et al*., 2014).

Consumers have also woken up to the negative side effects of too much sugar and are now demanding alternative options. While NAS are still in use they have lost market share to the natural plant-derived sweetener stevia since its introduction as a table top sweetener in 2009. In general there is a shift towards naturally-derived sweeteners as these are perceived as healthier. This includes sweeteners such as honey, agave, sugar alcohols such as xylitol and monk fruit. Of the range of sweeteners available stevia has taken the lead. Over the last year the number of products containing stevia has increased by 73 percent with over 2100 product launches internationally. Of these the beverage industry has been the largest affected (Olivo, 2014).

2.4.1.2 Bioactive ingredients: phenolic compounds

Bioactive compounds are defined as extra-nutritional constituents that naturally occur in plant-derived foods and lipid-rich products in very small quantities (Kris-Etherton *et al*., 2002). Ingredients which are considered to be bioactive include: prebiotics, probiotics, phenolic compounds,

phytosterols, phytostanols, bioactive peptides, and bioactive carbohydrates (Arvanitoyannis &Van Houwelingen-Koukaliaroglou, 2005). They contribute towards the health benefits of foods because they help to prevent chronic diseases and increase the performance and well-being of the consumer beyond the established role of macronutrients such as protein, carbohydrates and fats in nutritional function (Arvanitoyannis &Van Houwelingen-Koukaliaroglou, 2005). In particular natural ingredients with strong antioxidant activity are sought after for use in novel functional beverages (Sun-Waterhouse, 2011). Products containing phenolic compounds have been spotlighted due to their various health benefits, and these products can either be used in their natural form or to fortify other products (Servili *et al*., 2011).

In the past research focused on the antioxidant potential of phenolic compounds and their ability to prevent tissue damage by excess free radicals when the body's natural defence systems cannot keep up (Leiro *et al*., 2003). However, with further research it has been found that the situation is more complex than this. It has been postulated that many of the health-promoting properties of polyphenols may be independent from their antioxidant capacity and may exert their therapeutic benefits by interacting with key signal transduction pathways involved in disease processes (Fraga *et al*., 2010; Törrönen *et al*., 2012). A significant number of clinical trials and cohort studies have highlighted the link between consumption of dietary phenolic compounds and the prevention of several major chronic diseases such as hypertension, stroke and cardiovascular disease (Morand *et al*., 2011), type 2 diabetes, non-alcoholic fatty liver disease and cancer (Pérez-Jiménez *et* al., 2011; Bahadoran *et al*., 2013) as well as obesity (Fernández-Sánchez *et al*., 2011; Savini *et al*., 2013).

Understanding the multifaceted role of diet in the prevention of chronic diseases is difficult due to the wide variety of bioactive compounds consumed in the average diet, many of which could modify a number of processes that are related to these diseases (Egert & Rimbach, 2011). One of the major factors under investigation at present is whether phenolic compounds are more bioavailable in extract form when taken as supplements or consumed as part of their natural food matrix (Egert & Rimbach, 2011). It has also been found that certain compounds have a synergistic effect. In some cases a particular phenolic compound may not have a significant effect in isolation. When used in combination with other compounds they form an interlinked defensive system through a variety of biochemical mechanisms to protect the body against the effects of oxidative stress (Herranz-López *et al.*, 2012).

When developing functional beverages which contain phenolic compounds there are certain factors which need to be taken into consideration. Before formulating fortified beverages as with all functional foods it is essential to ensure that the bioactive compounds are stable throughout all steps

of processing and storage (Harbourne *et al*., 2013). The stability of phenolic compounds is affected by factors such as pH, heat, light and the presence of oxygen and moisture (Ortiz *et al*., 2008; Harbourne *et al*., 2013). These compounds are therefore very susceptible to degradation during processing (De Vos *et al*., 2010). One of the proposed means of improving stability would be to encapsulate these compounds using polymeric carrier compounds which will be discussed in a later section.

As with the development of any food product, a consumer driven approach should be taken when developing functional beverages. While consumers have a positive image of healthy food products and are actively seeking foods in the 'naturalness' and 'wellness' categories, taste remains the most important selling point – this is particularly true in the Western market (Sun-Waterhouse *et al*., 2011). Conversely, in Japan functionality is superior to taste in functional foods because they are regarded as a separate product category (Siró *et al.*, 2008). When developing beverages which contain high levels of phenolic compounds it is important to note that they have been associated with sensory attributes such as bitterness and astringency (Drewnowski & Gomez-Carneros, 2000). Therefore, the effect of the incorporated natural ingredient on sensory properties and consumer acceptance should always be taken into consideration (Sun-Waterhouse *et al*., 2011).

2.4.2 Current market trends

In order for a product to succeed in the market in the long run, it is important that it also complies with consumer beliefs and wants (Siró *et al*., 2008). It is therefore important to highlight some of the most important trends in the health food industry at present. The most successful products and ingredients are those that identify with a variety of market trends (Mellentin, 2014).

Products which are marketed with health benefits are often placed into three categories. The 'high in' category promotes foods with beneficial properties. Examples include high in protein, fibre or omega-3. The 'low in' category limits the consumption of food ingredients perceived as unhealthy such as low in sodium, sugar and saturated fats. The 'free-from' category caters for people with intolerances to certain ingredients as well as consumers who believe these ingredients to be harmful. Examples of 'free-from' foods include gluten-free and dairy-free products (Mellentin, 2014).

Naturally functional refers to foods or food ingredients that contain natural and intrinsic health benefits. This remains one of the most important trends in the marketing of health foods. Consumers are moving towards "less processed" products which are perceived to possess naturally health-promoting properties (Mellentin, 2014). Plant food supplements have been used for their health-promoting properties long before the emergence of modern medicine as we know it today (Bucchini

et al., 2011). World-wide there is resurgence or renaissance of the use of foods to reduce the risk of disease and plant extracts to treat disease. Needless to say there is a growing demand for natural health products which have been proven effective and safe and are of high quality (Bucchini *et al*., 2011).

The term antioxidant has been frequently used in the food industry to relate the health properties of many plant-based foods and is now well understood by the consumer to have high value for health. As the effectiveness of antioxidants becomes better understood there is an emerging trend towards the use of condition-specific antioxidants (Becker, 2013). The effectiveness of a compound is not based merely on its antioxidant activity, but may be used to treat specific diseases. For instance ergothioneine, an amino acid, has been found to target joint tissue as opposed to simply possessing antioxidant properties. Products containing antioxidants are now being marketed according to a specific use highlighting the need for scientific substantiation (Becker, 2013).

It is also important to understand the factors which influence consumers in order to develop products to suit their specific needs (Corbo *et al*., 2014). Ozen *et al*. (2012) performed a systematic review on the worldwide consumption behaviour of functional foods. It was found that, in general, females were more interested in functional foods. Overall education was a major contributor towards healthy eating habits, including the consumption of functional foods. Older consumers with health issues were also more inclined towards the consumption of functional foods.

One of the most successful products on the South African market at the moment is the breakfast cereal FUTURELIFE® (www.futurelife.co.za). It has been a huge success due to the fact that it complies with numerous consumer needs. For instance it has a low glycaemic index (GI), a scale which denotes a person's glycaemic response to specific foods, where 100 represents the standard, an equivalent amount of glucose. Furthermore it is gluten- and dairy-free, high in protein and dietary fibre and has been fortified with vitamins, minerals, 19 amino acids and omega-3 and -6. This complies with the drive to formulate foods which have multiple health benefits in a single product (Siró *et al*., 2008). It is presented in a convenient and on-the-go format which is suitable for an active lifestyle thereby conforming to a variety of consumer needs and desires (Anon, 2015b).

It goes without saying that the costs involved in the development of functional foods are considerably higher than conventional foods, because a number of factors need to be taken into consideration such as clinical trials and research into the bioactive compounds and their stability. However the prices of such foods are higher than conventional foods and can therefore have a higher profit margin. This makes them an attractive sector to invest in for the long run (Siró *et al*., 2008).

With the current global lifestyle-disease epidemic the search for new and innovative products containing bioactive compounds has become more important than ever before. It is also an important means by which the food industry can address these challenges.

2.5 New product development opportunities: Iced tea

Tea (*Camellia sinensis*) is the second most consumed beverage after water worldwide (Sinija & Mishra, 2008). Ready-to-drink (RTD) iced tea is a popular form of consuming this beverage due to its convenience and perceived health benefits. In 2013 it was reported that while consumption of black tea was rising moderately (3.4% annually) production of green tea and herbal teas was growing rapidly at 11% and >15% respectively (Bender, 2014). A recent report by the American Botanical Society showed that the consumption of RTD teas increased by 4.4% from 2013 to 2014. Based on the current sales figures and trends in the beverage market – sales in this sector are predicted to continue rising over the next few years (Anon, 2015c). Increased consumption of tea is driven by the positive media coverage which reports on research validating the health benefits of consuming tea, particularly green tea. This is mainly due to the fact that these products are very high in phenolic compounds which possess a host of health benefits as previously discussed. It is also spurred on by continuing development of convenience products and speciality niche products (Anon, 2015d).

Instant tea beverages and premixes for ready-made iced tea are commonly produced from powdered tea extracts which are added to sugar, citric acid and ascorbic acid (Ortiz *et al*, 2008). Joubert *et al*. (2009) tested eight commercially produced rooibos iced tea products for the presence of three prominent marker compounds found in rooibos extract. The results showed that one of the samples did not contain any of the marker compounds, while some of the others contained no detectable levels of at least one of the three marker compounds (Joubert *et al*., 2009). This calls into question the quality of mass produced products and the effects of processing on the beneficial compounds. Furthermore most iced tea beverages fall into the category of sugar sweetened beverages and even if they contain adequate quantities of beneficial bioactive compounds they still have negative implications in terms of excess sugar consumption as previously described (Brownell *et al*., 2009). Recent iced tea product launches have also focused on kilojoule and sugar reduction with particular focus on stevia as an alternative (Anon, 2015d).

Iced tea products present a lot of potential for the delivery of the beneficial compounds found in the indigenous South African tea product honeybush. It also presents an opportunity to give consumers a healthy alternative to the unhealthy beverages which saturate the market and thereby improve consumption habits.

2.5.1 Honeybush tea (*Cyclopia spp.*)

Following in the footsteps of the rooibos tea industry, the launch of honeybush tea onto the global market has sparked much interest in the consumption and health benefits of this indigenous South

African herbal tea. The story of these two herbal teas illustrates the vital role of research in the transition from a local product to one with an international market (Joubert *et al*., 2011).

Cyclopia species form a part of the unique fynbos biome which is distributed along relatively untouched coastal regions and mountainous areas in the Western and Eastern Cape (Du Toit *et al*., 1998; Joubert *et al.*, 2008a). Commonly known as honeybush due to its distinctive sweet honey-like taste and aroma, this herbal tea has long been consumed by the local inhabitants of areas where the *Cyclopia* spp. grows naturally. It remained a cottage industry until the mid-1990s when efforts to expand the industry started (Joubert *et al.*, 2011). During this time the Agricultural Research Council of South Africa embarked on a research programme which aided in the commercialisation of *Cyclopia* spp. Initially most of the research focused on agricultural aspects and agroprocessing (Joubert *et al*., 2011). With successful commercialisation, and a thorough understanding of the health benefits of this product, the focus has shifted towards the production of standardised extracts and preparations for the nutraceutical industry (Bosman, 2014; Du Preez, 2014).

There are 23 species of *Cyclopia* recorded to date, three of which are commercially relevant (Joubert *et al.*, 2011). *Cyclopia genistiodes* and *C. subternata* have been successfully cultivated due to the fact that they are fast growing crops which can be harvested within a year under ideal growing conditions (Joubert *et al.*, 2011). *Cyclopia intermedia* is still harvested almost exclusively from the wild (Joubert *et al.*, 2011). Other species which are currently under investigation for commercialisation include *C. maculata* and *C. longifolia.*

Consumption as herbal tea remains the primary demand for honeybush (Joubert *et al.*, 2011). At present the industry has grown to a point where the present international demand for honeybush far exceeds the current production capacity. The most recent report by the Department of Agriculture, Forestry and Fisheries (DAFF) on the market value of honeybush reported that over 200 tons are produced annually with 50 tons being consumed locally and the remainder being exported (Anon, 2013d).

A report by Kaiser Associates (2010), entitled "Rooibos and Honeybush Market Development Programme Framework" that was commissioned by the Western Cape Department of Economic Development and Tourism, identified production of honeybush extracts, as potential value-adding opportunities for the local agro-processing industry. Honeybush extracts are considered to be an intermediate product which can be further developed into a range of other applications in the food and beverage, nutraceutical and cosmetics industries. International regulations require extracts to contain minimum phenolic content and ORAC (Oxygen Radical Absorbent Capacity) values.

However at present most locally produced extracts are of sub-standard quality, have lower ORAC values, little standardisation and poor solubility making them unsuitable for their intended applications (Kaiser Associates, 2010).

The production of value-added products fits in with the most recent strategic development programme set out by DAFF. Agro-processing was identified as a key area of development to address issues such as food security, job creation and rural and economic development which are still major challenges facing South Africa. One of the outlined aims was to increase the percentage of processed rather than unprocessed agricultural products for the export market as this would increase returns to the South African economy. Amongst others rooibos tea and beverages were identified as specific strategic value-added chains to be improved and invested in (Anon, 2013d).

Therefore stakeholders in industry identified the need for research support in product development, new product innovation and improving quality of products.

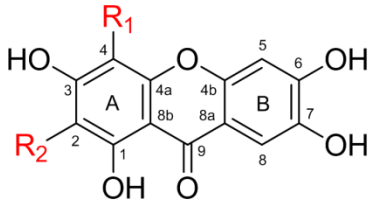
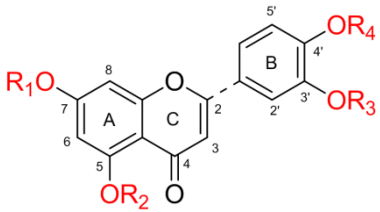
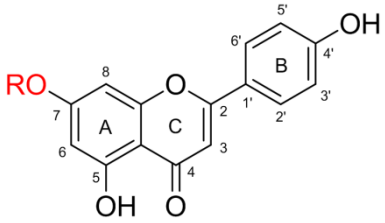
2.5.2 Phenolic compounds found in *C. subternata*

Research into honeybush as a commercial entity emerged during a time when consumers were becoming more health conscious and aware of the benefits of natural substances, particularly antioxidants (Joubert *et al.*, 2011). Focus on the phytochemical composition has contributed towards the commercial success of the honeybush industry by improving its marketing potential (Kamara *et al.*, 2004). A large portion of research performed on *Cyclopia* spp. has been devoted to the phenolic profiling of the most important commercial species. To date the most prominent phenolic compounds have been identified and quantified in *C. subternata* (Kamara *et al*., 2004; De Beer *et al*., 2009; De Beer *et al*., 2012; Kokotkiewicz *et al*., 2012), *C. genistoides* (Kokotkiewicz *et al*., 2013; Beelders *et al.*, 2014), *C. maculata* (Schulze *et al*., 2014), *C. intermedia* (Ferreira *et al*., 1998; Kamara *et al*., 2003) as well as *C. longifolia* (Schulze *et al*., 2015).

The phenolic composition of *Cyclopia* varies depending on the species, growing conditions and harvesting conditions (De Beer *et al.*, 2012; Schulze *et al*., 2014; Beelders *et al*., 2014; Joubert *et al*., 2014). Notably, the phenolic content, particularly xanthones and benzophenones are affected by factors such as the seed source as well as the harvesting conditions. Harvesting during summer resulted in higher concentrations of these compounds in the leaves of *C. genistoides* (Joubert *et al*., 2014). This can be explained by the conditions which stimulate plants to accumulate phenolic compounds including extreme temperatures, high solar radiation, ultraviolet radiation and drought (Matsuki, 1996).

A high temperature chemical oxidation processes (so-called ‘fermentation’) is used to produce the characteristic sweet taste and aroma as well as brown colour which make honeybush tea distinctive. Unfortunately this process also decreases the phenolic content and antioxidant activity of the source material as seen in Table 2.1 (Joubert *et al.*, 2008a; De Beer *et al.*, 2012; Schulze *et al*., 2014). Table 2.1 gives the structures of the major phenolic compounds, compares the percentage phenolic content of four different varieties of *Cyclopia* spp. and illustrates the detrimental effect of fermentation on the concentration of compounds. Therefore green (or unfermented) honeybush has entered the market with particular focus on its potential use for standardised extracts (De Beer *et al.*, 2012).

Table 2.1 Content (g/100 g extract) of major phenolic compounds in unfermented honeybush aqueous extracts from four different *Cyclopia* spp. and effect of fermentation. Adapted from Joubert *et al.* (2011).

Structure	Name	*C. genistoides*	*C. intermedia*	*C. sessiliflora*	*C. subternata*
	Xanthones				
	Mangiferin	9.55	4.35	4.67	2.73 [a]
	(R_1= glc; R_2 = H)	(−83%)	(−97%)	(−87%)	(−98%) [b]
	Isomangiferin	2.72	1.40	1.69	0.86
	(R_2= glc; R_1= H)	(−59%)	(−81%)	(−54%)	(−83%)
	Flavanones				
	Hesperidin	0.71	0.62	0.74	0.62
	(R_1= rut; R_3 = H, R_4= CH_3)	(−56%)	(−47%)	(−49%)	(−61%)
	Eriocitrin	Traces	0.13	0.32	0.32
	(R_1= rut; R_3,R_4= H)	(−100%)	(−77%)	(−38%)	(−63%)
	Eriodictyol glucoside	nd	0.07	nd	0.35
	(1 of R_1, R_2, R_3, R_4=glc; rest=H)		(−100%)		(−100%)
	Flavone				
	Scolymoside (R=rut)	Traces (−100%)	0.04 (−100%)	0.06 (−100%)	0.68 (−71%)

glc, glucopyranosyl ; nd, not detected; rut, rutinosyl.
[a] Average content in aqueous extract from unfermented tea.
[b] Percentage decrease in content of extract with fermentation of plant material

Of the commercialised species of *Cyclopia*, *C. subternata* has been selected for development into nutraceutical extract. The most important compounds detected in *C. subternata* include mangiferin and isomangiferin (xanthones), hesperidin and eriocitrin (flavanones) and scolymoside (flavone) (De Beer *et al*., 2009; De Beer *et al*., 2012; Kokotkiewicz *et al*., 2012). Recently additional compounds namely 3-hydroxyphloretin-3′,5′-di-*C*-β-hexoside and phloretin-3′,5′-di-*C*-β-glucoside (dihydrochalcone), iriflophenone-3-*C*-β-glucoside (benzophenone) and isorhoifolin (flavone) were identified (De Beer *et al*., 2012; Kokotkiewicz *et al*., 2012).

A number of phenolic compounds identified in *Cyclopia* spp. have received attention for their health benefits when found in other sources. Some of the health benefits of major compounds found in *C. subternata* have been outlined in Table 2.2. As previously explained, phenolic compounds sometimes have a synergistic effect when combined with other phenolic compounds. In the case of *C. subternata* the cumulative effect of the various phenolic compounds has resulted in the patenting of aqueous extracts for anti-diabetic properties (Mose Larsen *et al*., 2008). The evidence for the health claims of *Cyclopia* spp. was further substantiated by Dudhia *et al*. (2013) who reported the potential anti-obesity effects of *C. subternata* and *C. maculata* extracts during *in vitro* studies.

The most important phenolic compound detected in *Cyclopia* spp. is mangiferin. It has attracted considerable attention due to its numerous pharmacological activities as reviewed by Vyas *et al.* (2012). Nutraceutical extracts containing high levels of mangiferin are produced from mango bark and leaves (*Mangifera indica* L.) (Leiro *et al*., 2003). According to Leiro *et al.* (2003) mangiferin is a potent antioxidant which operates primarily by scavenging free radicals. It has therefore been investigated for therapeutic use in preventing and treating conditions caused by oxidative stress and has been demonstrated to have anti-diabetic, anti-mutagenic, anti-carcinogenic, anti-inflammatory and hypolipidaemic properties amongst others (Vyas *et al.*, 2012). A recent article reported that in order to ingest the same amount of mangiferin consumed in one cup of *C. genistoides* one would have to consume eight mangoes (Schulze *et al*., 2015).

To date most research has focused on the production of standardised aqueous extract of *Cyclopia* spp. (Bosman, 2014; Du Preez, 2014). However for practical applications such as instant iced tea a powdered extract is required. To date there has been no research on the stability of powdered *Cyclopia* extracts. In order for the consumer to reap the benefits associated with honeybush it is of paramount importance that the bioactive compounds are able to survive conditions related to the production, shelf-life and product applications.

Table 2.2 Documented health benefits of important phenolic compounds found in *C. subternata*.

Class of compounds	Compound	Health benefits	References
Xanthones	Mangiferin	Anti-obesity	Guo *et al*., 2011
		Anti-diabetic	Li *et al*., 2010
		Treatment of inflammatory and neurodegenerative disorders	Miura *et al*., 2001
		Enhancement of recognition memory	Leiro *et al*., 2003
		Potent antioxidant	Pardo Andreu *et al*., 2010
		Anti-carcinogenic	Vyas *et al*., 2012
Flavanones	Eriocitrin	Decreases VLDL and LDL in rats on a high fat and high cholesterol diet	Minato *et al*., 2003 Miyaki *et al*, 1997 & 2006
	Hesperidin	Improves endothelial function	Rizza *et al*., 2011
		Reduces inflammation	
		Favourably modifies lipid profiles of patients with metabolic syndrome (hypolipidaemic activity)	Garg *et al*., 2001 Morand *et al*., 2011
		Reduces permeability and fragility of blood vessels	Ahmed *et al*., 2012
		Vascular protective effects	
		Anti-diabetic activity	
Flavones	Scolymoside	Aldose reductase inhibitor	Lattanzio *et al*., 2009 Jung *et al*., 2011
Benzophenone	Iriflophenone-3-glucoside	Inhibits triglyceride synthesis	Feng *et al*., 2011
		α-glucosidase inhibitor	Zhang *et al*.,2011

2.6 Challenges in production of natural extracts

Natural extracts containing high concentrations of health-promoting substances are widely used in the food and beverage, nutraceutical and cosmetics industries (Sansone *et al*., 2011a). At present the use of natural products as a therapeutic alternative is increasing due to the perception that it is safe (Bott *et al.,* 2010). Joubert *et al.* (2010) identified green or unfermented honeybush as source material for the preparation of such extracts due to high concentrations of phenolic compounds.

There are a number of challenges associated with the production of natural extracts for use as food ingredients and nutraceuticals. One of the major challenges is the natural variation in composition due to factors such as climate, harvesting period, postharvest processing and storage conditions (Bott *et al.,* 2010). Phenolic compounds often occur in very low concentrations in their natural form, making the formulation of standardised products with therapeutic claims difficult (Da Rosa *et al*., 2014). It is very important to be able to standardise these products to ensure that adequate dosages of the bioactive compounds are administered (Bott *et al.,* 2010). This is a somewhat complex process due to the fact that herbal extracts (unlike synthetic drugs) contain a myriad of complex compounds with an array of different physicochemical properties (Bott *et al.,* 2010). For example, *C. subternata* was shown to have considerable variation in phenolic composition which hampers standardisation (De Beer *et al*., 2012).

There are also a number of technical hurdles which need to be overcome in order for natural extracts to be used in food and nutraceutical applications. In their unprocessed form natural extracts are often sticky, highly susceptible to degradation and practically difficult to work with (Sansone *et al*., 2011a). Many compounds have poor or limited solubility in water (Munin & Edwards-Lévy, 2011). One of the most prominent features of hesperidin, a major *C. subternata* phenolic compound (De Beer *et al*., 2012) is its low solubility especially in aqueous systems (Tomás-Navarro *et al*., 2014). Another problem is that phenolic compounds often have an unpleasant bitter taste or astringent mouthfeel (Munin & Edwards-Lévy, 2011). High levels of bitter phytonutrients may be beneficial for health, but may not be acceptable to the consumer, posing a problem to designers of functional foods (Drewnowski & Gomez-Carneros, 2000). For instance Theron (2012) found a correlation between high concentrations of mangiferin in honeybush and the bitter taste present in certain *Cyclopia* species. Complexation of bitter compounds with cyclodextrins is one of the techniques which has been used to mask the bitterness of these compounds (Szejtli & Szente, 2005).

The structure and functionality of polyphenolic compounds makes them sensitive to temperature, pH, light and oxygen (Fang and Bhandari, 2010), thus impacting on their stability during

processing and storage (Harbourne *et al.*, 2013; Sun *et al.*, 2014). These properties are further complicated by interactions with other food components, as well as conditions in the gastrointestinal tract such as pH, enzymes and presence of other nutrients (Fang and Bhandari, 2010). In many cases the properties of phenolic compounds which contribute towards their health benefits are also the properties which are responsible for their lack of long-term stability (Munin & Edwards-Lévy, 2011). Microencapsulation has been applied as a method to extend the stability of bioactive compounds such as polyphenols. The range of applications for encapsulated polyphenols is extensive and includes domains such as food, pharmaceutical and cosmetic, as well as agricultural products, industrial chemicals, biotechnology and biomedical industries (Munin & Edwards-Lévy, 2011).

2.6.1 Encapsulation

Encapsulation is a term which is broadly used in literature to describe both encapsulation and immobilisation technologies (De Vos *et al.*, 2010). Encapsulation refers to a technology in which the bioactive compound is completely enveloped in the encapsulating matrix without any protrusions (De Vos *et al.*, 2010). This is important for applications such as the protection of probiotics as these cells are sensitive to leakage should they be exposed (De Vos *et al.*, 2010). Immobilisation on the other hand is a technology in which the bioactive compound is immersed, but not necessarily enveloped in the matrix of the encapsulating agent (De Vos *et al.*, 2010). Some of these compounds may protrude from the surface of the matrix and be exposed to environmental conditions (De Vos *et al.*, 2010). Many encapsulation techniques result in immobilisation of the bioactive compounds, however this is not a commonly used term and to avoid confusion the term encapsulation will be used. Microencapsulation is the process in which minute particles are enveloped in a coating material or immersed in the continuous polymeric matrix to produce small capsules with various functional properties (Gharsallaoui *et al.*, 2007), creating a physical barrier to sensitive bioactive compounds in order to protect them from adverse environmental conditions (De Vos *et al.*, 2010).

2.6.2 Benefits of encapsulating foods

The benefits of encapsulating foods have been widely discussed in literature and were first summarised by Shahidi and Han (1993) and later revised by Desai & Park (2005a) into seven main advantages.

The most important feature of encapsulated nutraceuticals is their protection from environmental conditions which cause degradation. Encapsulation also reduces the evaporation or transfer rate of core material to the outside environment. The physical properties of the original material can be modified to make it easier to handle, store, transport and incorporate into other

products. An important extension of this is that unpleasant flavours can be masked. Bioactive materials, which are required in small quantities, can be diluted in order to achieve uniform dispersion in the final product formulation. Components which are typically very reactive with each other can also be separated by a physical barrier. Finally, encapsulation allows for the controlled release of the core material under specific environmental conditions in product applications or in the gastrointestinal tract. Encapsulation is therefore an important technology which makes the development of functional foods possible.

2.6.3 Selecting encapsulating techniques

Various encapsulation techniques have been developed in order to protect nutraceutical compounds from deterioration (Fang & Bhandari, 2010). These methods can be divided into three categories namely physical, chemical or physicochemical processes (Murugesan & Orsat, 2011). Table 2.3 gives the microcapsule morphology of the various techniques, as well as the most important steps in the process. Amongst others these include spray-drying, freeze-drying, coacervation, spray-cooling, fluid bed coating and extrusion technologies (De Vos *et al.*, 2010).

When selecting an encapsulating technique it is important to consider the physicochemical and molecular characteristics of the bioactive compounds to be protected (De Vos *et al.*, 2010). It is also mandatory that the encapsulation procedure is able to protect the compounds throughout processing, storage and transport (De Vos *et al.*, 2010). Furthermore the encapsulated bioactive compounds should be in a suitable form for the product application without interfering negatively with the taste or texture of the final product (De Vos *et al.*, 2010).

The simplest encapsulation technique involves making a solution containing the substance which needs to be encapsulated as well as the wall material followed by freeze-drying or spray-drying (Da Rosa *et al.*, 2014).

Table 2.3 Summary of important encapsulation techniques and major steps in the process. Adapted from Desai & Park (2005a); Murugesan & Orsat (2011) and Fang & Bhandari (2010).

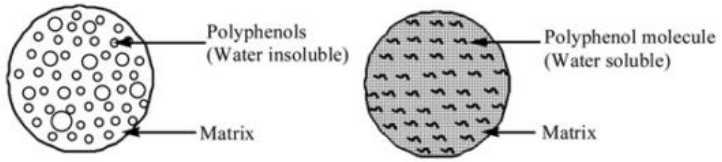
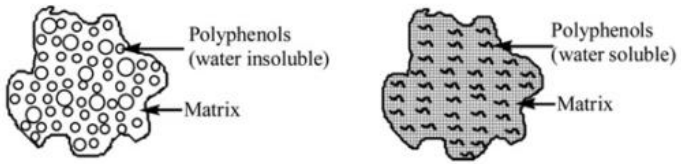
Category	Encapsulation technique	Particle morphology	Major steps in encapsulation
Physical process	Spray-drying		Preparation of the dispersion Homogenisation of the dispersion Atomisation of the feed solution Dehydration of the atomised particles
	Freeze-drying		Mixing of core in a coating solution Freeze-drying of the mixture
	Spray-cooling and chilling		Preparation of the dispersion Homogenisation of the dispersion Atomisation of the feed dispersion
	Fluidised-bed coating		Preparation of coating solution Fluidisation of core particles Coating of core particles

Table 2.3 continued

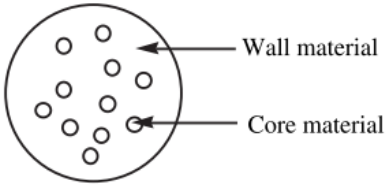
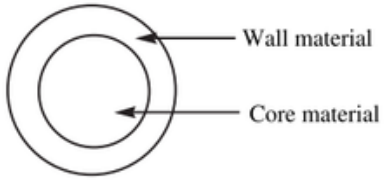
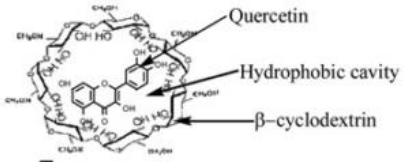
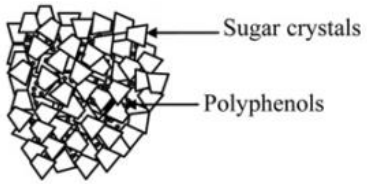
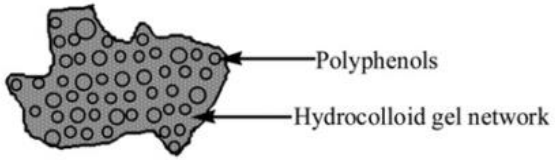
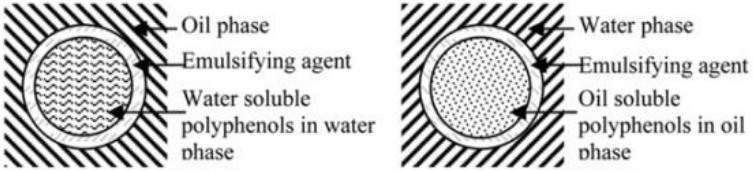
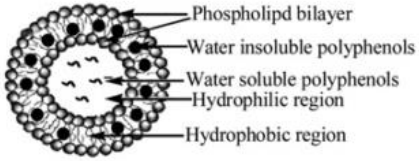
Category	Encapsulation technique	Particle morphology	Major steps in encapsulation
Physical process	Extrusion		Preparation of molten coating solution Dispersion of core into molten polymer Passing of core-coat mixture through dehydrating liquid
	Centrifugal extrusion		Preparation of core solution Preparation of coating material solution Co-extrusion of core and coat solution through nozzles
Chemical process	Molecular inclusion		Preparation of complexes by mixing or grinding or spray-drying
	Cocrystallisation		Preparation of supersaturated sucrose solution Adding of core into supersaturated solution Emission of substantial heat after solution reaches the sucrose crystallisation temperature

Table 2.3 continued

Category	Encapsulation technique	Particle morphology	Major steps in encapsulation
Physicochemical process	Coacervation		Formation of a three-immiscible chemical phase Deposition of the coating Solidification of the coating
	Emulsion		Two immiscible liquids are added. One of the liquids is dispersed as small droplets in the other
	Liposome entrapment		Micro-fluidisation Ultrasonication Reverse-phase evaporation

2.7 Spray-drying

Drying is considered to be one of the oldest forms of food preservation and is commonly used for the preservation of medicinal herbs (Harbourne *et al*., 2013). Drying of plant material is an important step in inhibiting metabolic processes which cause degradation of bioactive compounds (Harbourne *et al*., 2013). Traditionally herbs were air-dried in the sun which was not very effective because this method is slow and metabolic processes which result in degradation continue for an extended period of time (Harbourne *et al*., 2013). Drying remains an important part of preserving herbal extracts and technological advances have now resulted in methods which allow natural extracts to be dried quickly while still maintaining the quality of the raw material.

Although a number of microencapsulation techniques have been developed, spray-drying remains the oldest and most widely used technique (Gharsallaoui *et al*., 2007). It was developed in 1933 and was used to encapsulate flavours using gum Arabica as a wall material (Murugesan & Orsat, 2011; Gharsallaoui *et al*., 2007). Since then spray-drying technology has developed and is now widely used in the food industry for drying and encapsulating fruit extracts, essential oils, flavours, enzymes and herbal extracts (Cortéz-Rojas & Oliveira, 2012). It is also used in the pharmaceutical industry for the purpose of microencapsulation, drying of thermally sensitive products and improving the flow properties of granules (Baldinger *et al*., 2012).

It is currently considered to be the most important industrially used drying method due to the fact that it is a low cost, one-step, continuous process which requires minimal handling of the product (Chiou & Langrish, 2007; Fang & Bhandari, 2011). The resulting product is a fine powder of consistent quality with reduced bulk weight and particle size which is beneficial for transportation and storage purposes (Sasone *et al*., 2011a). While the process is considered wasteful in terms of energy, the costs of equipment and production are considerably lower than other technologies such as freeze-drying, which is 30-50 times more expensive (Gharsallaoui *et al*., 2007).

2.7.1 Spray-drying process

Spray-drying is one of the most simple encapsulation techniques (Da Rosa *et al*., 2014). The end product quality is dependent on the interaction between processing parameters and the composition of the feed solution (Murugesan & Orsat, 2011). There are several processing parameters which have an effect on powder properties – the most significant parameters include the air inlet temperature, the air flow rate and the feed concentration and rate (Baldinger *et al*., 2012).

The basic spray-drying process entails the following: Firstly, a solution containing the active ingredient and encapsulating compound is prepared. The solution is then pumped into the spray-dryer and atomised via a nozzle under pneumatic pressure or a spinning disk configuration (Fig. 2.2) (Gharsallaoui *et al*., 2007; Munin & Edwards-Lévy, 2011). A heated gas (air or nitrogen) is then brought into contact with the atomised feed in order to evaporate the solvent (Munin & Edwards-Lévy, 2011).

The atomisation process converts liquid feed into equally sized tiny droplets which causes uniform heat and mass transfer during the drying process (Gharsallaoui *et al*., 2007; Murugesan & Orsat, 2011). The reduction in particle size and even dispersion in the drying gas increases the surface area of the particles exponentially which results in a very fast drying process (Munin & Edwards-Lévy, 2011). The rate of heat and mass transfer is dependent on the droplet diameter as well as the relative velocity of the air and droplets (Murugesan & Orsat, 2011). The size of the droplets is dependent on the amount of energy supplied during the atomisation process, the more energy provided, the finer the droplets (Gharsallaoui *et al*., 2007). The fast drying rate enables moisture removal without disrupting the integrity of the microcapsule which forms (Murugesan & Orsat, 2011). This provides relative control of the particle size distribution, an important powder characteristic (Murugesan & Orsat, 2011). The typical shape of spray dried particles is spherical, with a mean size range of 10-100 µm (Fang & Bhandari, 2010).

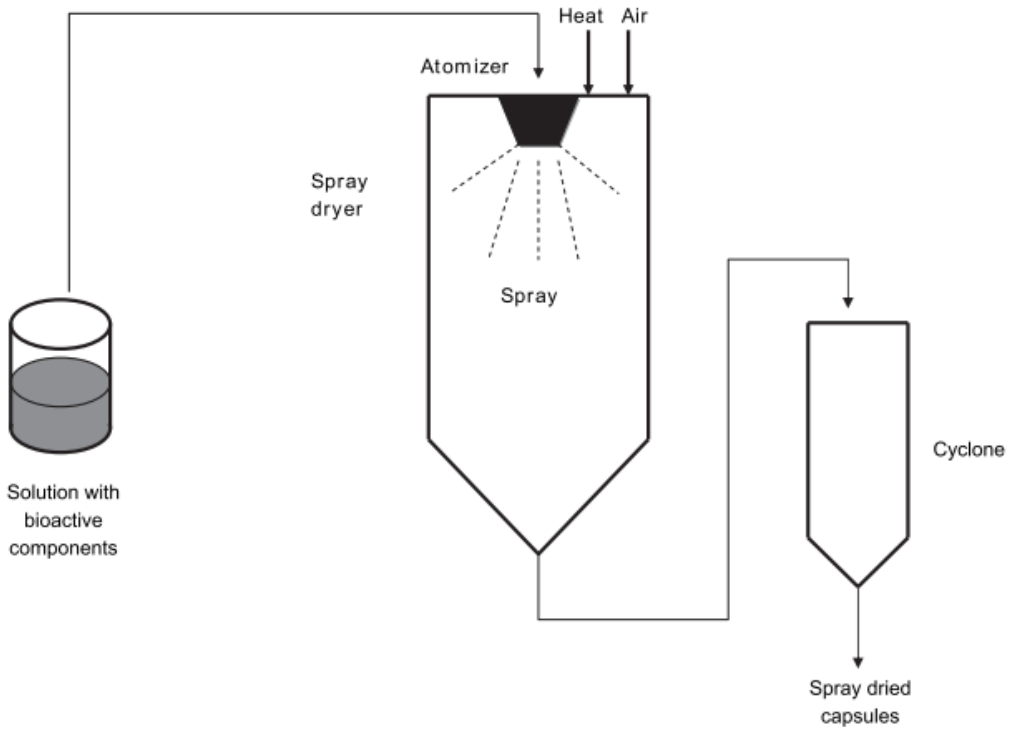


Figure 2.2 Schematic presentation of spray-drying process.

Baldinger *et al.* (2012), spray-drying a mixture of mannitol and trehalose, noted that the drying temperature has an effect on the morphology of the particles. When lower air inlet temperatures (110 °C) were used the particles were more spherical, whereas at higher air inlet temperatures (220 °C) evaporation occurred more quickly. This resulted in a rough and uneven surface which led to agglomeration as depicted in the scanning electron microscope (SEM) images seen in Fig.2.3.

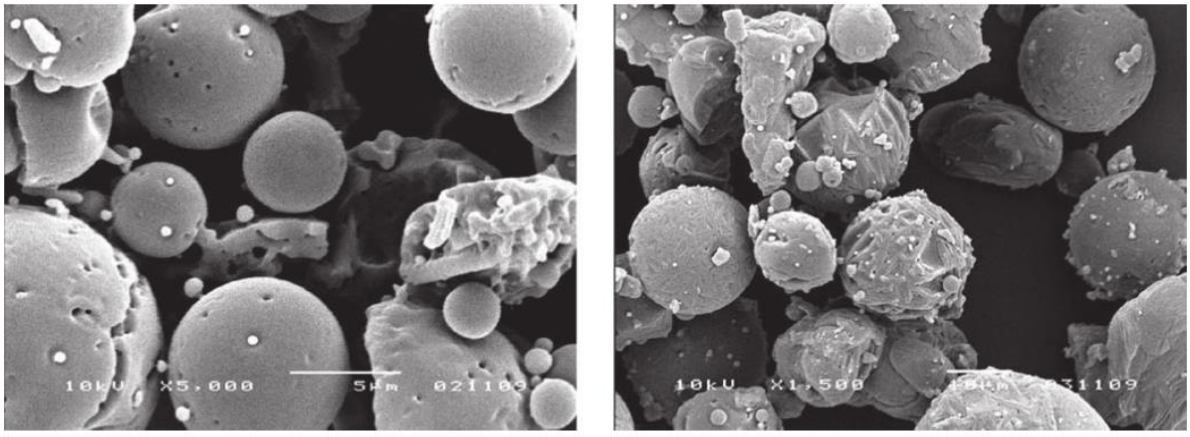


Figure 2.3 SEM images of spray-dried particles of a mannitol-trehalose mixture at different inlet temperatures 110 °C (left) and 220 °C (right) (Baldinger *et al.*, 2012).

The direction of the airflow plays an important role in the drying kinetics and particle behaviour of the powder (Murugesan & Orsat, 2011). Most spray-dryers have a co-current flow in which the air and the atomised particles enter and travel in the same direction through the machinery (Murugesan & Orsat, 2011). In a few rare cases counter-current and mixed-current flow are used, although very little is known about their drying kinetics (Murugesan & Orsat, 2011). As the particles dry they fall to the bottom of the drying chamber and are removed with the outgoing air (Murugesan & Orsat, 2011). Separation takes place in a cyclone which collects the powder while letting the moist air pass through (Murugesan & Orsat, 2011).

The main parameters to be controlled during spray-drying are the inlet temperature and feed flow rate, which determines the outlet temperature (Cortéz-Rojas & Oliveira, 2012). These parameters directly affect powder properties such as moisture content and retention of phenolic compounds (Cortéz-Rojas & Oliveira, 2012). The air inlet temperature is usually determined by balancing two factors, namely the temperature which can safely be used without damaging the product or creating operating hazards and the comparative cost of heat sources (Gharsallaoui *et al*., 2007).

The powder which is formed is usually amorphous in nature, which makes it rubbery and sticky (Murugesan & Orsat, 2011). This creates interesting challenges during the recovery of the product as it can lead to particles agglomerating and sticking to the walls of the equipment thereby lowering the yield (Murugesan & Orsat, 2011). This is a particular problem in products such as fruit juices which have low-molecular-weight sugars and organic acids

(Murugesan & Orsat, 2011). These components are hygroscopic and have low glass transition temperatures (T_g) leading to stickiness (Murugesan & Orsat, 2011). The amorphous versus crystalline state of powders as well as the glass transition of powders will be discussed in more detail in the characterisation of powders.

2.7.2 Spray-drying of natural extracts

The drying of natural extracts is quite a complex process, because these extracts contain a wide variety of different substances with an array of physical and chemical properties (Cortéz-Rojas & Oliveira, 2012). Extracts in powder form have a number of advantages over those in liquid form, i.e higher stability and easier handling, storage and transport (Couto *et al*., 2012). Spray-drying increases the shelf-life of the product by reducing the water activity which prevents the growth of microorganisms (Chiou & Langrish, 2007). It also allows complex mixtures to be standardised so that active compounds are administered in known quantities (Bott *et al.*, 2010). Spray-drying had been used to successfully encapsulate a number of different nutraceutical ingredients such as natural extracts, isolated phenolic compounds, vitamins and lipids as summarised in Table 2.4.

In general, most thermal processes lead to the degradation of phenolic compounds (Harbourne *et al*., 2013; Irina & Mohamed, 2012), but there are cases where heat processing is beneficial, for example, roasting of cashew nuts resulted in increased levels of extracted phenolic compounds (Chandrasekara & Shahidi, 2011). In many cases high inlet temperatures are used to spray-dry plant extracts, with typical air inlet temperatures varying between 140 and 180 ºC (Ersus & Yurdagel, 2007; Obón *et al*., 2009). However, the small particle size and large surface-to-volume ratio lead to rapid drying and the cooling caused by evaporation ensures that the core temperature never exceeds 100 ºC, making the process generally suitable for sensitive products (Fang &Bhandari, 2011). Since certain phenolic compounds are particularly heat sensitive, carrier molecules are used to microencapsulate the bioactive molecules by immobilising them in a polymeric matrix (Sasone *et al*., 2011a). Joubert *et al*. (2009) showed that spray-drying at an inlet temperature of >200 ºC did not significantly decrease the aspalathin content of a rooibos extract. The same extract, when subjected to sterilisation temperatures and heating times, caused significant losses of aspalathin.

The effect of spray-drying on different phenolic compounds showed that the heat sensitivity of compounds varies (Irina & Mohamed, 2012). A number of factors play a role in heat sensitivity of compounds such as the structure of the compound as well as the structure of the food matrix. The food matrix could act as a barrier towards heat treatment or induce the degradation (Irina & Mohamed, 2012). Apart from temperature, the stability of phenolic compounds throughout the drying process is also dependent on factors such as pH and the presence of oxygen.

Pure mangiferin, the major xanthone of *Cyclopia* spp., is susceptible to degradation during spray-drying, but its stability can be improved by micro-encapsulation. De Souza *et al.* (2013a) showed that the nature of the polysaccharide carrier and the use of a surfactant affect the retention of mangiferin during spray-drying. For instance mangiferin spray-dried with pumpkin pectin as carrier retained more than double the amount of mangiferin than when chitosan was used as a carrier.

Knowledge of the stability of bioactive compounds in *C. subternata* during processes such as spray-drying is limited to mangiferin. Previous studies have shown that the prolonged heating of moist *Cyclopia* plant material at high temperatures (70-90 ºC) during processing significantly reduced the total phenolic content, as well as mangiferin, isomangiferin, eriocitrin and hesperidin (Joubert *et al*., 2008a; Schulze *et al*., 2014; Beelders *et al*., 2015).

2.7.3 Carriers

Carriers have been shown to play a significant role in the stability of bioactive compounds, as well as the physical properties of the resulting powder (Munin and Edwards-Lévy, 2011). When selecting a carrier it is important to consider the following: Firstly, it should be easy to handle when producing the feed solution i.e. it should be soluble in the applicable solvent and have good film-forming and rheological properties (Desai & Park, 2005a). The carrier should be compatible with the substance which needs to be encapsulated. This includes being able to emulsify the active material in the feed solution and seal and protect it completely once dry without reacting with it chemically during processing and prolonged storage (Desai & Park, 2005a). It is clear from various studies that different carriers are suitable to specific bioactive substances. For instance Sun-Waterhouse *et al*. (2013) tested four different encapsulating

agents on the polyphenols vanillin and quercetin. It was found that vanillin had a much better encapsulating efficiency than quercetin. Furthermore, for powders containing vanillin, inulin had the highest encapsulating efficiency followed by methyl β-cyclodextrin (MβCD), hydroxypropyl-methyl cellulose (HPMC) and then sodium alginate. On the other hand inulin, MβCD and HPMC all had similar encapsulating efficiencies for powders containing quercetin.

Another important consideration is that it should be compatible with the format required for the final application as it should be able to protect the active material against environmental conditions while still being able to dissolve and release it at the appropriate time (Desai & Park, 2005a). When developing products for the nutraceutical industry it is also essential to take the health benefits or detriments of the carrier into consideration as it will be consumed as part of the final product. It would be counterproductive to encapsulate an anti-diabetic substance such as mangiferin (Li *et al*., 2010; Vyas *et al*., 2012) in a carrier such as maltodextrin, which has a large glycaemic response (Englyst *et al*., 1996). Naturally when developing products for the food industry with much smaller profit margins than the pharmaceutical industry, cost is one of the biggest factors which cannot be ignored and the carrier of choice should therefore be financially viable (Silva *et al*., 2013).

It would be unrealistic to expect a single carrier to possess all of these properties therefore different carriers can be used alone or in combination depending on the application and chemical profile of the phenolic compound (Silva *et al*., 2013). The most common carriers used in spray-drying include modified starches, cellulose derivatives, gums and synthetic polymers (Sansone *et al*., 2011a). The advantages and disadvantages of widely used carriers have been summarised in Table 2.4. Two carriers investigated in this study will be discussed in more detail.

2.7.3.1 Maltodextrin and corn syrup solids

Maltodextrin and corn syrup solids are the most common carriers used in the food industry, predominantly due to the fact that they are cheap, versatile and readily available (Silva *et al*., 2013). They are very easy to handle due to the fact that they are water-soluble and have low viscosity, making them suitable for a variety of applications (Silva *et al*., 2013). Maltodextrin and corn syrup solids are produced through the partial hydrolysis of starch and are composed

mainly of D-glucose, maltose, oligosaccharides, and polysaccharides (Cortés-Rojas & Oliveira, 2012). They are characterised in degrees of dextrose equivalents (DE), indicating the extent of hydrolysis and a measure of the total reducing power of the sugars present relative to a dextrose (D-glucose) standard, on a dry mass basis. Starch hydrolysates with DE values below 20 are referred to as maltodextrins (Sun *et al*., 2010). Corn syrup solids are defined as dried glucose syrup in which the reducing sugar content is 20 DE or higher. Maltodextrins with DE values between 10 to 20 DE are widely used for the encapsulation of anthocyanins and phenolic acids (Silva *et al*., 2013).

They are also widely used in the spray-drying of fruit juice-based products because they increase the overall T_g and reduce stickiness and deposition of powder to the walls of the spray-dryer (Langrish *et al*., 2007; Fang & Bhandari, 2011; De Souza *et al*., 2013a). On the other hand Sansone *et al*. (2011a) reported that maltodextrin has a lower glass transition temperature than other carriers. This could lead to crystal formation at high temperatures which induces disruption of the matrix, agglomeration and deterioration of bioactives.

One of the concerns about maltodextrin, pointed out in the previous section, is its highly refined nature, which induces a similar metabolic response to sugar. This makes maltodextrin unsuitable for the production of health products which aim to reduce sugar content.

2.7.3.2 Inulin

Inulin has attracted the attention of the food and pharmaceutical industries for its health benefits. It is a fructan which is linked by β (2-1) glycosidic bonds and contains either a terminal β-D-fructose or a β-D-glucose. The degree of polymerisation (DP) ranges between 2-60 units and averages at about 12 units (Kawai *et al*., 2011). It is widely used in the food industry as a low-calorie sweetener, fat replacer, non-digestible soluble fibre and to improve the organoleptic properties of foods. Inulin is usually produced by spray-drying which produces an amorphous powder with good handling properties (Ronkart *et al*., 2009). Its physical properties such as morphology, solubility, rheology, T_g and vapour sorption have been well characterised in previous studies (Ronkart *et al*., 2009; Kawai *et al*., 2011; Mensink *et al*., 2015). These properties are largely dependent on the DP of the powder and the amount of water present in the system (Mensink *et al*., 2015).

Inulin, together with substances such as lignin and oligosaccharides, is considered to be a prebiotic. When added to food they increase the dietary fibre content and are fermented to produce short-chain fatty acids by bacteria in the small intestine of mono-gastric mammals such as humans. This stimulates the growth of bifidobacteria which contribute towards a healthy gut, the suppression of pathogenic bacteria and an increase in immunity. Soluble fibres also improve mineral absorption, lower tryglycerides/cholesterol, decrease faecal transit time, increase stool weight and prevent constipation (Arvanitoyannis &Van Houwelingen-Koukaliaroglou, 2005, Corbo *et al*., 2014). Apart from their prebiotic features inulin and oligofructose have been shown to increase calcium absorption and therefore improve bone mineral content and density (Bosscher *et al*., 2006).

Soluble fibres such as inulin have previously been successfully incorporated into functional beverages and foods (Siró *et al*., 2008). The addition of these products is seen as an important way of addressing nutritional deficiencies in dietary fibre (Corbo *et al*., 2014). It should however be noted that if the concentration of added inulin is too high in functional beverages it could contribute negative sensory attributes such as grittiness (Sun-Waterhouse *et al*., 2010; Sun-Waterhouse, 2011). Villegas *et al.* (2009) showed that the acceptability of a prebiotic low-fat beverage formulation containing inulin was higher when the inulin was of short chain type (2 to 10 monomers) compared to inulin with long-chain length (>23 monomers).

Table 2.4 Summary of carriers used during the spray-drying of various nutraceutical ingredients.

Carrier	Properties	Nutraceutical	Reference
Maltodextrin & corn syrup solids	Good oxidative stability Cheap Good binding to flavour and fat Highly soluble Low hygroscopicity Poor emulsifying capacity & stability Undesired taste alterations Unnatural additive Low glass transition temp	*Hibiscus sabdariffa* L.	Sansone *et al.*, 2011a
		Bayberry (*Myrica rubra Sieb.* et Zucc)	Fang & Bhandari, 2011
		Colour extract of red Bordo grapes	De Souza *et al*., 2013b
		β-carotene	Desobry *et al*. 1997
		Cactus pear (*Opuntia streptacantha*)	Rodríguez-Hernández *et al*. 2005
Cyclodextrin	Increases solubility and bioavailability Produces powder with low a_w* Fast dissolution rate (9-14 s) Unnatural additive	Vanillin and quercetin	Sun-Waterhouse *et al.*, 2013
Chitosan	Hydrophilic Biocompatible Biodegradable Mucoadhesive properties – improves pharmacokinetics in digestive tract Positively charged – moderate to poor drug retaining capacity	Mangiferin	De Souza *et al.*, 2013a
		Vitamin C	Desai & Park, 2005b
		Olive leaf extract	Kosaraju *et al*., 2006
Pectins	Negatively charged – good drug retaining capacity Helps to maintain a healthy digestive system Well-formed powder with moisture content suitable for storage Hygroscopic – must be stored at RH# below 50% to prevent caking and microbial growth Highly viscous – therefore difficult to pump	Mangiferin	De Souza *et al.*, 2013a
		Hibiscus sabdariffa L. extract	Chiou & Languish, 2007
		M. officinalis, F. ancylantha & *T. farfara* extracts	Sansone *et al.*, 2011a
		Fish oil	Drusch, 2007
Inulin	Prebiotic health benefits Low glycemic impact Produces powder with very low a_w Fast dissolution rate (15-13 s) Could contribute negative sensory attributes if concentration is too high	Vanillin and quercetin	Sun-Waterhouse *et al.*, 2013
Alginates	Produces powder with low a_w Medium dissolution rate (48-55 s) Used in controlled release delivery systems	Vanillin and quercetin	Sun-Waterhouse *et al.*, 2013

Table 2.4 continued

Modified starches eg. Capsul ™	Good volatile retention Excellent stability Emulsification properties	Orange essential oil and acetic acid	Silva *et al*., 2013
Modified cellulose, e.g. Microcrystalline cellulose	Produces powder with low a_w pH dependent solubility – used in controlled release delivery systems Forms amorphous matrices Good flow properties Slow dissolution rate (68-166 s) Hygroscopic therefore could affect storage stability	Vanillin and quercetin *Bidens pilosa* L. Naringenin and quercetin Black carrot (*Daucus carota* L.)	Sun-Waterhouse *et al.*, 2013 Cortés-Rojas *et al*., 2014 Sansone *et al*., 2011b Ersus and Yurdagel 2007
Gums, e.g. Gum arabica	Natural High flavour retention Emulsifying properties Prevents oxidation of encapsulated lipids	Coffee extracts Linoleic acid Cardamom oleoresins	Rodrigues and Grosso, 2008 Minemoto *et al*. 2002 Krishnan *et al*. 2005
Whey protein isolate and skim milk powder	Retention of volatiles is affected by concentration of carrier used as well as the interactions between the carrier and the type of ester encapsulated Water soluble Emulsification properties Smooth surface morphology with no cracking or indentation	Ethyl butyrate and ethyl caprylate Caraway (*Carum carvi* L.) essential oil Essential oil of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and sweet marjoram (*Majorana hortensis* L.)	Rosenberg & Sheu, 1996 Bylaitë *et al*., 2001 Baranauskiene *et al*., 2006
Colloidal silicon dioxide, e.g. Aerosil® 200	Large specific surface area High glass transition temperature Good flow properties Hygroscopic High concentrations decreased the phenolic content of *Apeiba tibourbou*	*Bidens pilosa* L. *Apeiba tibourbou*	Cortés-Rojas *et al*., 2014 Couto *et al*., 2012

[#]RH= relative humidity , [*]a_w = water activity

2.8 Effects of storage on powders

It is of utmost importance to consider the interaction between a multi-component product and its environment when developing a nutraceutical product (Ortiz *et al*., 2008) so that its quality and efficacy can be maintained throughout its shelf-life. Establishment of adequate storage conditions is therefore essential to prevent the occurrence of physicochemical reactions, water adsorption and the growth of spoilage microorganisms (Bott *et al.,* 2010). Stability testing measures quality changes in herbal extracts as a function of time under different storage conditions such as temperature, relative humidity and oxygen content (Bott *et al.,* 2010). The outcomes of these tests establish the shelf-life and suitable storage conditions of the product (Bott *et al.,* 2010).

Research has shown that phenolic compounds are sensitive to various processing conditions and degrade over time if not stored under the correct conditions. Encapsulation of drugs, nutraceuticals and food ingredients has been reported to extend the shelf-life due to the fact that wall/coating material acts as a physical and permeable barrier towards oxygen and other factors which cause degradation over time (Sansone *et al*., 2011b). It is therefore of interest to model the physicochemical response of powder extract and micro-encapsulated extract under a range of storage conditions.

During storage of powders state transformations often occur (Bott *et al.,* 2010). Spray-dried powders are in an amorphous (glassy) or metastable state, and if not stored correctly, could change to a crystalline state (Müller *et al*., 2015). The stability of the carrier during storage is dependent on its T_g and susceptibility to crystallisation (Sansone *et al*., 2011a). The T_g of maltodextrins varies from 100 to 243°C according to their DE and water content (Goula and Adamopoulos, 2008). The T_g decreased with increasing moisture content due to the plasticising effect of water (Tonon *et al*., 2009). Low-DE maltodextrins have lower T_g values than high-DE maltodextrins at the same moisture content (Goula & Adamopoulos, 2008). A carrier with a low T_g such as maltodextrin could cause crystallisation at higher storage temperatures, leading to disruption of the wall matrix which would result in the release and degradation of encapsulated actives (Sansone *et al*., 2011a). Furthermore this causes agglomeration and caking of microparticulate powders (Sansone *et al*., 2011a).

Native inulin is in a semi-crystalline state (Zimeri & Kokini, 2002). Most commercially available types of inulin are amorphous as they are produced by spray-drying (Ronkart *et al*., 2009; Mensink *et al*., 2015) and undergo glass transition during dehydration/rehydration and freeze/thaw processing. There have been various studies on the T_g of inulin, showing that its T_g depends the degree of polymerisation (DP) (Ronkart *et al*., 2009) and moisture content (Zimeri & Kokini, 2002). At moisture contents of 4% (dry weight) low (DP = 7) and high (DP = 27) molecular weight inulin have T_g values > 320°C (Kawai *et al*., 2011). Compared to smaller carbohydrates like sucrose and fructose, inulin has a much higher T_g, but other glucans of similar molecular weight have even higher T_g values (Mensink *et al.,* 2015). Low moisture contents retain the amorphous state, but at a_w > 0.75 inulin recrystallised, reaching the crystallinity level of native inulin (Zimeri & Kokini, 2002). Kawai *et al*. (2011) found that high molecular weight inulin showed semi-crystalline behaviour in comparison to lower molecular weight inulin which was completely amorphous. They also confirmed that T_g decreased with increasing moisture content.

2.8.1 Interaction of ingredients

A critical factor to consider in the development of functional beverages is the effect of other ingredients on the stability of bioactive compounds (Corbo *et al*., 2014). Interactions with other ingredients could lead to reactions which cause precipitate formation, oxidation, insolubility or degradation which would compromise the functionality of bioactive compounds (Corbo *et al*., 2014). The addition of other ingredients could also affect the relative humidity at which deliquescence occurs and thus stability of the powder (Kwok *et al.,* 2010; Stoklosa *et al*., 2012).

Deliquescence is the first-order phase transition process whereby a substance dissolves in atmospheric moisture when the relative humidity (RH) reaches a certain critical value, the deliquescence RH (RH_0). Below the RH_0, a deliquescent solid adsorbs only a minimal amount of water, but when the ambient RH reaches the RH_0, water begins to condense on its surface and the solid starts to dissolve in the condensed moisture. The water activity is lowered by the presence of the dissolved solute, and further absorption of water occurs until a saturated solution forms. If there is more than one deliquescent substance present in a mixture, the RH_0 is lower than the lowest RH_0 of the individual substances (Kwok *et al*., 2010), leading to some level of dissolution at relatively low RH conditions. Dissolution arising as a result of

deliquescence will impact the chemical and physical stability of complex food systems. Deliquescence is important in terms of product stability, as it will enhance the degradation of labile food ingredients, given that chemical reactions occur much more readily in solution. RH fluctuations will lead to cycles of deliquescence and efflorescence (crystallisation), which will contribute to particle agglomeration and caking (Mauer & Taylor , 2010).

Ortiz *et al*. (2008) studied the effect of different iced tea dry ingredients and RH conditions (0-85% RH) on catechin stability in green tea powder over a period of three months. The study showed that the formulation as well as the interaction between RH and formulation significantly increased catechin degradation over time. Degradation increased as RH increased and was exacerbated at ≥ 58 % RH by the presence of citric acid and at ≥ 75 % RH by the presence of ascorbic acid. While catechin stability was maintained at ≤ 43 % RH caking was still observed at these RH values.

The pharmaceutical industry places a lot of importance on the compatibility of bioactive compounds and other excipients (or ingredients) within the formulation step of a new drug (Chadha & Bhandari, 2014). The techniques used to determine compatibility are also relevant in the development of nutraceutical products (Li *et al*., 2014) and will be discussed in further detail in the following section.

2.8.2 Accelerated storage

The degradation of substances in the solid-state is usually a very gradual process, especially in crystalline form (Bott *et al.*, 2010). It is therefore normal to accelerate this process by storing these substances at high temperatures and relative humidity conditions (Bott *et al.*, 2010). Under accelerated storage conditions chemical reactions and changes in physical stability are accelerated so that predictions regarding the shelf-life of the product can be made and/or changes introduced to prolong shelf-life (Waterman & Adami, 2005). In the food industry products are designed to undergo minimal changes during their normal distribution and storage. By using accelerated storage conditions, the shelf-life and thus when a product will have to be withdrawn from the market, could be predicted (Corradini & Peleg, 2007). In the pharmaceutical industry standardised storage conditions have been stipulated for the testing of solid-state drugs. Ambient conditions include 25 °C at 60 % RH and accelerated conditions

include 40 °C at 75 % RH for a period of six months. Accelerated conditions also imitate extreme environmental conditions such as those experienced at the tropics.

Cortés-Rojas *et al.* (2014) in a shelf-life study of spray dried *Bidens pilosa* L. extract demonstrated that samples stored under conditions which exposed them to the relative humidity of the storage cabinets showed rapid degradation of flavonoids. Samples which were stored in sealed packaging were stable for up to a year. Degradation was shown to be more dependent on the uptake of water, although temperature also had an effect on certain compounds. This illustrated the importance of packaging and correct storage conditions to preserve bioactive compounds in dried extracts (Cortés-Rojas *et al.*, 2014).

Moraga *et al*. (2012) stored freeze-dried grapefruit at different relative humidity conditions and tested the samples at month three and six. It was found that the degradation of flavonoids was linked to an increase in RH. It was also concluded that in order to maintain stability during long term storage the amorphous matrix must be guaranteed by preventing uptake of moisture.

2.9 Characterisation of powders

During the spray-drying of natural extracts a number of physical and chemical changes take place, depending on stability of the bioactive constituents, as previously described. It is therefore important to characterise the powders to determine if product requirements have been met (Chiou & Langrish, 2007). It is also important to assess interactions between different components within a formulation to determine if they are compatible (Chadha & Bhandari, 2014).

Two factors which have the biggest impact on the stability of solid state products are temperature and moisture (Chadha & Bhandari, 2014). High temperatures increase the rate of degradation (Bott *et al.*, 2010). Water acts as a plasticiser which increases molecular mobility, enhances reaction rates and alters product quality. Amorphous solids are generally more soluble and less stable than their crystalline counterparts and may undergo crystallisation if enough water is present to act as a plasticiser (Bott *et al.*, 2010). The moisture content not only has an effect on the amorphous state of the powder which in turn has an effect on the stability of the bioactive compounds, but it also affects a number of other physical properties such as dissolution rate and flowability (Chiou & Langrish, 2007). Major changes in samples can usually be observed visually though changes in colour, aggregation of powders and any other physical changes which indicate incompatibility of ingredients and degradation of the product. However, more in-depth analyses are required to explain why these changes occur.

When characterising powders it is important to take the following into consideration: moisture content, compound retention (e.g. phenolic retention), flowability, dissolution efficiency and size analysis (Chiou & Langrish, 2007; Stoklosa *et al*., 2012). In the pharmaceutical industry a number of techniques have been developed to detect incompatibilities between active ingredients and excipients used in formulations as well as to define other physicochemical properties of the product. These include thermal methods such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), differential thermal analysis (DTA) and isothermal microcalorimetry. Spectroscopy methods include X-ray powder diffraction (XRPD) and Fourier transform-infrared spectroscopy (Chadha & Bhandari, 2014). Methods used in this study will be described in detail.

2.9.1 Compound stability and functionality

2.9.1.1 HPLC analysis

High-performance liquid chromatography (HPLC) is the most widely recognised method of quantifying phenolic compounds. This technique is also suitable for routine quantitative and qualitative analysis as a method of quality control of plant material. Many reviews have dealt with the principles and/or the application of HPLC in the analysis of plant extracts and these will not be discussed here. A considerable body of research has been devoted to developing appropriate HPLC methods for *Cyclopia* species, specifically those of commercial importance (De Beer & Joubert, 2010). Most recently species-specific HPLC methods have been developed to accommodate inter-species variation in the phenolic profile of *C. subternata* (De Beer *et al*., 2012), *C. maculata* (Schulze *et al*., 2014), *C. genistiodes* (Beelders *et al*., 2014) and *C. longifolia* (Schulze *et al*., 2015). The new HPLC method for *C. subternata* (De Beer *et al*., 2012) overcame the limitations of the previous generic method (De Beer & Joubert, 2010), including the co-elution of unidentified compound(s) with isomangiferin and a complicated integration process due to the elution of eriocitrin, scolymoside and phloretin-3',5'-di-*C*-β-glucoside on a polymeric hump.

Measuring the stability of natural substances can be quite challenging because of their complexity (Bott *et al.*, 2010). They contain thousands of different substances some of which have defined therapeutic activity and some of which remain unknown (Bott *et al.*, 2010). One of the best ways of determining the efficacy is to use quality markers, which have been proven to have pharmacological activity (Bott *et al.*, 2010).

In order to analyse compound stability throughout the spray-drying process the extract is tested before and after spray-dying. A range of compounds which are found in significant quantities and which possess therapeutic benefits are selected as quality markers. Comparing the concentrations of these compounds before and after spray-drying indicates the degradation of these important polyphenols. Information on stability of specific polyphenol constituents of plant extracts is however scarce as changes in total polyphenol content and/or the antioxidant capacity (Ersus & Yurdagel, 2007; Da Silva *et al*., 2011; Nunes *et al*., 2015) are mostly determined.

2.9.1.2 Total antioxidant capacity and total polyphenol content

While HPLC determines the concentration of specific compounds the less-specific total antioxidant capacity (TAC) and total polyphenol content (TP) assays are used as a form of routine analysis to standardise herbal extracts. Not only are the methods relatively simple and fast, but adaptation to 96-well plate format allow for high through-put (Zhang *et al*., 2006; Medina-Remón *et al*., 2009).

The first method for determining TP content dates as far back as 1912 and was originally called the Folin and Denis method. This method was later updated by Folin and Ciocalteau in 1927 and adapted by Singleton and Rossi in 1965. Minor variations to their method have been used such as an increase in reaction temperature to shorten the reaction time (Cicco *et al*., 2009; Magalhães *et al*., 2010) and the use of other standards as alternative to gallic acid, but basically the classical method as employed today remained more or less the same. The Folin-Ciocalteau assay is based on the principle that phenolic compounds are oxidised in an alkaline medium containing molybdenum and tungsten to produce a bright blue complex. The intensity of this complex is measured at 765 nm and the total content of phenolic compounds is calculated in gallic acid equivalents (Bajcan *et al*., 2013). In addition to being a quick method, it is easy to use and the solvents are affordable and easy to source (Zhang *et al*., 2006). The Folin-Ciocalteu method has been extensively applied to characterise plant extracts, including rooibos (Joubert *et al*., 2008b; Joubert & De Beer, 2012; Joubert *et al*., 2012) and honeybush (De Beer *et al*., 2012; Joubert *et al*., 2008b; Joubert *et al*., 2012).

Several TAC assays have been described in literature. One of these is the method based on the ability of antioxidants to reduce DPPH radicals ($DPPH^{\bullet}$) which is measured by a colour change from purple to yellow. The decrease in absorbance is measured spectrophotometrically at 515 nm (Tirzitis & Bartosz, 2010; Krishnaiah *et al*., 2011). This method is easily reproducible due to the fact that $DPPH^{\bullet}$ is a stable free radical which is commercially available and does not have to be produced before the assay like other radicals such as $ABTS^{\bullet+}$ (Tirzitis & Bartosz, 2010). It also has the advantage of being sensitive enough to detect active ingredients at low concentrations (Hsu *et al*., 2005). The method has also been adapted to 96-well micro-plate format (Arthur *et al*., 2011) and applied for analysis of *Cyclopia* extracts (De Beer *et al*., 2012; Beelders *et al*., 2015).

While both of these methods remain important for quality control of natural extracts they are not specific for phenolics and may suffer from interference by various no-phenolic reducing substances. Furthermore these analysis are not an indication of the efficacy of the antioxidants and cannot be used to make health claims (Becker, 2013). This requires more in-depth analysis of the bioavailability and mechanism of action as this differs from compound to compound (Becker, 2013). It is important to note that the degradation of phenolic compounds due to heating does not necessarily result in a direct correlation with a decrease in antioxidant activity. In some cases the degradation products have an equal or higher antioxidant activity than the original phenolic compound (Buchner *et al*., 2006; Murakami, *et al*., 2004). Interactions can also occur with the food matrix which could cause the antioxidant activity to be enhanced, reduced or remain the same (Irina & Mohamed, 2012).

2.9.2 Moisture content and water activity

Water is considered to be the most important plasticiser or mobility enhancer in hydrophilic food components. Controlling the moisture content of foods has therefore been one of the most ancient forms of food preservation (Al-Muhtaseb *et al*., 2002). Water content and water activity are two of the most significant factors which play a role in the stability of powdered extracts.

2.9.2.1 Moisture content

Moisture content determination is one of the most frequently performed analysis on food products due to the critical role that it plays on stability. While there are various methods of determining moisture content, as reviewed by Mathlouthi (2001), the most common method is the desiccation method. Moisture content is expressed as a percentage of the total weight of the product and is measured by drying the sample at elevated temperatures until no loss is observed. This can be done using an automated moisture analyser or in an oven. One of the biggest drawbacks of this method is the fact that weight loss could be caused by the volatility of gases other than water (Mathlouthi, 2001). The moisture content alone is not a good indication of product stability because it does not give an indication of the nature of the water that exists within the product. Therefore water activity measurements are also required to ascertain the shelf-life of the product (Mathlouthi, 2001). The aim of spray-drying is to decrease the moisture content of the powder significantly in order to optimise its stability. It is therefore an important

factor to take into consideration when selecting the spray-drying conditions of the product and serves as a quick indicator when something has gone wrong in the system.

2.9.2.2 Water activity

Water exists in food in different forms which are based on its interactions with the various food components (Andrade *et al.*, 2011). Water activity is a term which describes the degree to which water is bound within a food system (Al-Muhtaseb *et al*., 2002). It is understood that water exists with either hindered or unhindered mobility. Free or bulk water within a system has the ability to act as a solvent and increases the rate of deleterious reactions within food products. Water which is bound to other food constituents via hydrogen bonds stronger than those with other water molecules exhibits physical properties significantly different from those of free water (Al-Muhtaseb *et al*., 2002). Some of these characteristics include lower water vapour pressure, higher binding energy, reduced mobility, inability to freeze at low temperatures and unavailability as a solvent.

Water activity therefore gives an indication of the amount of water available for chemical, biochemical and microbiological reactions which could lead to deterioration (Andrade *et al.*, 2011). It is defined as the ratio of water vapour pressure in the food system (Pv) and the pure water vapour pressure ($Pv\ sat$) at a constant value of pressure and temperature (Andrade *et al.*, 2011) as expressed with the following equation:

$$a_w = \frac{Pv}{Pv\ sat}$$

Therefore water activity of food equals the relative humidity of the air surrounding it divided by 100, which means that equilibrium has been reached (Andrade *et al.*, 2011).

Water activity is an important criterion used to determine the quality and safety of food products (Cortés-Rojas & Oliveira, 2012). Reducing the water activity of foods is used to control degradation reactions and the growth of spoilage microorganisms (Cortés-Rojas & Oliveira, 2012). It is important to aim for a water activity value below 0.5 because at water activities above this level microorganisms are able to grow. This is particularly important in the drying process which causes changes in the water binding, dissociation and solubility of

the compounds (Cortés-Rojas & Oliveira, 2012). Cortés-Rojas and Oliveira (2012) concluded that at a set inlet temperature the variation in water activity between samples was dependent on the feed composition and was related to the hygroscopicity of the product.

2.9.2.3 Moisture sorption isotherms

While water activity is a useful measure of the behaviour of moisture in food, it is limited in its ability to predict shelf-life under different relative humidity and temperature conditions. Therefore it is necessary to establish moisture sorption isotherms (MSI) in order to account for the behaviour of food products under a range of relative humidities that it may be exposed to during storage (Mathlouthi, 2001). MSI describe the relationship between water activity and the equilibrium moisture content of a food product at a constant temperature (Gabas *et al*., 2007). Knowledge of MSI is important for food processing methods such as drying, storage and packaging (Gabas *et al*., 2007). They are used to calculate drying times, predict behaviour of ingredients on mixing, select the correct packaging, model moisture changes during storage and estimate shelf-life stability, all of which are important for food powders (Gabas *et al*., 2007; Anon, 2011). MSI gives information about the mechanisms of moisture uptake in foods, as well as the interactions between food components and water (Gabas *et al*., 2007).

About 270 mathematical models exist to describe the moisture sorption behaviour of foods (Anon, 2011; Al-Muhtaseb *et al*., 2002). Some of them were developed around theories of sorption mechanisms, while others are simply empirical or semi-empirical. The sorption model is selected based on the degree of fitting to the experimental data, as well as the physical meaning of the model (Gabas *et al*., 2007). Andrade *et al.* (2011) described the uses and limitations of the most common models which are used for food systems, including the Langmuir, Brunauer, Emmett and Teller (BET) and Iglesias-Chirife equations, as well as the Oswin, Smith, Halsey, Henderson, GAB and Peleg models. The most commonly used models are the BET and GAB models. BET is frequently used in the food industry, however it is only applicable at $a_w \leq 0.5$. Therefore the GAB model is considered to be the most useful when characterising isotherms across the entire water activity range. Both of these models have coefficients which provide the monolayer moisture content (Anon, 2011).

Molecular monolayer water refers to the amount of water which is strongly adsorbed to specific sites on the surface of the material (De Souza *et al*., 2013a). It is an important parameter given by the models and is considered to be a critical value above which water is more available for chemical reactions and degradation (De Souza *et al*., 2013a). Studies have shown that the moisture in the monolayer decreased as the concentration of carriers such as maltodextrin and gum Arabic increased. These carriers influence the ratio of hydrophilic/hydrophobic sites, thereby increasing the sorption of water to these sites (De Souza *et al*., 2013a).

When analysing solid sorbent materials, the phenomena of moisture sorption hysteresis should be taken into consideration. Moisture sorption hysteresis is the situation whereby two different paths exist for the absorption and desorption isotherms (Labuza & Altunakar, 2007). An adsorption isotherm is obtained by placing a completely dry material into various atmospheres of increasing relative humidity and measuring the weight gain due to moisture uptake. A desorption isotherm is the inverse whereby a completely wet material is placed into the same relative humidity conditions and the weight loss is measured (Al-Muhtaseb *et al*., 2002). When hysteresis occurs the desorption curve usually lies above the adsorption curve and a closed hysteresis loop is formed (Fig. 2.4). The extent of hysteresis is related to the state and nature of the product constituents. When hysteresis occurs it is usually an indication of structural or conformational rearrangement which affects the availability of favourable polar sites and therefore may hinder the movement of moisture (Al-Muhtaseb *et al*., 2002; Anon, 2011).

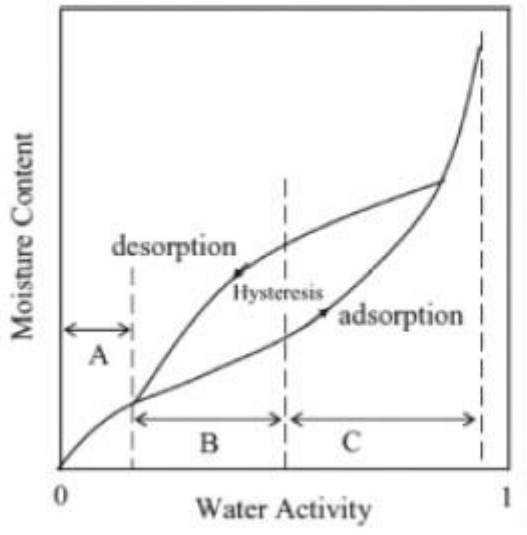


Figure 2.4 Sorption isotherm for a typical food product showing hysteresis (Andrade *et al*., 2011).

In the typical food product a generalised moisture sorption isotherm can be divided in three sections as seen in Fig. 2.4. Section A represents the monolayer or strongly bound water. Most dried foods are considered to be the most stable within this region. Region B represents water molecules which are less firmly bound and exist as multi-layers above the monolayer. Here the water is held in the solid matrix by capillary action and is available as a solvent for low-molecular weight solutes and some biochemical reactions. Water in region C possesses all of the properties of bulk water and forms the fluid phase in high moisture materials. Under these conditions food products are more susceptible to microbial growth and other deteriorative reactions (Al-Muhtaseb *et al*., 2002).

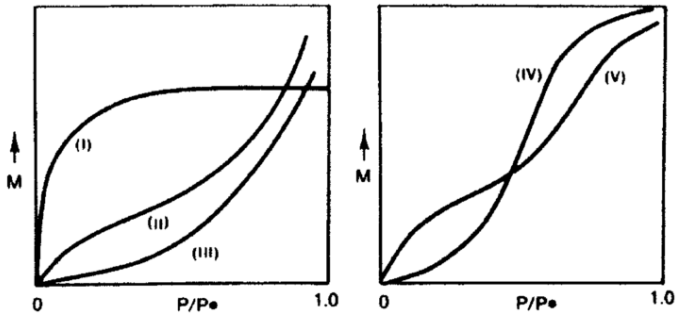


Figure 2.5 Five types of van der Waals adsorption isotherms (Al-Muhtaseb *et al*., 2002).

Based on the van der Waals theory of adsorption of gases on various solid substrates, adsorption isotherms were classified by Brunauer (1945) into five general types (Fig. 2.5). Type I and Type II have been called the Langmuir and the sigmoid shaped adsorption isotherms respectively. The other three do not have any specified names. It is important to note that Types II and III are closely related to Types IV and V, except that the maximum adsorption occurs at a pressure lower than the vapour pressure of the gas. The moisture sorption isotherms of most foods are nonlinear and are generally classified as sigmoidal Type II isotherms (Al-Muhtaseb *et al*., 2002).

Sinija & Mishra (2008) studied the MSI of instant green tea powder and green tea granules. It was found that the isotherms presented a sigmoid shape and were therefore classified as type II. As seen in Fig. 2.6 the equilibrium moisture content (EMC) increased with

decreasing temperature at a constant relative humidity. This was explained by the higher excitation state of water molecules at a higher temperature which decreases the attractive forces between them. Furthermore as expected the EMC increased with increasing RH.

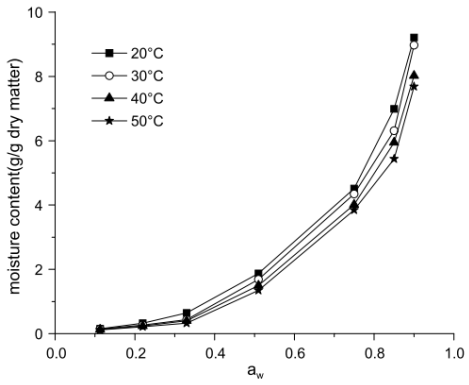


Figure 2.6 Adsorption isotherms of instant iced tea powder at different temperatures (Sinija & Mishra, 2008).

Nogueira *et al*. (2007) studied the MSI of inulin which represented a type III curve (non-sigmoidal) and followed similar adsorption trends to high sugar foods. This is an indication that the inulin had a low DP or was enriched with low molecular weight sugars because the MSI of inulin usually represents type II curves (Ronkart *et al*., 2006). The monolayer values for inulin were also calculated as 0.0522, 0.0528 and 0.0505 g/g dry basis at 25 ºC, 35 ºC and 45 ºC, respectively using the BET model (Nogueira *et al*., 2007).

The MSI of powder blends such as sugar and citric acid have been studied in detail by Kwok *et al*. (2010) and Stoklosa *et al*. (2012). Stoklosa *et al*. (2012) observed that moisture adsorption increased with a decrease in particle size. Smaller particle size was also linked to a greater hysteresis loop. In this case the hysteresis was attributed to capillary condensation in the pores – smaller pores result in more difficulty in removing water.

2.9.3 Thermal analysis

Foodstuffs undergo a number of physical, chemical and biological changes throughout the stages of processing, storage and distribution. Understanding these phase transitions is useful for characterising their quality and creating optimised processing conditions (Silva *et al*., 2006). Thermal analysis is a method of determining the temperatures at which transitions take

place and is a standard method used to study the stability of substances with pharmaceutical potential (Šimon *et al.*, 2004). It is often used as a quick assessment for physicochemical incompatibility between ingredients in the formulation step because it does not require the long storage time and multiple sample preparation associated with other testing methods (Chadha &Bhandari, 2014).

One of the most important characteristics that thermal analysis determines is the T_g. Glass transition temperature is defined as the temperature at which an amorphous system changes from the glassy to liquid-like rubbery state (Silva *et al.*, 2006). In the glassy state, molecular mobility is low due to the high viscosity of the matrix therefore eliminating diffusion controlled reactions (Silva *et al.*, 2006).

Conversion of spray-dried powders from a high energy amorphous state to low energy crystalline state has been linked to the T_g and is exacerbated by the presence of moisture (Chiou & Langrish, 2007; Shrestha *et al.*, 2007). Fig. 2.7 shows the moisture induced crystallisation of (-)-epigallocatechin gallate powder at different relative humidity conditions. It is characteristic for amorphous powders to exhibit substantial moisture uptake under inadequate storage conditions (Bott *et al.*, 2010). This has negative implications for the stability of the powder due to the fact that crystallisation could cause disruption of the wall matrix and release of the encapsulated phenolic compounds (Sansone *et al.*, 2011a). Furthermore it causes agglomeration and caking in microparticulate powder which reduces flowability (Sansone *et al.*, 2011a; Chiou & Langrish, 2007).

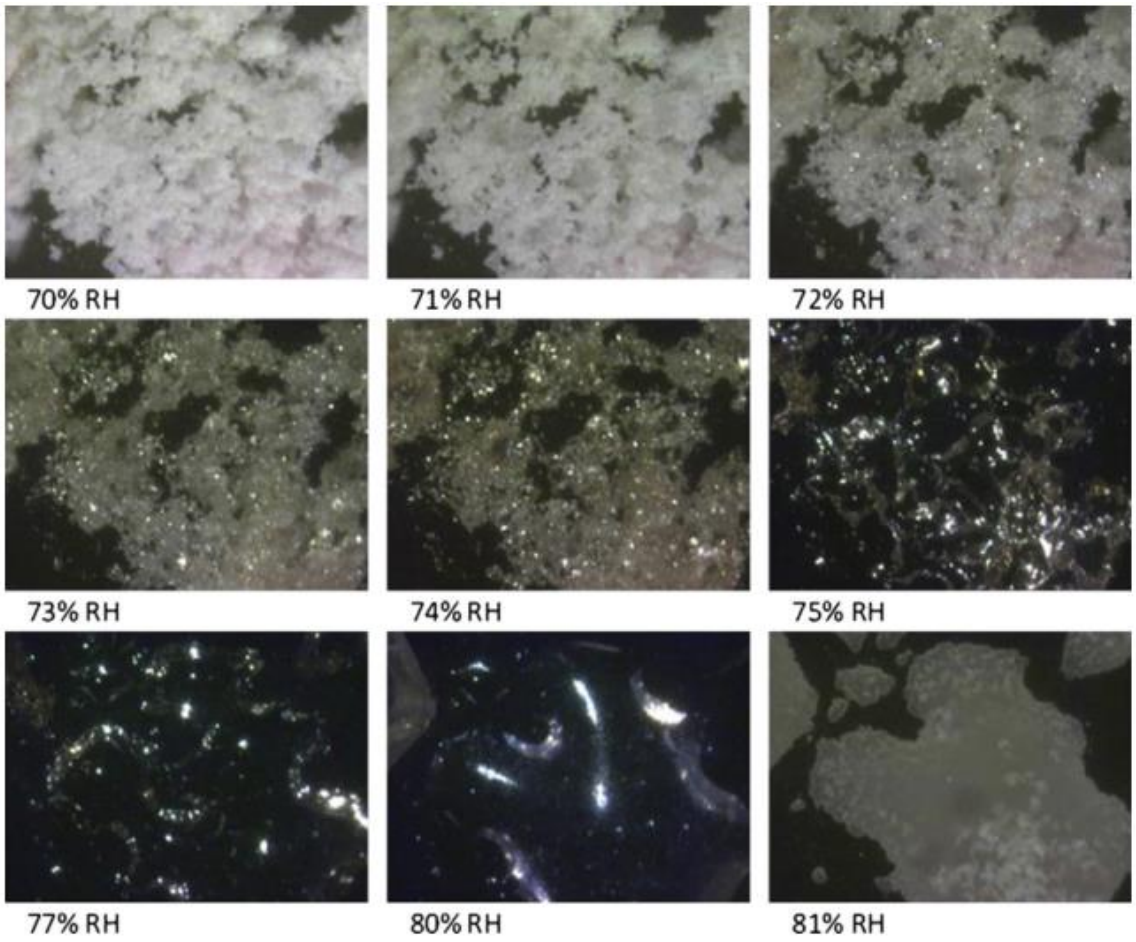


Figure 2.7 Moisture induced crystallisation of amorphous (-)-epi-gallocatechin gallate (Li *et al*, 2014).

It is also important to note that the T_g is different for each specific material (Shrestha *et al*., 2007). In order to reduce stickiness, carriers with a high molecular weight are sometimes added to spray-dried products to increase the T_g (Silva *et al.*, 2006). Therefore T_g can be taken as a reference parameter to characterise powder properties, quality, stability and safety (Silva *et al.*, 2006).

2.9.3.1 Differential Scanning Calorimetry (DSC)

DSC is an important thermal analytical technique which has been used to screen for incompatibilities in pharmaceutical products for more than 50 years (Chadha & Bhandari, 2014). It measures the change in heat capacity of a material as it goes through a heating cycle

usually from 30 – 300 °C. It can be used to determine the melting behaviour, heat of fusion, purity, polymorphism, T_g and crystallisation temperature of a substance (Shrestha *et al*., 2007; Šimon *et al*., 2004). While this method has many advantages it should be noted that the interpretation of data may not always be straightforward. For instance samples are exposed to very high temperatures (up to 300 °C) and interactions which are observed at these temperatures may not be relevant to samples stored under ambient conditions (Chadha & Bhandari, 2014). However it can give valuable information relating to the effect of heating at high temperatures such as those experienced during spray-drying.

2.9.3.2 Thermal Gravimetric Analysis (TGA)

TGA is used to study the solid-state kinetics of a sample by measuring its weight while it is heated at a constant rate or held at a constant temperature (Khawam & Flanagan, 2006). TGA can be used to indicate the formation of complexes between phenolic compounds and carriers (Hădărugă *et al*., 2012). Da Rosa *et al.* (2014) confirmed the encapsulation of blackberry phenolic extracts in β-cyclodextrin, chitosan, xanthan and hydro-gel matrixes using TGA. This was achieved by comparing the loss in mass of the complex with a loss of mass of the pure extract (physical mixture). It was found that the microcapsules had a lower mass loss than the pure extract (Da Rosa *et al.*, 2014).

2.9.3.3 Differential Thermal Analysis (DTA)

DTA is performed with a piece of equipment which combines DSC and TGA and measures both thermograms at the same time. These methods have the benefits of being rapid, requiring small amounts of sample and being able to easily detect changes in polymorphic form and conversions from amorphous to crystalline form. However they are not effective when thermal changes are very small. They also have shortcomings in that they are unable to resolve overlapping thermal events that occur at the same temperature. It should also be noted that certain test materials may exhibit properties which make the interpretation of data difficult (Chadha & Bhandari, 2014).

2.9.3.4 Isothermal Microcalorimetry

Calorimetry is a technique which is used for direct determination of rate of heat production as well as heat and heat capacity as a function of temperature and time. Microcalorimetry is a powerful tool which allows for the detection of instabilities between two substances, such as active pharmaceutical ingredients and excipients (Aucamp, M., 2014, Department of Pharmaceutical Sciences, North West University, personal communication, 23 July 2014). This is based on the principle that almost all physical and chemical processes are accompanied by heat exchange within their environment (Chadha & Bhandari, 2014; Briggner *et al*., 1994). Analysis can be performed on a Thermal Activity Monitor (TAM) which is a highly sensitive piece of equipment. It detects minute amounts of heat which are dissipated or absorbed in the µW range (Chadha & Bhandari, 2014). Other advantages include its ability to detect reactions which occur very slowly (Aucamp, M., 2014, Department of Pharmaceutical Sciences, North West University, personal communication, 23 July 2014).

The thermal activity of the individual components as well as the mixture is measured individually where after the output of the mixture is compared to the "non-interaction" curve, calculated from the individual components. If an experimentally significant difference is observed, the mixture components are considered to be potentially incompatible, e.g. in the case of a bioactive extract formulated with other ingredients in a food product (Chadha & Bhandari, 2014).

It is important to note that the heat flow data could contain contributions from either one reaction or several reactions. It should therefore be used as a screening method at the start of development of a product to indicate potential incompatibilities. It can therefore be used to reduce the number of samples which are required for testing in time-consuming shelf-life stability trials, involving HPLC and other methods (Chadha & Bhandari, 2014).

This method could therefore be useful to determine whether there were interactions between the carriers and the extract during spray-drying. It could also be helpful during the formulation step of an iced tea to determine if the different ingredients are compatible.

2.9.4 X-ray powder diffraction

XRPD is a direct measure of the crystal form of a product and can be used to determine whether the spray-dried powder is in crystalline or amorphous state (Chadha & Bhandari, 2014; Cortés-

Rojas & Oliveira, 2012). The output is a plot of intensity versus diffraction angle (2ºθ). Substances in crystalline states possess a characteristic reflection pattern (see ketoprofen (KT) in Fig. 2.8), which allows it to be distinguished from other substances. Amorphous substances (see polyvinylpyrrolidone K30 **(**PVP) in Fig. 2.8) are confirmed by the lack of defined peaks which produces a typical halo pattern (Bott *et al.,* 2010). This occurs due to the fact that molecules in an amorphous state have broad angles and are randomly oriented (Bott *et al.,* 2010). In a study performed by Baldinger *et al.* (2012) on spray-dried mannitol and trehalose, characteristic reflections for β-mannitol were observed in the XRPD pattern while no reflections correlating to trehalose were observed. This showed that the spray-drying process resulted in crystalline mannitol and amorphous trehalose.

X-ray diffraction can also be used to track the recrystallisation of spray-dried amorphous powders (Briggner *et al*., 1994) as well as test for interactions between substances. Tit *et al.* (2011) performed a compatibility study of KT with PVP and magnesium stearate (MS). When comparing the X-ray diffractograms of the physical mixtures of KT with PVP (Fig.2.8A) and MS (Fig.2.8B) with those of its individual components, it is evident that some of the peaks present in the individual components disappeared, while others showed a change in intensity therefore indicating interaction.

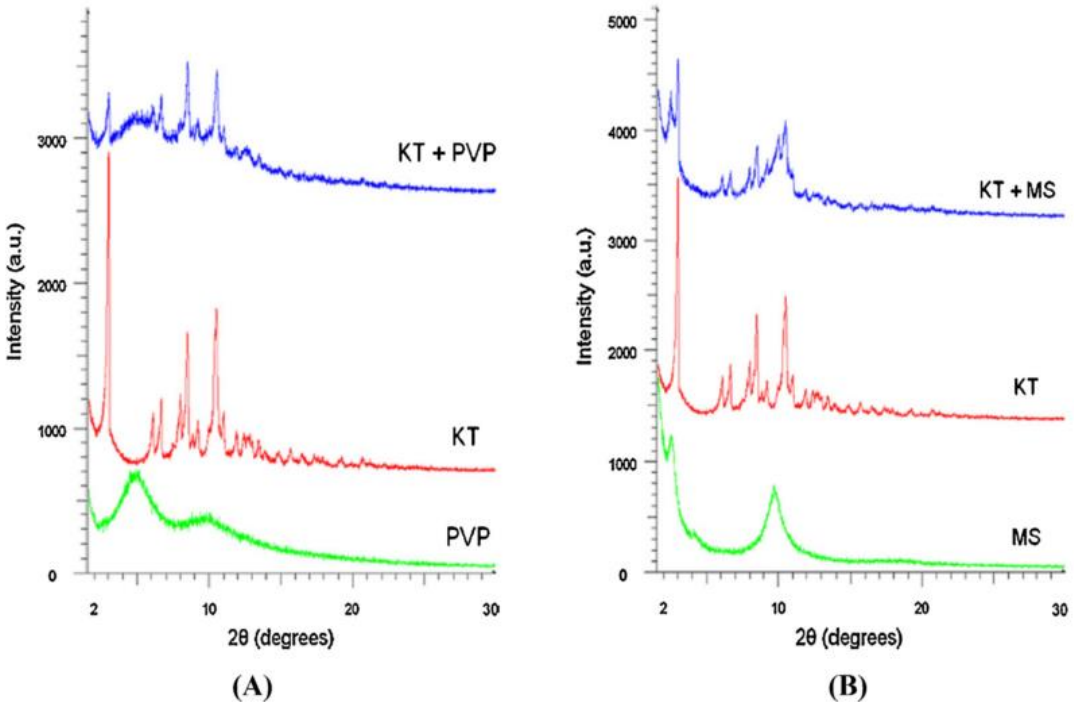


Figure 2.8 (A) X-ray diffractogram of PVP, KT and 1:1 blend of KT and PVP. (B) X-ray diffractogram of PVP, KT and 1:1 blend of KT and MS (Tit *et al*., 2011).

The degree of crystallinity affects a number of powder properties such as stickiness, porosity, flowability and solubility (Cortés-Rojas & Oliveira, 2012). The extent of crystallinity is related to interplay between the drying conditions and the glass transition temperature of the constituents (Cortés-Rojas & Oliveira, 2012). When products have a low glass transition temperature there is a big difference between the temperature of the drying gas and T_g which results in crystallisation during spray-drying (Cortés-Rojas & Oliveira, 2012). However, as noted previously, amorphous powders are usually formed during the spray-drying of complex mixtures such as herbal extracts (Cortés-Rojas & Oliveira, 2012).

Ronkart *et al*. (2006) used X-ray diffraction to study the crystallinity changes of spray-dried inulin during storage at different relative humidities at 20°C. At the start of the storage experiment and at low RH only one peak was present, indicating the amorphous nature of the native inulin. The inulin remained in this state up to 0.56 a_w conditioning. With an increase in RH the diffractograms showed more peaks, the increasing intensities and peak areas correlated with the development of crystallinity in the sample (Fig.2.9). The crystallisation process was facilitated by the presence of moisture in the sample which increased the molecular mobility within the system and decreased the T_g to below the storage temperature. In this case it dropped from 48 to 15 °C.

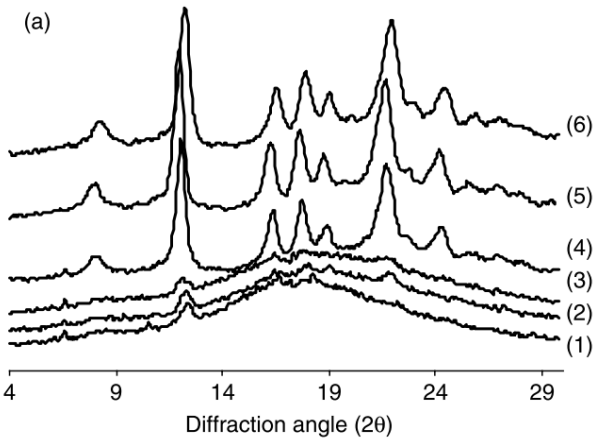


Figure 2.9 Powder X-ray diffractograms of inulin obtained on adsorption isotherm in the water activity range of 0.06–0.93. The different saturated salts used were P_2O_5 (1) LiCl, (2) $MgCl_2$, (3) NaBr, (4), NaCl, (5) and KNO_3 (6).

2.9.5 Wettability

The ability of food powders to dissolve and disperse in water is very important to ensure that they are easy to use. Wetting is a precursor to dissolution and the wettability of a solid material thus has a significant effect on its dissolution rate. It also plays a role in the interaction of the different ingredients when in contact with water (Hogekamp & Schubert, 2003). During the measurement of the contact angle the powders are pressed flat. A droplet of purified water is then dropped onto the surface while a video records the interaction between the water and the surface of the powder. The first frame to feature a well-defined vibration-free drop is analysed to determine the contact angle (Stieger, N., 2014, Department of Pharmaceutical Sciences, North West University, personal communication, 23 July). Contact angle is measured through the liquid phase and is inversely proportional to wettability (Yuan & Lee, 2013):

$$Contact\ angle \propto \frac{1}{Wettability}$$

This method has found previous application in powder formulation of pharmaceuticals but has not been widely used for food products (Kiesvaara *et al.*, 1993; Prestidge & Tsatouhas, 2000). When analysing the results, the lower the contact angle, the greater the wettability of a powder. Contact angles below 90º correspond to high wettability. With a contact angle < 90° the fluid will spread over a large area on the surface, while contact angles > 90° generally means that wetting of the surface is unfavourable. The fluid will minimise its contact with the surface and form a compact liquid droplet (Yuan & Lee, 2013).

2.9.6 Colour

Colour is an important quality attribute of dried natural extract because it is also perceived as an indicator of quality to the consumer and a change in colour is associated with degradation of active ingredients in pharmaceutical products (Hetrick *et al.,* 2013). Due to the complexity of plant extracts, many compounds are prone to oxidation and hydrolysis which is reflected in colour changes (Cortés-Rojas *et al*., 2014). This is particularly prevalent in samples which absorb water during storage (Cortés-Rojas *et al*., 2014). Inadequate processing and storage conditions in functional foods may lead to a loss of bioactive compounds and result in colour

changes and it is therefore important to measure the rate of change of these parameters during storage (Harbourne *et al.*, 2013).

Instrumental colour measurements are more objective than visual measurements made by the human eye (Hetrick *et al.*, 2013). Tristimulus colorimetry is an instrumentation method whereby the parameters for colour perception namely illumination, sample and observer are standardised and well defined (Hetrick *et al.*, 2013).

Several colour coordinate systems have been developed to describe the colour of an object (Pathare *et al*., 2013). One of the most commonly used methods in the food industry is the Commission Internationale de l'Eclairage's (CIE) L*a*b* system. CIELAB is a uniform space which is defined by two colour coordinates a* and b*, as well as a psychometric index of lightness L* (Fig.2.10). The parameter a* takes positive readings for reddish colours and negative readings for greenish ones while the parameter b* takes positive readings for yellowish colours and negative readings for bluish ones. L* is an approximate measurement of luminosity according to which each colour can be considered equivalent to a value on the grey scale, between black and white (Pathare *et al*., 2013).

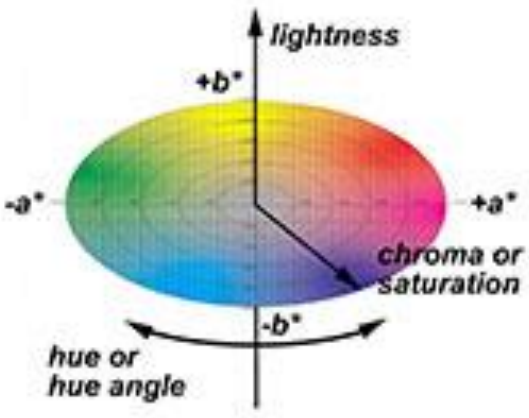


Figure 2.10 (CIE) L*a*b* colour space system.

Chroma (C*) is considered to be a quantitative value for colourfulness which contrasts the degree of difference between a hue and the same degree of lightness on the grey scale. The

higher the chroma is the higher the degree of colourfulness as perceived by humans (Pathare *et al*., 2013). It is calculated as follows:

$$C* = \sqrt{a*^2 + b*^2}$$

Hue angle (h*) is considered to be a qualitative attribute of colour according to which colour is defined as reddish or bluish etc. (Pathare *et al*., 2013). It is used to define a specific colour with reference to the correlating grey colour with the same lightness using the following formula:

$$h* = tan^{-1}\left(\frac{b*}{a*}\right)$$

An angle of 0° or 360° represents red hue, whilst angles of 90°, 180° and 270° represent yellow, green and blue hues, respectively (Pathare *et al*., 2013). The difference in colour between a control and a stored sample can be calculated as total colour difference (ΔE) (Pathare *et al*., 2013) according to the following formula:

$$\Delta E = \sqrt{\Delta a*^2 + \Delta b*^2 + \Delta L*^2}$$

2.10 Conclusions

Research into the health benefits of *Cyclopia* spp. (honeybush) has come about at a time when consumers are looking towards natural, bioactive substances as a means of enhancing their health and well-being. This indigenous species possesses a host of possibilities in terms of product development for the functional food and nutraceutical industries. The production of spray-dried extracts of honeybush is an important step in improving the stability and versatility of this product. Furthermore the value-adding aspect of the process is of economic importance as it increases returns on the international market and supports an industry which provides valuable employment opportunities in rural areas.

The development of honeybush extract into an instant iced tea with reduced sugar content is valuable in providing a healthy alternative to the sugar sweetened beverages which saturate the market. The development of such products is an important step in addressing the

global obesity epidemic provided that the stability of bioactive compounds can be ensured from production to the point of consumption.

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Chapter 3

Effect of spray-drying and formulation on the chemical and physicochemical stability of green *C. subternata* extract

3.1 Abstract

Green or "unfermented" *Cyclopia subternata*, containing bioactive phenolic compounds, has been identified for the production of value-added extracts for use in the nutraceutical industry. A hot water extract of green *C. subternata* was spray-dried, using corn syrup solids and inulin as carriers at three treatment levels (25, 50 and 75%). The effect of spray-drying on the individual phenolic content, total polyphenol content and the total antioxidant capacity of the treatments were determined. Physicochemical properties of the resulting spray-dried powders were characterised in terms of moisture content, water activity, moisture sorption isotherm and objective colour measurement. Isothermal microcalorimetry was used to determine if the carriers interacted with the extract. Simultaneous differential thermal analysis and thermogravimetry were applied to measure the phase transition temperatures of the powders. X-ray powder diffraction measurements were performed to confirm the crystalline or amorphous nature of the spray-dried powders. Spray-drying produced fine, light brown, free-flowing powders which were amorphous in nature. The moisture content and water activity of the powders fell within the range of the monolayer moisture values as calculated, using the BET model and moisture sorption isotherm data. Inulin produced similar powder characteristics to corn syrup solids. Both carriers were compatible with *C. subternata* extract, with the exception of mixtures containing 75% corn syrup solids. Heating conditions during spray-drying had a negligible effect on the bioactive polyphenol content and the radical scavenging capacity of the extract. Therefore spray-drying was considered to be a suitable method of producing dried honeybush extracts. Inulin, a natural prebiotic and fibre, was found to be a suitable substitute for corn syrup solids for the production of health products.

3.2 Introduction

In the ongoing search for novel sources of bioactive compounds *Cyclopia* species have been identified for the production of value-added extracts for use in the nutraceutical industry (Joubert *et al.*, 2011). Better known as honeybush, the "fermented" form is commonly consumed as herbal tea, while the green or "unfermented" form is preferred for the production of extracts due to higher concentrations of polyphenols (Joubert *et al.*, 2008; De Beer *et al.*, 2012a; Schulze *et al*., 2014; Beelders *et al*., 2015). Of particular interest is the xanthone, mangiferin, which has been found in high concentrations in *Cyclopia* spp. This compound is sought after due to its potent antioxidant capacity and other well-documented health benefits which include anti-diabetic, anti-mutagenic, anti-carcinogenic, anti-inflammatory and hypolipidaemic properties (Vyas *et al.*, 2012). *Cyclopia subternata* also contains substantial quantities of isomangiferin, a regio-isomer of mangiferin, and 3-hydroxyphloretin-3′,5′-di-*C*-β-hexoside, a dihydrochalcone, and iriflophenone-3-*C*-β-glucoside, a benzophenone. Other phenolic glycosides include, amongst others, the flavanones, hesperidin and eriocitrin, the dihydrochalcone, phloretin-3′,5′-di-*C*-β-glucoside, and the flavone, scolymoside (De Beer *et al*., 2012a; Kokotkiewicz *et al*., 2012). Aqueous extracts of *C. subternata* have been demonstrated to have anti-diabetic (Mose Larsen *et al*., 2008; Schulze *et al*., 2015) and anti-obesity properties (Dudhia *et al*., 2013), further substantiating interest in honeybush for the production of nutraceutical products.

A common application of tea extracts is the production of ready-to-drink beverages (Ortiz *et al*., 2008). Stability of phenolic compounds in such products is normally poor as shown for the rooibos dihydrochalcone, aspalathin (Joubert *et al*., 2010; De Beer *et al*., 2012b) and green tea polyphenols (Su et al., 2003). An alternative convenience product is dry instant iced tea mixes which need to be reconstituted in water before consumption. Iced tea is a growing sector of the functional beverage market and presents a convenient format for delivering polyphenols to consumers, as well as providing an alternative for unhealthy sugar sweetened beverages, which saturate supermarket shelves (Anon, 2015). For these purposes a stable, free-flowing powder is required.

Converting natural extracts to dry powder form makes them easier to handle, increases their stability during storage and allows for standardised dosage forms to be administered

(Sansone *et al.*, 2011; Murugesan & Orsat, 2011; Bott *et al*., 2010). Amongst the techniques used to dry natural extracts, spray-drying is used extensively (Gharsallaoui *et al*., 2007). It is common practice to spray-dry natural extracts with carriers for the purpose of protecting phenolic compounds throughout the process, as well as improving the physicochemical properties of the powder (Munin and Edwards-Lévy, 2011). A common carrier used by industry in a variety of food products, including dry mixes and beverages, is corn syrup solids. Similar to maltodextrin, it is widely used in industry due to the fact that it is cheap, versatile and readily available (Silva *et al*., 2013). However, it is highly refined and has a similar metabolic effect to that of sugar, which makes it unsuitable for products designed for diabetics (Gross *et al*., 2004). Inulin, on the other hand, is considered a prebiotic fibre, stimulating the growth of beneficial bifidobacteria species (Kolida *et al.*, 2002) and thus provides a healthy alternative for use in functional beverages (Siró *et al*., 2008; Sun-Waterhouse, 2011; Dehghan *et al.*, 2014). To date there has been no research on the effects of spray-drying with or without carriers on honeybush extracts.

The main objectives of this study were thus to determine the effect of spray-drying, as well as that of different carriers and carrier levels, on the chemical and physicochemical properties of green *C. subternata* extract. This was done with the purpose of creating a powder with suitable characteristics for application in an instant iced tea powder mixture. In order to assess the spray-drying process and to determine if suitable product requirements had been met, compatibility of the carrier and extract were evaluated. In addition, adsorption and desorption moisture isotherms, amorphous to crystalline behaviour and thermal characteristics of the spray-dried powders were determined to understand the behaviour and the properties of the powder. Colour of the powders was defined in terms of CIELab colour space parameters. HPLC-DAD was used to quantify phenolic content and total antioxidant capacity of the extract was evaluated in terms of its DPPH radical scavenging ability.

3.3 Materials and methods

3.3.1 Chemicals and reagents

Authentic reference standards with purity > 95% were obtained from Sigma-Aldrich (mangiferin, hesperidin, maclurin, iriflophenone-3-*C*-β-D-glucoside), Extrasynthese (Genay, France: luteolin) and Phytolab (Vestenbergsgreuth, Germany: eriocitrin, vicenin-2). Sigma-Aldrich (St Louis, MO, USA) supplied 2,2-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, HPLC gradient grade acetonitrile and analytical grade glacial acetic acid. Merck-Millipore (Darmstadt, Germany) was the supplier of analytical grade methanol, Folin-Ciocalteau's phenol reagent and sodium carbonate. Deionised water, prepared using an Elix water purification system (Merck-Millipore), was further purified to HPLC grade using a Milli-Q Reference A+ System (Merck-Millipore).

3.3.2 Plant material and carriers

Unfermented *C. subternata* plant material (400 kg), sourced from Cape Honeybush Tea Company (Mosselbay, South Africa) was batch extracted in an industrial extraction plant using a percolator-type extraction vessel. Purified water was preheated to 93 °C and introduced at the top of the vessel to give a final a solid-solvent ratio of 1:10 (m.v^{-1}). The resulting extract was continuously siphoned from the bottom of the vessel, passed through a heat exchanger to maintain the extraction temperature and re-introduced at the top of the vessel to circulate it for 35 min. On completion of extraction the extract was drained, centrifuged and concentrated under vacuum using a plate evaporator. Finally it was vacuum dried at 40 °C for 24 h. An aliquot of the extract powder was used for the treatments. Using this extract ensured that the extract composition in all the treatments was the same.

Star Dri 200 corn syrup solids with 20-23 dextrose equivalents (DE) was kindly donated by Tate and Lyle (Cape Town, South Africa). Orafti HP inulin, a long-chain inulin food additive derived from chicory roots (*Cichoriumintybus*) with 21-26 degree of polymerisation (DP), was procured from Savannah Fine Chemicals (Pty) Ltd (Gardenview, South Africa).

3.3.3 Preparation of treatments

Corn syrup solids (CS) and inulin (IN) were added to the vacuum-dried extract as separate treatments before spray-drying (BSD). Each carrier was added at three treatment levels, namely at 25%, 50% and 75% of the total solids content, as shown in Table 3.1. A control sample consisting of pure extract was also spray-dried. Each of the treatments were replicated 4 times, except the control (8 replicates), in a completely randomised design. The pure extract was also analysed before spray-drying to determine the effect of spray-drying on its individual phenolic compound content, total polyphenol content and antioxidant capacity.

Table 3.1 Levels of extract and carrier in different treatments.

Treatment code	% Extract	% Corn syrup solids	% Inulin
Pure extract	100	-	-
CS25	75	25	-
CS50	50	50	-
CS75	25	75	-
IN25	75	-	25
IN50	50	-	50
IN75	25	-	75

The spray-dryer feed solution was prepared by accurately weighing off the extract and carriers in the predefined ratios (Table 3.1) to obtain a total mass of 40 g. Deionised water (400 mL) heated to *ca* 55 °C was stirred on a magnetic stirrer as the powders were slowly added. Stirring continued until the powders were completely dissolved (*ca.* 10 min).

3.3.4 Spray-drying conditions

Spray-drying was conducted using a Büchi B-290 mini spray-dryer (Büchi Labortechnik AG, Flawil, Switzerland), set up with a glass cyclone separator to collect the powder. The spray-dryer was fitted with a 1.5 mm nozzle and the feed solution was sprayed in a co-current direction using air as a drying medium. The following operating conditions were employed: inlet temperature, 180 °C, aspirator rate, 100% (35 $m^3.h^{-1}$), peristaltic pump speed, 25% (*ca.* 7.5 $mL.min^{-1}$), atomisation air rotameter, 40 mm (667 $L.h^{-1}$), and nozzle cleaner, 8 strikes per min. The outlet temperature varied depending on the composition of the solution (*ca.* 90-100 °C). Once the experiment was run to completion the system was allowed to continue running with the heating turned off until the inlet temperature fell below 70 °C. The powder was then

collected and weighed off in amber vials. The total yield was determined and the vials were stored in a desiccator with silica gel in a cool dry place until further analysis.

The percentage yield was calculated using the following equation:

$$\%\ Yield = \frac{m_{ASD}}{m_{BSD}} \times 100$$

Where m_{ASD} is the mass of powder recovered after spray-drying and m_{BSD} is the mass of powder solids in the feed solution.

3.3.5 Characterisation of powders

3.3.5.1 Moisture content and water activity

The moisture content was determined gravimetrically using an HR73 Halogen Moisture analyser (Mettler Toledo, Greifensee, Switzerland). Approximately 2 g of sample was spread out on an aluminium foil dish and dried gently at 100 °C for 60 min. The moisture content was expressed as a percentage of the total mass of the product (wet basis). It was then converted to percentage dry basis using the following equation:

$$M_d = \frac{M_w}{(100 - M_w)} \times 100$$

Where M_d is the moisture content on a dry basis and M_w is the moisture content on a wet basis.

Water activity (a_w) was determined using a Novasina LabMASTER-aw electric hygrometer (Novasina, Lachen, Switzerland). The equipment was calibrated regularly using 33% and 58% salt standards (Novasina) according to the instructions provided by the manufacturer.

3.3.5.2 Moisture sorption isotherms

Moisture sorption analyses were performed using a VTI-SA vapour sorption analyser (TA Instruments, New Castle, Delaware, USA). The microbalance was calibrated prior to each vapour sorption run with a 100 mg standard weight. The microbalance was set to zero prior to weighing of the sample into the stainless quartz container. The sample was carefully placed into the sample holder and care was taken to evenly distribute it. A pre-dying step was

performed on each sample at 40 °C until the weight fluctuated by no more than 0.001 g. During the analysis the temperature was set at a constant 25 °C. The percentage relative humidity (% RH) was programmed to ramp from 5 to 75% RH during the adsorption phase, followed by a desorption step (inverse of adsorption) and a second adsorption step. The program criteria were set to 0.0001% weight change or 2-minute stability of weight gained or lost before the program would continue to the next set parameter.

The data were fitted to the classical Brunauer, Emmett and Teller (BET) sorption isotherm suitable for low water activities (Timmerman *et al*., 2001), using the following equation:

$$\frac{a_w}{(1-a_w)m} = \frac{1}{m_0} + \left[\frac{c-1}{m_0 c}\right] a_w$$

Where m is the moisture (dry basis) in g/100 g solids at a_w (a_w = equilibrium RH (%)/100) and temperature T, and m_0 is the monolayer value in the same units, while c is the surface heat constant given by: $c = e^{Q_s/RT}$ where Q_s is the excess heat of sorption (Jmol^{-1}), R is the gas constant (8.314 x 10^7 J K^{-1} mol^{-1}) and T is the temperature in Kelvin (K) (Labuza & Altunakar, 2007).

3.3.5.3 Quantification of major phenolic compounds

High performance liquid chromatography with diode array detection (HPLC-DAD) was performed to quantify the major phenolic compounds in the samples before and after spray-drying according to the validated *C. subternata* method described by De Beer *et al*. (2012a). Analysis was conducted in duplicate on an Agilent 1200 series instrument consisting of an in-line degasser, quaternary pump, autosampler, column thermostat and DAD (Agilent Technologies Inc., Santa Clara, CA, USA). Separation was performed at 30 °C on a reverse phase Gemini-NX C18 column (dimensions: 150 x 4.6 mm; particle size: 3 µm; pore size: 110 Å) (Phenomenex, Torrance, CA, USA). Mobile phases, 2% acetic acid (A) and acetonitrile (B), were used for gradient separation at a flow rate of 1 mL.min^{-1}: 0–2 min (8% B), 2–27 min (8–38% B), 27–28 min (38–50% B), 28–29 min (50% B), 29–30 min (50–8% B), 30–40 min (8% B). Stock solutions of the samples were prepared by accurately weighing off the powder using a 5-decimal balance and dissolving in water (final concentration *ca.* 6 mg.mL^{-1}). Aliquots

of these solutions were frozen at –20°C until analysis. A six-point calibration curve was set up using a mixture of authentic standards dissolved in DMSO and diluted with HPLC water. Ascorbic acid solution (0.1 mL) (10% w/v) was added to 1 mL of the standard mixtures and 1 mL of each sample stock solution to prevent oxidation of phenolic compounds during analysis. The mixtures were filtered through Millipore Millex-HV syringe filters (0.45 µm pore-size) and the filtered standard and sample solutions injected in duplicate in 5-15 µL and 15 µL quantities, respectively. UV-Vis spectra were recorded for each sample in the range of 200–450 nm. Compounds were identified based on retention time and the UV-Vis spectra of the available authentic standards. 3-Hydroxy-phloretin-3′,5′-di-*C*-hexoside and iriflophenone-3-*C*-β-D-glucoside-4-*O*-β-D-glucoside were tentatively identified based on comparison of relative retention time and UV-Vis spectra from De Beer *et al.* (2012a) and Beelders *et al*. (2014), respectively. Iriflophenone-3-*C*-β-D-glucoside, eriocitrin and hesperidin were quantified at 288 nm, while mangiferin, maclurin-3-*C*-β-D-glucoside, vicenin-2 and scolymoside were quantified at 320 nm. Due to scarcity of some reference standards, isomangiferin, scolymoside, iriflophenone-3-*C*-β-D-glucoside-4-*O*-β-D-glucoside and phloretin-3′,5′-di-*C*-β-D-glucoside were quantified using previously determined response factors with compounds present in the calibration mixture. 3-Hydroxy-phloretin-3′,5′-di-*C*-hexoside was quantified as phloretin-3′,5′-di-*C*-β-D-glucoside equivalents due to unavailability of a reference standard. The compound concentration was expressed as g compound per 100 g pure extract (recalculated to dry basis) in order to directly compare treatments, irrespective of different carrier levels.

3.3.5.4 Total polyphenol assay

The total polyphenol content (TPC) of the samples before and after spray-drying was determined using the Folin-Ciocalteau method adapted for micro-plate reader format by Arthur *et al*. (2011). A gallic acid calibration curve was set up with values ranging from 1 to 10 µg.mL^{-1}. The samples were diluted to 1.8 mg extract.mL^{-1} to obtain an absorbance value within the range of the calibration curve. Triplicate 20 µL aliquots of assay control (deionised water), gallic acid standard solutions and samples were transferred to a 96-well polystyrene flat-bottomed microplate. A Gilson (Middleton, USA) multi-channel pipette was then used to transfer 100 µL Folin-Ciocalteau reagent (10 x diluted) and 80 µL Na_2CO_3 solution (7.5% w.v^{-1}), respectively, to each well. The reaction mixtures were mixed for 30 s at 1000 rpm using an Eppendorf MixMate (Hamburg, Germany) and then transferred to an incubator at 30 °C for 2

h. The absorbance was measured at 765 nm and the TPC was expressed as g gallic acid equivalents (GAE) per 100 g pure extract (dry basis).

3.3.5.5 Total antioxidant capacity assay

The free radical scavenging activity of the samples before and after spray-drying was determined as described by Arthur *et al.* (2011). DPPH$^{\bullet}$ reagent was prepared by dissolving 5 mg DPPH$^{\bullet}$ in 100 mL methanol, the absorbance of the solution was measured at 515 nm and the solution diluted until an absorbance value between 0.68-0.72 was obtained for assay standardisation purposes. Trolox was used to set up a calibration curve ranging from 1 to 10 µg.mL^{-1}. The samples were diluted to 1.8 mg extract per mL to obtain an absorbance value within the range of the calibration curve. Triplicate 30 µL aliquots of the assay blank (deionised water), Trolox standard solutions and samples were pipetted into a deep-well plate. A Gilson multi-channel pipette was then used to transfer 270 µL DPPH$^{\bullet}$ reagent to each of the wells. The plate was sealed with a silicone sealing mat, mixed for 30 s at 1650 rpm on an Eppendorf MixMate and incubated for 2 h in the dark to allow scavenging of DPPH$^{\bullet}$ by the antioxidants. An aliquot of the reaction mixture (200 µL) of each deep well were then transferred to a polystyrene flat-bottomed microplate and the absorbance measured at 515 nm. The % inhibition was calculated as follows:

$$\%\ inhibition = \frac{(A_c - A_s)}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the standard or samples.

The % inhibition of the Trolox standard solutions was plotted against their concentration, giving a linear regression curve. This curve was used to calculate the total antioxidant capacity of the extracts expressed as µmole Trolox.g^{-1} pure extract.

3.3.5.6 Isothermal microcalorimetry

A 2277 Thermal Activity Monitor (TAM III) (TA Instruments, USA), equipped with an oil bath with a stability of ± 100 µK over 24 h, was used during this study. The temperature of the calorimeter was maintained at 60 °C. For compatibility studies the heat flow is measured for the single components as well as the mixtures. The observed calorimetric outputs for the single

components were summed to give a theoretical response of the mixture. This calculated hypothetical response represents a calorimetric output that would be expected if the materials do not interact with each other. If the materials interact the measured calorimetric response of the mixture will differ from the calculated theoretical response. Results were expressed in $\mu W.g^{-1}$ of the single component or mixture.

3.3.5.7 Simultaneous thermogravimetry / differential thermal analysis (DTG)

A Shimadzu DTG-60 instrument (Kyoto, Japan) was used to record differential thermal analysis (DTA) and thermogravimetry (TG) simultaneously. The former measures the heat flux (µV) of the powders during heating and is suited for the determination of characteristic phase transition temperatures, while the latter measures mass loss (mg). Samples (3-5 mg) were accurately weighed in open aluminium cells. The samples were heated from 25 to 300 °C with a heating rate of $10°.min^{-1}$ and nitrogen gas purge of 35 $mL.min^{-1}$. Spray-dried samples were compared to pure inulin and corn syrup solids.

3.3.5.8 X-ray powder diffraction (XRPD)

Powder X-ray diffraction measurements were performed to confirm the crystalline or amorphous nature of the spray-dried samples, as well as the individual carriers. A PANalytical (Almelo, Netherlands) Empyrean X-ray diffractometer with a PIXcel3D detector was used to record XRPD patterns at ambient temperature. Samples were evenly distributed on a zero background sample holder. The measurement conditions for all the scans were set as follows: target, Cu; voltage, 40 kV; current, 40 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; scanning speed, $2°.min^{-1}$ (step size, 0.02°; step time, 1.0 s).

3.3.5.9 Objective colour measurement

CIE L*a*b* objective colour measurements were performed on the spray-dried samples using a Konica Minolta CM-5 spectrophotometer (Osaka, Japan). L*, a* and b* values were measured directly in reflectance mode using a 152 mm integrating sphere. Measurement conditions were standardised on illuminant D65 with diffuse illumination and 8° and 10° viewing and observer angles, respectively. Auto-calibration was performed using a built-in standard white calibration plate and manual zero calibration was performed with a zero calibration box (black inverted cone cylinder) prior to measurements. Powders were placed in

quartz cuvettes and illuminated (30 mm diameter measurement area) from the bottom of an optical glass tube cell cup (CR-A502, Ø 60 mm, 40 mm depth, Konica Minolta) during measurements. The colour of each sample was measured in duplicate. The powder was removed, mixed and returned to the cuvette between measurements. Chroma and hue values were automatically calculated using SpectraMagic NX Pro colour data software (Konica Minolta Inc.). The difference in colour (ΔE) between the control and treatment samples was calculated using the following equation:

$$\Delta E = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}}$$

3.3.5.10 Statistical analysis

The experimental design was completely random with four replicate experiments for each of the 8 treatments, namely pure extract (before spray-drying; BSD), pure extract (after spray-drying), CS25, CS50, CS75, IN25, IN50 and IN75. Univariate analysis of variance was performed on each measured variable using the GLM (General Linear Models) procedure of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). The Shapiro-Wilk test was performed on the standardised residuals from the model to test for normality (Shapiro & Wilk, 1965). Outliers were removed when the standardised residual for an observation deviated by more than three standard deviations from the model value. Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

3.4 Results and Discussion

3.4.1 Spray-drying conditions

Preliminary studies were conducted on the spray-dryer to determine suitable processing conditions. Different combinations of inlet temperature, feed concentration and pump speed were tested. The outputs were assessed based on powder yield, moisture content and phenolic retention. It was found that increasing the inlet temperatures from 170 to 180°C produced powders with higher yields and lower moisture content without having a noticeable impact on the phenolic retention (data not shown). Increasing the feed concentration from 10% to 15% had the opposite effect on the yield and moisture content, while increasing the pump speed

from 15% to 25% had no effect on the yield, moisture content and phenolic retention (data not shown). Therefore a lower feed concentration (10%) and higher pump speed (25%) were selected.

3.4.2 Powder yield and general characteristics

All powders produced were fine, light brown and free-flowing. Table 3.2 gives the CIELab colour values for the different treatments. The powders became lighter with increasing concentrations of both inulin and corn syrup solids as these products are white powders. The powder recovery ranged from 61.3% to 65.2% (Table 3.2) with an average 63% for all the samples. The highest yield was obtained for the IN25 (65.2%). Losses were attributed to powder deposits which accumulated on the walls of the spray-drying chamber. The typical yield from a spray-dryer when drying small quantities is between 20 and 50% (Krishnaiah *et al*., 2014) and powder yields above 50% are accepted as suitable criteria for efficient drying (Bhandari *et al.*, 1997). Low yields are obtained when the product has a low glass transition temperature (T_g) and the rubbery and thermoplastic nature of such a product causes stickiness (Krishnaiah *et al*., 2014). This "rubbery" state of an amorphous solid occurs when the drying temperature is above the T_g (Bhandari & Howes, 1999). Addition of high molecular weight carriers with high T_g values (Kawai *et al.*, 2011; Tonon *et al*., 2009) usually facilitates drying, especially in the case of sugar-rich extracts with low T_g values (Krishnaiah *et al.*, 2014).

Table 3.2 The colour measurements and yields of powders after spray-drying. Treatments were composed of *C. subternata* extract spray-dried with corn syrup solids (CS) and inulin (IN) at levels of 25 %, 50% and 75% of the total soluble solids.

Treatment	L*	a*	b*	C*	h	ΔE	Product yield
Pure extract	68.8 f	10.6 a	30.0 a	31.8 a	70.5 g	0.0 d	62.2 b
CS25	71.8 e	9.0 b	27.7 c	29.1 b	72.1 f	4.2 c	61.5 b
CS50	74.8 d	7.6 c	25.7 d	32.5 c	73.5 d	8.0 b	61.4 b
CS75	80.6 a	4.9 d	21.5 e	22.0 d	77.1 a	15.6 a	61.3 b
IN25	72.0 e	9.1 b	28.7 b	30.2 b	72.4 e	3.8 c	65.2 a
IN50	75.3 c	7.4 c	25.7 d	26.8 c	73.9 c	8.4 b	63.5 ab
IN75	80.1 b	5.1 d	21.1 e	21.8 d	76.4 b	15.4 a	62.5 ab
CS100	98.9	-0.2	2.0	2.0	95.6		
IN100	97.7	-0.3	4.7	4.7	93.8		

Means in the same column with the same letter are not significantly different ($p \geq 0.05$).
CS100 and IN100 were measured separately for the purpose of comparison.

The yields obtained in this present study are not only considered satisfactory and validate that the spray-drying conditions were suitable, but indicate that the *C. subternata* extract and extract mixtures had high T_g values. The high yields from this present study may be explained by the fact that honeybush extract does not contain high concentrations of low molecular weight sugars which are the primary cause of stickiness on the walls of the spray-drying chamber, resulting in low product yields (Bott *et al*., 2010).

3.4.3 Crystalline vs. amorphous state determination

XRPD is a direct measure of the crystal form of a product and can be used to determine whether the spray-dried powder is in a crystalline or amorphous state (Cortés-Rojas & Oliveira, 2012; Chadha & Bhandari, 2014). Fig. 3.1 shows the diffractogram of pure *C. subternata* extract displaying a typical halo pattern with no distinct peaks, characteristic of amorphous powders. The other powders showed similar diffractograms (Addendum A), indicating that all the spray-dried powders were in the amorphous state.

The amorphous nature of the powders may be attributed to a combination of the spray-drying process and the fact that it is a complex natural substance (Cortés-Rojas & Oliveira, 2012). Amorphous powders are often produced in the spray-drying process, mainly due to insufficient time for crystallisation given the rapid drying conditions (Bhandari & Howes, 1999). Rapid cooling of substances prevents preliminary nucleation and crystal growth which

results in the formation of an amorphous glass (Kasapis, 2006). Frick and Richter (1995) explained that glassy systems are thermodynamically unstable, but derive kinetic stability from their high viscosity. In spite of the thermodynamic instability of amorphous powders, they are usually more soluble than their crystalline counterparts – which is an important characteristic in product applications (Bott *et al*., 2010) such as an iced tea dry mixture. Furthermore, knowledge of the amorphous nature of the powder is an important prerequisite for understanding its behaviour in further analysis and its stability and storage requirements.

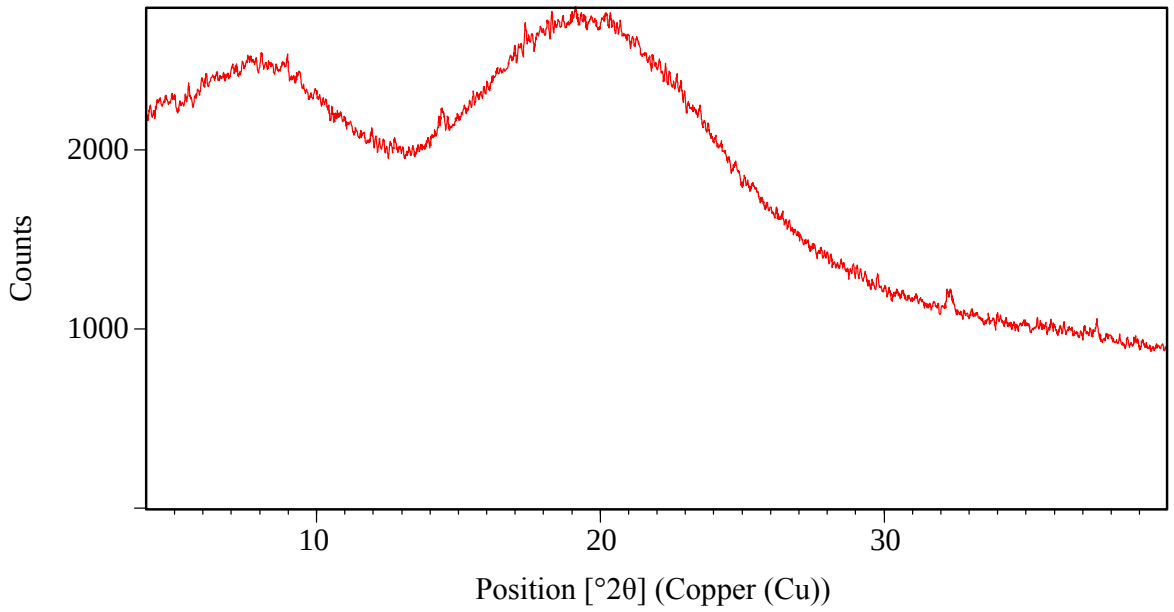

Figure 3.1 X-ray powder diffractogram showing amorphous nature of spray-dried pure *C. subternata* extract as indicated by the typical halo pattern and the absence of distinct peaks.

3.4.4 Phase transition temperatures

In a glassy amorphous form molecular mobility is higher due to the higher free energy state in which it exists. In order to ensure product stability over long storage periods this state should not alter with time (Bhandari & Howes, 1999). Given the confirmed amorphous nature of the spray-dried extract and extract-carrier mixtures, insight into conditions that may compromise the amorphous state are required. In particular, relative humidity and high temperatures (Bhandari & Howes, 1999) result in phase transitions which lead to caking of such powders (Fang & Bhandari, 2011).

Thermal analysis gives information about changes in material properties as a function of temperature. Both TG and DTA were employed to analyse the thermal behaviour of individual mixture components as this helps to explain the behaviour of these components within the mixtures. While TG only measures changes caused by mass loss, DTA registers changes in material where no mass loss occurs, e.g. crystal structure changes, melting behaviour and glass transition. Fig. 3.2 shows the thermogram obtained from DTA of pure inulin. The red curve denotes the DTA thermogram which illustrates a change in heat capacity as the substance is heated. The DTA displayed a melting endotherm between 220 and 300 °C, but it was not sensitive enough to detect the T_g of inulin. Additional analysis using DSC was performed to supplement this data and a T_g was detected between 50.3 and 59.2 °C (Addendum A). These results agree with those repoted in literature. For instance Schaller-Povolny *et al.* (2000) studied the glass transition behaviour of inulin over a range of different molecular weights and water activities. At water activity of 0.33, Orafti HP inulin with an average degree of polymerisation (DP) of 23 (the same product used in this study) had a glass transition temperature of 56.9 °C. It should be noted that the T_g of carbohydrate polymers decreases with an increase in water activity (Tonon *et al*., 2009).

Concurrent to the DTA thermogram for inulin the TG thermogram, denoted by the dark blue curve (Fig. 3.2), was also obtained. It shows a gradual change in the mass of inulin, due to moisture loss throughout the heating process until *ca* 220 °C, where after a rapid decrease in mass was observed due to thermal degradation of the sample coinciding with the melting endotherm.

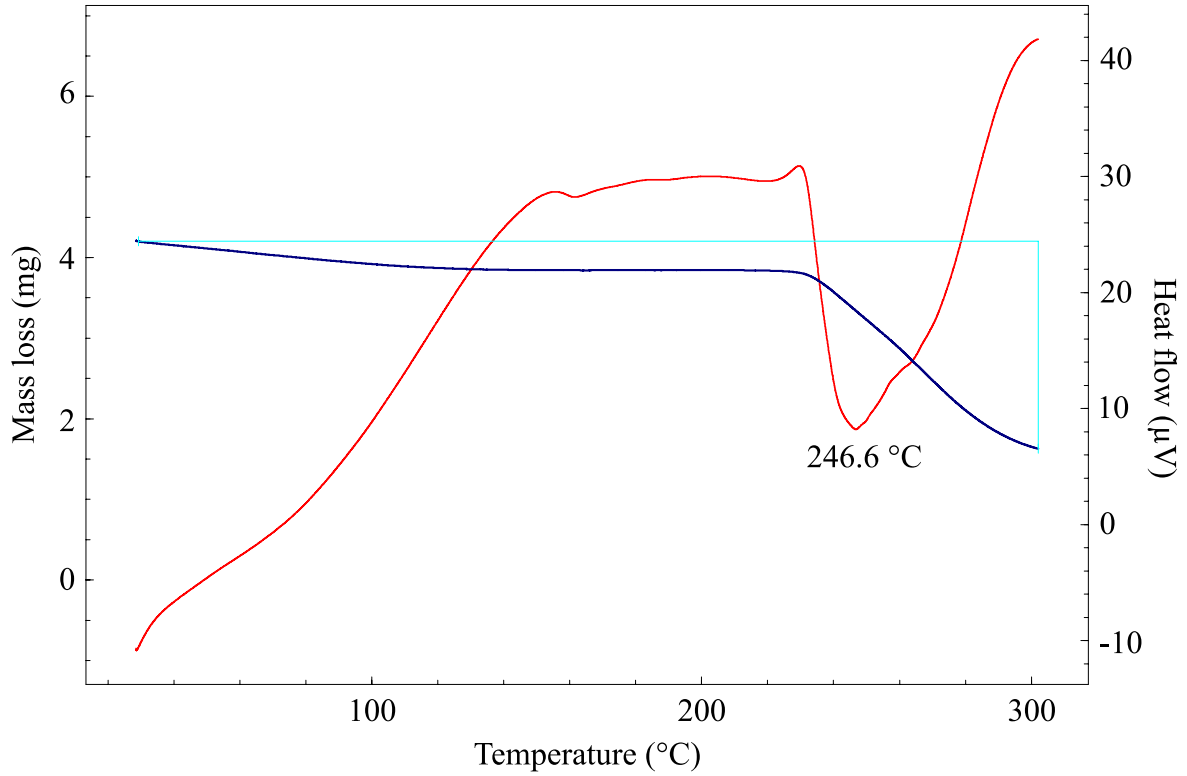

Figure 3.2 Differential thermal analysis (red curve) and thermogravimetric analysis (blue curve) of pure inulin (IN100).

Fig. 3.3 shows the DTA thermogram of pure corn syrup solids with DE 20-23, which had a melting endotherm at 242 °C. No distinct T_g was measured for corn syrup solids using DTA or DSC. During the analysis of large molecules it is common for water to evaporate, causing a broad endotherm, which masks the T_g (Aucamp, M., 2015, Department of Pharmaceutical Sciences, North-West University, personal communication, 02 November 2015). Previous studies have reported that the T_g of anhydrous maltodextrin with 20 dextrose equivalents (DE) was 141 °C (Roos & Karel, 1991), whilst Avaltroni *et al*. (2004) calculated a T_g value of 111.85 °C for dry maltodextrin of 19 DE. The presence of water affects the T_g of maltodextrin as for other biopolymers with its T_g decreasing with increasing water content (Roos & Karel, 1991; Schaller-Povolny *et al.*, 2000; Avaltroni *et al*., 2004). Molecular weight also plays a role with an increase in DE causing an increase in T_g (Avaltroni *et al.*, 2004). It is expected that the spray-dried corn syrup solids would have a T_g within a similar range as reported by these groups, because of similar DE values. Storage at low relative humidity is therefore recommended to prevent phase transition that would lead to caking.

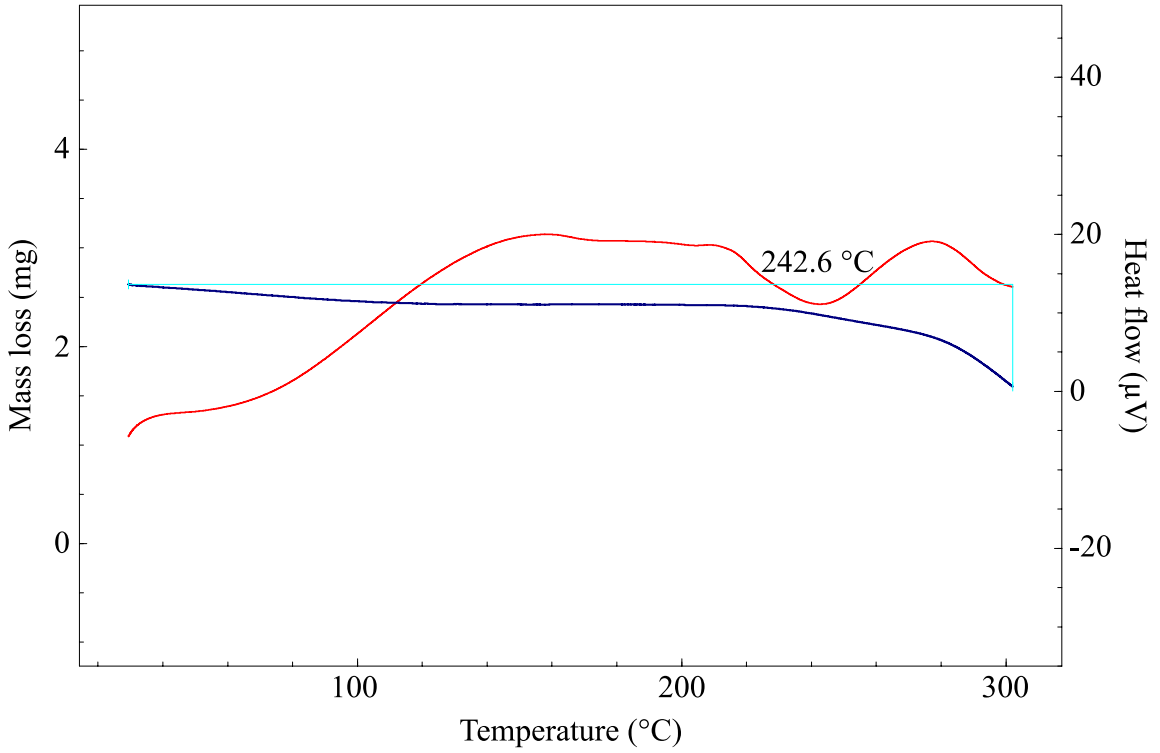

Figure 3.3 Differential thermal analysis (red curve) and thermogravimetric analysis (blue curve) of pure corn syrup solids (CS100).

Fig. 3.4 depicts the DTA thermogram of pure extract showing no distinct thermal events. This may be attributed to the fact that *C. subternata* extract is a natural substance which contains a complex mixture of different components. It is possible that glass transition reactions took place in some of the compounds across a variety of temperatures, however, the reactions were too small to be detected. Similar results were obtained during the characterisation of spray-dried propolis extract, a complex resin collected by bees (da Silva *et al*., 2011). Mass loss of *C. subternata* extract when heated from 25 to 300 °C was 24.17%, thus substantially less than that observed for inulin and corn syrup solids under similar heating conditions. This is a further indication that the extract was more thermo-stable than these carbohydrate polymers. Apart from the phenolic composition of *C. subternata* extract very little is known about the other constituents, comprising the bulk of the extract.

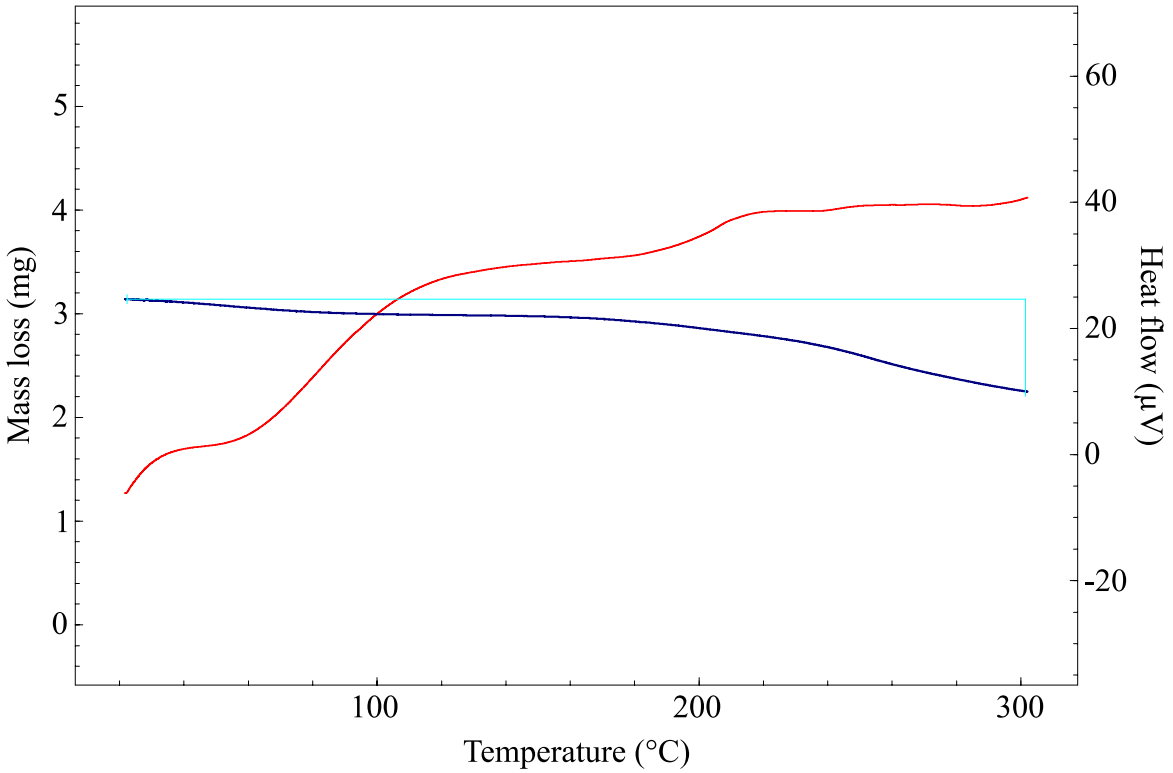

Figure 3.4 Differential thermal analysis (red curve) and thermogravimetric analysis (blue curve) of pure *C. subternata* extract.

Fig. 3.5 compares the DTA thermograms of pure inulin (IN100) with those of IN25, IN50 and IN75. The thermograms of pure inulin and IN75 both displayed melting endotherms at 246.68 °C and 232.01 °C, respectively, while none was observed for IN25 and IN50. It is thus the inulin content of the powder that determines its thermal properties as the higher the inulin content, the more it behaved like pure inulin and vice versa.

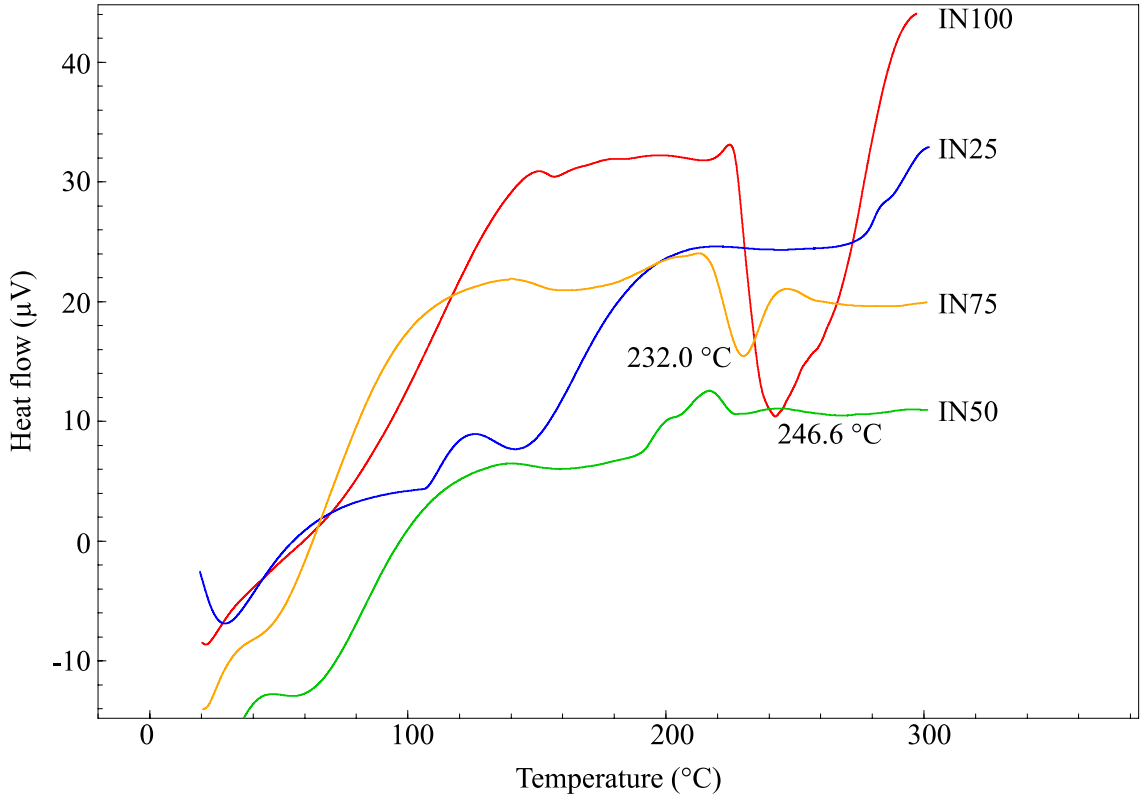

Figure 3.5 Differential thermal analysis of pure inulin (IN100), as well as samples containing inulin at levels of 25, 50 and 75% (IN25, IN50 and IN75).

Fig. 3.6 shows the thermograms of pure corn syrup solids (CS100) and the mixtures, CS25, CS50 and CS75. None of the thermograms showed a distinct T_g. The lack of clear T_g for mixtures of corn syrup solids and *C. subternata* extract is attributed to the complex nature of the extract as explained previously. Even pure corn syrup solids had no clear T_g as also previously indicated (Fig. 3.2).

Melting endotherms are observed in substances that are in crystal form. This is illustrated by the multiple melting behaviours of polyimide. Ratta (1999) reported that polyimide displayed melting endotherms at 360°C and 416°C. Both of these melting endotherms (endothermic) were preceded by crystallisation reactions (exothermic). The first one was preceded by glass transition at *ca*. 230°C and a re-crystallisation exotherm reaction took place between the two melting endotherms (Ratta, 1999). Although no clear T_g was detected for the pure corn syrup solids it is probable that it went through a glass transition reaction due to the fact that a melting endotherm at *ca.* 242°C was detected. A similar

phenomenon was observed for IN75 (Fig. 3.5). This could be attributed to the fact that the mixture is made up of predominantly inulin (75%).

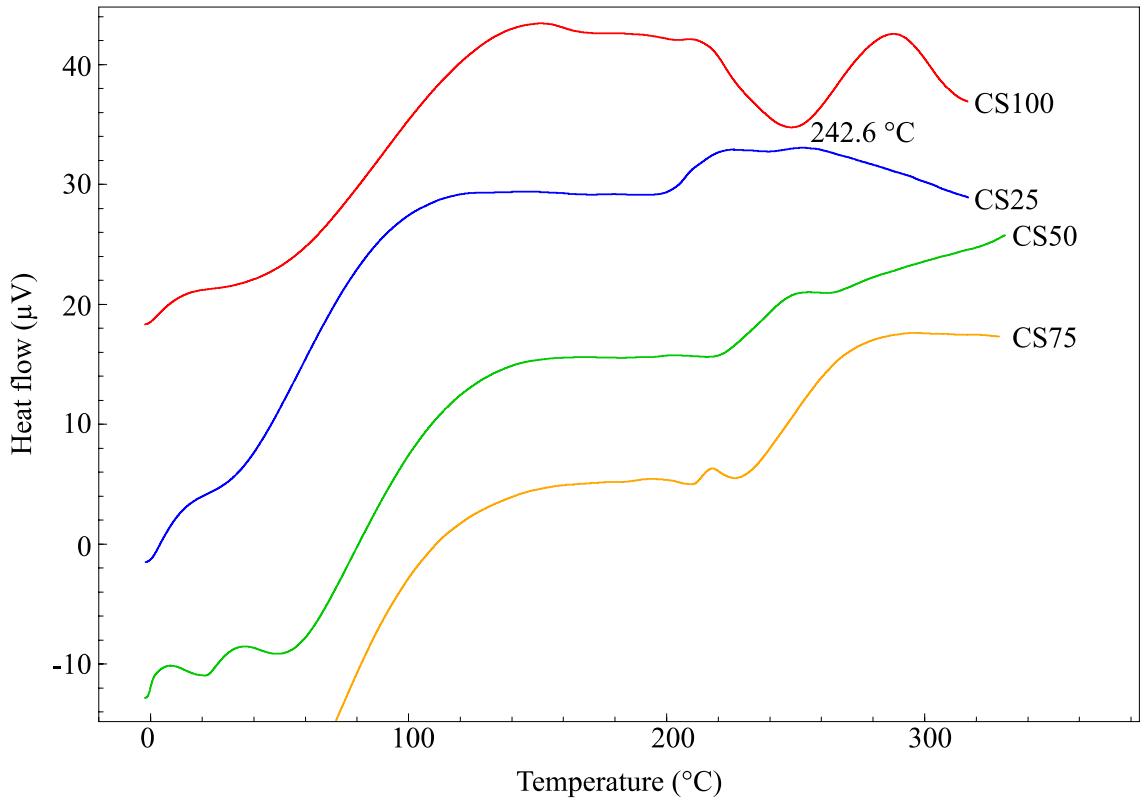

Figure 3.6 Differential thermal analysis thermograms for pure corn syrup solids as well as samples containing corn syrup solids at levels of 25, 50 and 75% (CS25, CS50 and CS75).

The TGA thermograms measured the mass loss of samples throughout the heating cycle. Table 3.3 summarised the mass loss of samples due to moisture loss and degradation. Initial weight loss was attributed to moisture loss and further weight loss was attributed to degradation of the sample due to high temperatures rather than a specific thermal event.

Table 3.3 Mass loss due to loss of moisture and degradation of compounds in spray-dried samples as measured by thermogravimetric analysis. Treatments were composed of pure *C. subternata* extract, spray-dried inulin (IN100) and corn syrup solids (CS100), as well as mixtures of extract and IN or CS at carrier levels of 25, 50 and 75% (IN25, IN50, and IN75/ CS25, CS50 and CS75).

Treatments	Mass loss due to moisture (%, wet basis)	Mass loss due to degradation (%, wet basis)	Total mass loss (%, wet basis)
Pure extract	6.58	24.17	30.90
IN25	8.55	28.53	37.11
IN50	3.83	33.61	39.04
IN75	7.02	44.35	52.05
IN100	8.63	52.04	60.83
CS25	6.74	29.96	36.86
CS50	8.69	36.42	45.57
CS75	6.90	34.97	41.69
CS100	7.53	30.56	38.80

TGA results showed that inulin was much more thermally sensitive to degradation than corn syrup solids (Table 3.3). Inulin showed a mass loss due to degradation of 52.04%, while the value was only 30.56% for corn syrup solids. The pure extract was more thermally stable than either of the carriers with a mass loss due to degradation of only 24.17%. As the levels of inulin and corn syrup solids increased in the samples, the percentage weight loss also increased. It is however, important to note that degradation took place at temperatures above 200 ºC which are higher than those experienced within the spray-dryer, further confirming that it is a suitable processing method for extract mixtures.

It is unlikely that samples will be exposed to temperatures as high as those of the T_g of their carriers during storage. However, should these samples be exposed to high RH conditions it could lead to a decrease in their T_g. This will be explained in more detail in the following section.

3.4.5 Relationship between moisture content and water activity

Water content, water activity and the relationship between the two are some of the most significant factors, which play a role in the stability of powdered extracts. While the powders were free flowing directly after spray-drying, exposure to high RH conditions could result in decreases in T_g, resulting in agglomeration, caking, etc. due to phase transition. Fig.3.7 shows the moisture sorption isotherm (MSI) of pure *C. subternata* extract after spray-drying. The MSI

produced a distinct sigmoidal shaped curve characteristic of type II isotherms (Labuza & Altunakar, 2007). During the analysis samples were exposed to a range of RH conditions with maximum RH of 75% as samples deliquesced irreversibly at RH levels above 75%. Deliquescence is the process whereby a powder absorbs moisture from the atmosphere until it dissolves in the absorbed water and forms a solution (Mauer & Taylor, 2010).

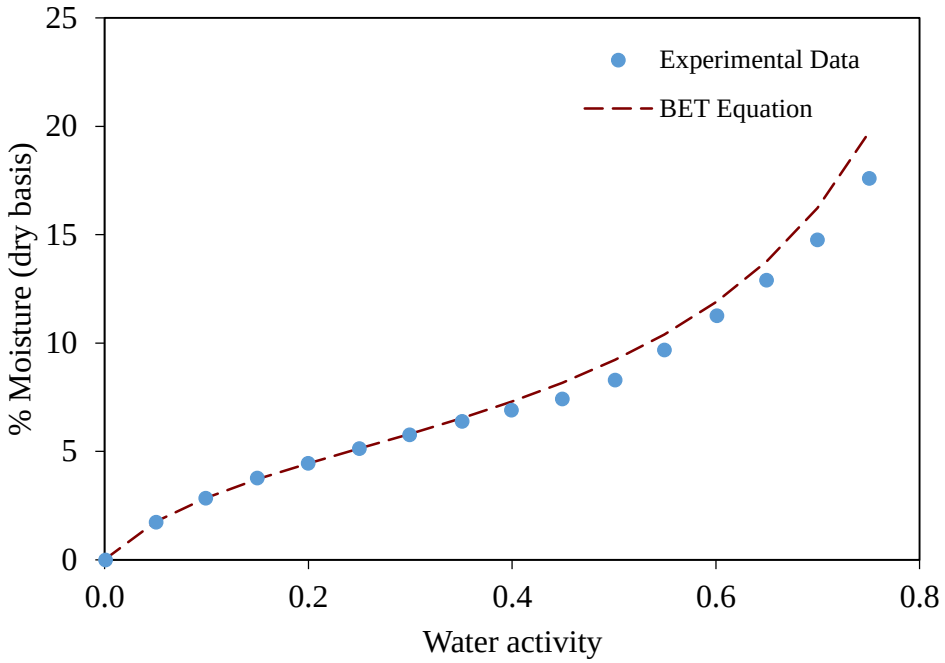

Figure 3.7 Moisture sorption isotherm of spray-dried pure *C. subternata* extract fitted with the BET sorption model.

The BET sorption model was applied to the raw and transformed MSI data as seen in Fig. 3.7 and Fig. 3.8, respectively. The constants derived from the BET model for each treatment are shown in Table 3.4. The model was found to have a good fit with R^2 values above 0.97 for each of the curves. The model fitted the data well, particularly at water activities below 0.4 (Fig. 3.7). This is due to the fact that the BET model is suited to low a_w foods and works particularly well within the range of 0-0.55 a_w (Labuza & Altunakar, 2007).

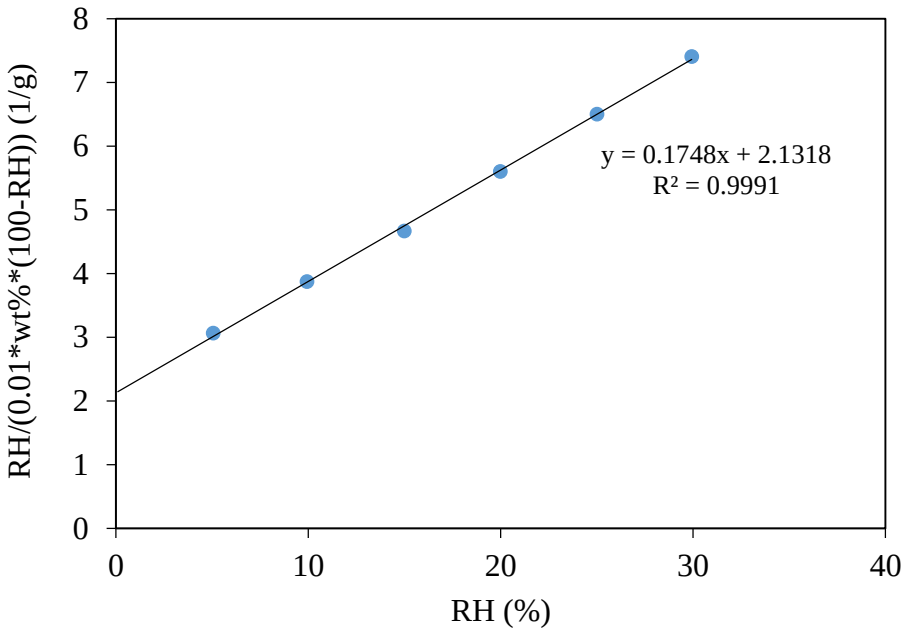

Figure 3.8 Transformed moisture sorption isotherm data of spray-dried pure *C. subternata* extract fitted with the BET sorption model (RH = relative humidity).

One of the most important features of the BET model is its ability to give the monolayer moisture content of a food product. The monolayer moisture content (M_0) refers to the maximum amount of water which can be strongly adsorbed to specific sites on the surface of the material (Labuza & Altunakar, 2007). It is an important parameter and is considered to be a critical value above which water is more available for chemical reactions and degradation (de Souza *et al*., 2013). The M_0 of dried foods usually fall within the range of 0.2-0.3 water activity (Labuza & Altunakar, 2007). Table 3.4 gives the M_0 value of each of the mixtures and the pure extract as calculated using the BET model. The M_0 values (4.77- 6.02 g per 100 g solids) fall within the range of 0.2-0.3 a_w as seen on the MSI (Fig. 3.7). The samples containing 50 and 75% inulin had higher M_0 values than the corresponding corn syrup solids samples. Schaller-Povolny *et al*. (2000) found inulin, similar to that used in the present study, to have an M_0 of 7.3 g per 100 g solids. They compared it with data from literature which reported that different maltodextrins had a M_0 below 6.6 g per 100 g.

Table 3.4 Constants and R^2 values of moisture sorption data obtained at 25 °C for spray-dried pure *C. subternata* extract, as well as mixtures of extract and carriers, inulin (IN) and corn syrup solids (CS) at various levels (25, 50 and 75%), using the BET sorption model.

Sample	C	M_0 (g H_2O / 100 g dry solids)	R^2
Pure extract	9.1996	5.10	0.999
IN25	7.4986	4.77	0.979
IN50	7.8808	5.66	0.997
IN75	7.2533	6.02	0.999
CS25	8.5111	5.04	0.993
CS50	9.2564	4.94	0.995
CS75	8.7550	4.98	0.995

C = constant in BET sorption model; M_0= monolayer moisture content (% dry basis)

Fig. 3.9 shows the average water activity of the samples directly after spray-drying. The water activity of the samples did not exceed 0.25. Samples containing inulin had an overall lower water activity than corresponding samples containing corn syrup solids.

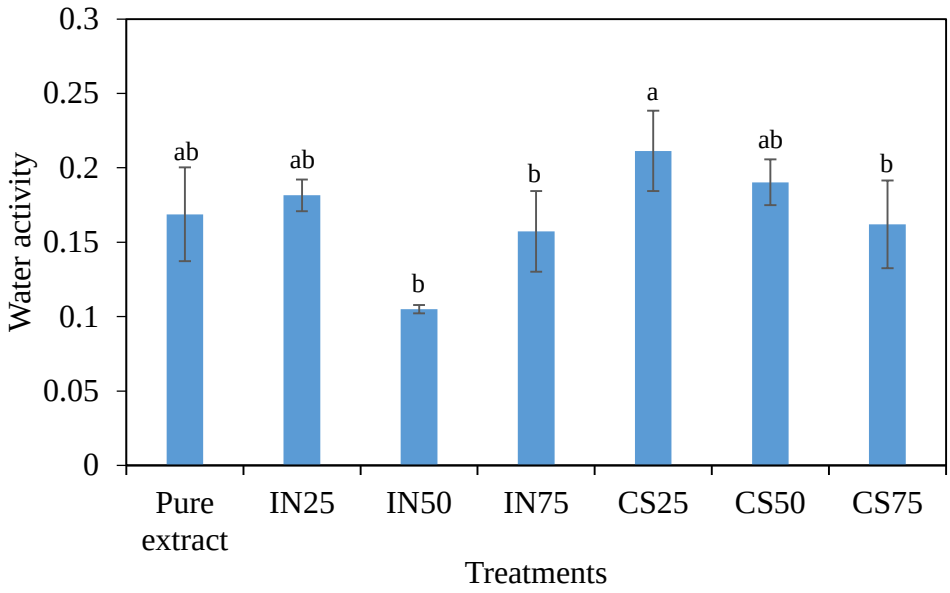

Figure 3.9 Water activity (mean ± standard deviation; n = 4) of pure *C. subternata* extract, as well as samples containing 25, 50 and 75% inulin (IN25, IN50, IN75) and 25, 50 and 75% corn syrup solids (CS25, CS50,CS75) after spray-drying.

Fig. 3.10 shows the monolayer moisture content for each sample compared to the measured moisture content on a dry basis. In each case the moisture content was lower than the monolayer value. From these results it can be seen that the moisture content and water

activity of the powders fall within the range of the monolayer value and can therefore be considered shelf-stable if stored under the correct conditions (Labuza & Altunakar, 2007). The BET monolayer value of pure *C. subternata* extract was similar to that demonstrated for spray-dried green tea extract (4.2 g per 100 g; Donlao & Siriwattanayotin, 2012). The predicted monolayer moisture content represents the maximum water content of a food during storage to ensure stability and depends on the temperature, decreasing with an increase in temperature (Akoy *et al.*, 2013).

The moisture sorption isotherms can help in the prediction of adequate storage conditions (Gabas *et al*., 2007). With a_w values of less than 0.25, it is recommended that spray-dried honeybush powders are stored below 30% RH humidity. RH conditions higher than this will result in the absorption of moisture, increasing a_w levels to the range where physical state changes start to occur.

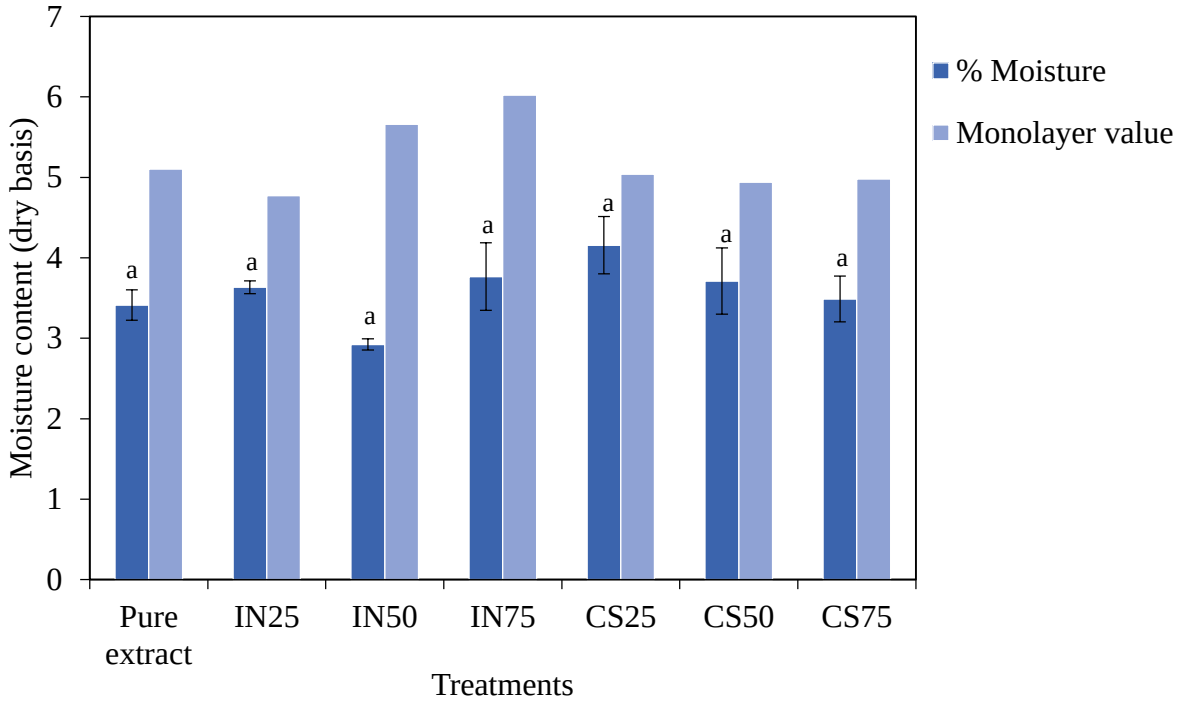

Figure 3.10 Comparison of moisture content (mean ± standard deviation; n = 4) of pure *C. subternata* extract, as well as samples containing 25, 50 and 75% inulin (IN25, IN50, IN75) and 25, 50 and 75% corn syrup solids (CS25, CS50, CS75) after spray-drying and BET monolayer values calculated from the moisture sorption isotherm data (n = 1) of the same spray-dried powders.

The stability of amorphous, low moisture foodstuffs such as powders is dependent on the interplay between monolayer moisture content and the glass transition temperature. In powdered foods this is related to the transition from glassy to rubbery state as determined by glass transition temperature of the powder (Bhandari & Howes, 1999). In most powdered foods this occurs in the range of 0.35-0.45 a_w (Labuza & Altunakar, 2007).

During the analysis of the moisture sorption isotherms the first adsorption phase was performed by ramping the RH from 0 to 75%. This was followed by a desorption phase where the RH was ramped back to 0%, and a second adsorption phase. Fig. 3.11 shows the adsorption and desorption curves for the pure *C. subternata* extract and samples containing inulin in increasing levels. Fig. 3.12 provides the same information for the samples containing corn syrup solids at increasing levels. Hysteresis, i.e. the phenomenon when the desorption curves lie above the adsorption curves, was particularly noticeable for powders containing the highest levels (75%) of the two carriers, while the pure *C. subternata* extract showed very little indication of hysteresis. The hysteresis loops for the samples containing the same levels of carrier in the mixture were more or less of the same size, indicating that their extent of water binding is more or less the same.

When hysteresis occurs it is usually an indication of structural or conformational rearrangement which affects the availability of favourable polar sites and therefore may hinder the movement of moisture out of the sample (Al-Muhtaseb *et al*., 2002). A number of theories to explain the hysteresis phenomenon have been proposed in the literature, however, no qualitative prediction is available (Labuza & Altunakar, 2007). Interpretations of hysteresis differ between products and are based on capillary condensation of porous solids, phase changes for non-porous solids or structural changes of non-rigid solids (Kapsalis, 1987).

The presence of hysteresis in the samples containing carriers is likely an indication that the samples have transitioned from glassy to rubbery state with the absorption of water. Transition occurs because water acts as a plasticiser which drives the reaction of glass transition forward by decreasing the glass transition temperature. The addition of water to an amorphous, hydrophilic glass decreases the glass transition temperature toward that of pure water, which is around -135 °C (Roos, 2007). As sugary materials change from a glassy state to a rubbery

state on adsorption, some sugars can crystallise and collapse can occur, both of which means less water adsorption (Labuza & Altunakar, 2007).

Increasing levels of carrier resulted in more pronounced hysteresis as seen in Fig. 3.11 and Fig. 3.12. It can therefore be assumed that it is the carriers that undergo transition and not the pure extract. Plasticisation of these polysaccharides occurs when their surface comes into contact with high RH conditions resulting in agglomeration and caking of the powders (Mathlouthi & Rogé, 2003). In the presence of high RH conditions the powders could become a sticky mess, rendering them completely unusable.

In practical terms it is essential that these powders are stored under the correct conditions. If moisture absorption takes place it could lead to changes in the structure of the powder which cannot be reversed by subsequent drying. Furthermore the sorption properties of inulin are similar to corn syrup solids, thus inulin may be useful in providing similar functionality as corn syrup solids (Schaller-Povolny *et al.*, 2000). In addition, inulin contains far fewer kilojoules (6.3 $kJ.g^{-1}$) compared to corn syrup solids (16.3 $kJ.g^{-1}$) (Roberfroid, 1999; Schaller-Povolny *et al*., 2000) and is therefore more suitable, considering that the aim of this study is to produce a reduced kilojoule powder iced tea mixture.

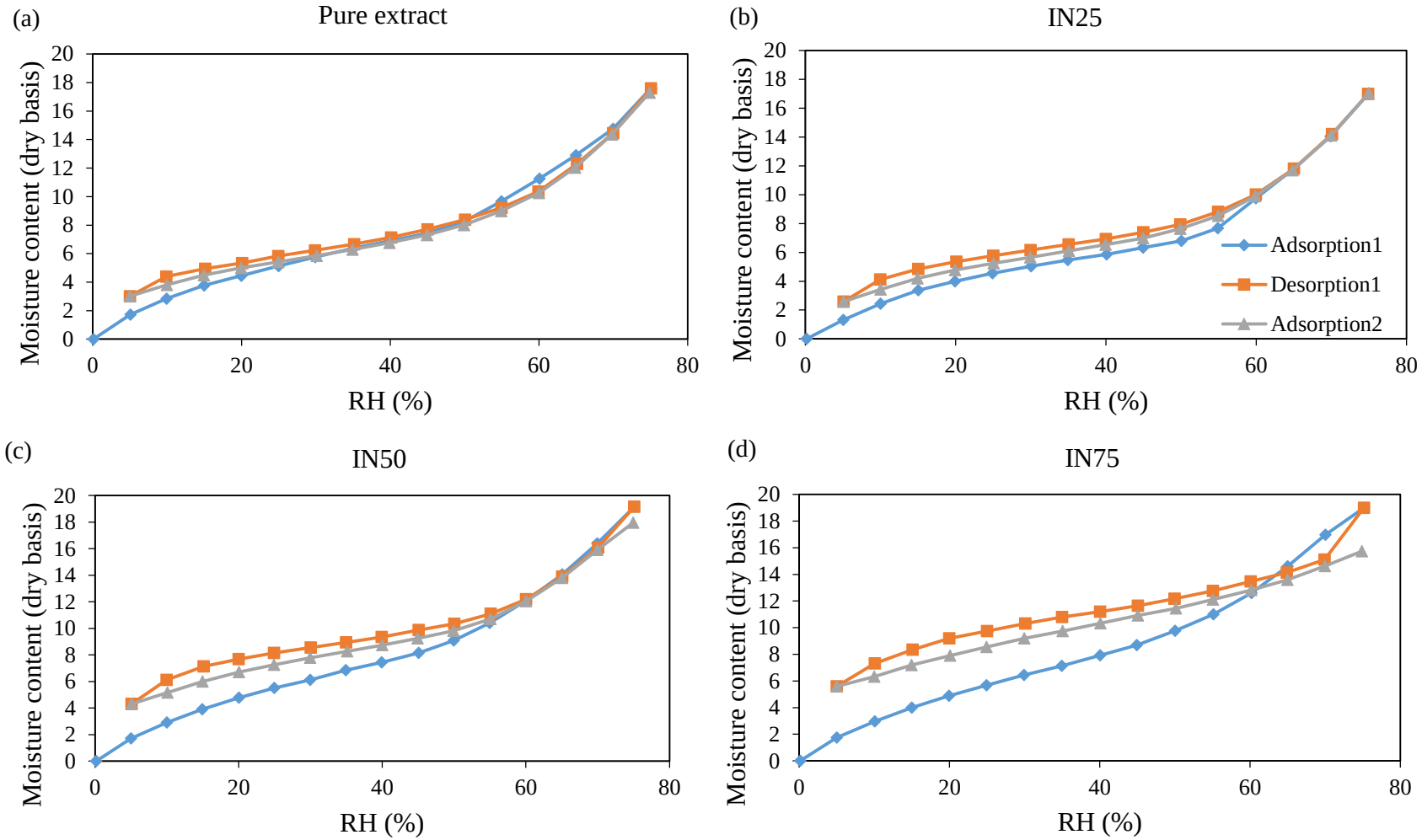

Figure 3.11 Moisture sorption isotherms for spray-dried pure *C. subternata* (a), as well as samples containing 25% (b), 50% (c) and 75% (d) inulin (IN25, IN50 and IN75) showing first adsorption, desorption and second adsorption at 25 °C (RH = relative humidity).

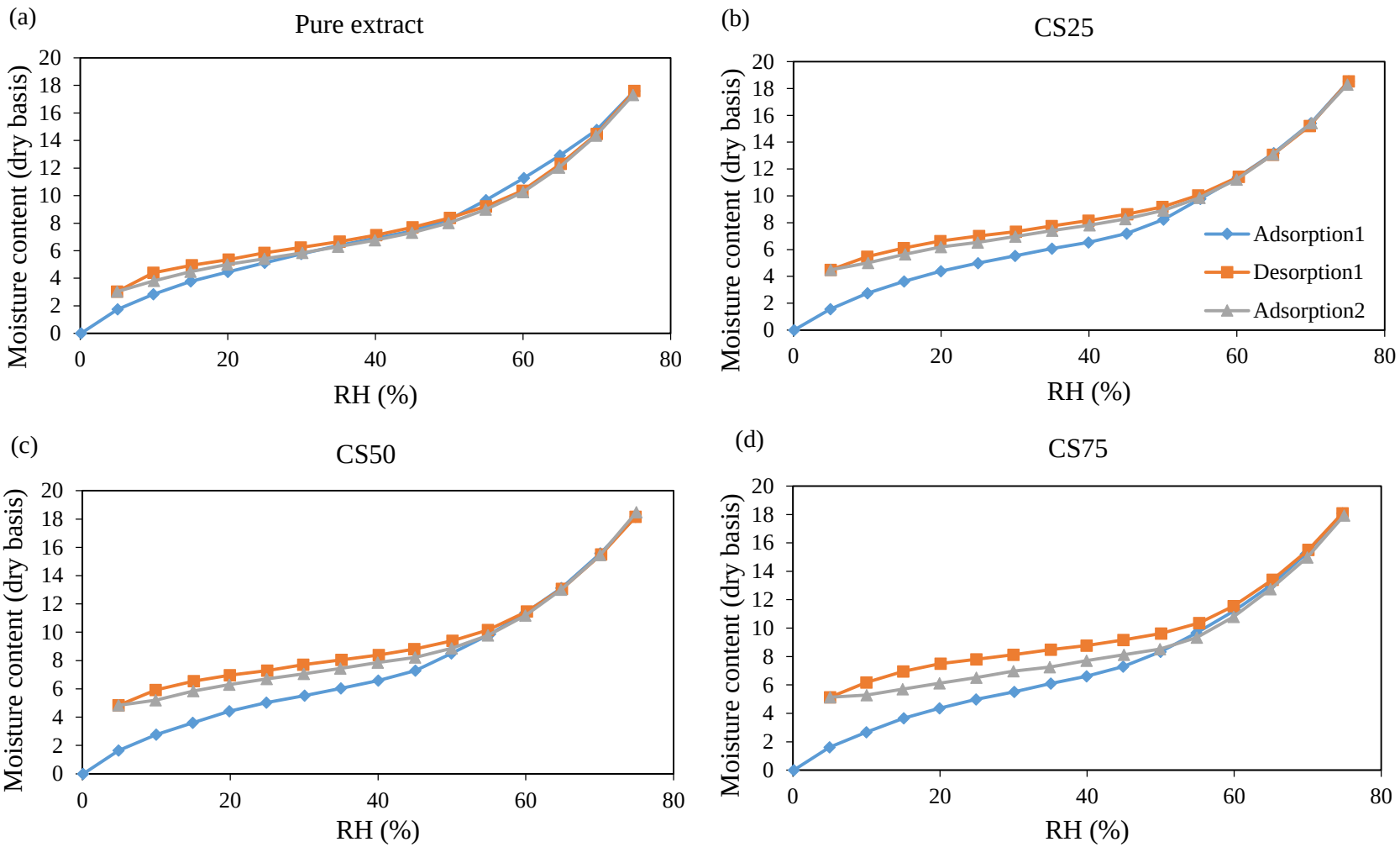

Figure 3.12 Moisture sorption isotherms for spray-dried *C. subternata* (a), as well as samples containing 25% (b), 50% (c) and 75% (d) maltodextrin (CS25, CS50 and CS75) showing first adsorption, desorption and second adsorption at 25 °C (RH = relative humidity).

3.4.6 Compatibility of *C. subternata* extract with carriers

Microcalorimetry is a useful tool which is generally used to detect incompatibility between active pharmaceutical ingredients with other ingredients, but it can be used to detect incompatibility in any powder mixture. It measures the heat flow or thermal activity of individual components, which make up a mixture (Briggner *et al*., 1994; Chadha & Bhandari, 2014). Fig. 3.13 shows the heat flow data of the individual components which make up treatment CS50, i.e. pure spray-dried *C. subternata* extract and corn syrup solids DE 20-23.

From these results a theoretical curve for the mixture was calculated and compared to the measured heat flow of the actual mixture (Fig. 3.14). If a difference is observed, the components are considered to be potentially incompatible with each other. Fig. 3.14 shows that the interaction curve (difference between theoretical and measured) is very close to zero. Therefore no interaction was identified and no incompatibility exists between corn syrup solids and pure *C. subternata* extract when they are combined in a ratio of 1:1. Similar results were observed for CS25, IN25, IN50 and IN75 (Addendum A).

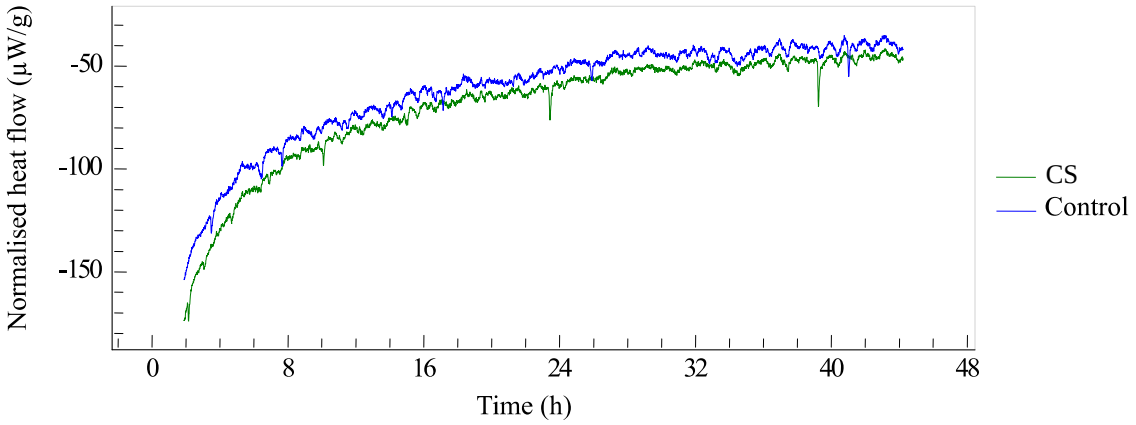

Figure 3.13 Graph depicting the heat flow data of individual components, corn syrup solids and pure *C. subternata* spray-dried extract of sample CS50.

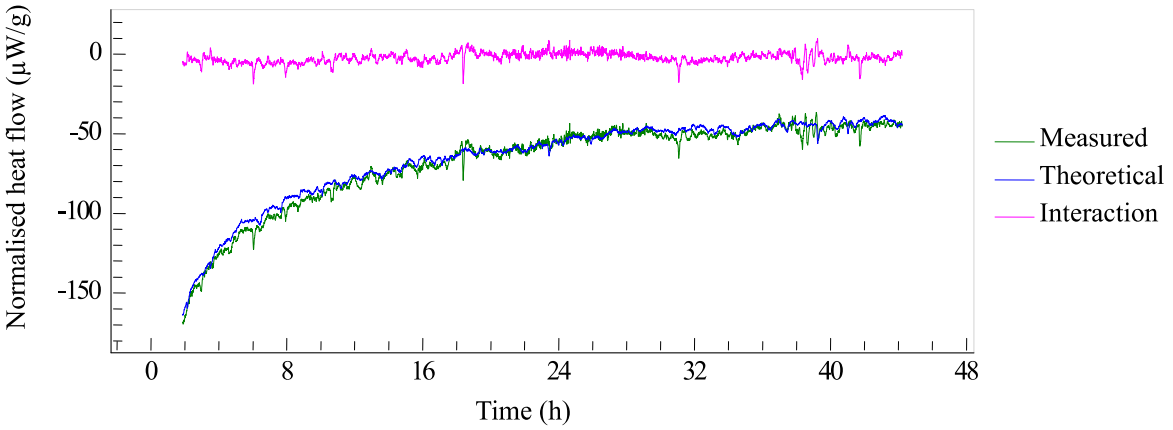

Figure 3.14 Graph depicting the measured and theoretical heat flow data for sample CS50 consisting of a 1:1 ratio (m/m) of pure *C. subternata* extract and corn syrup solids, as well as the interaction between the two components (difference between theoretical and measured heat flow).

Sample CS75, however, gave different results than the other sample mixtures. Fig. 3.15 shows the heat flow data for the components of CS75, while Fig. 3.16 depicts the measured and theoretical heat flow data for the combination of corn syrup solids and the *C. subternata* extract (3:1). A heat flow difference of 922 µW.g^{-1} was calculated between the measured and theoretical data. This is considered a significant difference and is an indication of a possible incompatibility between the two components when mixed in a combination that contains 75% corn syrup solids.

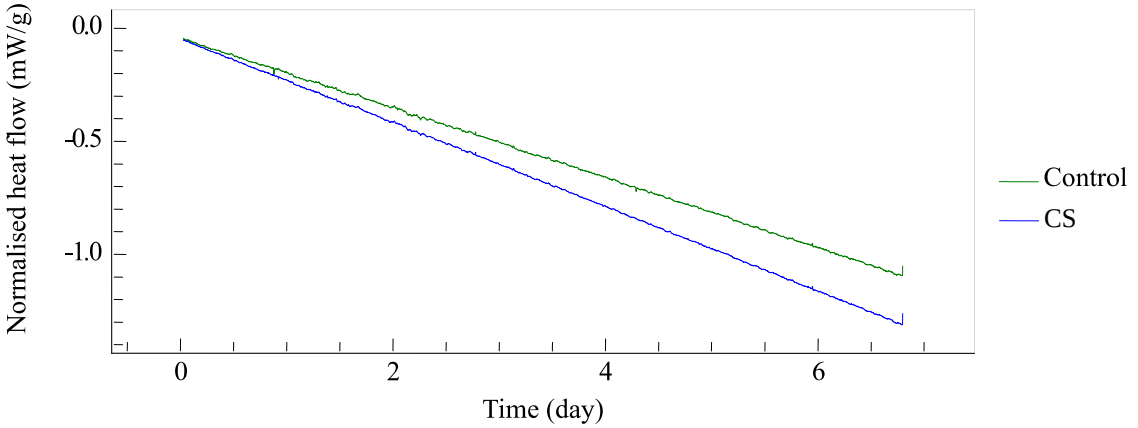

Figure 3.15 Graph depicting the heat flow data of individual components, corn syrup solids and pure *C. subternata* spray-dried extract, that make up sample CS75.

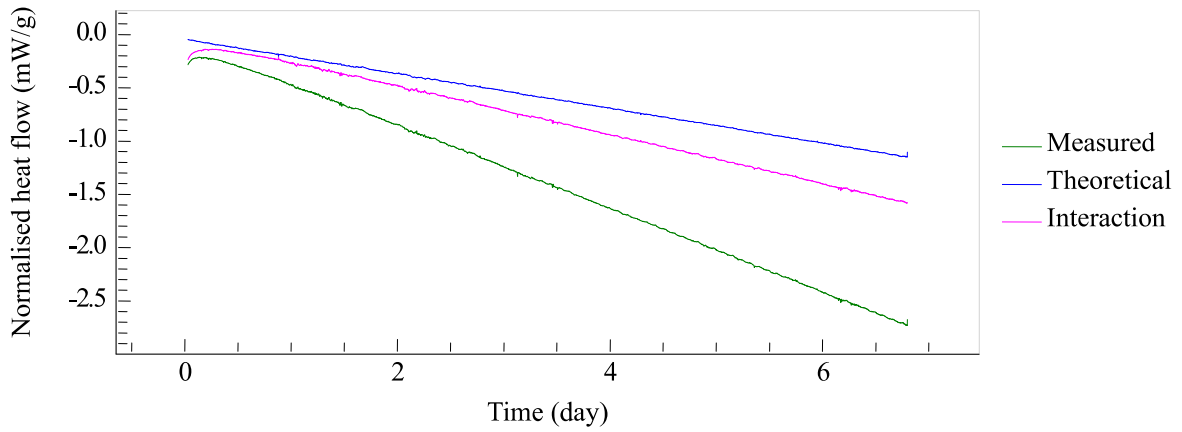

Figure 3.16 Graph depicting the measured and theoretical heat flow data for sampe CS75 consisting of 1:3 ratio of *C. subternata* extract spray-dried with corn syrup solids, as well as the interaction between the two components (difference between theoretical and measured heat flow).

From these results it is not possible to conclude what is causing the interaction between the corn syrup solids and *C. subternata* extract mixed in a ratio of 3:1. However, isothermal microcalorimetry is able to detect possible problems with incompatibility of a mixture that would arise during product storage without having to conduct lengthy shelf-life trials. This helps in the decision making process during the early phases of product development. On these premises CS75 is considered unsuitable as an option for further development into an instant iced tea due to the detected incompatibility.

3.4.7 Retention of phenolic compounds

One of the concerns during the spray-drying processes is the degradation of important bioactive phenolic compounds. Results of studies vary in terms of phenolic retention during spray-drying. This depends on the specific phenolic composition, since some sub-classes of polyphenols are more labile to heat and oxidation. The analytical procedure employed also affects results. Colorimetric methods such as the Folin-Ciocalteu method for quantification of total polyphenol content are less sensitive than when individual compounds are quantified by HPLC. Fang and Bhandari (2011) obtained 96% retention of total polyphenols and 94% retention of total anthocyanins during the spray-drying of bayberry extract performed at an inlet temperature of 150 °C and an outlet temperature of 80 °C. On the other hand Bott *et al.* (2010) reported 22.9% degradation of flavonoids during the spray-drying of *Passiflora alata*

extracts at an inlet temperature of 150 °C. Furthermore, Ersus and Yurdagel (2007) showed that increasing the inlet/outlet temperatures (inlet temperatures = 160, 180, 200 °C; outlet temperatures = 67, 118, 131 °C) caused greater anthocyanin degradation during the spray-drying of black carrot (*Daucuscarota L*.) extract.

Table 3.5 shows the concentration of individual polyphenols, total polyphenol content and total antioxidant capacity of *C. subternata* extract before and after spray-drying. The total polyphenol content and total antioxidant capacity showed no significant differences between the extracts before and after spray-drying. The HPLC results showed slightly, but significantly ($p \geq 0.05$) higher contents of most compounds after spray-drying. All of the compounds were present in spray-dried extracts in concentrations which did not vary by more than 10% of the concentration in the original extract before spray-drying. These discrepancies may be attributed to heterogeneous dispersion of phenolic compounds within the batch of vacuum dried extract, as well as minor experimental errors, which were incurred at different stages of sample preparation. From these results it can be concluded that the spray-drying process did not detrimentally affect the concentration of phenolic compounds in the extract and mixtures.

Previous studies have shown that the bioactive xanthone, mangiferin, is sensitive to degradation during the spray-drying process. When pure mangiferin was spray-dried onto different natural polymers at inlet and outlet temperatures of 160 ºC and 80 ºC, respectively, degradation was substantial and the retention of mangiferin ranged from 16–44% (De Souza *et al*., 2013). In the present study mangiferin did not degrade, possibly due to the natural matrix conferring protection to mangiferin during spray-drying.

Beelders *et al*. (2015) studied the thermal degradation of major xanthones and benzophenones in *C. genistiodes* plant material at 90 °C over 16 h. Over this period the concentration of mangiferin decreased by 55.04 %, but in the first 60 min mangiferin degraded by only 17.09 %. It was also found that some compounds were more stable than others. For instance iriflophenone-3-*C*-β-D-glucoside-4-*O*-β-D-glucoside only degraded by 11.73 % over 16 hours, compared to 42.78% degradation of the monoglucoside, iriflophenone-3-*C*-β-D-glucoside.

In this study it is expexted that the minimal degradation of these phenolic compounds occurred due to the short passage time through the spray-dryer. The amount of time that the droplets spent in the spray-dryer was in the range of a few seconds. During the drying process evaporation of water from the atomised particles also has a cooling effect so that the core temperature of the particles never exceeded 100 °C (Fang & Bhandari, 2011). It is therefore safe to assume that the highest temperatures to which the particles were exposed resembled those of the outlet temperature. In the present study the final outlet temperature ranged between 95 and 102 °C. In light of the results obtained by Beelders *et al* (2015), the time and temperature combination that the extract was exposed to in the spray-dryer should not cause detectable phenolic degradation.

The addition of a carrier did not appear to play a crucial role in protecting the phenolic compounds during processing, because the phenolic compounds in the pure extract did not undergo degradation in the first place. It can therefore be concluded that the spray-drying process is suitable for producing dried extracts of *C. subternata*.

Table 3.5 Concentration of phenolic compounds (g/100g extract (dry basis); mean ± standard deviation; n = 4) from independent replicates of pure *C. subternata* extract before and after spray-drying (SD), as well as samples containing 25%, 50% and 75% inulin (IN25, IN50, IN75) and 25%, 50% and 75% corn syrup solids (CS25, CS50, CS75) after spray-drying.

Compound	Before SD	Pure extract	IN25	IN50	IN75	CS25	CS50	CS75
Maclurin-3-*C*- β-D-glucoside	0.048 bc ± 0.001	0.048 bc ± 0.001	0.048 ab ± 0.001	0.046 d ± 0.000	0.045 d ± 0.000	0.049 a ± 0.001	0.047 c ± 0.000	0.048 abc ± 0.001
Mangiferin	0.493 d ± 0.008	0.518 bc ± 0.015	0.533 a ± 0.005	0.528 ab ± 0.004	0.519 b ± 0.004	0.528 ab ± 0.007	0.535 a ± 0.004	0.506 c ± 0.002
Isomangiferin	0.317 e ± 0.005	0.337 bc ± 0.009	0.343 ab ± 0.004	0.339 abc ± 0.004	0.333 cd ± 0.002	0.343 ab ± 0.004	0.346 a ± 0.003	0.328 d ± 0.001
Vicenin-2	0.401 e ± 0.007	0.426 bc ± 0.011	0.432 ab ± 0.005	0.428 abc ± 0.004	0.421 cd ± 0.003	0.433 ab ± 0.006	0.436 a ± 0.004	0.414 d ± 0.002
Scolymoside	1.041 e ± 0.019	1.093 cd ± 0.045	1.168 bc ± 0.015	1.150 c ± 0.017	1.065 cd ± 0.007	1.123 a ± 0.010	1.106 ab ± 0.009	1.093 de ± 0.010
Iriflophenone-3-*C*-β-D-glucoside-4-*O*-β-D-glucoside	0.841 e ± 0.003	0.912 a ± 0.004	0.908 ab ± 0.011	0.895 bc ± 0.009	0.882 d ± 0.005	0.911 a ± 0.008	0.912 ab ± 0.015	0.893 c ± 0.002
Eriocitrin	0.594 d ± 0.01	0.623 bc ± 0.013	0.629 ab ± 0.007	0.639 a ± 0.012	0.626 ab ± 0.000	0.634 ab ± 0.008	0.639 a ± 0.003	0.611 c ± 0.002
3-Hydroxy-phloretin-3′,5′-di-*C*-hexoside	0.732 e ± 0.014	0.782 ab ± 0.009	0.791a ± 0.011	0.775 bc ± 0.006	0.762 d ± 0.006	0.785 ab ± 0.007	0.781 ab ± 0.010	0.766 cd ± 0.009
Phloretin-3′,5′-di-*C*-β-D-glucoside	1.173 d ± 0.018	1.254 a ± 0.016	1.261 a ± 0.015	1.244 ab ± 0.009	1.223 c ± 0.007	1.255 a ± 0.014	1.261 a ± 0.012	1.234 bc ± 0.005
Hesperidin	0.602 d ± 0.01	0.639 ab ± 0.018	0.651 a ± 0.006	0.644 ab ± 0.004	0.631 bc ± 0.004	0.648 a ± 0.010	0.652 a ± 0.005	0.621 c ± 0.002
Iriflophenone-3-*C*- β-D-glucoside	0.322 c ± 0.005	0.339 b ± 0.01	0.350 a ± 0.003	0.344 ab ± 0.002	0.328 c ± 0.003	0.339 b ± 0.011	0.353 a ± 0.007	0.346 ab ± 0.003
Total antioxidant *	2342 a ± 38.139	2387 a ± 209	2435 a ± 158	2422 a ± 85	2452 a ± 201	2447 a ± 52	2277 a ± 234	2378 a ± 79
Total polyphenols#	29.72 ab ± 0.52	30.94 ab ± 1.27	29.60 ab ± 0.97	29.22 a ± 1.64	30.94 ab ± 0.47	29.54 ab ± 2.62	30.95 ab ± 1.39	31.52 b ± 0.54

Means in the same row with the same letter are not significantly different ($p \geq 0.05$).
*Total antioxidants in Trolox equivalents (µmole Trolox/g pure extract).
#Total polyphenols in gallic acid equivalents (g gallic acid/100 g pure extract)

3.5 Conclusions

Spray-drying was demonstrated to produce adequate yields of powdered dried green *C. subternata* honeybush extract with sufficiently low moisture content and a_w to ensure stability under the correct storage conditions. Furthermore, the heating process had a negligible effect on the phenolic compounds resulting in good retention. Inulin as carrier produced similar powder characteristics to corn syrup solids. Inulin with prebiotic properties and relatively low energy value can therefore be considered a suitable substitute for the production of low energy health products, such as a powdered instant iced tea product. Both of these carriers were compatible with *C. subternata* extract, with the exception of the mixture containing 75% corn syrup solids.

3.6 References

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Chapter 4

Effect of formulation on the shelf-life stability of dry iced tea powder mixtures containing green *C. subternata* extract

4.1 Abstract

Development of iced tea powder formulations with reduced sugar content is of interest for the functional food industry, but shelf-life of such mixtures is a concern. Green *Cyclopia subternata* (honeybush) was chosen as functional base due to its potential health-promoting benefits. The aim of this study was to determine the effect of storage conditions and product formulation on the stability of iced tea powder mixtures containing green *C. subternata* extract. Spray-dried *C. subternata* extract and five formulations containing mixtures of *C. subternata* extract with various combinations of inulin, sugar, xylitol, stevia and 6:1 citric acid – ascorbic acid mixtures were subjected to a six month shelf-life stability trial at ambient (25 °C/60% relative humidity (RH)) and accelerated (40 °C/75% RH) storage conditions, representing the recommended temperature and RH storage conditions for climatic zone II. Isothermal microcalorimetry was also used to assess the effect of raised relative humidity (RH) conditions (25 °C/55% RH and 40°C/75% RH) on the stability of the mixtures.

X-ray diffraction and differential thermal analyses showed no significant differences in the physical structure of the powders before and after 6 months of storage. Isothermal microcalorimetry also detected no incompatibilities in the mixtures in the absence of raised RH conditions. Powders were therefore stable in terms of physical characteristics at ambient temperatures (25 °C) and showed adequate phenolic retention, although small losses were observed, especially in the presence of citric acid – ascorbic acid mixture. However, when the powders were stored at 40 °C, the presence of the acids drastically increased the degradation of phenolic compounds and caused physicochemical changes in the mixtures resulting in prominent colour changes in the samples. Changes in available moisture content and a_w over time contributed to physical and chemical changes within the powders, as well as the release of moisture due to dehydration of citric acid at 40 °C. At 25 °C/55% RH spray-dried *C.*

subternata extract started to deliquesce while mixtures containing sugar and xylitol remained stable. At 40 °C/75% RH deliquescence occurred in samples containing xylitol due to the deliquescence lowering effect of raised temperature conditions (40 °C). The addition of sugar and xylitol to *C. subternata* extract improved the wettability of the powder and therefore made it easier to dissolve.

The stability of *C. subternata* extract in a standard, and reduced kilojoule iced tea formulations was adequate when the mixtures were stored in dry powder form at ambient conditions (25 °C) and in the absence of increased RH conditions.

4.2 Introduction

Numerous scientific studies have shown a positive association between the consumption of sugar-sweetened beverages and an increase in weight gain and obesity in both adults and children (Ludwig *et al*., 2001; Bray *et al*., 2004; Ebbeling *et al*., 2006; Malik *et al*., 2006; Bleich *et al*., 2009). The main concern with their consumption is that they contribute high amounts of sugar to the diet with very little nutrient compensation for the total energy intake (Malik *et al*., 2006).

Iced tea products are a growing sector in the functional beverages market (Anon, 2015a; Anon, 2015b) and present a convenient vehicle for the delivery of the bioactive phenolic compounds found in the indigenous South African herbal tea, honeybush (*Cyclopia* spp.) (Joubert *et al.*, 2011). Major phenolic compounds, present in *Cyclopia* infusions, belong to the subclasses, xanthones, flavanones, flavones, benzophenones and dihydrochalcones (Schulze et al., 2015). Several of the compounds, most notably mangiferin, a xanthone, are postulated to contribute towards the anti-diabetic and anti-obesity properties of honeybush tea (Muller *et al*., 2011; Dudhia *et al*., 2013). Recently, Beelders *et al*. (2014) demonstrated that the *Cyclopia* benzophenones inhibit mammalian α-glucosidase and were marginally effective in increasing glucose uptake *in vitro*. Inhibition of α-glucosidase delays the breakdown and absorption of carbohydrates in the gut, preventing postprandial hyperglycaemia (a sharp rise in blood sugar after meals), a common and serious problem faced by many people with Type 2 diabetes (Van de Laar *et al*., 2006). A reduced-sugar or sugar-free beverage containing *C. subternata* extract

would thus give consumers a healthier alternative to the high sugar content beverages which currently saturate the market.

The stability of phenolic compounds is affected by factors such as pH, heat, light and the presence of oxygen and moisture (Ortiz *et al*., 2008; Harbourne *et al*., 2013). Combined these factors have resulted in the oxidation of catechins in ready-to-drink iced tea products containing green, tea thereby decreasing their physiological activity and affecting their visual appeal (Zimeri & Tong, 1999; Ortiz *et al*., 2008). Joubert *et al.* (2009) found that sterilisation of ready-to-drink rooibos iced tea formulations significantly decreased the content of the major flavonoids. An alternative product format which could provide improved stability of phenolic compounds is iced tea powder mixtures (Ortiz *et al*., 2008). These products are usually formulated with food ingredients such as sugar and citric acid and can be conveniently packed in a single serving "stick pack". Such a product needs to be reconstituted in water before consumption, implying that physical characteristics such as wettability, dispersion and solubility of the mixture are also important.

During the development of functional foods it is important to determine the optimum shelf-life to ensure that the quality and efficacy of the bioactive compounds are maintained to the point of consumption (Bott *et al*., 2010; Fang & Bhandari, 2011; Sansone *et al*., 2011; Cortés-Rojas *et al.*, 2014). To date the only research performed on the stability of *Cyclopia* phenolic compounds during storage focussed on their stability in the "unfermented" plant matrix (Joubert *et al*., 2010; Alexander, 2015). Generally, no information is available on the effects of storage, ingredient interactions and moisture content on these compounds in a formulated dry mixture. It is well known that the presence of water can trigger degradation of bioactive ingredients and changes in the physicochemical properties of powders (Moraga *et al*., 2012; Cortés-Rojas *et al.*, 2014). For this reason accelerated storage conditions at relative high temperature and relative humidity are used to test the shelf-life of products. For products aimed at the South African market (climatic zone II) accelerated storage at 40 °C/75% RH and normal storage at 25 °C/60% RH is recommended according to the ICH Guidelines (Q1 Scientific, 2015). Products should also be tested in the final packaging intended for use.

The aim of this study was therefore to address two of the major challenges associated with the development of a 'healthy' convenience iced tea product. The first objective was to

replace sugar with a healthy alternative, and secondly, to develop a reduced-kilojoule stable dry iced tea mixture containing ingredients commonly used in iced tea applications. The effect of formulation on product stability was studied by subjecting the mixtures and pure powdered *C. subternata* extract to storage at 25 °C/60% RH and 40 °C/75% RH for 6 months. Stability of the phenolic compounds and physiochemical stability of the powders were determined.

4.3 Materials and methods

4.3.1 Chemicals and reagents

Authentic reference standards for HPLC analysis, HPLC solvents and water purification systems have been previously described in Chapter 3 (section 3.3.1). Orafti HP inulin was procured from Savannah Fine Chemicals (Pty) Ltd (Gardenview, South Africa). Food grade citric acid monohydrate, ascorbic acid, xylitol and stevia were kindly donated by Cape Food Ingredients (Westlake, South Africa). Sucrose was purchased from a local supermarket.

4.3.2 *Cyclopia subternata* extract

Bulk unfermented *C. subternata* plant material was batch extracted, using a percolator-type extractor and vacuum-dried as described in Chapter 3 (section 3.3.2). The extract was reconstituted in water for preparation of spray-dried extract or extract-carrier mixture.

4.3.3 Spray-drying conditions

Spray-drying of *C. subternata* extract and extract-inulin mixture was conducted on a Büchi B-290 mini spray-dryer (Büchi Labortechnik AG, Flawil, Switzerland) as described in Chapter 3 (section 3.3.4).

4.3.4 Preparation of treatments and storage conditions

Different formulations were prepared to form six treatments (Table 4.1), which were tested in the shelf-life stability trial. Treatment 1 (T1) represented 100% spray-dried *C. subternata* extract; treatment 2 (T2) a 75:25% spray-dried mixture of *C. subternata* extract and inulin (IN25); treatment 3 (T3) IN25 and sugar; for treatment 4 (T4) the sugar was replaced by a mixture of xylitol and stevia. For treatments 5 and 6, respectively, the same ingredients were used as for treatments 3 and 4, except that citric acid and ascorbic acid were added. Although

the percentage extract and inulin differed in treatments 2 to 6 to accommodate the other ingredients, their ratio remained the same in the final beverage once reconstituted with water.

Table 4.1 Composition of treatments per 100 g of powder formulations.

Component	T 1	T2	T 3	T 4	T 5	T 6
Extract	100	75	2.81	4.13	2.75	4
Inulin	-	25	0.93	1.37	0.91	1.33
Sugar	-	-	96.26	-	94.15	-
Xylitol	-	-	-	94.45	-	91.43
Stevia	-	-	-	0.05	-	0.05
Citric acid	-	-	-	-	1.88	2.74
Ascorbic acid	-	-	-	-	0.31	0.45

The sugar, xylitol, citric acid and ascorbic acid were ground separately to a fine powder, using a Fritsch GmbH Pulverisette ball mill (Idar-Oberstein, Germany) where after the powder was sieved to ensure a small particle size (< 210 µm; Endecotts Ltd, London, England). The ingredients for each formulation were then accurately weighed off using a Zeiss West 3 decimal balance (Sartorius AG, Goettingen, Germany). For mixing a specially designed glass mixing vessel which was attached to the driving mechanism of a rotary evaporator (Büchi Labortechnik AG) was used. Aliquots (*ca*. 5 g) of the mixtures representing the different treatments were weighed into screw-cap amber vials sealed with silicon PTFE seals. Four replicates of each treatment were prepared for each storage condition × time combination. The powders were stored at normal (ambient) (25 °C/60% RH) and accelerated (40 °C/75% RH) storage conditions, using temperature and humidity controlled storage cabinets (SMC Scientific Manufacturing cc., Table View, South Africa). Light was prevented from entering the cabinet by blocking the glass viewing door of the cabinets with a non-transparent material. The treatments were sampled at time 0 and once a month for a period of six months.

4.3.5 Characterisation of powders

4.3.5.1 Assessment of wettability

Measurements were performed on a Krüss DSA100 Drop Shape Analyzer (KRÜSS GmbH, Hamburg, Germany) according to the static sessile drop method with ultrapure water as liquid phase. Samples were prepared by pressing the powder samples to 2 tons in a 13 mm die. Video

files were recorded and the first frame to feature a well-defined vibration-free drop was analysed using Krüss DSA4 software. Due to the heterogenous nature of the samples (Treatments), Circle Fitting analysis was found to give the best results. The contact angle was measured through the liquid phase and is inversely proportional to wettability (Yuan & Lee, 2011):

$$Contact\ angle \propto \frac{1}{Wettability}$$

4.3.5.2 Monthly analysis

Samples taken at monthly time-points were analysed for moisture content, water activity, retention of phenolic compounds (HPLC) and colour according to the methods described in Chapter 3 (section 3.3.5).

Samples for colour analysis were prepared by dissolving the powders in water to the concentration at which they would be consumed (1.75 g extract.L^{-1}). Colour measurements were performed using the same parameters as Chapter 3 (section 3.3.5) with the exception that the colour meter was operated in transmittance mode and 10 mm plastic cuvettes (Greiner Bio-one, Monroe, USA) were used for the sample solutions.

4.3.5.3 Analysis at start and end of storage

The phase transition temperatures and the crystalline vs. amorphous nature of the samples were investigated before and after the storage period using simultaneous thermogravimetry (TG)/ differential thermal analysis (DTA), as well as X-ray powder diffraction (XRPD) analysis as described in Chapter 3 (section 3.3.5).

4.3.5.4 Analysis before storage

The moisture sorption isotherms and compatibility of the mixtures were tested at 25°C using a vapour sorption analyser and isothermal calorimetry as described in Chapter 3 (section 3.3.5).

Isothermal microcalorimetry of the mixtures was also undertaken to test for shelf-life stability by exposing them to 25 °C/55% RH and 40 °C/75% RH. The sample was maintained at a reproducible and well defined RH throughout the experiment by using micro-hygrostats.

The micro-hygrostats, containing saturated solutions of $Mg(NO_3)_2$ and NaCl, which correspond to RH values of 55% and 75%, were placed inside crimp-top glass ampoules containing the samples, immediately before sealing. For control purposes the mixtures were also sealed in the glass ampoules and exposed to 25 and 40°C, but without adjusting the RH through the use of micro-hygrostats.

4.3.6 Statistical analysis

The experimental design was completely random with four independent replicate experiments for each of the 12 treatment combinations. The treatment design was a factorial with six formulations tested at two storage conditions and two storage periods (0 and 6 months). Observations were made on all four replicates of each of the 12 treatment combinations before and after 6 months storage.

Univariate analysis of variance was performed on all variables assessed using the GLM (General Linear Models) Procedure of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). The Shapiro-Wilk test was performed on the standardized residuals from the model to test for normality (Shapiro & Wilk, 1965). In cases where there was significant deviation from normality outliers were removed when the standardized residual for an observation deviated with more than three standard deviations from the model value. Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

4.4 Results and discussion

4.4.1 Development of iced tea formulations

In Chapter 3 spray-drying of green *C. subternata* extract with two different carriers, namely corn syrup solids and inulin at three treatment levels 25%, 50% and 75%, was described. Samples containing inulin were selected for the development of instant iced tea formulation, because of its reported health benefits and lower energy value (Dehghan *et al*., 2014).

The formulation for ready-to-drink (RTD) green rooibos iced tea (Joubert *et al*., 2010), comprising 1.75 g rooibos extract, 1.2 g citric acid, 0.2 g ascorbic acid and 60 g sucrose per litre, was used as the starting point for the development of green honeybush instant iced tea

powder. When samples containing inulin were tested in the standard formulation it was found that the mixture IN25, containing 25% inulin, dissolved well at the level of extract (1.75 g/L) required. However, IN50 was more difficult to dissolve and IN75 did not dissolve completely in cold water, resulting in undesirable lumps with a gritty texture. While the inclusion of dietary fibre may improve the health profile of this beverage, grittiness is an undesirable sensory attribute and should therefore be avoided to ensure consumer acceptance (Sun-Waterhouse *et al*., 2010; Sun-Waterhouse *et al*., 2013). With this in mind IN25 was selected for further development into a dry iced tea mixture. In the standard formulation this related to 2.33 g IN25 per litre of iced tea solution (Table 4.2).

Xylitol and stevia were selected as alternative sweeteners to sugar in view of the aim to produce a low kilojoule beverage product. The sweetness of xylitol is equal to that of sugar, while stevia is 1000 times sweeter (Lanton, B., 2014, Owner, Cape Food Ingredients, personal communication, 28 July). Stevia also has a bitter aftertaste (Hellfritsch *et al*., 2012), so that a mixture of these two were evaluated. The selected ratio (2:1) of these two sweeteners was tested by an informal sensory panel and was considered acceptable. In the final formulation xylitol replaced two thirds of the sugar content with stevia replacing the other third (Table 4.2).

Table 4.2 Final composition of treatments (g) used to produce 1litre of iced tea.

Component	T1	T2	T3	T4	T5	T6
Extract	1.75	1.75	1.75	1.75	1.75	1.75
Inulin	-	0.58	0.58	0.58	0.58	0.58
Sugar	-	-	60.00	-	60.00	-
Xylitol	-	-	-	40.00	-	40.00
Stevia	-	-	-	0.02	-	0.02
Citric acid	-	-	-	-	1.20	1.20
Ascorbic acid	-	-	-	-	0.20	0.20

Table 4.3 shows the contribution of the different sweeteners to the total energy content of the iced tea formulations compared to one serving (330 mL) of a typical carbonated beverage and a typical ready-to-drink iced tea on the market. T5, the formulation containing sugar,

contributes 322.7 kJ per serving which is nearly half that of a typical carbonated beverage (570.7 kJ). T6, the formulation containing the alternative sweeteners, contributes only 132 kJ per serving, which translates into 59% less energy than T5 and 79% less energy than the average carbonated beverage.

Table 4.3 Comparison of the contribution of different sweeteners to the total energy content of honeybush iced tea formulations and popular beverages on the market. Treatments were composed of pure *C. subternata* extract spray-dried with 25% inulin (IN25) + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

Formulation	Energy contributed by sweeteners	Total energy per L	Total energy per serving (330 mL)
Typical carbonated beverage	Sugar: 16.3 kJ x 106.1 g	1729.4 kJ	570.7 kJ
Typical iced tea	Sugar: 16.3 kJ x 76.8 g	1251.8 kJ	413.1 kJ
T5 (with sugar)	Sugar: 16.3 kJ x 60 g	978 kJ	322.7 kJ
T6 (alternative sweeteners)	Xylitol: 10.0 kJ x 40 g Stevia: 0 kJ x 0.02 g	400 kJ	132 kJ

4.4.1.1 Assessment of wettability

The ability of iced tea powder formulations to dissolve and disperse in water is very important to ensure that it is easy to use. Wetting is a precursor to dissolution and the wettability of a solid material thus has a significant effect on its dissolution rate. It also plays a role in the interaction of the different ingredients when in contact with water (Hogekamp & Schubert, 2003). The contact angle method has found previous application in powder formulation of pharmaceuticals but has not been widely used for food products (Kiesvaara *et al*., 1993; Prestidge & Tsatouhas, 2000). Table 4.4 gives the recorded wettability values of the individual ingredients, as well as the different treatments in descending order. When analysing the results, the lower the contact angle, the greater the wettability of a powder. The contact angles of all samples and ingredients were below 90º (Table 4.4) which corresponds to high wettability. With a contact angle < 90° the fluid will spread over a large area on the surface, while contact angles > 90° generally means that wetting of the surface is unfavourable. The fluid will minimize its contact with the surface and form a compact liquid droplet (Yuan & Lee, 2013).

Interesting behaviour was observed for T1 and T2. During their analysis it was found that when a water droplet was applied to the powder it would initially spread, indicating rapid wetting, but then the contact angle would increase, as seen by the droplet rising slightly (Fig. 4.1). This kind of behaviour would make the pure extract and IN25 very difficult to dissolve in a glass of water, because a viscous coating forms around the outside of the powder resulting in lumps of powder. The penetration of water into the lump is impaired by the highly viscous layer of gel-like consistency forming when particles start to dissolve while still in close proximity of each other. The outer layer effectively confines the undissolved powder to the interior of the lump. Similar results were observed for spray-dried rooibos powders containing maltodextrin and agglomeration techniques were investigated as a means of overcoming these challenges (Joubert, 1988).

In the present study it was observed that the addition of the ingredients such as xylitol, sugar, citric acid, and ascorbic acid improved the wettability of the mixtures by decreasing the contact angle of T3 to T6 to less than 26°. Xylitol, with the lowest contact angle of all the ingredients, was slightly more wettable than sugar, and substantially more wettable than stevia. It can therefore be concluded that the iced tea formulations possess suitable wettability properties resulting in easy dissolution of the powders.

Table 4.4 Wettability (contact angle (º) ± standard deviation**)** of iced tea powder formulations and the individual ingredients. Treatments were composed of pure *C. subternata* extract (T1), *C. subternata* extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

Sample	Contact angle (º) ± Std dev
T1	45.3 ± 2.27
T2	43.6 ± 3.42
T3	24.5 ± 1.96
T4	23.0 ± 0.97
T5	25.8 ± 2.64
T6	23.6 ± 1.14
Sugar	24.6 ± 1.09
Xylitol	16.0 ± 1.02
Citric acid	22.4 ± 1.08
Ascorbic acid	24.8 ± 0.33
Stevia	56.5 ± 6.17

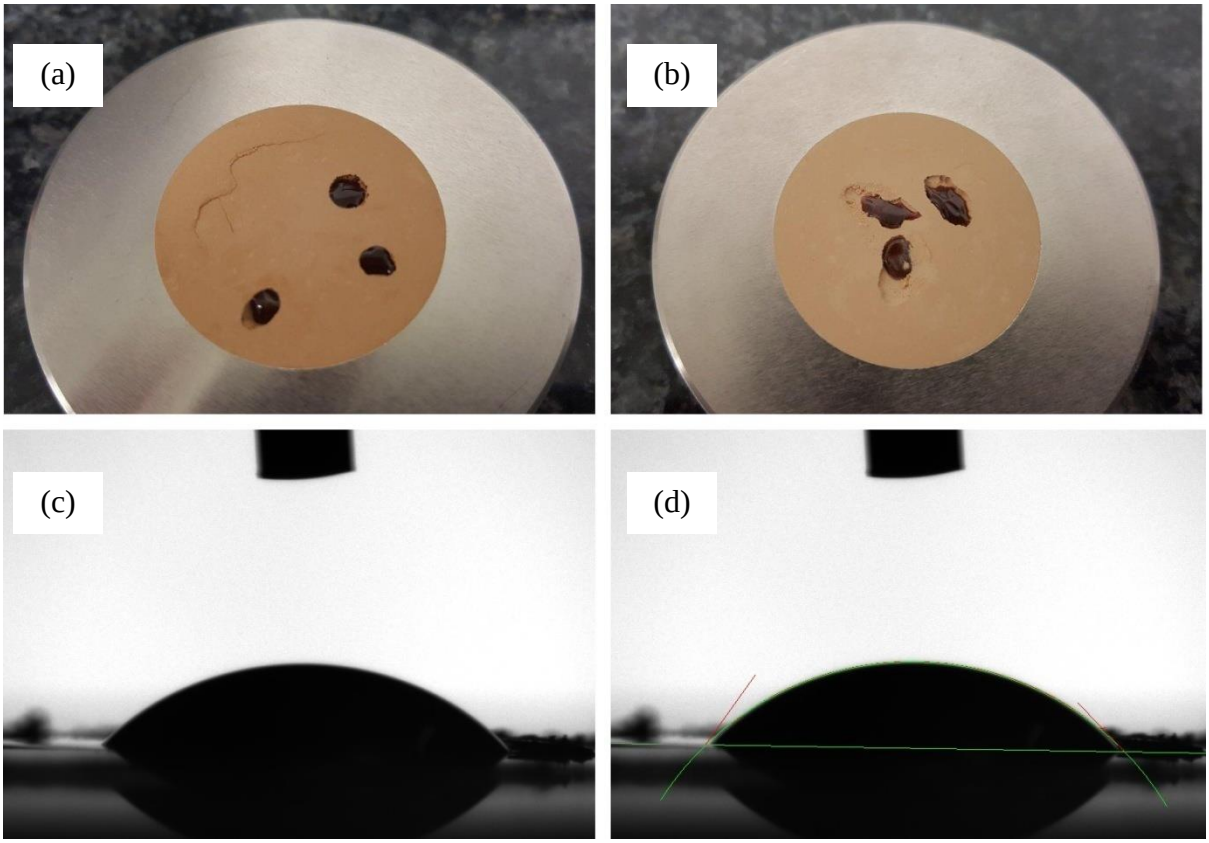


Figure 4.1 Photos of wettability experiment. (a) is pure spray-dried *C. subternata* extract (T1) and (b) is the extract spray-dried with 25% inulin (T2). (c) Shows a screen shot of the video used to record the wettability angle of T1 and (d) shows the way in which the contact angle was determined.

4.4.2 Storage stability

4.4.2.1 Change in colour and visual appearance

Observing a change in colour and visual appearance is one of the first and most obvious means of determining if powders have degraded during storage. Under adverse storage conditions natural extracts are prone to hydrolysis and oxidation which is often reflected in colour changes (Cortés-Rojas *et al.*, 2014). It is therefore important to determine if a change in colour is correlated with a loss of bioactive ingredients in functional foods (Harbourne *et al.*, 2013; Hetrick *et al.*, 2013).

At the start of the storage experiment the powders were free-flowing and off-white or tan coloured (Table 4.5). Over the six month storage period the powders changed colour to various degrees (Table 4.5). When powders were reconstituted to a concentration of 1.75 g

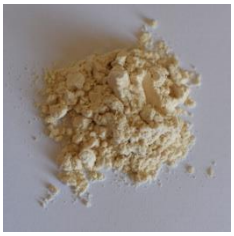
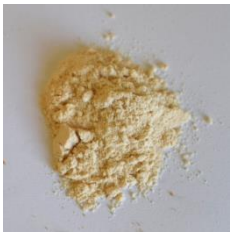
extract.L^{-1} the solution was light yellow in colour as indicated by hue angle (Fig. 4.2). Little change in total colour (as indicated by colour difference), hue and chroma was observed for T1 to T4 when stored at 25 and 40 °C. Addition of citric acid and ascorbic acid (T5 and T6) exacerbated the change as observed for the solution, mostly by the decrease in hue, indicating a shift from yellow to red. This change was less for T5 containing sugar instead of the alternative sweeteners. T5 also contained a lower concentration of extract than T6 within the powder, although the amount of extract in the solution and thus beverage as would be consumed was the same.

At 40 °C the change in colour was accelerated (Fig.4.2) and within the first month of storage the samples containing citric and ascorbic acid (T5 and T6) reached hue values lower than after 6 months at 25 °C. Over the 6 month storage period the hue angle of the reconstituted beverage decreased from 86° to 65° for T5 and from 86° to 60° for T6. These powders turned bright orange (Table 4.5) and once diluted the liquid was also visibly darker.

Samples from T5 and T6 also started to agglomerate and became sticky and difficult to work with when they were stored at 40 °C. By the end of six months the powders containing citric acid and ascorbic acid, stored at 40 °C, had fused together and were no longer free-flowing (Table 4.5). The reasons behind these physical changes will be discussed further in following sections.

Table 4.5 Change in visual appearance of powder samples before and after 6 months storage at 25 °C/ 60% RH and 40 °C/ 75% RH. Treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

Treatments	Before storage	After storage at 25 °C	After storage at 40 °C
T1			

T2			
T3			
T4			
T5			
T6			

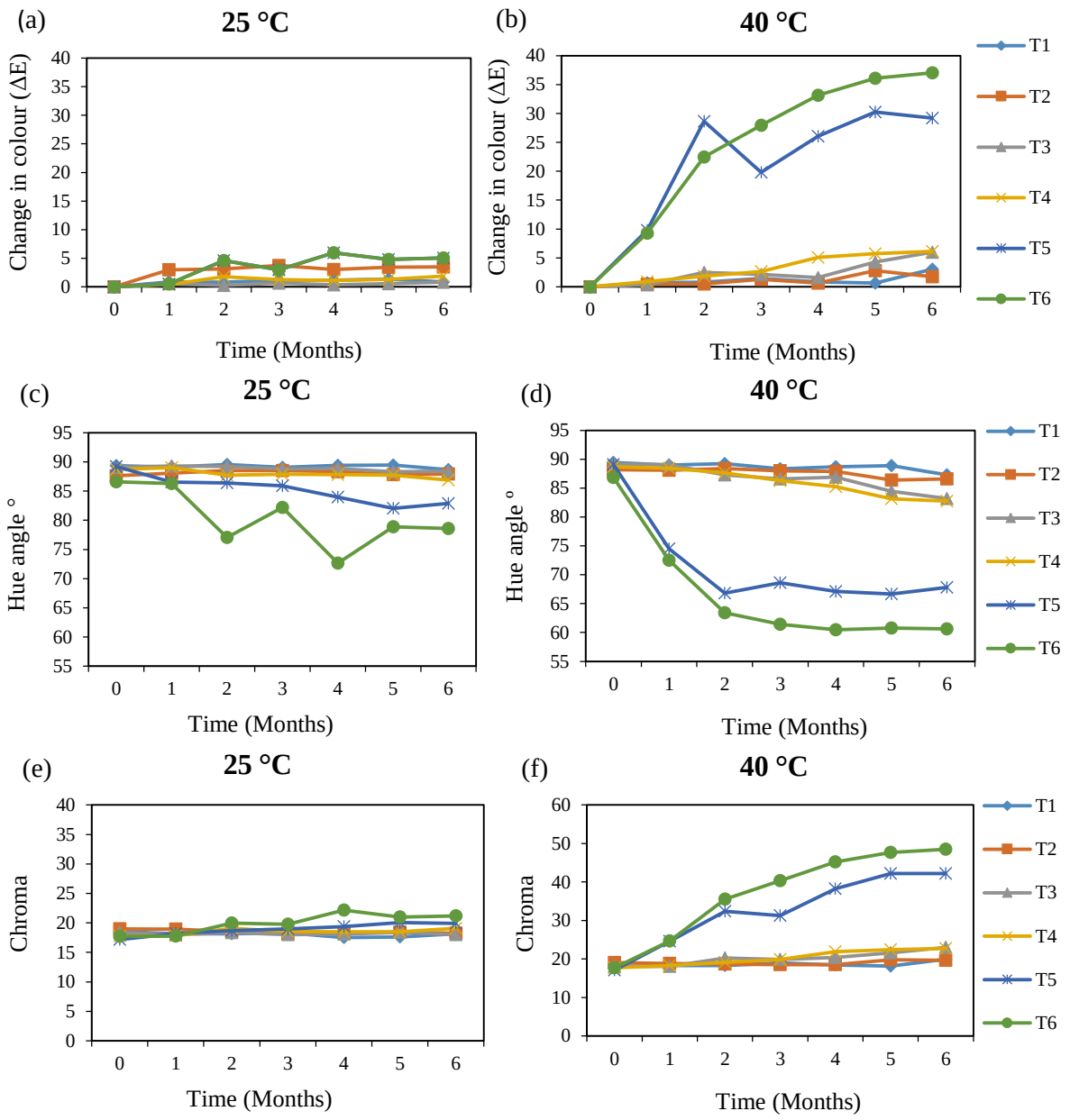


Figure 4.2 Change in colour (ΔE) (a & b), hue angle (c & d) and chroma (e & f) of the reconstituted powders at beverage strength. The powder samples were stored for six months at 25 °C/ 60% RH and 40 °C/ 75% RH. Treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

4.4.2.2 Crystalline vs. amorphous state

At the start of the storage experiment X-ray powder diffraction (XRPD) was used to determine the amorphous vs. crystalline nature of the individual components, as well as that of the different mixtures. The mixtures were then retested at the end of the six month storage stability period to determine if the components within the mixtures had interacted and to assess whether the components had undergone any physical state changes.

Fig. 4.3 shows the XRPD of the individual components, which made up the different mixtures. The extract spray-dried with 25% inulin (IN25) was completely amorphous thus showing a completely diffused XRPD pattern, with an absence of intense sharp peaks. Stevia was also largely amorphous in nature although two small peaks were detected in its XRPD pattern by the software, although not evident on the graph. These peaks are not considered large enough to be significant, but do indicate a very slight degree of crystallinity and/or the possible presence of a small percentage of a crystalline impurity. Ascorbic acid, citric acid, sugar and xylitol all displayed distinctive crystalline diffraction patterns.

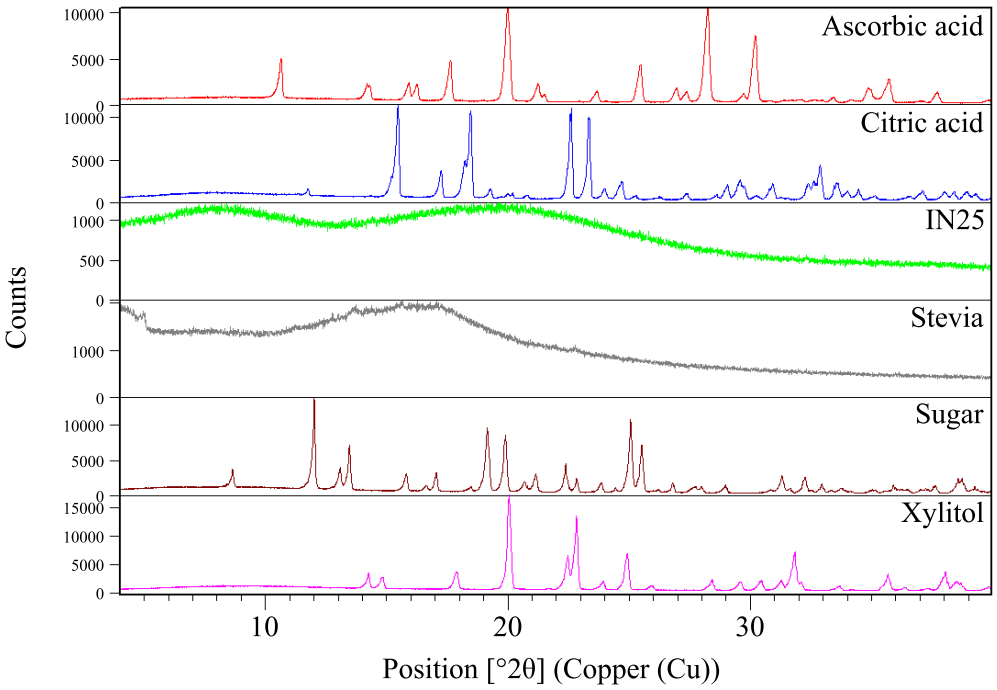


Figure 4.3 X-ray diffractograms of individual components, ascorbic acid, citric acid, *C. subternata* extract spray-dried with 25% inulin (IN25), stevia, sugar and xylitol, used in iced tea powder formulations.

Fig.4.4 shows calculated and recorded X-ray diffractograms for T5 compared to that of sugar. From these results it can be seen that the calculated and experimental patterns were a good match, but also that they were almost identical to the main ingredient i.e. sugar (94.15%). Ascorbic acid was present at a very low concentration (0.31%) and was therefore not detected in the mixture. Citric acid was present in a slightly higher amount (1.88%) and the calculated pattern predicted one small peak, distinctive from the pattern of sugar, at approximately 23.3 2°θ. Magnification of the area showed a peak which is smaller than would be considered significant (Fig. 4.5).

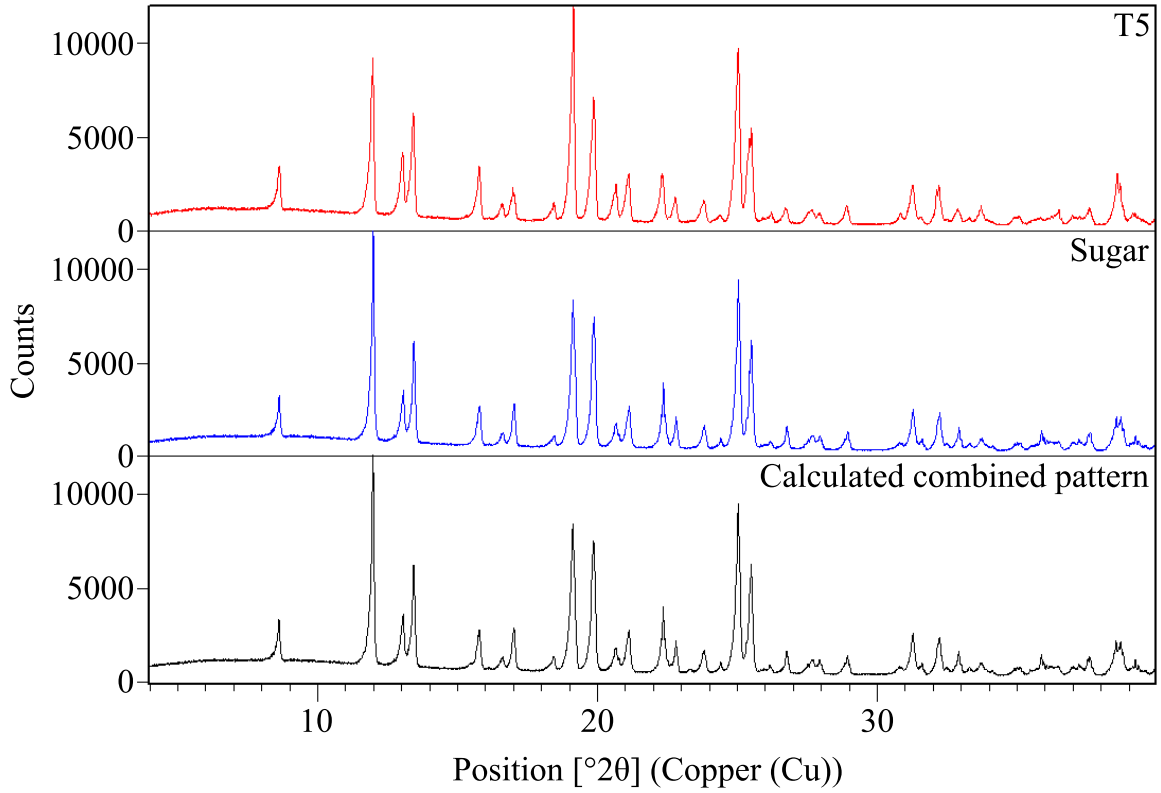


Figure 4.4 X-ray diffractograms of the calculated and recorded patterns of T5 before storage (containing *C. subternata* extract spray-dried with 25% inulin (IN25) + sugar + citric acid + ascorbic acid) compared to the recorded pattern of sugar.

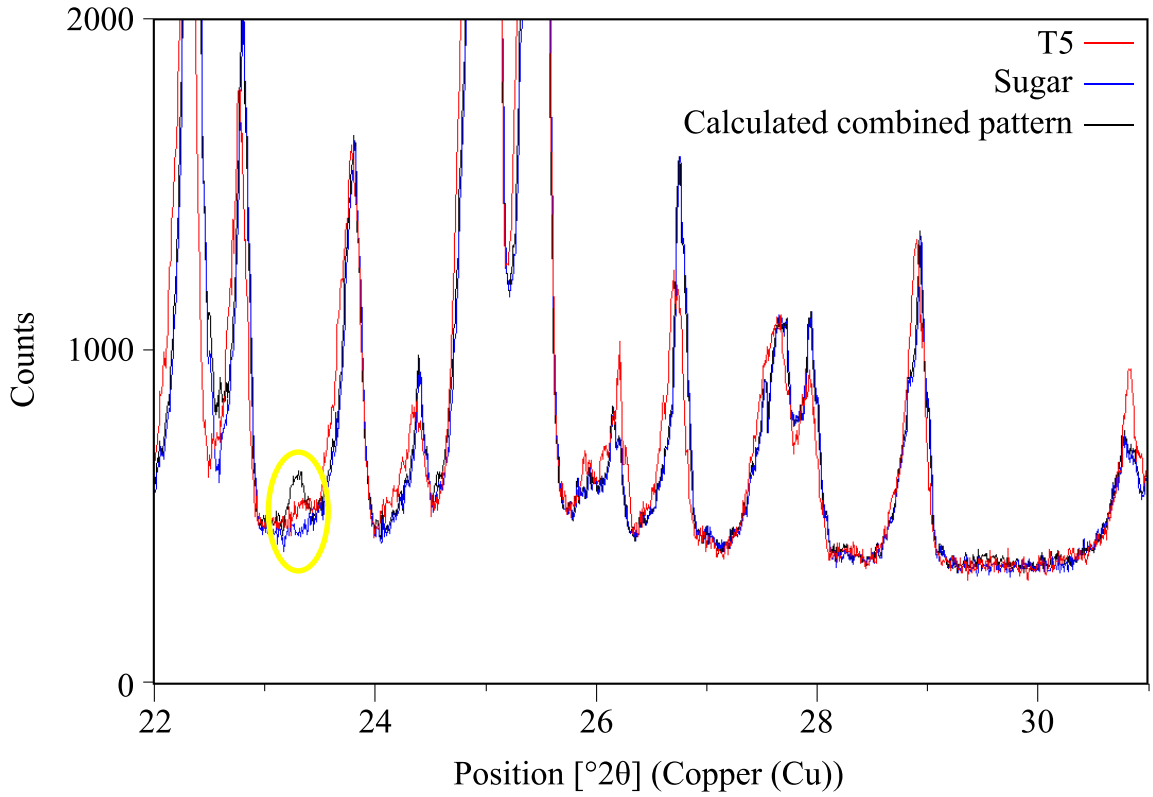


Figure 4.5 Magnified view of the overlay of the X-ray diffractograms for sugar (blue) compared to the calculated (black) and recorded (red) response for T5 (containing extract spray-dried with 25% inulin (IN25) + sugar + citric acid + ascorbic acid). Peak attributed to citric acid indicated in yellow elipse.

Fig. 4.6 shows the calculated and recorded XRPD patterns of T6, as well as the recorded patterns for xylitol. Once again it was found that the calculated and experimental XRPD patterns were very similar and that patterns were almost identical to the main ingredient i.e. xylitol (91.43%). Ascorbic acid was not detected due to its low concentration (0.45%). As for citric acid (2.74%), the calculated XRPD pattern predicted three small peaks, distinctive from the pattern of xylitol, at approximately 15.3, 18.3 and 23.2 2°θ (Fig. 4.7). Magnification of the experimental pattern in those regions showed a peak only at 18.3 2°θ (Fig. 4.7). However, the experimental XRPD pattern had an additional peak (Fig. 4.7), not matching the calculated XRPD pattern, but corresponding with a citric acid peak at 26.2 °2θ. The differences between the calculated and experimental data may be due to the inability to mix the powders completely homogenously.

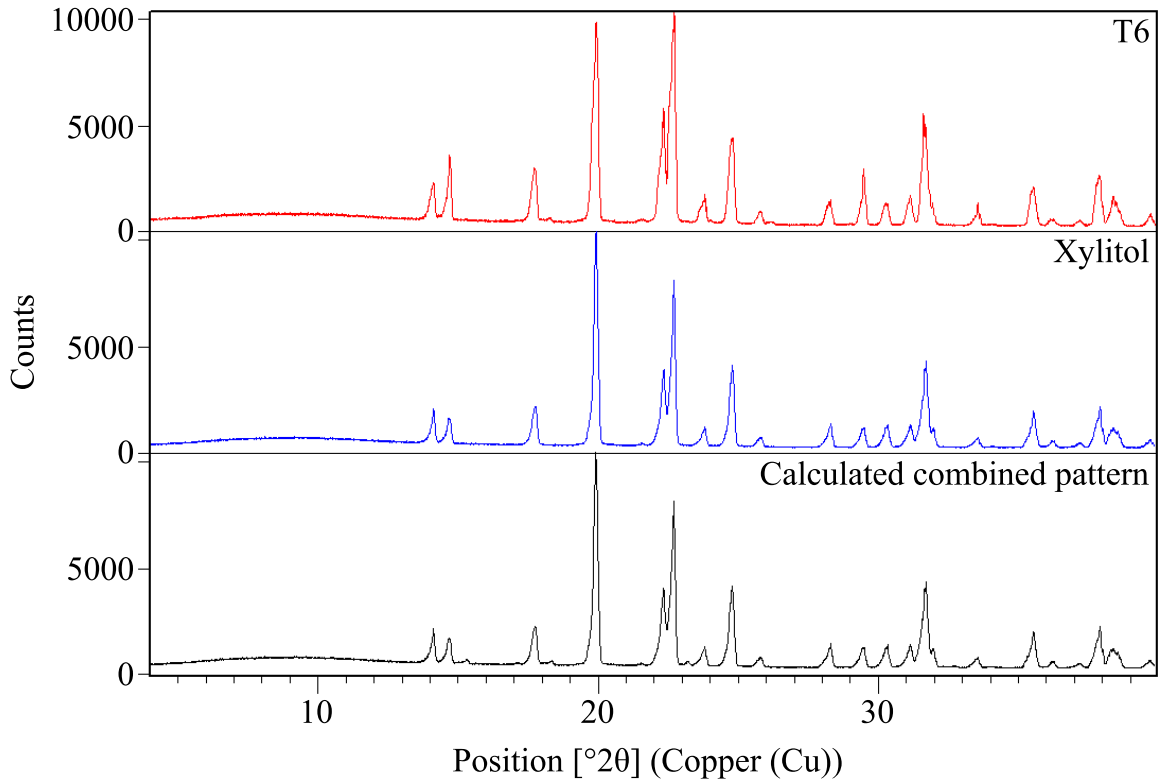


Figure 4.6 X-ray diffractograms of the calculated and recorded patterns of T6 before storage (containing *C. subternata* extract spray-dried with 25% inulin (IN25) + xylitol + stevia + citric acid + ascorbic acid) compared to the recorded pattern of xylitol.

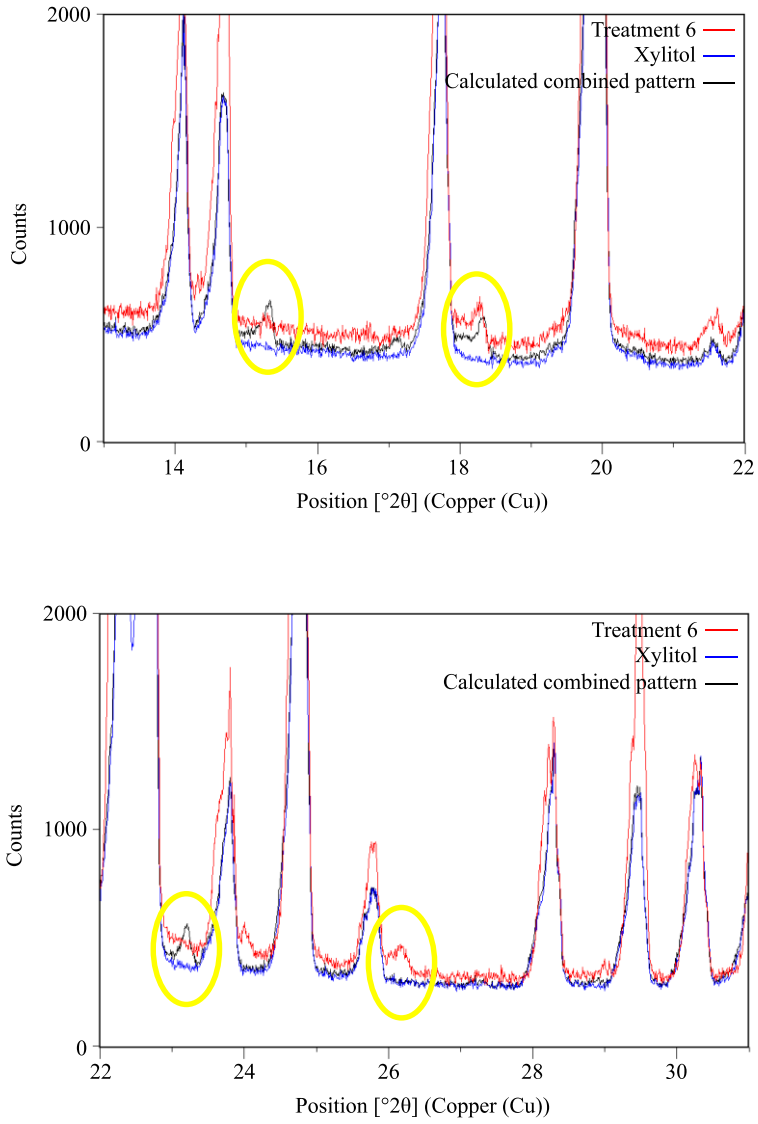


Figure 4.7 Magnified view of the overlay of the X-ray diffractograms for xylitol (blue) compared to the calculated (black) and recorded (red) response for T6 (containing *C. subternata* extract spray-dried with 25% inulin (IN25) + xylitol + stevia + citric acid + ascorbic acid). Calculated peaks are highlighted in purple and detected peaks in yellow.

At the end of the storage stability period it was observed that there were minor differences in the XRPD patterns of the samples before and after storage at 25 °C/ 60% RH and 40 °C/ 70% RH for T5 (Fig. 4.4, Fig. 4.8a) and T6 (Fig. 4.6, Fig. 4.8b). Some treatments exposed to the higher temperature and humidity conditions had XRPD patterns of overall lower intensity, most notably T6 stored at 40 °C/ 70% RH (Fig. 4.8b). A decrease in overall peak intensity could be attributed to higher moisture content. However, these changes were not significant enough to constitute structural changes within the samples.

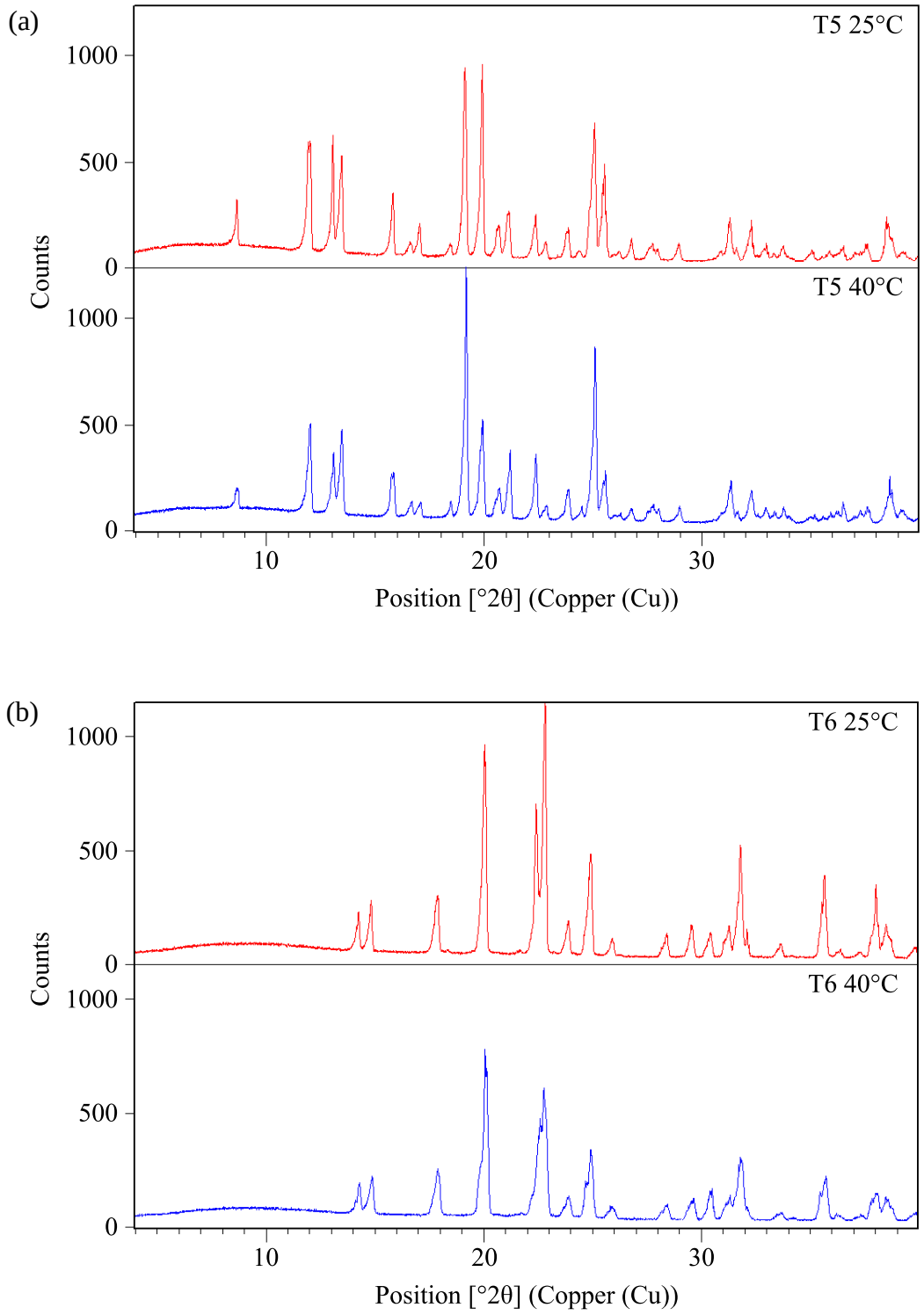


Figure 4.8 X-ray powder diffraction diffractograms of T5 (a) and T6 (b) after six month storage at 25 °C/ 60% RH (red) and 40 °C/ 75% RH (blue). Treatments were composed of *C. subternata* extract spray-dried with 25% inulin (IN25) + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

4.4.2.3 Thermal analysis

Differential thermal analysis supported the data elucidated by the XRPD analysis. From the thermograms depicted in Fig. 4.9 and Fig. 4.10 it can be seen that the sugar, xylitol, citric and ascorbic acid were all in a crystalline form as substantiated by the presence of the distinct melting endotherms. The thermogram of T5 compared to IN25 changed due to the presence of sugar, the major constituent of this mixture (94.15%). It was also influenced by the presence of citric acid as seen by the first melting endotherm which correlated with the second melting endotherm of citric acid.

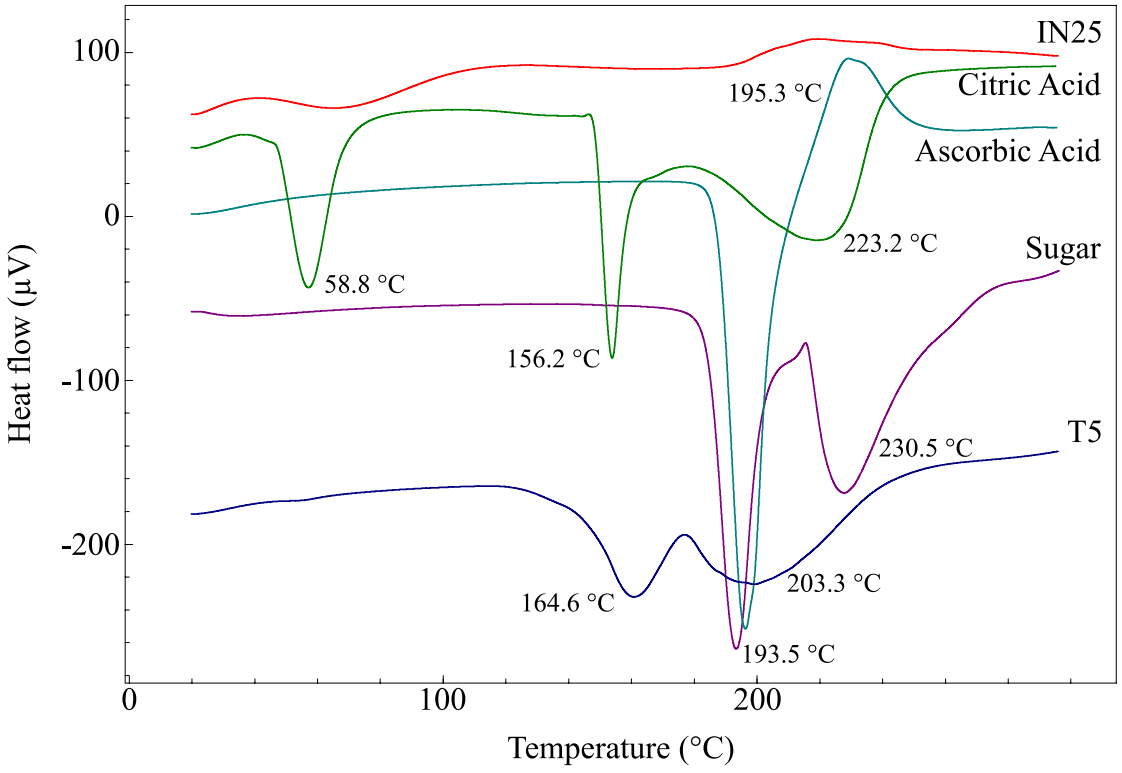


Figure 4.9 Differential thermal analysis (DTA) of T5 as well as its individual components, i.e. *C. subternata* extract spray-dried with 25% inulin (IN25), sugar, ascorbic acid and citric acid, before storage.

Similarly the melting endotherm of IN25 was changed by the addition of xylitol (Fig. 4.10), the major constituent of T6 (91.43%). Unlike T5, the thermogram of T6 did not appear to be influenced by the presence of citric acid.

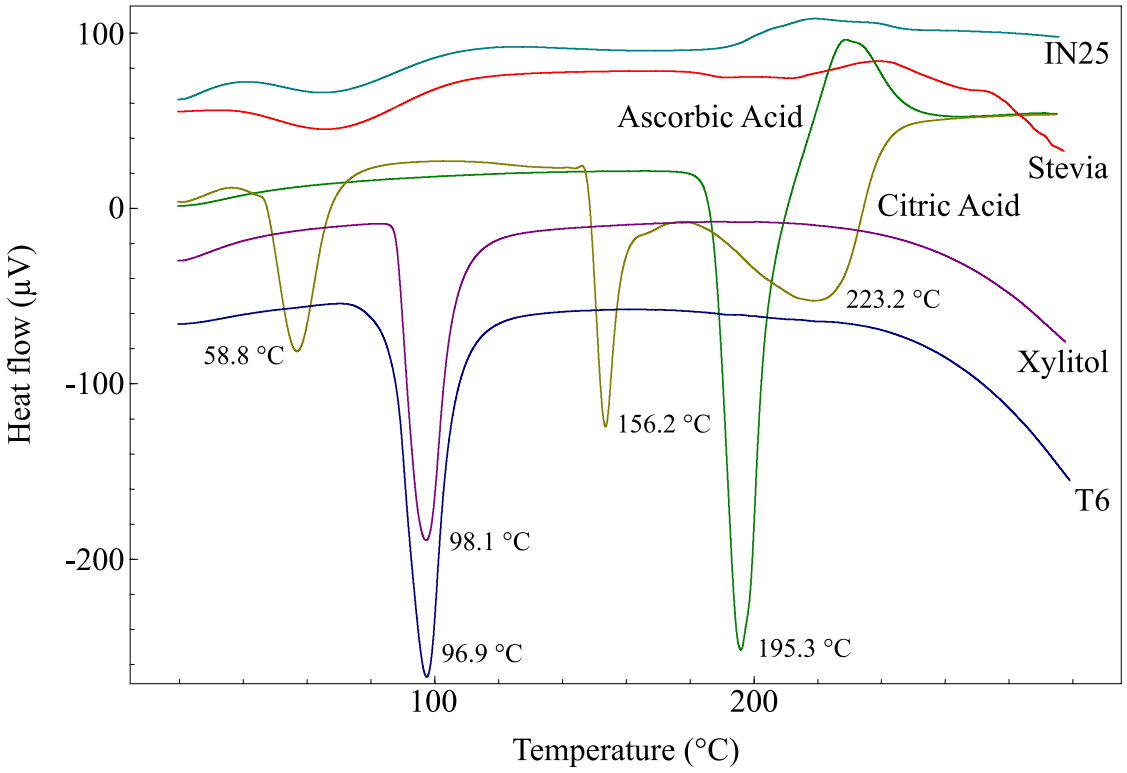


Figure 4.10 Differential thermal analysis (DTA) of T6 and its individual components, i.e. *C. subternata* extract spray-dried with 25% inulin (IN25), xylitol, stevia, ascorbic acid and citric acid, before storage.

The samples were retested at the end of the six month storage period. Comparing the thermograms of T5 before and after storage (Fig. 4.9 and Fig. 4.11, respectively), as well as the thermograms of samples stored at 25 °C/ 60% RH and 40 °C/ 70% RH, it would appear that there were no major changes to the thermal properties of these mixtures over time or as a result of difference in storage temperature. Similar results were obtained for T6 (Fig. 4.10 and Fig. 4.12).

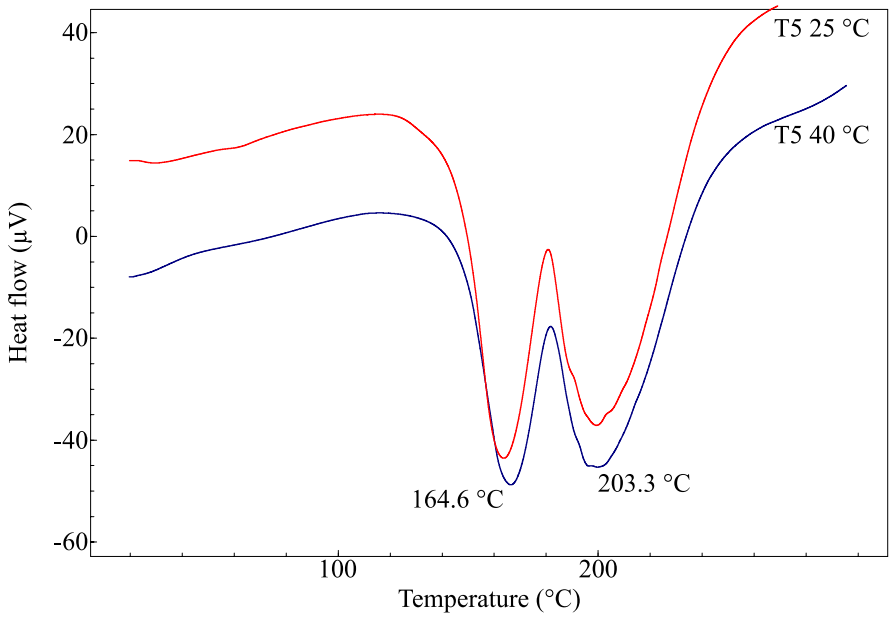


Figure 4.11 Differential thermal analysis of T5 (consisting of *C. subternata* extract spray-dried with 25% inulin + sugar + citric + ascorbic acid) after 6 months storage at 25 °C/ 60% RH (red) and 40 °C/ 75% RH (blue).

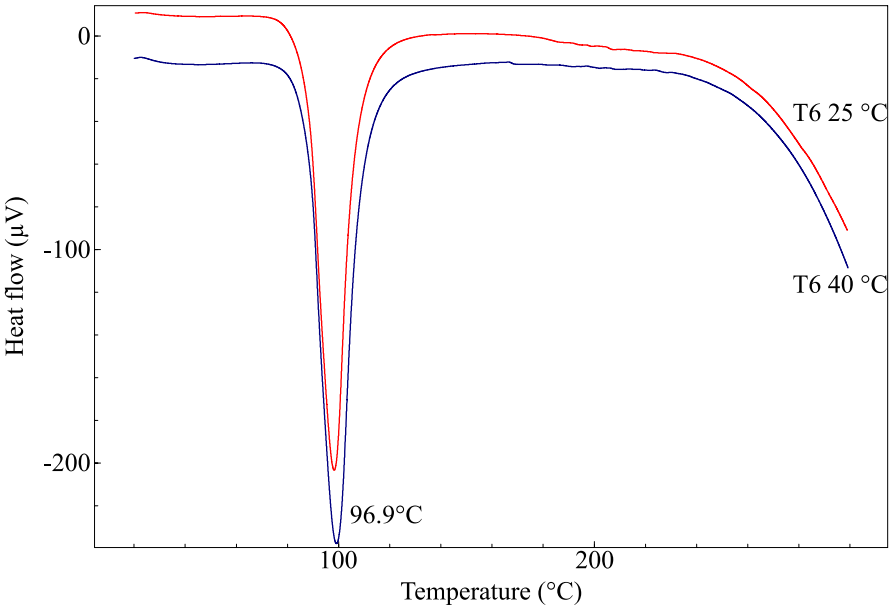


Figure 4.12 Differential thermal analysis thermograms of T6 (consisting of *C. subternata* extract spray-dried with 25% inulin + xylitol + stevia + citric + ascorbic acid) after 6 months storage at 25 °C/ 60% RH (red) and 40 °C/ 75% RH (blue).

4.4.2.4 Compatibility of components within mixtures

Isothermal microcalorimetry was used at the start of storage to assess the compatibility of the mixtures at 25 °C and 40 °C (control samples without adjusted RH). This technique, an invaluable tool in solid-state applications, provides information on stability and compatibility of samples and ingredients. It is very useful to obtain information on chemical and physical stability of a mixture prior to long storage tests as it is a direct and sensitive measurement of reaction processes occurring within a sample, used in many instances in tandem with HPLC analysis (Phipps & Makin, 2000). For T3, exposed to 40 °C, an interaction integral of -1.06 $J.g^{-1}$ and interaction average heat flow of -13.66 $\mu W.g^{-1}$ with an interaction error of ±33.71 $\mu W.g^{-1}$ were calculated. The higher interaction error is an indication that a possible incompatibility may exist. Fig. 4.13 depicts the heat flow data measured for the individual components that constitute T3. The interaction curve, calculated from the measured and theoretical curves showed a slight slope (Fig. 4.14), an indication of possible incompatibility between honeybush extract, inulin and sugar that may occur during long periods of storage at 40 °C.

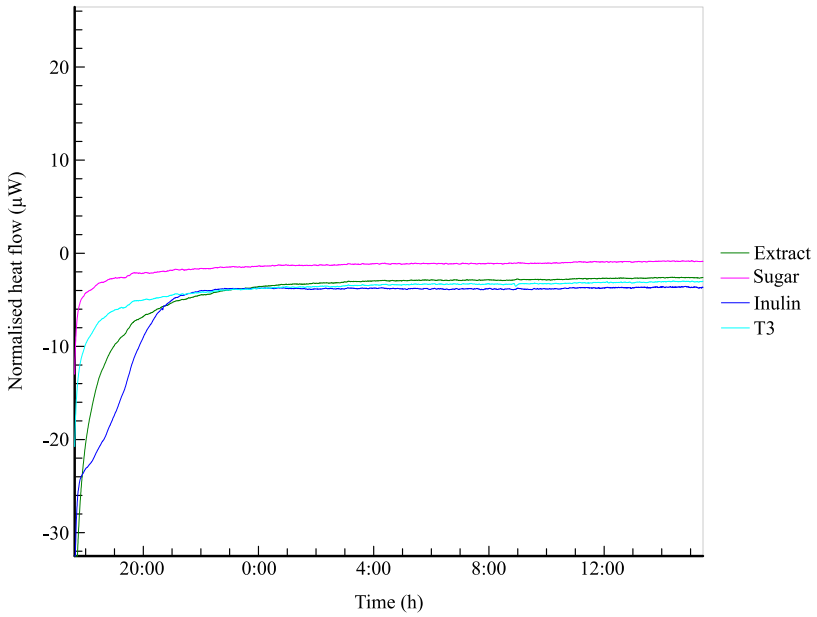


Figure 4.13 Graph depicting heat flow for individual components, sugar (magenta), *C. subternata* extract (green) spray-dried with 25% inulin (blue), that constitute T3 (turquoise) during compatibility testing of mixtures at 40 °C (RH not adjusted) using isothermal microcalorimetry.

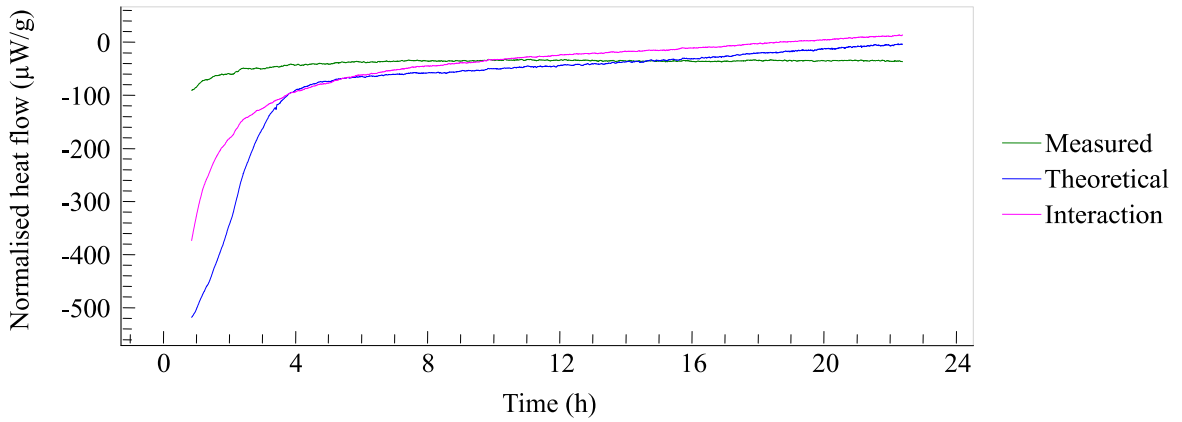


Figure 4.14 Graph depicting the measured (green) and theoretical (blue) heat flow data for T3 (*C. subternata* extract spray-dried with 25% inulin + sugar), as well as the interaction (magenta) between the two during compatibility testing of mixtures at 40 °C (RH not adjusted) using isothermal microcalorimetry.

No evidence of incompatibility between honeybush extract, inulin, sugar, citric acid and ascorbic acid in the ratios present in T5 was detected (Fig. 4.15 and Fig. 4.16). Similar results were observed for the other mixtures at 25°C and 40°C (Addendum B) and it was therefore concluded that in the control samples, which were not exposed to elevated RH conditions (through inclusion of micro-hygrostats in sample ampoules) no interactions should occur in the mixtures during storage, with the exception of T3. It is not clear why T5 that also contained high levels of sugar did not show similar interaction.

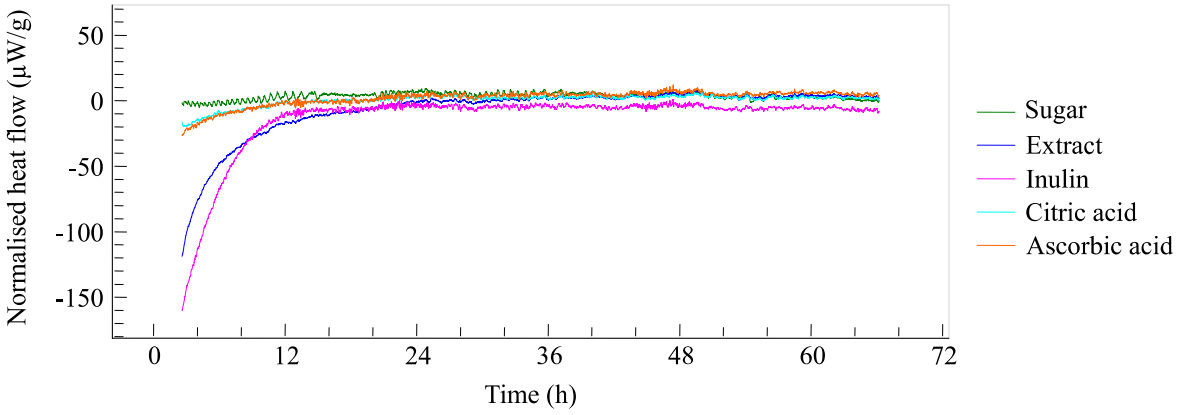


Figure 4.15 Graph depicting heat flow of individual components, *C. subternata* extract (blue) spray-dried with 25% inulin (magenta) + sugar (green) + citric acid (turquoise) + ascorbic acid (orange) that constitute T5 during compatibility testing of mixtures at 40 °C (RH not adjusted) using isothermal microcalorimetry.

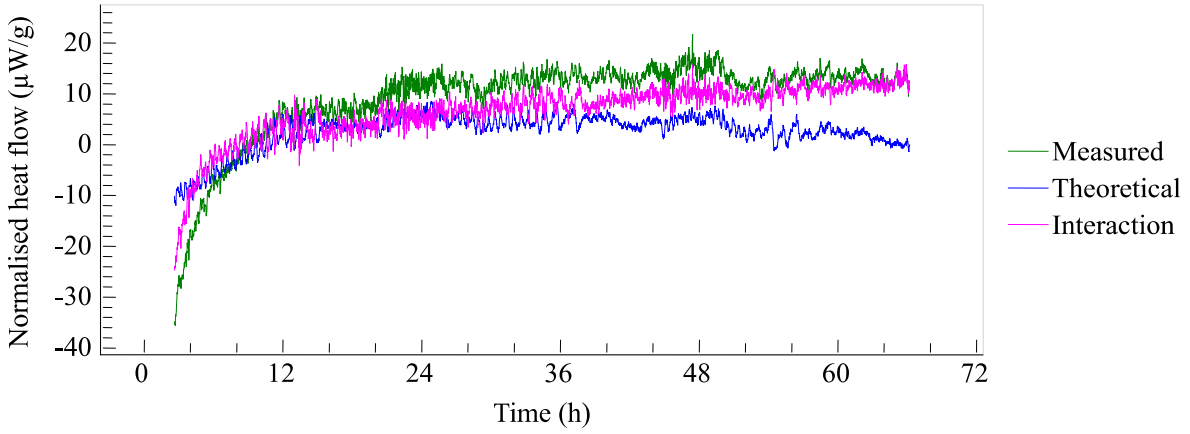


Figure 4.16 Graph depicting the measured (green) and theoretical (blue) heat flow data for T5 (*C. subternata* extract spray-dried with 25% inulin + sugar + citric acid + ascorbic acid), as well as the interaction (magenta) during compatibility testing of mixtures at 40 °C (RH not adjusted) using isothermal microcalorimetry.

4.4.2.5 Stability of mixtures in the presence of moisture

Water is considered to be the most important plasticiser or mobility enhancer in hydrophilic food components (Al-Muhtaseb *et al*., 2002). It was therefore important to assess the stability of the powders at normal and increased RH conditions as defined for climatic zone II (i.e. 25 °C/55% RH and 40 °C/75% RH) using isothermal microcalorimetry. During these experiments it was found that some of the powders succumbed to deliquescence. Deliquescence is the process by which a substance absorbs moisture from the atmosphere until it dissolves in the absorbed water and forms a solution. Deliquescence will thus enhance degradation of labile food ingredients, because chemical reactions occur much more readily in solution (Mauer & Taylor, 2010). It usually occurs in crystalline products and causes significant changes in the physical and chemical stability of powders (Mauer & Taylor, 2010; Stoklosa *et al*., 2012).

As previously indicated, isothermal microcalorimetry can also be applied to provide information on potential shelf-life instability of a product, since when a reaction takes place, heat will be generated or absorbed by the molecules reacting. By executing the measurements at the same temperature and RH conditions as those used for determining shelf-life stability, it provides an alternative technique to monitor the stability of solid state materials as it provides continuous real-time heat flow data at controlled user-specified conditions (Calahan *et al*., 2013). At both 25 °C and 40 °C in the absence of micro-hygrostats, the pure spray-dried extract (T1), which is in an amorphous state, appeared to be stable. However, it began to deliquesce

when the RH at these temperatures was controlled at 55% and 75% RH, respectively. Deliquescence was evident from the appearance of an exothermic peak, recorded as a positive signal, as depicted for the heat flow trace recorded at 40 °C (Fig. 4.17) and 25 °C (data not shown). At 25 °C/ 55% RH the total quantity of heat (*q)* released as a result of deliquescence was 164.31 mJ. At 40 °C/ 75% RH this process was accelerated and q = 60.96 mJ was calculated. These events indicated that the pure extract was unstable under conditions of increased relative humidity (≥ 55% RH).

From Fig. 4.17 it can be seen that the deliquescence of inulin is a two-step process. It was also clear that inulin had an effect on the deliquescence behaviour of T2. For pure inulin a heat integral of 928.57 mJ was calculated. Addition of inulin to the *C. subternata* extract increased the heat released by T2 substantially. The first heat integral of T2 was a combination of the exothermic peak of the extract (58.67 mJ) and the first heat output of inulin (182.62) (Fig. 4.17). Furthermore, the second exothermic peak of T2 (182.53 mJ) can be correlated with the second exothermic peak of inulin (928.57 mJ).

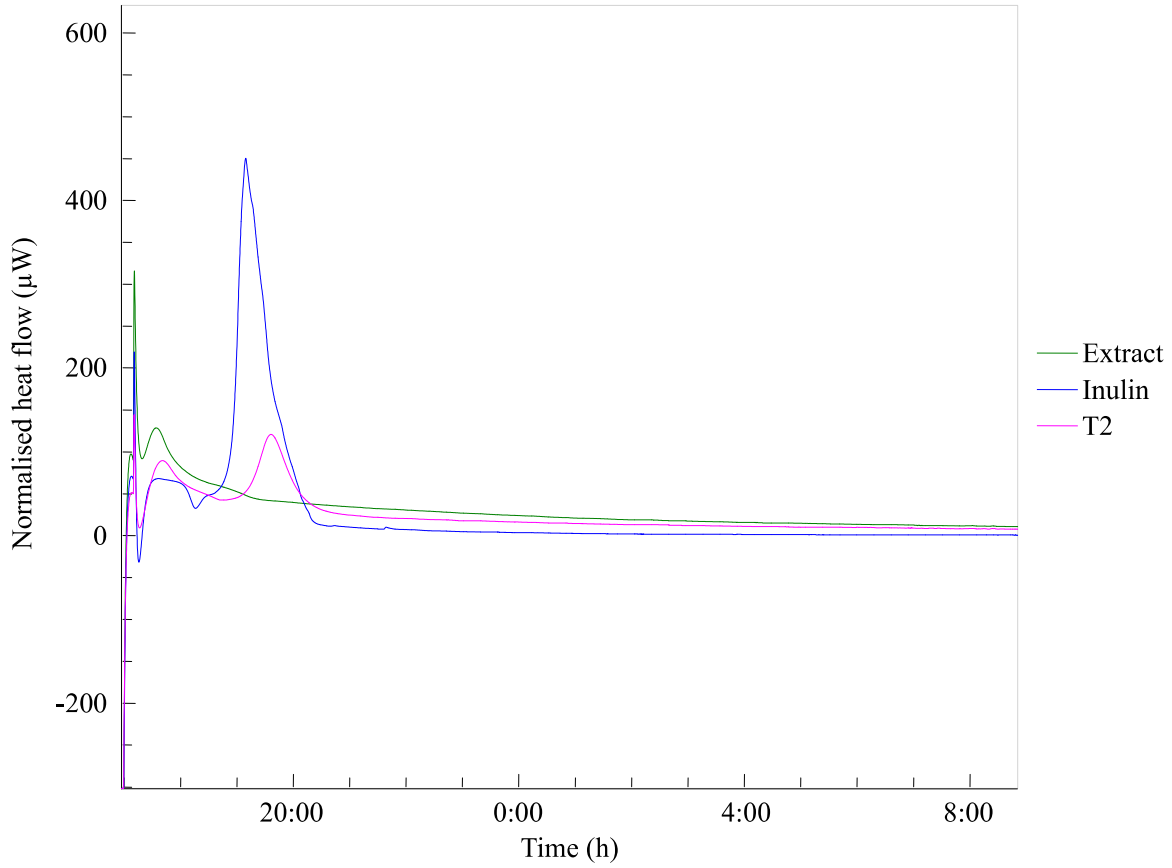


Figure 4.17 Heat flow response obtained for T2 (*C. subternata* extract spray-dried with 25% inulin; magenta), *C. subternata* extract (green) and inulin (blue) during stability testing at 40°C/75% RH.

Crystalline substances all have a specific deliquescence RH (RH_0) which is temperature dependent and unique to that crystalline solid (Stoklosa *et al.*, 2012). Sugar (85% RH), xylitol (77-79% RH), citric acid monohydrate (78% RH) and ascorbic acid (> 95% RH) all have a RH_0 above 70% at 25 °C as reported in the literature (Salameh *et al.*, 2006; Hiatt *et al.*, 2008; Lipasek *et al.*, 2013). When stored at 25 °C these ingredients did not deliquesce at relative humidities of 55% and therefore conferred a degree of stability to the mixtures which contained them (Sugar: T3 & T5; Xylitol: T4 & T6). This can be seen by the heat flow obtained for xylitol and T4 at 25 °C/55% RH, which exhibited no exothermic peaks (Fig. 4.18).

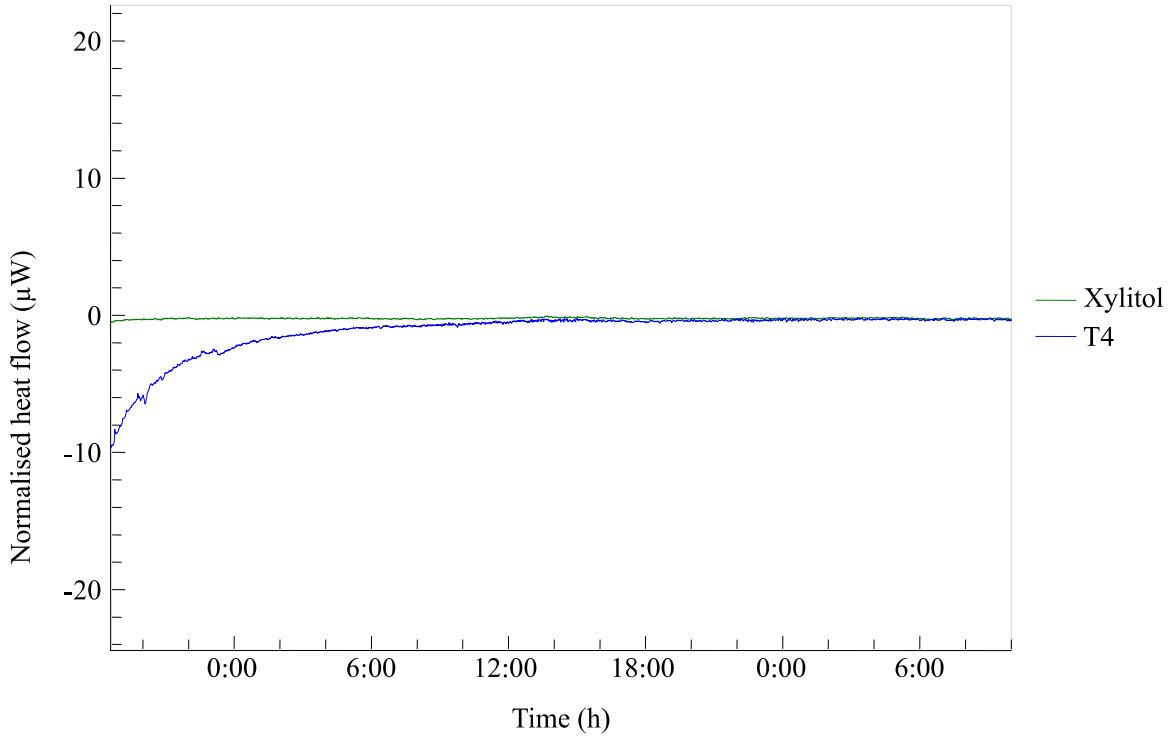


Figure 4.18 Heat flow response obtained by isothermal microcalorimetry for xylitol and T4 (*C. subternata* extract spray-dried with 25% inulin + xylitol + stevia), during stability testing at 25 °C/ 55% RH.

A number of factors have been shown to decrease the specific RH_0 of powders. Schaller-Povolny *et al*. (2000) demonstrated that the degree of polymerisation of inulin affects its RH_0. Furthermore the RH_0 of a substance with a positive heat of solution (which would lead to an increased solubility with increasing temperature) will decrease with rising temperature (Tang & Munkelwitz, 1996; Kelly & Wexler, 2006; Lipasek *et al*., 2013). Therefore at higher temperatures, a lower relative humidity will induce deliquescence in these substances. This was clearly observed for xylitol with its RH_0 (20 °C) at *ca*. 83% RH and RH_0 (40 °C) at *ca.* 68% RH (Lipasek *et al*., 2013). In the present study T6, containing xylitol, appeared to have undergone deliquescence at 40 °C/75% RH (Fig. 4.19). When stored at these conditions T6 showed a slow exothermic reaction (Fig. 4.20) that could be attributed to the combined deliquescence effect of the extract, inulin, xylitol and citric acid.

Figure 4.19 Photo showing the deliquescence of T6 (*C. subternata* extract spray-dried with 25% inulin + xylitol + stevia + citric acid + ascorbic acid) upon exposure to 40 °C/ 75 % RH (left) and 40°C in the absence of increased RH (right), with no deliquescence.

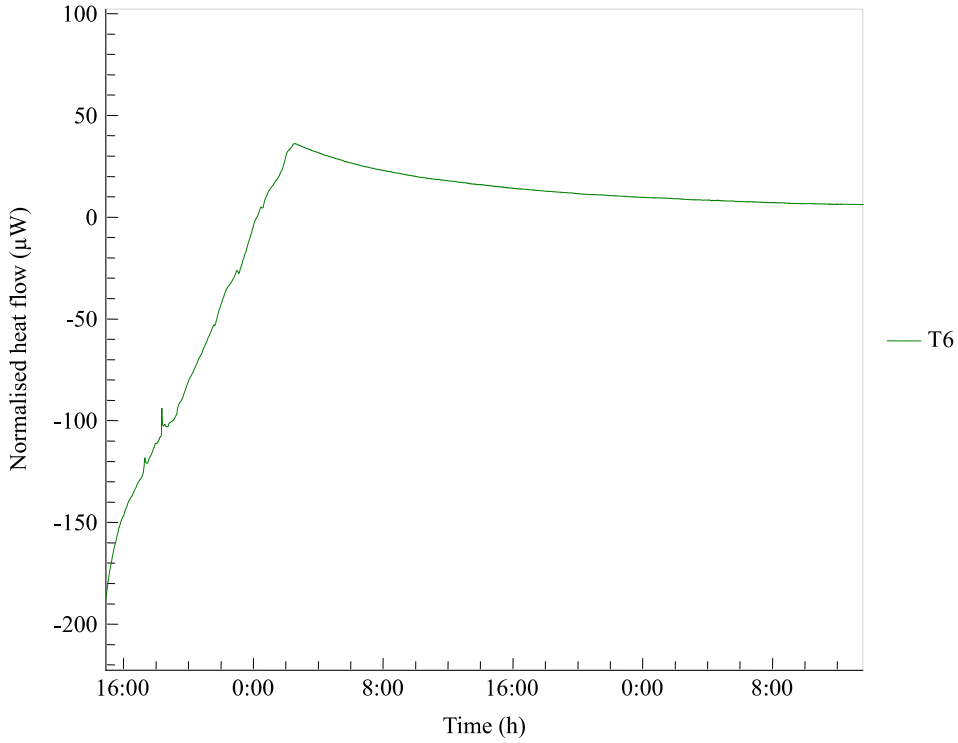


Figure 4.20 Heat flow response obtained by isothermal microcalorimetry for T6 (*C. subternata* extract spray-dried with 25% inulin + xylitol + stevia + citric acid + ascorbic acid) during stability testing at 40 °C/ 75% RH.

The second factor which causes lowering of deliquescence occurs when two or more deliquescent substances are combined. A new deliquescence point is formed, known as the RH_{0mix}, which is lower than the lowest RH_0 of the individual components and leads to some level of dissolution at relatively low RH conditions (Salameh *et al*., 2006; Kwok *et al*., 2010; Allan *et al*., 2015). This phenomenon has been clearly illustrated with sugar and citric acid during long periods of storage. In sucrose-citric acid blends, citric acid has been shown to hydrolyse sucrose in the presence of moisture. The by-products (fructose and glucose) of this hydrolysis reaction further decrease the RH_0 and cause further degradation and deliquescence of the mixture (Kwok *et al*., 2010). It was found that sucrose hydrolysis can occur below the mutual deliquescence RH of 64% and was observed for samples stored at 54% RH (Kwok *et al*., 2010). This is not only true for mixtures containing crystalline ingredients. Co-formulation of amorphous and crystalline solids also makes the blend more sensitive to moisture uptake as demonstrated for a sucrose-maltodextrin blend. Mixing of sugar with maltodextrin lowered the RH_0 of sucrose (Ghorab *et al*., 2014).

In this study samples containing citric acid and ascorbic acid and stored at 40 °C/ 70% RH showed drastic colour changes and visible signs of degradation such as deliquescence of the powders. At the start of storage the water activity of all of the powders was below 0.45. It therefore brings into question what caused an increase in available moisture in the sealed vials. At 40 °C a strong endothermic event was detected for citric acid indicating its dehydration (Fig. 4.21). Upon removal of the ampoule containing citric acid moisture was observed on the sides of the ampoule, therefore confirming dehydration. It was therefore postulated that the moisture released by citric acid monohydrate when stored at 40 °C might have caused degradation within the samples. Furthermore, it was demonstrated that the amorphous substances (*C. subternata* extract, inulin, stevia) had RH_0 values below 55%, which may have contributed towards deliquescence lowering in the presence of additional moisture.

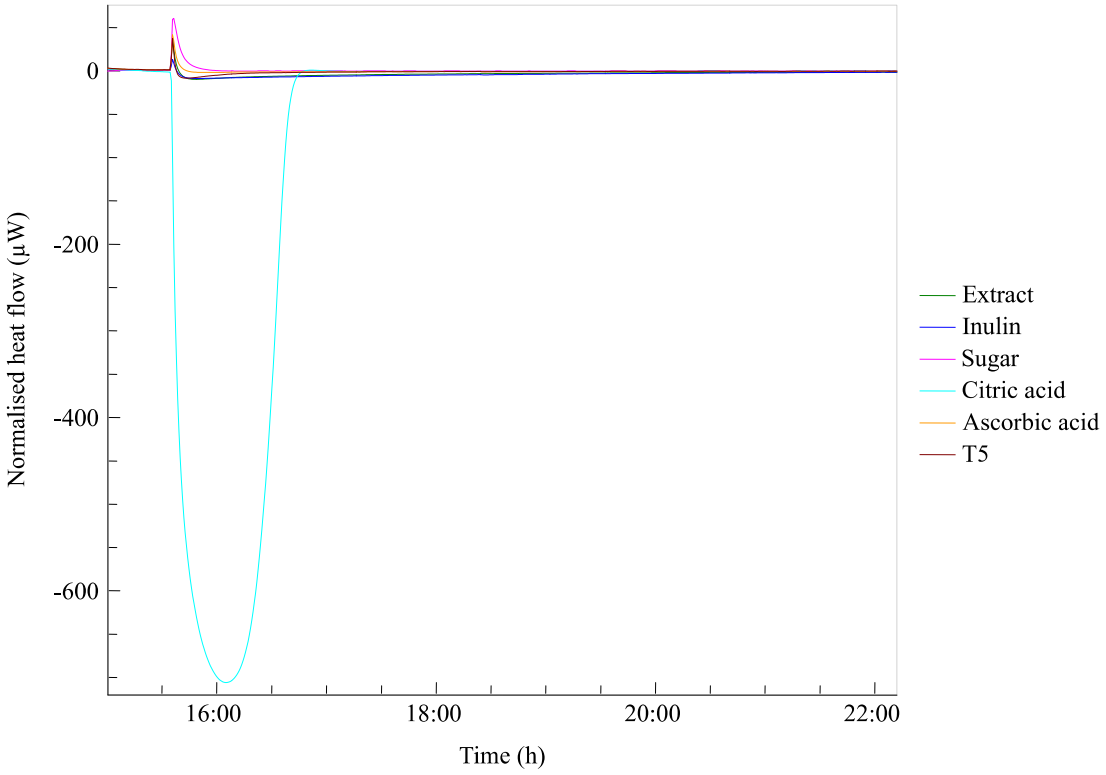


Figure 4.21 Heat flow response obtained by isothermal microcalorimetry for T5 (brown) and its individual ingredients, *C. subternata* extract (green) + inulin (blue) + sugar (magenta) + citric acid (turquoise) + ascorbic acid (orange), during stability testing at 40°C.

4.4.2.6 Relationship between moisture content and water activity

Moisture sorption isotherms (MSIs) are useful in predicting the behaviours of the mixtures in presence of different RH conditions. Knowledge of MSIs is also helpful in making predictions about storage conditions and the type of packaging needed (Gabas *et al*., 2007). For these reasons the MSI of each treatment was determined and the BET sorption model applied to the raw and transformed MSI data (for example T4 shown in Fig 4.22 and Fig. 4.23). The model was found to have a good fit with $R^2 > 0.95$ for each of the curves with the exception of T6 (Table 4.6). As previously demonstrated (Chapter 3) the BET model fitted the data well at water activities below 0.5 (Fig. 4.22). The MSIs of T1 and T2 were identical to that of the pure extract and IN25 which were discussed in the previous chapter. They will therefore not be covered in detail in this section.

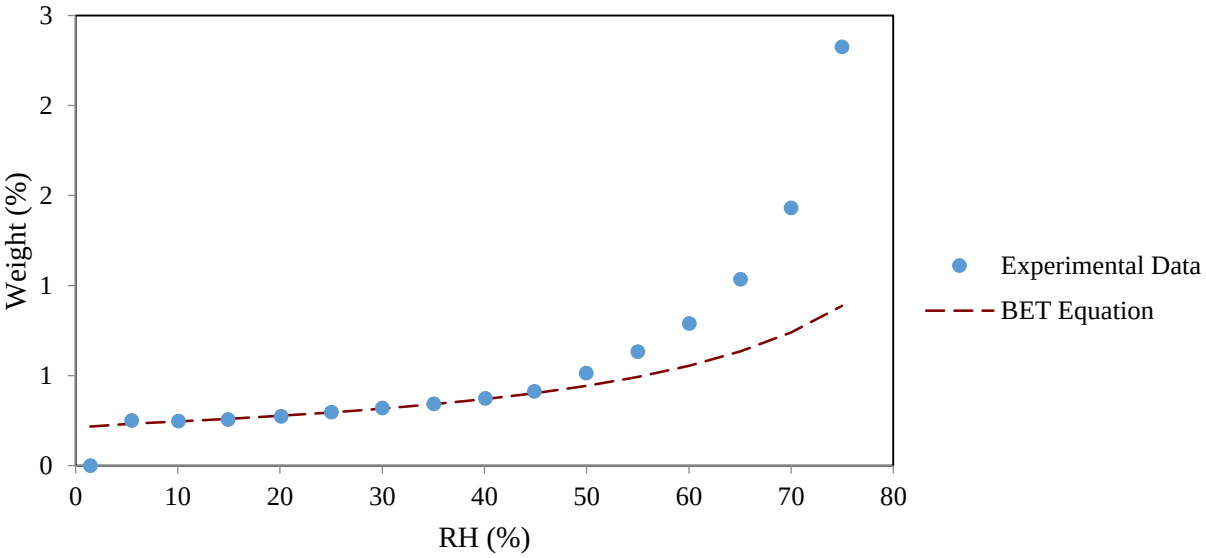


Figure 4.22 Moisture sorption isotherm of T4 (*C. subternata* extract spray-dried with 25% inulin + xylitol + stevia) fitted with the BET sorption model.

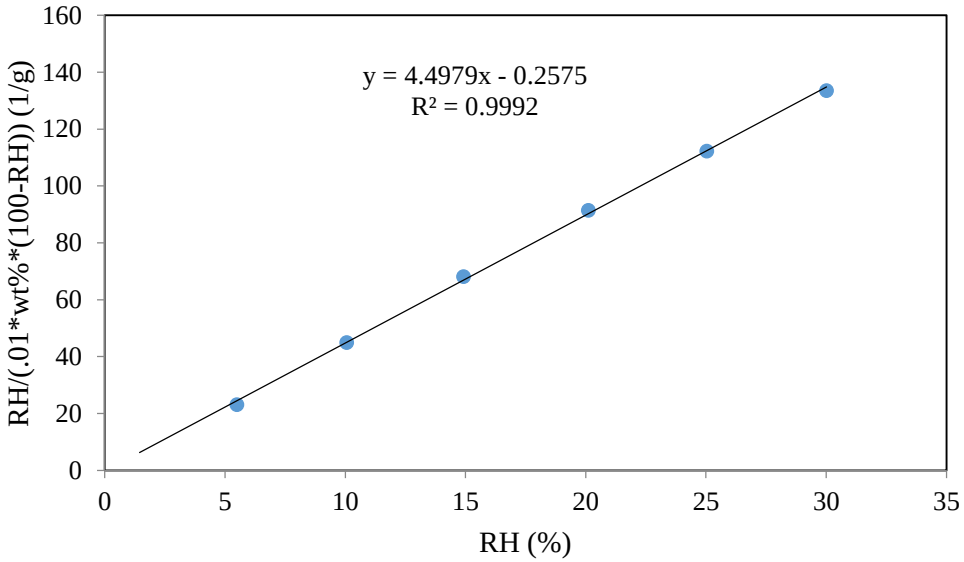


Figure 4.23 Transformed moisture sorption isotherm data of T4 (*C. subternata* extract spray-dried with 25% inulin + xylitol + stevia) fitted with the BETsorption model.

Table 4.6 Constants and R^2 values of moisture sorption data obtained for treatments at 25 °C using the BET equation. Treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + citric acid + ascorbic acid (T6).

Treatment	C	M_0 (g H_2O per 100 g solids)	R^2
T1	9.199	5.10	0.999
T2	7.4986	4.77	0.979
T3	15.2946	0.10	0.996
T4	1747.7573	0.22	0.999
T5	11.5550	0.09	0.958
T6	2.1939	0.13	0.044

C = constant in BET sorption model; M_0= monolayer moisture content (% dry basis)

The MSI of T3, T4, T5 and T6 (Fig. 4.24) all display a Type III curve according to the Brunauer classification (Labuza & Altunakar, 2007). This type of classification is typical of crystalline components such as sugar and correlates well with the composition of these mixtures as they are composed of predominantly crystalline substances, sugar and xylitol. In Type III curves the moisture uptake is very low until the crystals reach their deliquescent point at which they begin to dissolve in the absorbed moisture (Labuza & Altunakar, 2007). A minimal amount of moisture is absorbed to the crystalline surface, but when the RH_0 is reached, the amount of surface-absorbed water is enough to induce surface dissolution of highly water-soluble crystals to form a film of saturated solution around the crystals. Since its partial vapour pressure is lower than that of pure water, more moisture is taken up to maintain the same RH (Ghorab *et al*., 2014). With a deliquescent point of >75% RH for xylitol and 85% RH for sugar (Mauer & Taylor, 2010), the MSI data of the present study showed trends in agreement with these characteristics of xylitol and sugar. An increase in mass gain was observed for samples containing xylitol (T4 & T6) as they approach 75% RH (Fig. 4.24). On the other hand, samples containing sugar (T3 & T5) did not reach their deliquescence point and therefore did not absorb as much moisture (Fig. 4.24). It was also interesting to note that treatments containing a larger variety of ingredients (T5 & T6) started to absorb more water than those with fewer ingredients (Fig. 4.24). This in agreement with the theory of deliquescence lowering due to the presence of multiple ingredients within the mixtures (Lipasek *et al*., 2013; Ghorab *et al*., 2014).

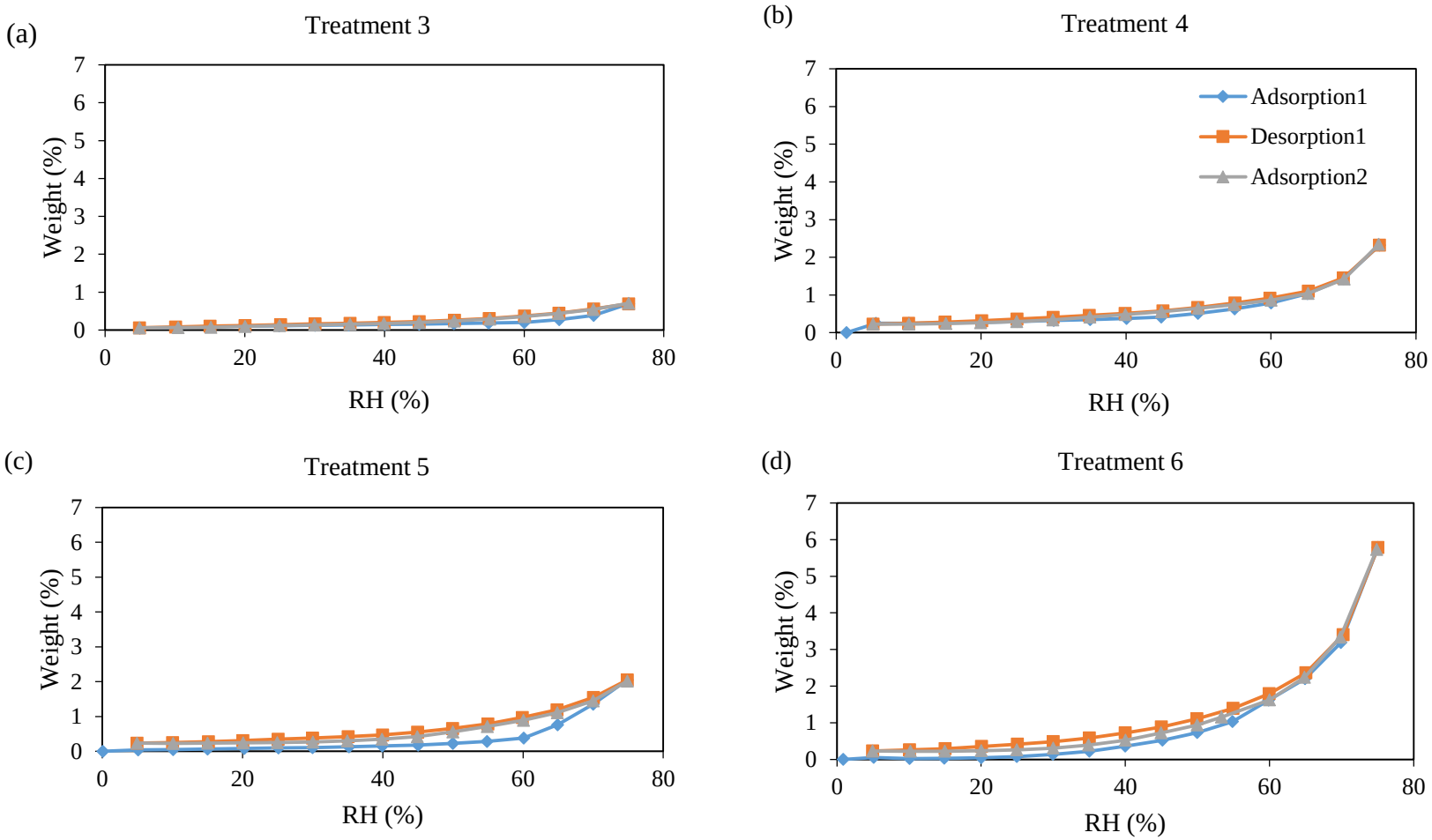


Figure 4.24 Moisture sorption isotherms for Treatments 3(a), 4(b), 5(c) and 6(d) showing first adsorption, desorption and second adsorption at 25 °C. Treatments were composed of *C. subternata* extract spray-dried with 25% inulin (IN25) + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

During storage of the mixtures for the shelf-life stability study it was observed that the moisture content and water activity (a_w) of the samples increased over time (Fig. 4.25). At the start of storage the amorphous spray-dried *C. subternata* extract (T1) and IN25 (T2) had much higher moisture contents than the samples containing crystalline sugar and xylitol. However the a_w of these treatments (T1 & T2) was lower than the others at the start of storage. It was therefore postulated that although the spray-dried extracts had higher moisture contents, this moisture was chemically bound at the start of storage and therefore not available for chemical and physical reactions. Over time the moisture content of these samples increased and this correlated with an increase in a_w. The one logical explanation for this phenomenon is that, even though the vials were closed by screw-cap, they appeared to not be properly sealed to provide an air-tight closure and as a result allowed moisture in from the storage environment. Their amorphous nature thus made them more susceptible to moisture adsorption. The other explanation is that the procedure followed for moisture determination was not adequate to allow complete determination of moisture and thus underestimated the moisture content of the amorphous materials. It is postulated that physicochemical changes brought about during storage may have resulted in a release of water and therefore more evaporation during the finite heating period employed during moisture determination. Ronkart *et al*. (2006) employed oven drying at 105 °C for 24 h to determine the moisture content of inulin, as opposed to the substantially shorter drying period at 100 °C for 60 min used in the present study. Ronkart *et al.* (2006) also pointed out that water included in crystals may or may not be completely detected by a technique such as oven drying. Inulin, however, remains amorphous up to water content of 15.7 g water per 100 g dry weight (Ronkart *et al*., 2009) so that phase transition does explain the results for T2. It was therefore concluded that further investigation using completely airtight packaging is required to provide the necessary insight into the behaviour observed.

The behaviour of the powders at raised RH conditions correlated with the data from their MSI. The amorphous spray-dried extract absorbed high amounts of moisture while the mixtures containing crystalline powders did not. However, from the a_w data it is clear that the amount of moisture available for chemical reaction was still high in mixtures containing sugar and xylitol as the main ingredients. These raised a_w conditions within the samples may help to explain the physical changes which were observed in the powders.

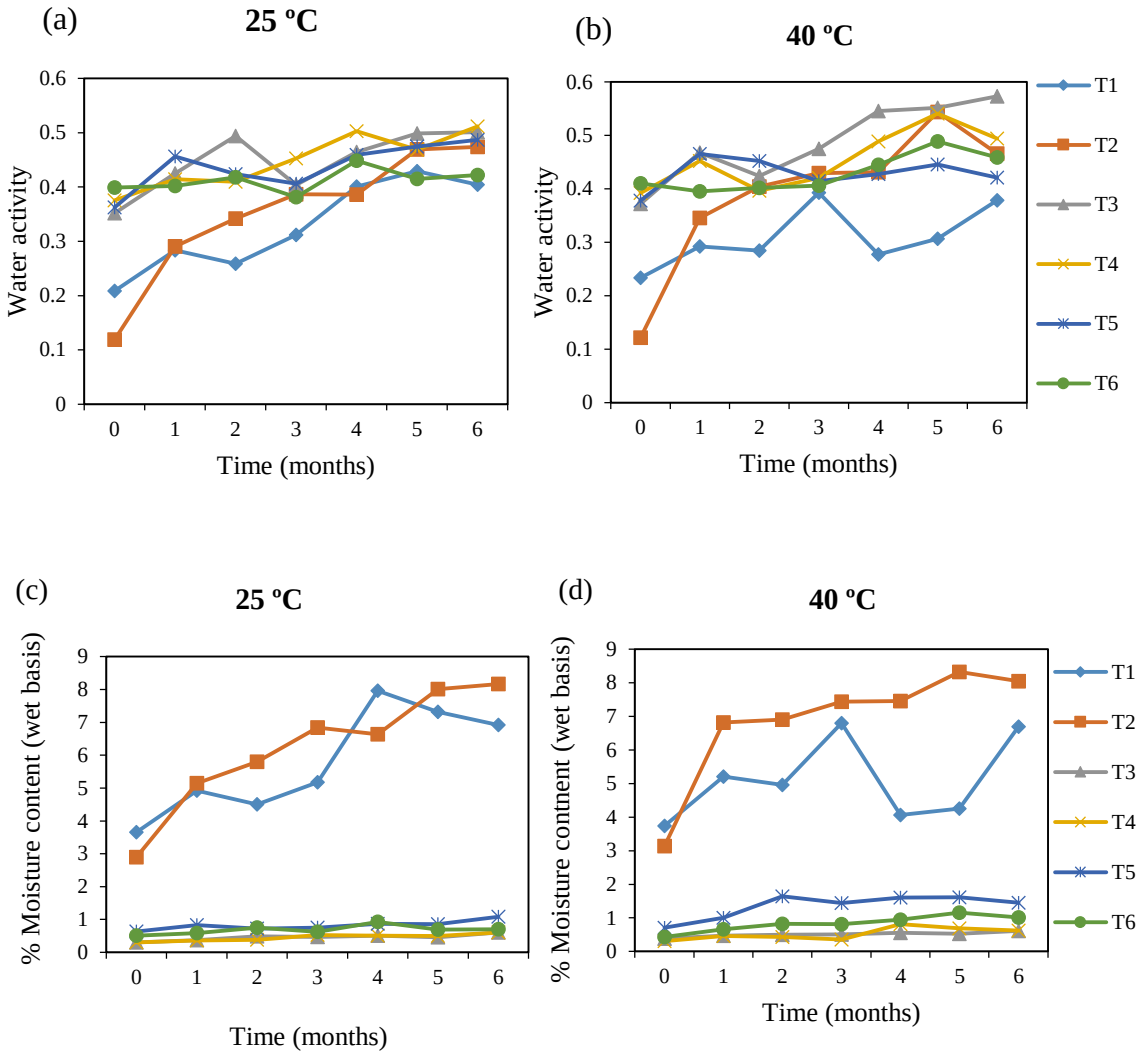


Figure 4.25 Water activity and moisture content (wet basis) of treatments throughout 6 month storage period at 25 °C/ 60% RH (a & c) and 40 °C/ 75% RH (b & d). Treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

4.4.2.7 Retention of phenolic compounds

In order for the consumer to reap the benefits associated with the consumption of phenolic compounds in honeybush tea it is important that they remain stable within the powder mixture from production until consumption. The following section deals with the stability of individual polyphenols during a six month storage stability trial, as well as the effects of formulation on phenolic stability. The stability of the major phenolic compounds in *C. subternata* varied depending on the temperature conditions, as well as the presence of different ingredients within the mixture. The concentrations of the compounds sometimes differed significantly between some of the treatments at the start of storage. This may have occurred due to a lack of homogenous mixing or slight errors during weighing. While this may not be noticeable in the visual appearance or taste of the product, it is detected by HPLC, which is a very sensitive analytical technique.

One of the most important classes of phenolic compounds found in *C. subternata* extracts are xanthones. In this study the concentrations of mangiferin and isomangiferin differed significantly ($p \geq 0.05$) before and after storage regardless of the treatment (Table 4.7). At 25 °C, the degradation of mangiferin and isomangiferin was minor (*ca.* 10%) for all the treatments (Fig. 4.26). However, at 40 °C, their degradation was accelerated by the presence of other ingredients, especially due to the presence of citric acid and ascorbic acid.

Previous studies have shown that xanthones are particularly sensitive to high temperatures such as those experienced during the chemical oxidation process (so-called "fermentation") used to produce traditional honeybush tea (Joubert *et al*., 2008). In this study it appears that isomangiferin the regio-isomer of mangiferin, was slightly more stable than mangiferin at 40 °C. These results agree with the study performed by Beelders *et al*. (2015) on high temperature oxidation of the plant material (*C. genistoides*), i.e. treatment with moist heat at 90 °C for 16 hrs, showing less degradation of isomangiferin than mangiferin.

Table 4.7 Concentration of mangiferin and isomangiferin (g per 100g extract; mean ± standard deviation; n = 4) at the beginning and end of 6 month storage. Treatments were stored at 25 °C/ 60% RH and 40 °C/ 75% RH in sealed vials and were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

		Mangiferin								Isomangiferin							
Treat	Temp (°C)	Month 0				Month 6				Month 0				Month 6			
T1	25	0.519	bc	±	0.004	0.467	de	±	0.010	0.327	b	±	0.003	0.301	cde	±	0.007
T1	40	0.510	bc	±	0.004	0.472	d	±	0.009	0.321	b	±	0.002	0.306	c	±	0.005
T2	25	0.522	b	±	0.005	0.451	ef	±	0.003	0.329	b	±	0.003	0.292	e	±	0.001
T2	40	0.514	bc	±	0.005	0.447	f	±	0.006	0.324	b	±	0.003	0.292	e	±	0.004
T3	25	0.518	bc	±	0.009	0.472	d	±	0.009	0.328	b	±	0.005	0.304	cd	±	0.004
T3	40	0.519	bc	±	0.012	0.421	g	±	0.038	0.329	b	±	0.007	0.296	de	±	0.011
T4	25	0.511	bc	±	0.006	0.459	def	±	0.018	0.323	b	±	0.004	0.304	cd	±	0.002
T4	40	0.543	a	±	0.004	0.428	g	±	0.026	0.349	a	±	0.003	0.304	cd	±	0.010
T5	25	0.512	bc	±	0.009	0.459	def	±	0.013	0.325	b	±	0.005	0.296	de	±	0.014
T5	40	0.514	bc	±	0.005	0.355	h	±	0.013	0.327	b	±	0.004	0.247	f	±	0.016
T6	25	0.504	bc	±	0.004	0.449	ef	±	0.016	0.321	b	±	0.003	0.296	de	±	0.008
T6	40	0.502	c	±	0.007	0.326	i	±	0.017	0.320	b	±	0.005	0.249	f	±	0.004

For each compound, means with the same letter are not significantly different ($p \geq 0.05$).

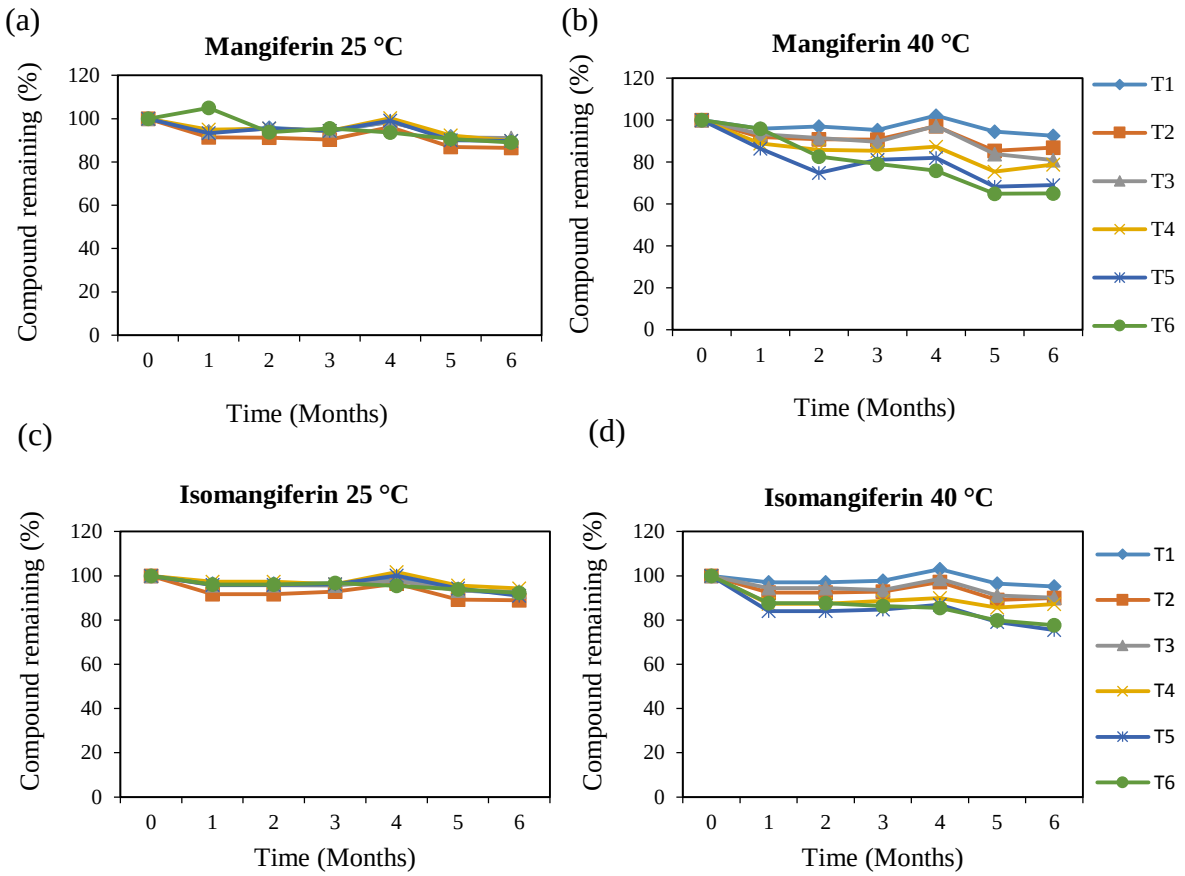


Figure 4.26 Percentage remaining mangiferin (a & b) and isomangiferin (c & d) during a six month storage trial at 25 °C/ 60% RH (a & c) and 40 °C/ 75% RH (b & d). The treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

For the flavones, scolymoside and vicenin-2, significant ($p < 0.05$) differences between their concentration before and after the six month storage period regardless of treatment were observed (Table 4.8). Vicenin-2 degraded substantially at 25 °C and 40 °C within the first month and the extent of degradation at 25 °C was similar for all the treatments over time (Fig. 4.27). With the exception of samples containing citric acid and ascorbic acid, the temperature conditions caused an equal amount of degradation of vicenin-2 for all the treatments. On the other hand, scolymoside was more stable at both 25 °C and 40 °C and was not drastically affected by the presence of citric acid and ascorbic at 40 °C (Fig. 4.27). This is unexpected as scolymoside (luteolin-7-*O*-rutinoside) contains a catechol group on the B-ring opposed to the monohydroxy group of vicenin-2 (apigenin-6,8-di-*C*-glucoside). Position of the sugars may also play a role as observed for the xanthone regio-isomers. In a study on the thermal

degradation of quercetin and rutin in aqueous model systems the stability of rutin (quercetin-3-*O*-rutinoside) over its corresponding aglycone was attributed to steric hindrance by the sugar moiety which prevented the formation of a carbanion on the 3-hydroxyl group (Makris & Rossiter, 2000). Beelders *et al.* (2015) demonstrated that iriflophenone-3-*C*-glucoside-4-*O*-glucoside was much more heat stable than the monoglucoside iriflophenone-3-*C*-glucoside.

Table 4.8 Concentration of vicenin-2 and scolymoside (g per 100g extract; mean ± standard deviation; n = 4) at the beginning and end of 6 month stability trial. Treatments were stored at 25 °C/ 60% RH and 40 °C/ 75% RH and were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

		Vicenin-2								**Scolymoside**							
Treat	**Temp (°C)**	**Month 0**				**Month 6**				**Month 0**				**Month 6**			
T1	25	0.163	bcd	±	0.002	0.132	jk	±	0.003	1.088	bc	±	0.008	0.969	e	±	0.021
T1	40	0.159	f	±	0.001	0.134	hij	±	0.002	1.070	cd	±	0.010	0.977	e	±	0.021
T2	25	0.166	b	±	0.002	0.128	l	±	0.001	1.090	bc	±	0.010	0.939	f	±	0.006
T2	40	0.166	b	±	0.001	0.129	l	±	0.001	1.069	cd	±	0.011	0.928	f	±	0.011
T3	25	0.165	bc	±	0.003	0.134	hij	±	0.002	1.097	B	±	0.020	0.990	e	±	0.015
T3	40	0.166	b	±	0.003	0.136	gh	±	0.001	1.096	B	±	0.026	0.971	e	±	0.023
T4	25	0.160	ef	±	0.003	0.135	hi	±	0.001	1.081	bc	±	0.013	0.989	e	±	0.009
T4	40	0.171	a	±	0.001	0.139	g	±	0.003	1.145	A	±	0.014	0.972	e	±	0.004
T5	25	0.162	de	±	0.003	0.133	ij	±	0.003	1.067	cd	±	0.018	0.982	e	±	0.024
T5	40	0.163	cd	±	0.002	0.109	m	±	0.003	1.074	bcd	±	0.013	0.902	g	±	0.034
T6	25	0.158	f	±	0.001	0.129	kl	±	0.004	1.056	D	±	0.014	0.992	e	±	0.018
T6	40	0.158	f	±	0.002	0.109	m	±	0.001	1.051	D	±	0.017	0.922	fg	±	0.010

For each compound, means with the same letter are not significantly different ($p > 0.05$).

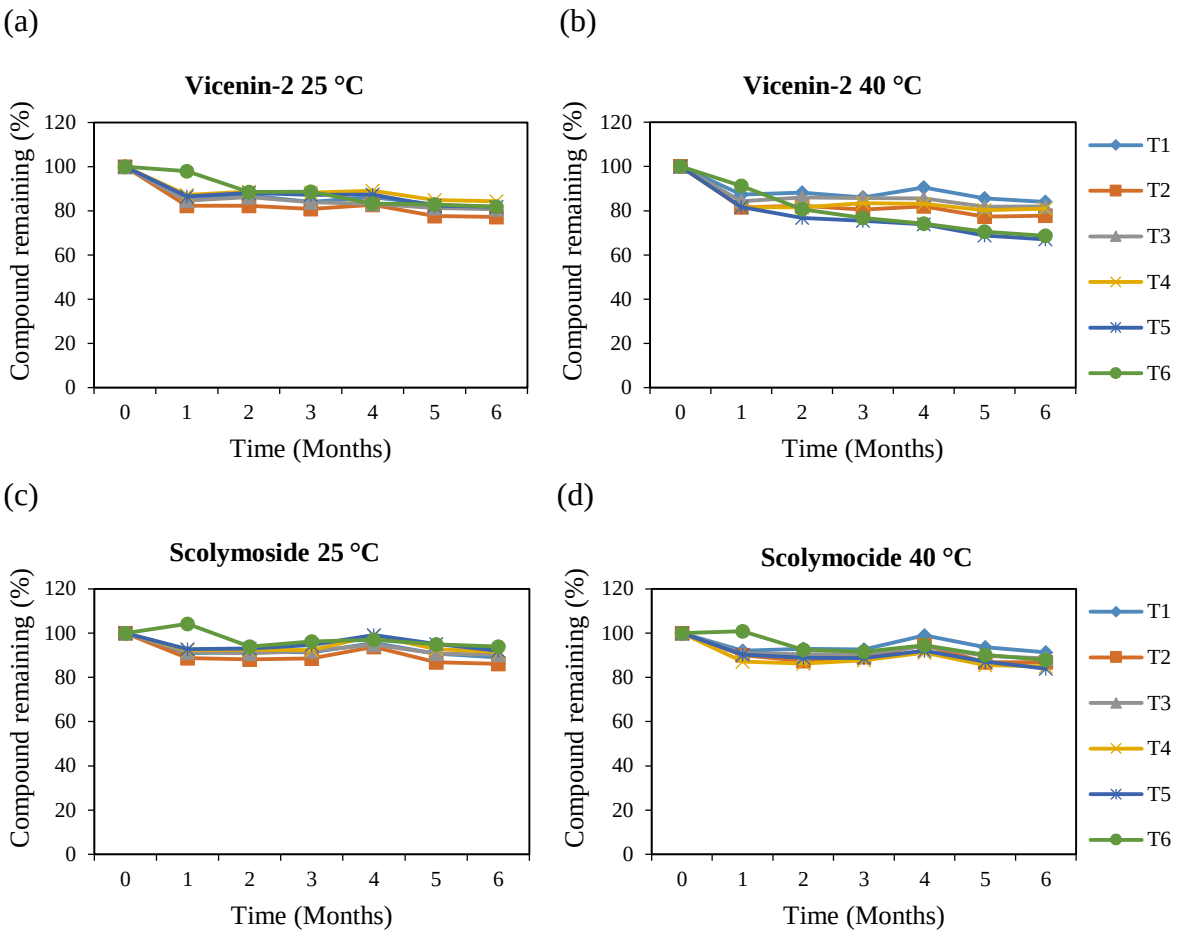


Figure 4.27 Percentage remaining vicenin-2 (a & b) and scolymoside (c & d) during a six month stability trial at 25 °C/ 60% RH (a & c) and 40 °C/75% RH (b & d). The treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

When studying the flavanones, eriocitrin and hesperidin, the concentration of eriocitrin was significantly ($p < 0.05$) different in the treatments before and after six months of storage (Table 4.9). This effect was not as pronounced in the concentration of hesperidin with only some treatment and time combinations decreasing significantly ($p < 0.05$) (Table 4.9). The degradation of eriocitrin followed a similar trend to that of the xanthones with the presence of citric acid and ascorbic acid at 40 °C accelerating this process. Hesperidin on the other hand was stable at both temperatures with a slight increase in degradation with the addition of xylitol and the acids (Fig. 4.28).

Converting OH to OCH_3 (B-ring) likely explains the higher stability of hesperidin over eriocitrin. The high stability of hesperidin is supported by findings by Dhuique-Mayer, *et al*. (2007) who reported that no degradation of hesperidin was observed during the heating of orange juice at 90 °C for up to 240 min. On the other hand previous studies have reported that the honeybush fermentation process (80 °C /24 h or 90 °C/16 h) significantly ($p < 0.05$) decreased the concentration of both eriocitrin and hesperidin in *Cyclopia* plant material (Joubert *et al*., 2008, Schulze *et al*., 2014). These conditions are by comparison extreme.

Table 4.9 Concentration of eriocitrin and hesperidin (g per 100g extract; mean ± standard deviation; n = 4) at the beginning and end of 6 month stability trial. Treatments were stored at 25°C/60% RH and 40 °C/75% RH and were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

Treat	Temp (°C)	Eriocitrin								Hesperidin							
		Month 0				Month 6				Month 0				Month 6			
T1	25	0.605	b	±	0.003	0.552	efgh	±	0.008	0.619	bc	±	0.006	0.598	fg	±	0.011
T1	40	0.598	bc	±	0.005	0.569	de	±	0.015	0.609	cdef	±	0.007	0.607	cdef	±	0.011
T2	25	0.608	b	±	0.003	0.544	ghi	±	0.007	0.622	b	±	0.004	0.586	gh	±	0.006
T2	40	0.597	bc	±	0.007	0.542	hi	±	0.004	0.610	bcdef	±	0.006	0.583	h	±	0.007
T3	25	0.602	b	±	0.016	0.553	efgh	±	0.005	0.617	bcd	±	0.011	0.609	cdef	±	0.010
T3	40	0.605	b	±	0.008	0.550	gfh	±	0.014	0.618	bcd	±	0.014	0.604	ef	±	0.016
T4	25	0.598	bc	±	0.007	0.562	efg	±	0.008	0.609	bcdef	±	0.006	0.617	bcd	±	0.003
T4	40	0.628	a	±	0.009	0.560	efgh	±	0.010	0.660	a	±	0.006	0.602	ef	±	0.003
T5	25	0.582	cd	±	0.011	0.528	i	±	0.023	0.611	bcde	±	0.009	0.601	ef	±	0.017
T5	40	0.581	cd	±	0.007	0.450	j	±	0.034	0.613	bcde	±	0.008	0.552	i	±	0.005
T6	25	0.570	de	±	0.007	0.549	fgh	±	0.019	0.606	def	±	0.007	0.612	bcde	±	0.015
T6	40	0.566	def	±	0.008	0.462	j	±	0.015	0.603	ef	±	0.009	0.549	i	±	0.005

For the same compound, means with the same letter are not significantly different ($p > 0.05$).

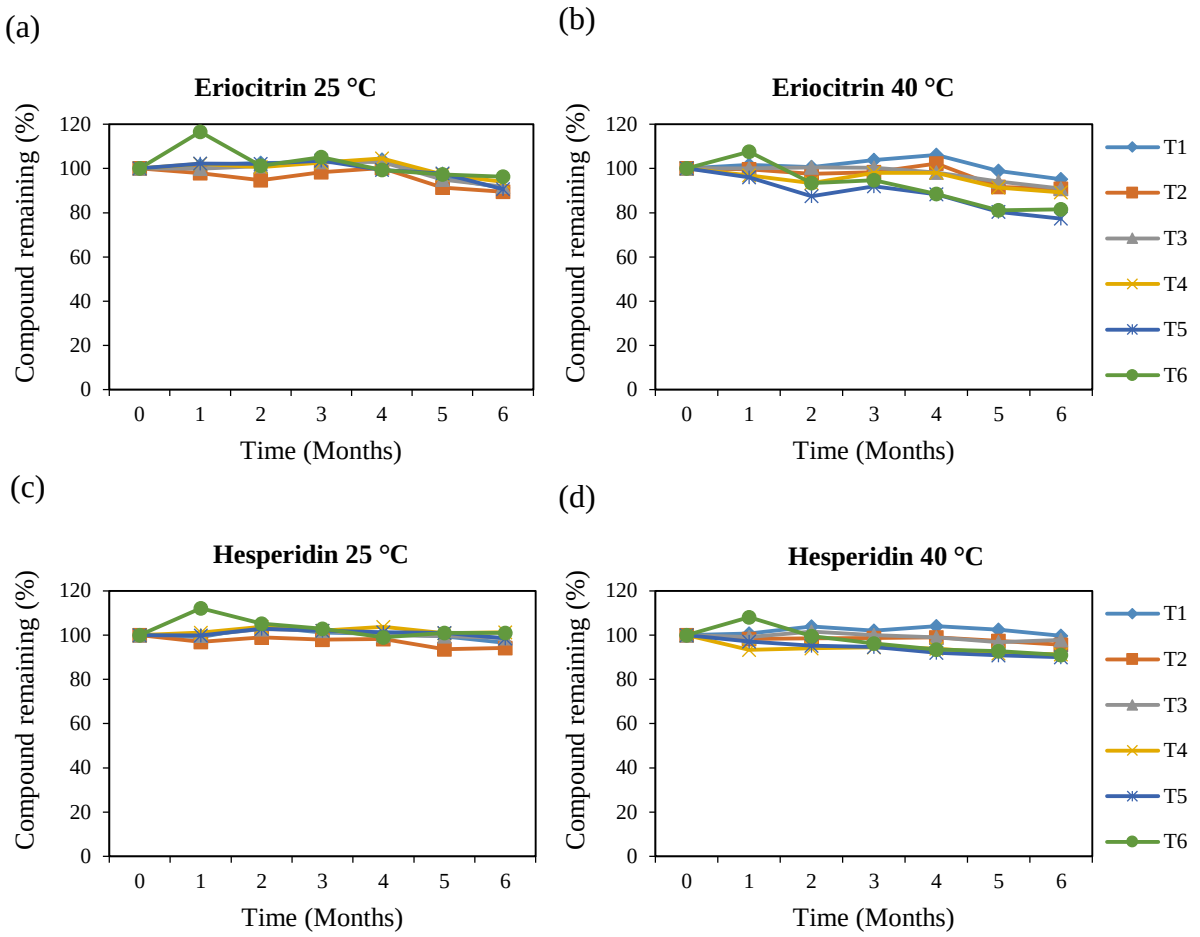


Figure 4.28 Percentage remaining eriocitrin (a & b) and hesperidin (c & d) during a six month stability trial at 25 °C/60% RH (a & c) and 40 °C/75% RH (b & d). The treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

The benzophenones in *C. subternata* extract produced varied results in terms of stability. For instance iriflophenone-3-*C*-glucoside-4-*O*-glucoside showed remarkable stability throughout storage. On the other hand, iriflophenone-3-*C*-glucoside and maclurin-3-*C*-glucoside degraded significantly ($p < 0.05$) at both temperatures regardless of the treatment (Table 4.10 and Fig. 4.29).

At 25 °C and 40 °C iriflophenone-3-*C*-glucoside degraded up to 21.4% and 49.6%, respectively after 6 months storage. In comparison to this, iriflophenone-3-*C*-glucoside-4-*O*-glucoside showed very little degradation at 25 °C and 40 °C with the exception of samples containing citric acid and ascorbic acid and stored at 40 °C. As previously mentioned Beelders *et al*. (2015) observed similar trends regarding relative stability and attributed the additional stability of iriflophenone-3-*C*-glucoside-4-*O*-glucoside to the presence of the additional O-linked glucopyranosyl moiety at C-4.

Maclurin-3-*C*-glucoside was present in very low concentrations in the samples (Table 4.10). The small peak areas for this compound increased the chance of error during the integration process. However, from the results in Fig. 4.29 it can be seen that this compound was very unstable and degraded extensively for all of the treatments (between 35.5 and 60.7%) over 6 months of storage. Degradation of maclurin-3-*C*-glucoside may be attributed to its high antioxidant capacity due to the presence of an additional hydroxyl (OH) group which increases the oxidation potential of this compound. Beelders *et al*. (2015) also observed that the presence of an additional hydroxyl group on maclurin-3-*C*-glucoside decreased thermal stability in comparison to iriflophenone-3-*C*-glucoside.

Table 4.10 Concentration of iriflophenone-3-*C*-glucoside-4-*O*-glucoside, iriflophenone-3-*C*-glucoside and maclurin-3-*C*-glucoside (g per 100g extract; mean ± standard deviation; n = 4) at the beginning and end of 6 month stability trial. Treatments were stored at 25 °C/60% RH and 40 °C/75% RH and were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

		Iriflophenone-3-*C*-glucoside-4-*O*-glucoside								**Iriflophenone-3-*C*-glucoside**								**Maclurin-3-*C*-glucoside**							
Treat	**Temp (°C)**	**Month 0**				**Month 6**				**Month 0**				**Month 6**				**Month 0**				**Month 6**			
T1	25	0.885	bcd	±	0.007	0.872	bcdef	±	0.017	0.373	b	±	0.004	0.320	f	±	0.006	0.069	a	±	0.000	0.044	e	±	0.001
T1	40	0.870	cdef	±	0.007	0.882	bcde	±	0.018	0.365	bcd	±	0.003	0.319	f	±	0.011	0.068	ab	±	0.001	0.044	ef	±	0.002
T2	25	0.892	b	±	0.007	0.847	gh	±	0.010	0.376	ab	±	0.003	0.311	fg	±	0.005	0.069	a	±	0.000	0.042	efg	±	0.001
T2	40	0.884	bcd	±	0.008	0.845	hi	±	0.006	0.373	b	±	0.003	0.295	ghi	±	0.011	0.067	abc	±	0.001	0.040	fgh	±	0.001
T3	25	0.867	defg	±	0.016	0.863	efgh	±	0.015	0.371	bc	±	0.006	0.309	fgh	±	0.017	0.065	cd	±	0.001	0.043	efg	±	0.002
T3	40	0.871	cdef	±	0.020	0.869	def	±	0.012	0.373	b	±	0.007	0.188	k	±	0.009	0.065	bcd	±	0.001	0.030	ij	±	0.009
T4	25	0.864	efgh	±	0.011	0.877	bcdef	±	0.004	0.370	bc	±	0.004	0.290	hi	±	0.027	0.065	cd	±	0.001	0.040	gh	±	0.004
T4	40	0.939	a	±	0.008	0.889	bc	±	0.012	0.392	a	±	0.009	0.215	j	±	0.016	0.071	a	±	0.001	0.028	j	±	0.003
T5	25	0.858	fgh	±	0.013	0.820	j	±	0.051	0.353	cde	±	0.005	0.289	i	±	0.028	0.064	cd	±	0.001	0.037	h	±	0.005
T5	40	0.868	def	±	0.006	0.710	k	±	0.009	0.344	e	±	0.005	0.182	k	±	0.035	0.064	cd	±	0.000	0.030	ij	±	0.000
T6	25	0.865	defgh	±	0.004	0.825	ij	±	0.022	0.344	e	±	0.004	0.297	ghi	±	0.010	0.063	d	±	0.000	0.042	efg	±	0.002
T6	40	0.862	efgh	±	0.014	0.676	l	±	0.013	0.347	de	±	0.005	0.213	j	±	0.007	0.063	d	±	0.001	0.032	i	±	0.002

For each compound, means with the same letter are not significantly different ($p > 0.05$).

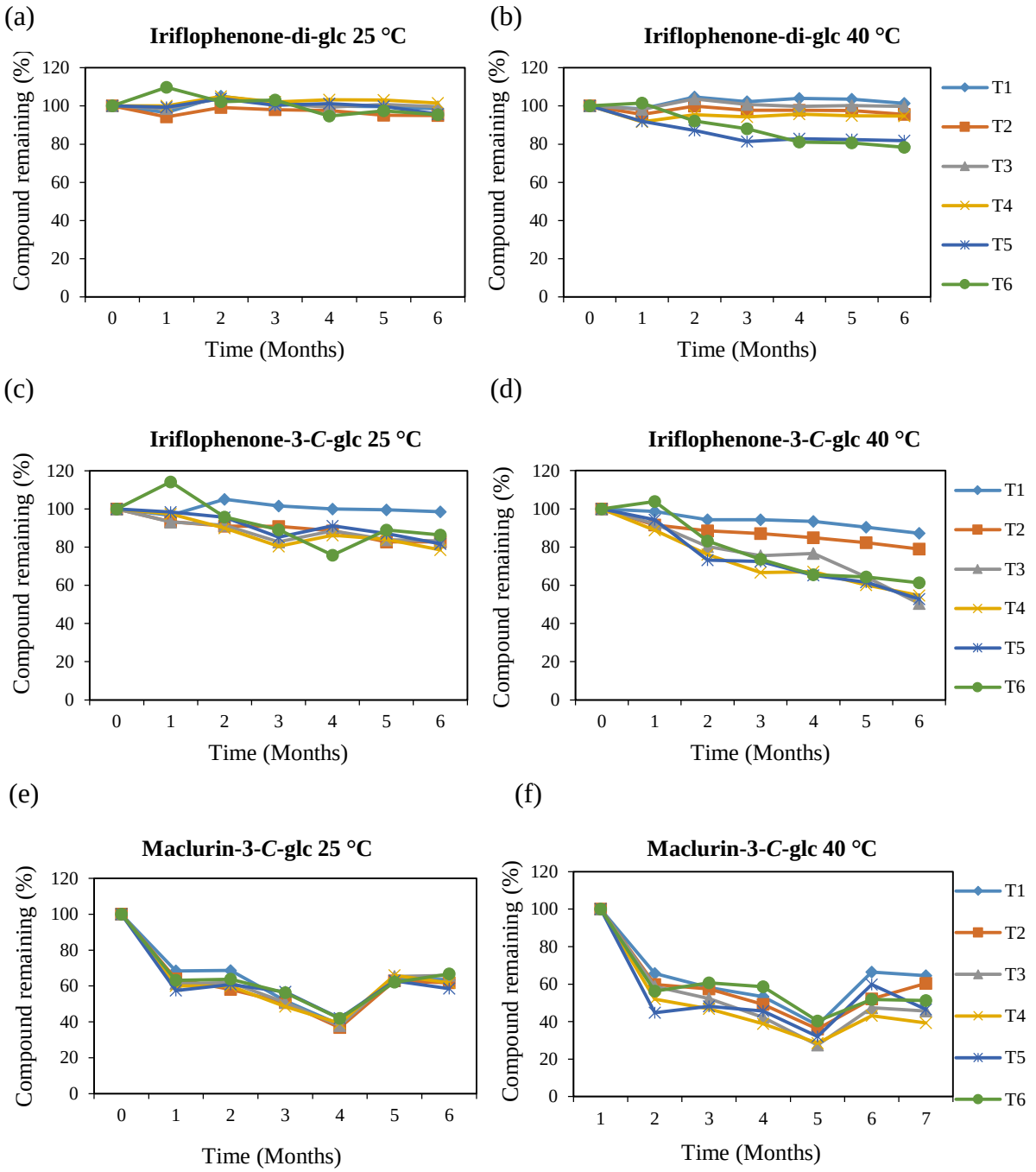


Figure 4.29 Percentage remaining iriflophenone-3-*C*-glucoside-4-*O*-glucoside (a & b), iriflophenone-3-*C*-glucoside (c & d) and maclurin-3-*C*-glucoside (e & f) during a six month stability trial at 25 °C/60% RH (a, c & e) and 40 °C/75% RH (b, d & f). The treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + citric acid + ascorbic acid (T6). glc = glucoside.

The dihydrochalcone, phloretin-3′,5′-di-*C*-glucoside, was present in the samples at high concentrations (Table 4.11) and storage decreased its concentration significantly. It showed higher stability at 25 °C and degradation was accelerated slightly by the presence of sugar, xylitol and inulin and more substantially by the presence of citric acid and ascorbic acid at 40 °C (Fig. 4.30), following similar trends to mangiferin.

Table 4.11 Concentration of phloretin-3′,5′-di-*C*-glucoside (g per 100g extract) at the beginning and end of 6 month stability trial. Treatments were stored at 25 °C/60% RH and 40 °C/75% RH and were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6).

		Phloretin-3′,5′-di-*C*-glucoside							
Treatment	Temp (°C)	Month 0				Month 6			
T1	25	1.236	bc	±	0.011	1.174	fghi	±	0.022
T1	40	1.213	bcde	±	0.009	1.175	fghi	±	0.038
T2	25	1.246	b	±	0.007	1.144	ijk	±	0.012
T2	40	1.223	bcd	±	0.012	1.110	kl	±	0.026
T3	25	1.220	bcd	±	0.020	1.168	ghi	±	0.018
T3	40	1.228	bcd	±	0.025	1.065	m	±	0.057
T4	25	1.216	bcde	±	0.012	1.177	fghi	±	0.011
T4	40	1.311	a	±	0.006	1.076	lm	±	0.016
T5	25	1.199	defg	±	0.020	1.154	hij	±	0.028
T5	40	1.208	cdef	±	0.014	0.850	n	±	0.049
T6	25	1.193	defg	±	0.009	1.126	jk	±	0.035
T6	40	1.184	efgh	±	0.020	0.846	n	±	0.027

Means with the same letter are not significantly different (p > 0.05).

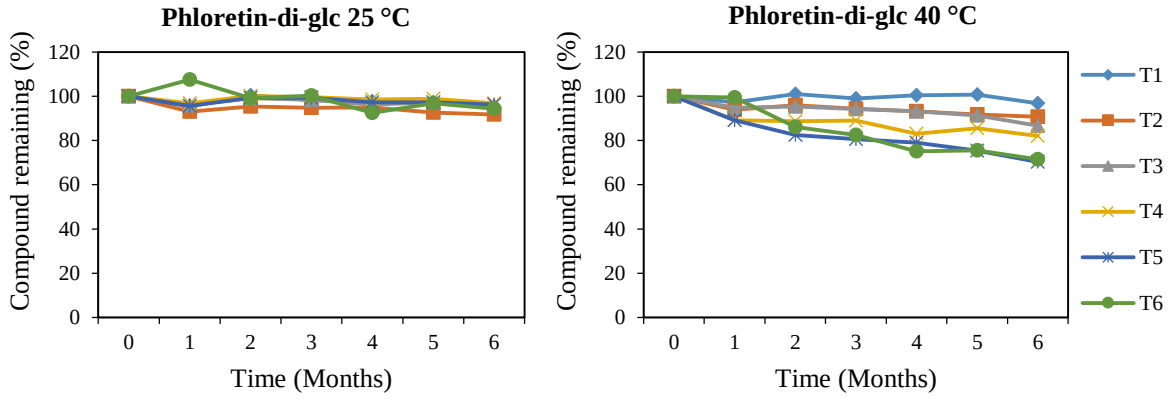


Figure 4.30 Percentage remaining phloretin-3′,5′-di-*C*-glucoside over six month stability trial at 25 °C/60% RH and 40 °C/75% RH. The treatments were composed of pure *C. subternata* extract (T1), extract spray-dried with 25% inulin (IN25) (T2), IN25 + sugar (T3), IN25 + xylitol + stevia (T4), IN25 + sugar + citric acid + ascorbic acid (T5) and IN25 + xylitol + stevia + citric acid + ascorbic acid (T6). glc = glucoside.

In the pure extract vicenin-2, iriflophenone-3-*C*-glucoside and scolymoside were the only compounds which degraded more than 10%. Overall this treatment was the most stable. The presence of inulin appeared to increase the rate of degradation during storage rather than having a protective effect. Overall degradation of phenolic compounds was accelerated most drastically by the presence of citric acid and ascorbic acid at 40 °C, and to a lesser extent, by the presence of sugar and xylitol at 40 °C. These results agreed with those obtained by Ortiz *et al*. (2008) in a study on the effect of formulation and environmental moisture on the stability of catechins in green tea powder. It was found that the chemical degradation of total and individual catechins in green tea powder formulations was significantly increased ($p < 0.0001$) by exposure to increasing RH, and the degradation was exacerbated at ≥ 58% RH by the presence of powdered citric acid and at ≥75% RH by the presence of ascorbic acid. Catechins degraded the most in formulations containing both acids. In the present study moisture content of the mixture increased to values above their M_0 value (Fig. 4.25), which would contribute to an acceleration of degradation reactions.

A study on the stability of (-)-epigallocatechin gallate during storage reported a change in colour that was correlated with oxidation of this compound (Li *et al*., 2014). Therefore the change in colour in the samples in the current study may be correlated with the oxidation of the phenolic compounds. Notably, samples containing citric acid and ascorbic acid and stored at 40 °C displayed the biggest colour change and the highest degradation of polyphenols. Colour changes may also be attributed to the degradation of ascorbic acid. Ascorbic acid is a highly unstable compound which has been shown to degrade during storage in dry powder form, particularly at increased temperature and RH conditions (Hiatt *et al*., 2010). In the presence of sugars the degradation of ascorbic acid produces 5-hydroxymethylfurfural which has been linked to the production of brown pigments in fruit juice (Shinoda *et al*., 2004; Kambo & Upadhyay, 2012). This compound was also detected after heat treatment of ready-to-drink green rooibos iced tea (Joubert *et al*., 2010).

4.5 Conclusions

The use of alternative sweeteners xylitol and stevia improved the health profile of an instant iced tea beverage. Xylitol produced similar results to sugar in terms of stability, although it was shown to have a lower RH_0 making the powder mixture more susceptible to changes such as degradation at raised RH conditions. In the absence of other ingredients pure *C. subternata* extract was very stable and appeared to be more stable than the extract that had been spray-

dried with inulin, especially in terms of phenolic retention. In the absence of elevated RH and at ambient temperatures (25 °C) the powders were physically stable with good retention of phenolic compounds for a period of six months. However when the powders were stored at 40 °C, the presence of citric acid and ascorbic acid, food ingredients commonly found in iced tea, drastically increased the rate of degradation of phenolic compounds and decreased the physical stability of the powder mixture resulting in prominent colour changes in the samples. Deliquescence could also be a problem if storage conditions are not optimum, thus highlighting the need for moisture impermeable packaging. Future studies should investigate stability of the powders in different types of packaging.

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Chapter 5

General discussion and conclusions

With the devastating effects of metabolic syndrome taking on global proportions there is an overwhelming moral, medical and economic imperative to address the problems associated with this condition (Anon, 2014). As the link between diet and health becomes clearer the responsibility of the food industry towards the consumer is becoming increasingly important. South Africa currently has the highest levels of obesity in sub-Saharan Africa and diabetes is the number one cause of death in the Western Cape (Anon, 2013). The health care costs associated with these conditions have a crushing effect on resource-poor countries, such as South Africa, which now have to cope with the double burden of chronic and infectious diseases (Malik *et al*., 2006).

One of the major contributors to this condition is an increase in the availability of cheap, over-processed and high energy foods (Baboota *et al.*, 2013). Sugar-sweetened beverages in particular are very high in sugar, have no nutritional value and have been directly linked to increases in obesity and diabetes (Ludwig *et al*., 2001; Bray *et al*., 2004; Ebbeling *et al*., 2006; Malik *et al*., 2006; Bleich *et al*., 2009). Improving the health profile of ready-to-drink beverages is two-fold. Firstly, it is important to reduce the number of kilojoules contributed by sugar. Secondly, the nutritional value of the product can be improved by incorporating bioactive ingredients which are able to prevent and/or treat conditions associated with metabolic syndrome (Corbo *et al.*, 2014).

The indigenous herbal tea honeybush, prepared from *Cyclopia* spp. fits these requirements perfectly. It is naturally low in energy and contains high concentrations of bioactive phenolic compounds (Joubert *et al*., 2011). In particular, aqueous extracts of *C. subternata* have been proven to possess anti-obesity effects (Dudhia *et al*., 2013) and have been patented for their anti-diabetic properties (Mose Larsen *et al*., 2008). During the development of functional beverages it is important to create a product which is convenient and has an appealing taste profile. At the same time it is essential to monitor the stability of the bioactive compounds from the point of production to consumption (Bott *et al*., 2010).

Previous research on *Cyclopia* species has focused on developing optimised extraction techniques with standardised concentrations of key phenolic compounds such as mangiferin and hesperidin (Bosman, 2014; Du Preez, 2014). The next step in transforming *Cyclopia* extracts to nutraceutical ingredients is drying them to produce stable free-flowing powders. Spray-drying is the most widely used method of drying natural extracts and is commonly used in industry due to the fact that the equipment is easily accessible and affordable in comparison to other techniques (Gharsallaoui *et al*., 2007; Chiou & Langrish, 2007; Fang & Bhandari, 2011; Cortéz-Rojas & Oliveira, 2012; Harbourne *et al*., 2013). However, to date there has been no research on the effects of this process on *Cyclopia* extracts.

In Chapter 3 (the first experiment) the aim was to determine the effects of spray-drying on the physical and chemical properties of green *C. subternata* extract. The addition of carriers was investigated as a means of increasing the stability of *C. subternata* extract throughout processing and storage. Corn syrup solids (CS) and inulin (IN) were compared at three treatment levels i.e. 25% (CS25 & IN25), 50% (CS50 & IN50) and 75% (CS75 & IN75) of the total soluble solids content. This was done with the aim of creating a stable dried extract which could be used in the production of a sugar-free instant iced tea powder. IN25 was selected for these purposes due to the fact that it dissolved easily.

In Chapter 4 (the second experiment) the aim was to test the stability of spray-dried green *C. subternata* extract when in contact with ingredients commonly used in powdered iced tea formulations during six months of storage at ambient (25 ºC/60% RH) and accelerated (40 ºC/75% RH) storage conditions. A conventional formulation containing sugar, citric acid and ascorbic acid was used as the starting point for the development of a healthier iced tea formulation. A reduced kilojoule formulation was developed using a combination of xylitol and stevia. From these two formulations six treatments were derived namely T1 (pure *C. subternata* extract), T2 (*C. subternata* extract spray-dried with 25% inulin, i.e. IN25), T3 (IN25 + sugar), T4 (IN25 + xylitol), T5 (IN25 + sugar + citric acid + ascorbic acid) and T6 (IN25 + xylitol + stevia + citric acid + ascorbic acid).

Physicochemical properties of all spray-dried extracts as well as the iced tea powders were characterised in terms of moisture content, water activity (a_w), moisture sorption isotherms and objective colour measurement. Isothermal microcalorimetry was used to determine if the ingredients interacted with each other. X-ray powder diffraction measurements were performed to confirm the crystalline or amorphous nature of the spray-dried powders.

Simultaneous differential thermal analysis and thermogravimetry were applied to measure heat flux and mass loss during heating. This was done to determine the thermal behaviour of the powders, particularly the glass transition temperature (T_g) of amorphous substances. Finally, the wettability of the powders was assessed to determine their ability to dissolve and disperse in water, an important attribute of convenient dry powder mixtures of iced tea products.

During the first experiment spray-drying parameters were selected based on the results from preliminary trials. The parameters included an inlet temperature of 180 ºC, outlet temperature of *ca.* 90-100 ºC, aspirator rate of 100% (35 m^3/h) and peristaltic pump speed of 25% (*ca* 7.5 mL/min). These parameters produced satisfactory results such as adequate product yields of *ca.* 63% and free-flowing powders with low moisture content (< 5% dry basis) and water activity (< 0.25 a_w). Although it was not within the scope of this study, future research to optimise spray-drying parameters could help to improve the product yield and make the process more efficient. Previous studies have used a "quality by design" approach to optimise the spray-drying process, analytical chemistry techniques, and biotechnology experiments using statistical methods such as response surface methodology (Bezerra *et al*., 2008; Baldinger *et al*., 2012; Lebrun *et al*., 2012; Steinberg & Bursztyn, 2012). It should also be noted that the results obtained on a laboratory spray-drier might not be the same as those obtained on a commercial spray-drier. In order to ensure the quality of spray-dried honeybush extracts on a commercial scale, it will be necessary to test quality parameters under conditions experienced in commercial production.

Carriers have played a crucial role in the stability of natural extracts such as *Hibiscus sabdariffa* L. and blackberry extracts (Chiou & Langrish, 2007; Da Rosa *et al*., 2014) as well as isolated polyphenols such as vanillin and quercetin (Sun-Waterhouse *et al*., 2013) during spray-drying. They are also commonly used in industry, with maltodextrin and CS being the most widely used due to the fact that they are cheap and versatile (De Vos *et al*., 2010; Silva *et al*., 2013). However, at present there is a trend in the health-food industry to move away from highly processed foods, especially refined carbohydrates such as maltodextrin and CS (Mellentin, 2014). This trend is reflected in literature with many studies investigating alternative carriers such as pectin, chitosan and alginates, which not only improve the stability of natural extracts, but also contribute additional health benefits to the final product (Chiou & Languish, 2007; Sansone *et al.*, 2011; De Souza *et al.*, 2013; Sun-Waterhouse *et al.*, 2013).

In the present study IN was investigated as an alternative carrier to CS. IN contributes towards the dietary fibre content of a product and has been proven to have prebiotic properties, stimulating the growth of beneficial Bifidobacteria species (Kolida *et al.*, 2002). During the preparation of the feed solution, at levels of 25 and 50% of the total solids, both of these carriers dissolved easily in water and formed a homogenous solution with the extract. However at 75% of the total solids inulin became more difficult to dissolve while CS still dissolved easily. After spray-drying the yields of the resulting powders were all above 60% and the treatments were very similar in colour and appearance, with powders becoming lighter as the concentration of carrier increased. Powders containing IN had a slightly lower moisture content and a_w than those containing CS.

Interestingly the presence of carriers did not appear to improve the stability of *C. subternata* extract during spray-drying, since the phenolic compounds were very stable even when the pure extract was spray-dried without any carriers present. It was postulated that the natural matrix of *C. subternata* extract may have conferred a degree of stability to the phenolic compounds throughout the spray-drying process. This was suggested due to the fact that the spray-drying process substantially degraded mangiferin when it was spray-dried in its isolated form (De Souza *et al*., 2013), while the present study showed complete retention of this compound in the absence of carriers. Future studies to spray-dry ultra-filtered and standardised extracts such as those produced by Bosman (2014) should proceed with caution as the large polymers removed during this process may contribute towards the stability of the extract. It is therefore important to elucidate the structure of these polymers. A recent study analysed the low molecular weight sugars present in honeybush tea, revealing new insights into its composition which could aid in future studies (Moldoveanu *et al*., 2015). For instance the presence of sugar alcohols such as sorbitol may contribute to a water activity lowering effect by binding available moisture.

The natural matrix of *C. subternata* extract also appeared to confer stability to the phenolic compounds in the dried powders throughout six months of storage. Pure spray-dried *C. subternata* extract was the most stable treatment in the storage stability trial. In this treatment (T1) most of the major phenolic compounds maintained acceptable stability (< 10% degradation) over the six month period, even at raised temperatures of 40 ºC.

The moisture sorption isotherms (MSI) of powders containing IN and CS were very similar to that of the pure *C. subternata* extract and displayed Type II curves. Increasing the

concentrations of these carriers resulted in more pronounced hysteresis within the powders upon subsequent desorption and adsorption cycles. While this may not have an effect on the powders when stored correctly (in the absence of moisture), if the powders were exposed to compromising conditions, moisture uptake would cause irreversible changes to the structure of the powders containing carriers (Al-Muhtaseb *et al*., 2002). Plasticisation of the polymer carriers occurs when their surface comes into contact with high relative humidity conditions resulting in agglomeration and caking of the powders (Mathlouthi & Rogé, 2003). On the other hand, the MSI of the powder iced tea formulations containing the crystalline substances sugar and xylitol behaved very differently from those of the spray-dried extract. The Type III MSI curves of these mixtures showed less moisture uptake than the spray-dried extract at RH below 75%. In Type III curves the moisture uptake is very low until the crystals reach their deliquescent point at which they begin to dissolve in the absorbed moisture (Labuza & Altunakar, 2007).

During the formulation of green honeybush iced tea an existing rooibos iced tea formulation was used as a starting point. The concentration of extract incorporated was based on the sensory appeal of the product. In order to make claims about the health benefits of the extract it would be necessary to determine the dosage of extract required to confer therapeutic benefit to the consumer (Sun-Waterhouse, 2011). Globally the use of health claims on functional beverages is being tightly regulated and in the EU all claims must be substantiated by scientific research which has been verified by the European Food Safety Authority (EFSA) (Reis, 2011). When developing a food product it is essential that the taste of the product is not compromised by the addition of functional ingredients. High levels of phenolic compounds have been associated with sensory attributes such as bitterness and astringency (Drewnowski & Gomez-Carneros, 2000). Therefore, it would be necessary to determine if the levels of extract required would have a negative impact on the sensory appeal of the product.

Citric and ascorbic acid are frequently added to ready-to-drink iced tea products for sensory purposes (Ortiz *et al*., 2008). In this study, the addition of citric and ascorbic acid (T5 & T6) appeared to drastically affect the stability of the powders when stored at 40 ºC. This was reflected in both the degradation of phenolic compounds as well as distinct changes to the colour and visual appearance of these powders. During investigation of the compatibility of the ingredients used in mixtures, a large endothermic curve was detected using isothermal microcalorimetry. This signified the dehydration of monohydrate citric acid at 40 ºC. The

release of moisture may have catalysed degradation reactions within the powders by increasing the amount of moisture available for chemical reactions. It would therefore be recommended that anhydrous citric acid be used in future studies.

It was unexpected that the presence of acids decreased the stability of phenolic compounds because low pH is known to confer stability to polyphenols and ascorbic acid has also been shown to improve the stability of green tea catechins as well as rooibos phenolic compounds in aqueous solutions (Peters *et al*., 2009; Beelders *et al*., 2012). On the other hand Ortiz *et al*. (2008) found that both citric acid and ascorbic acid caused degradation of catechins in dried green tea extract under increased RH conditions and that degradation was increased when both acids were present. The degradation of ascorbic acid may also have contributed towards the production of brown pigments within these treatments (Shinoda *et al*., 2004; Kambo & Upadhyay, 2012). It would be of interest to investigate whether citric acid or ascorbic acid alone had a larger effect on the stability of these mixtures and if alternative acids such as malic acid or fumaric acid may produce better results. These acids are naturally present in fruit and are frequently blended with citric acid and added to beverages to provide a more balanced acidity profile (Lanton, B., 2014, Owner, Cape Food Ingredients, personal communication, 28 July).

Two major concepts used to explain the stability of foods are water activity and glass transition (Sablani *et al*., 2007). From literature it is clear that these two concepts are inter-related and cannot be viewed in isolation (Rahman, 2010; Venir & Maltini, 2013). The concept of water activity is based on the binding nature of water molecules in the food matrix. At the monolayer value (as determined by the BET model), water is bound to the solid matrix and no deterioration reactions are expected (Rahman, 2010). The glass transition concept is based on the mobility of the matrix. Amorphous substances derive kinetic stability due to their high viscosity which limits the diffusivity of the reactants in the system (Rahman, 2010). The rate of diffusion, and therefore deterioration in amorphous systems is exacerbated by increases in temperature as well as the plasticisation effect of moisture. An amorphous substance is most stable below its T_g and molecular mobility increases 100-fold above its T_g (Rahman, 2010). This has negative implications for powders as they could start to crystallise which would cause a disruption of the matrix potentially leading to caking of the powders (Sansone *et al.*, 2011).

The results from this study provided valuable insights into the storage conditions required to maintain the stability of both spray-dried honeybush extract as well as powder

formulations of instant iced tea. X-ray powder diffraction revealed the amorphous nature of spray-dried *C. subternata* extract and the crystalline nature of the iced tea powders. During this study no distinct T_g was identified for the pure *C. subternata* extract, or treatments containing extract due to their heterogeneous compositions. However the T_g of the carriers IN and CS were detected at 50.4 – 59.2 °C and 155.7 °C, respectively. These results correlated well with those reported in literature (Roos & Karel, 1991; Schaller-Povolny *et al.*, 2000). From these results it could be seen that inulin had a lower T_g than CS and would therefore be more susceptible to glass transition under high temperature conditions.

Directly after production spray-dried *C. subternata* extracts had moisture contents below their BET monolayer values. However, MSI revealed that these powders absorbed moisture quickly in the presence of increased RH conditions. When the stability of the powders was tested in the presence of moisture it was observed that amorphous powders were much more susceptible to deliquescence than crystalline powders. At 55% RH IN, *C. subternata* extract and stevia started to deliquesce while the crystalline powders were stable. Of the crystalline powders used in the product formulations xylitol (RH_0 < 75% RH) was shown to have a lower deliquescence RH (RH_0) than sugar (RH_0 > 75% RH) which agreed with results in literature (Salameh *et al*., 2006; Hiatt *et al*., 2008; Lipasek *et al*., 2013). Formulations containing sugar would therefore be more stable at 75% RH.

During the wettability experiments it was found that the spray-dried powders were very difficult to dissolve on their own. When in contact with water these powders formed a highly viscous layer which effectively confined the undissolved powder to the interior of lumps. However, the addition of other ingredients such as sugar, xylitol and citric acid improved the wettability and therefore the ability of the mixtures to dissolve. These results have very important implications for the usability of the product as the powders need to be able to disperse easily in a glass of water before consumption.

From this study it is clear that adequate packaging material is essential for the stability of powder iced tea formulations. It is therefore recommended that such a product be stored in airtight and moisture-impermeable packaging which can provide consumers with a single serving of iced tea. Common packaging materials for this type of product include sachets made of flexible plastics which are lined with aluminium foil.

The use of xylitol and stevia improved the health profile of instant iced tea by decreasing the number of kilojoules for a serving of the beverage by more than half compared to consuming the formulation containing sugar. The bioactive phenolic compounds present in honeybush were stable throughout the spray-drying process. These compounds were also stable when the iced tea formulations were stored in dry powder form, under the correct storage conditions, namely in the absence of moisture and at ambient temperatures (25 ºC). However, the stability of the powders was compromised when stored at 40 ºC. A dry powder format presents a number of benefits over the ready-to-drink format in terms of reducing transportation costs and improving the shelf-life of bioactive compounds. Instant iced tea therefore presents a viable option for delivering the bioactive compounds in honeybush tea to consumers in a healthy and convenient format.

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Addendum A

Supplementary results pertaining to Chapter 3

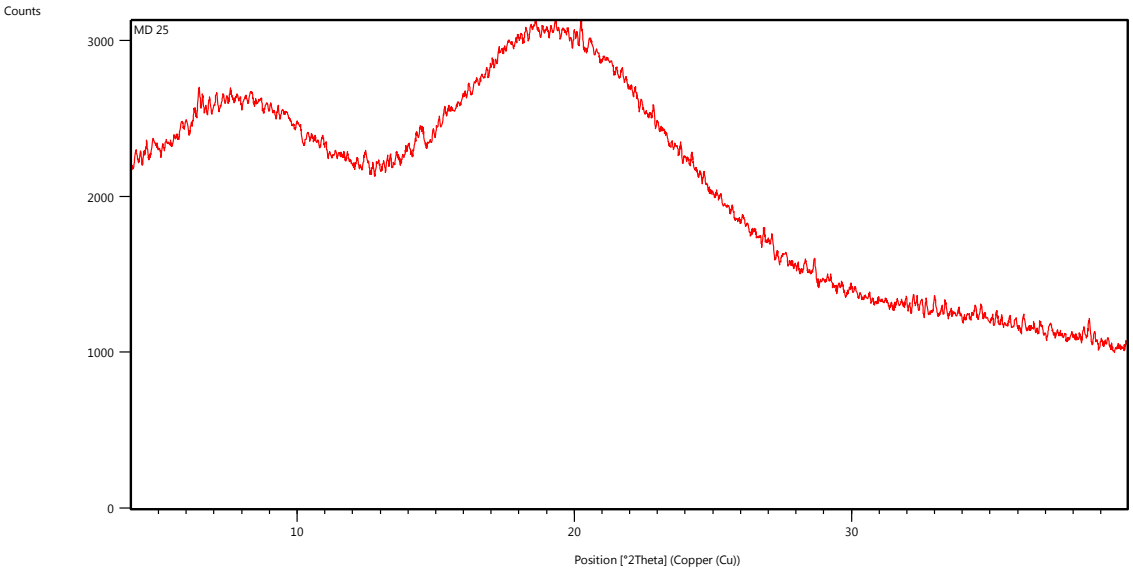

Figure A.1 X-ray powder diffractogram showing amorphous nature of *C. subternata* extract spray-dried with corn syrup solids in a ratio of 3:1 (CS25).

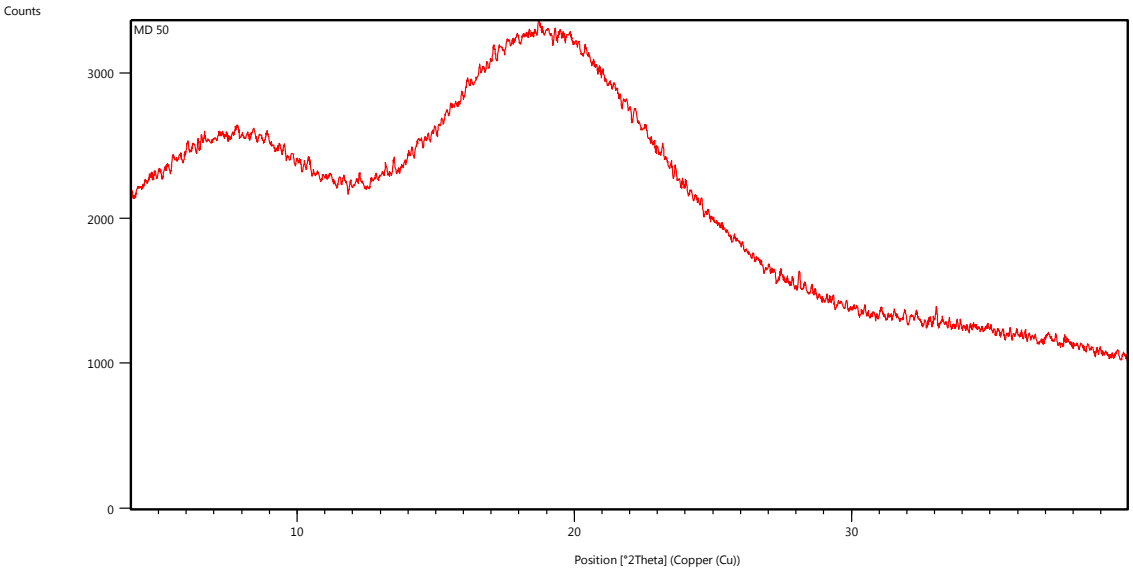

Figure A.2 X-ray powder diffractogram showing amorphous nature of *C. subternata* extract spray-dried with corn syrup solids in a ratio of 1:1 (CS50).

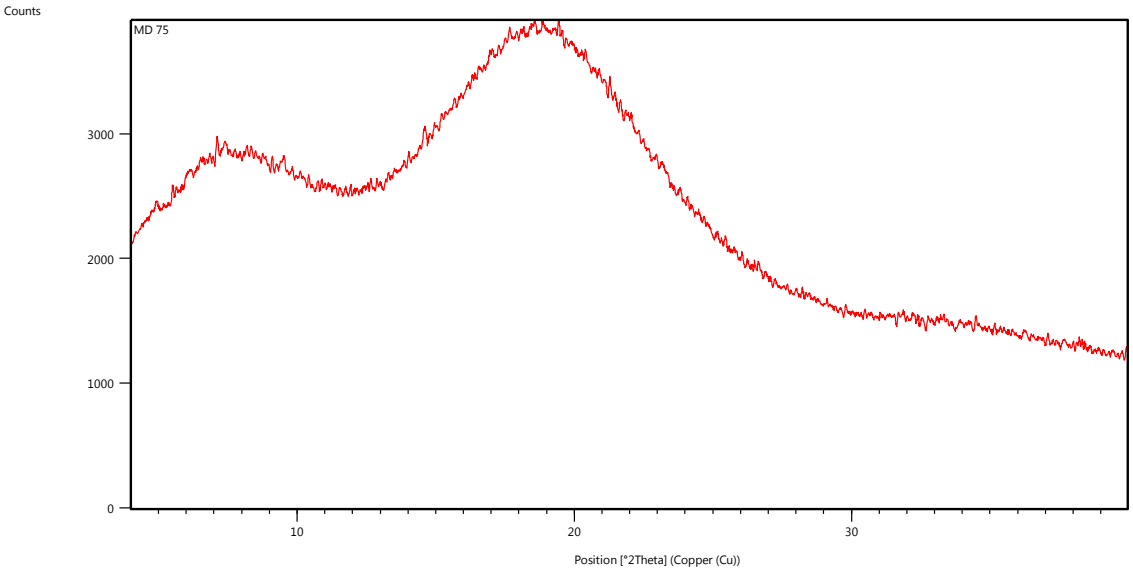

Figure A.3 X-ray powder diffractogram showing amorphous nature of *C. subternata* extract spray-dried with corn syrup solids in a ratio of 1:3 (CS75).

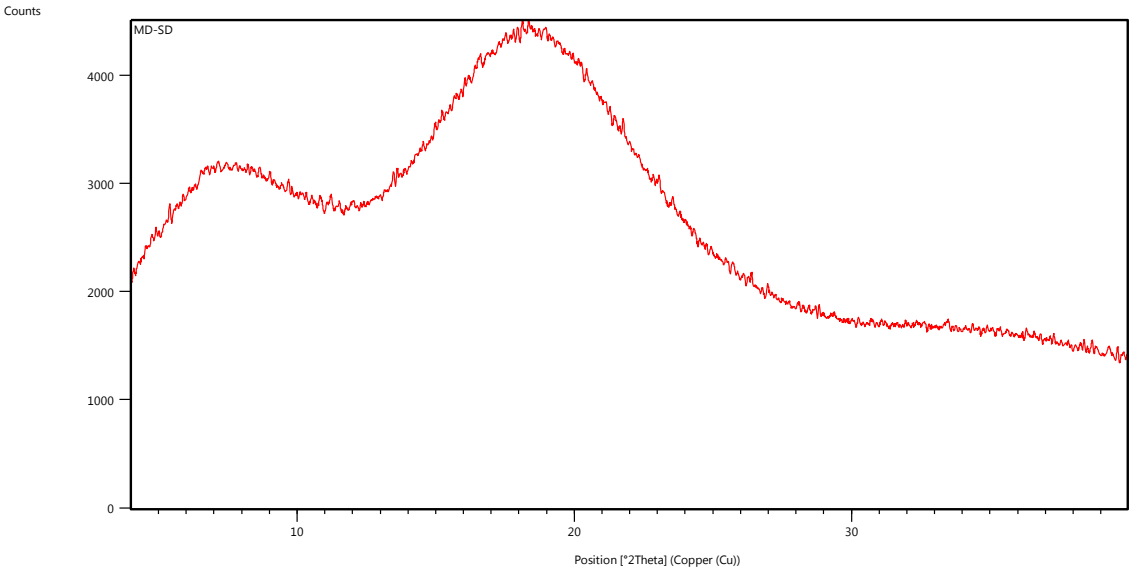

Figure A.4 X-ray powder diffractogram showing amorphous nature of pure corn syrup solids (CS100).

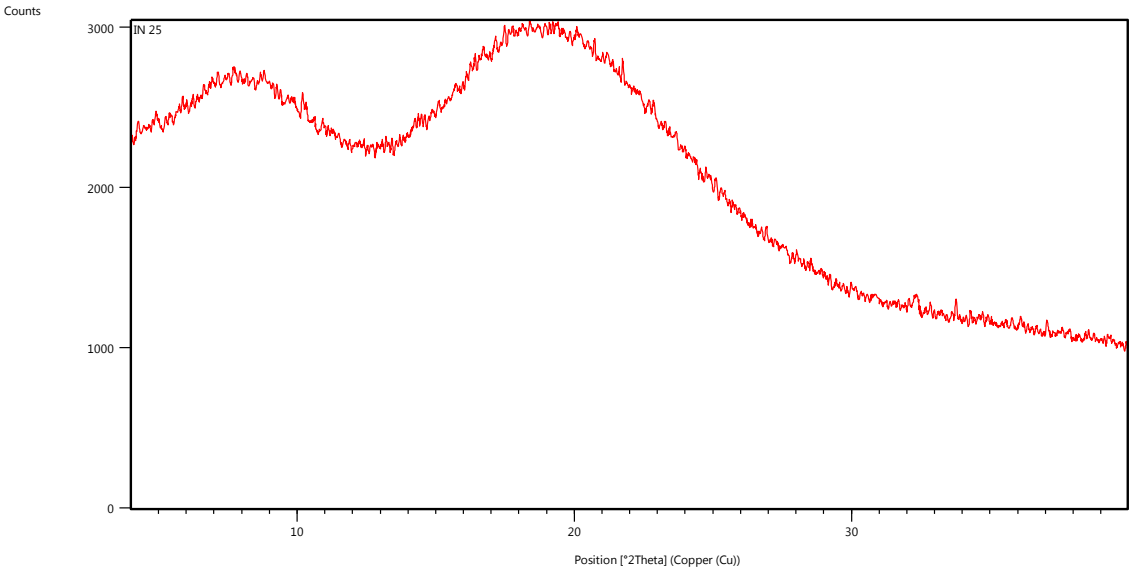

Figure A.5 X-ray powder diffractogram showing amorphous nature of *C. subternata* extract spray-dried with inulin in a ratio of 3:1 (IN25).

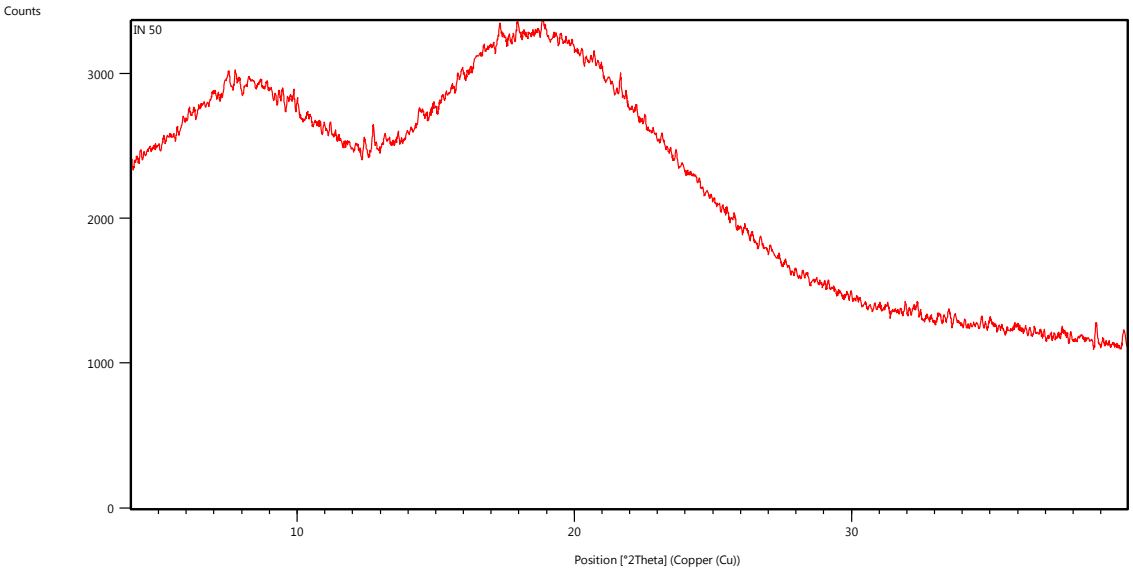

Figure A.6 X-ray powder diffractogram showing amorphous nature of *C. subternata* extract spray-dried with inulin in a ratio of 1:1 (IN50).

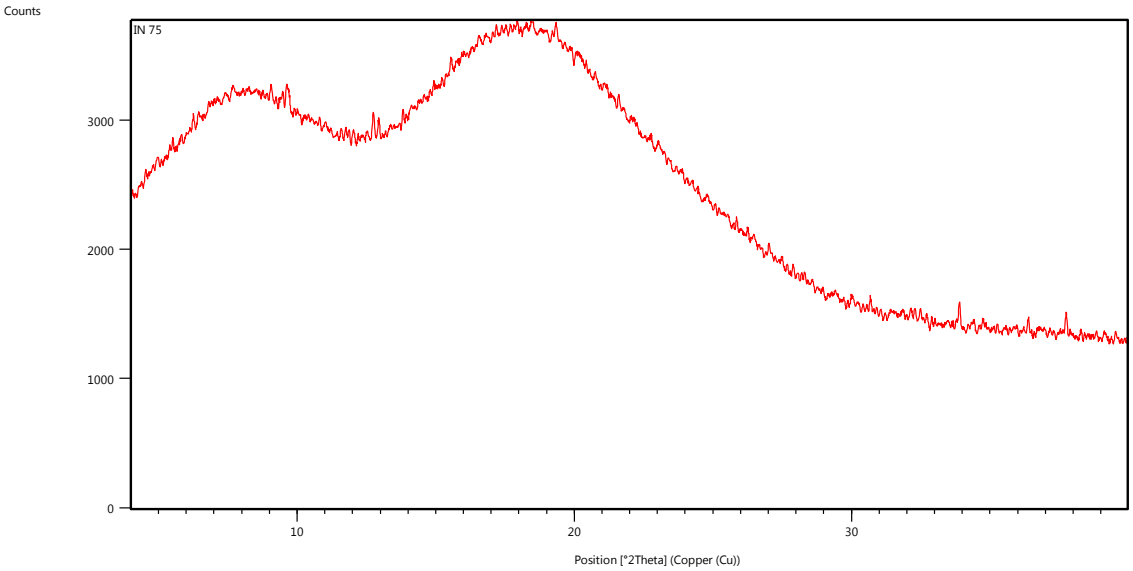

Figure A.7 X-ray powder diffractogram showing amorphous nature of *C. subternata* extract spray-dried with inulin in a ratio of 1:3 (IN75).

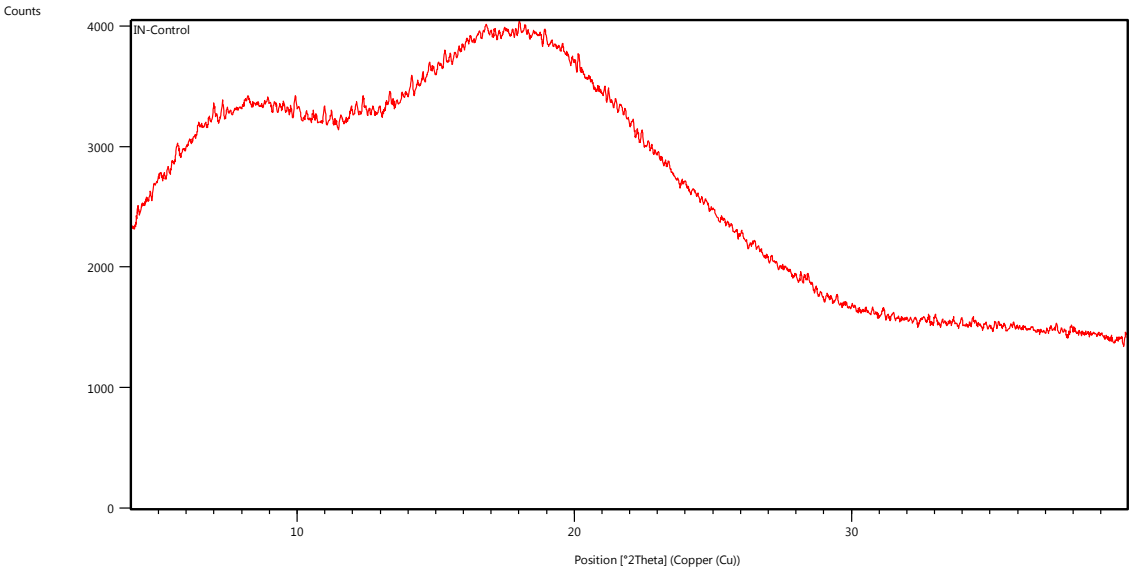

Figure A.8 X-ray powder diffractogram showing amorphous nature pure inulin (IN100).

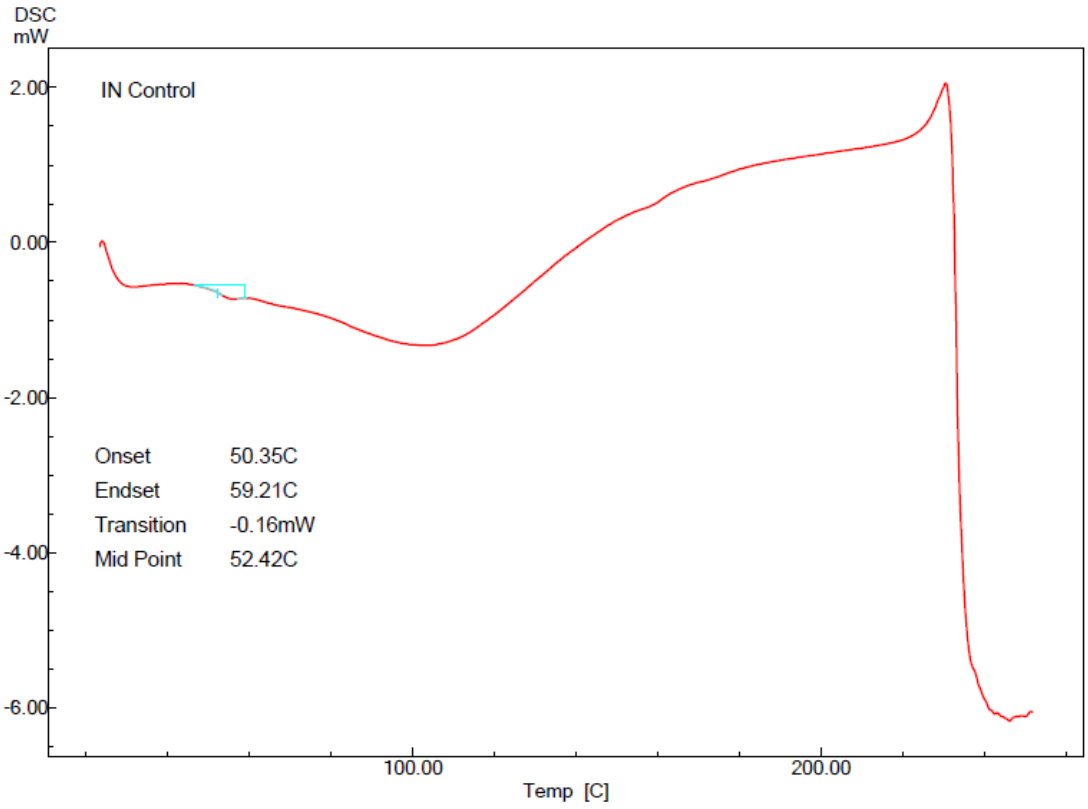

Figure A.9 Differential Scanning Calorimetry (DSC) thermogram of pure inulin showing glass transition between 50.4 and 59.2 ºC.

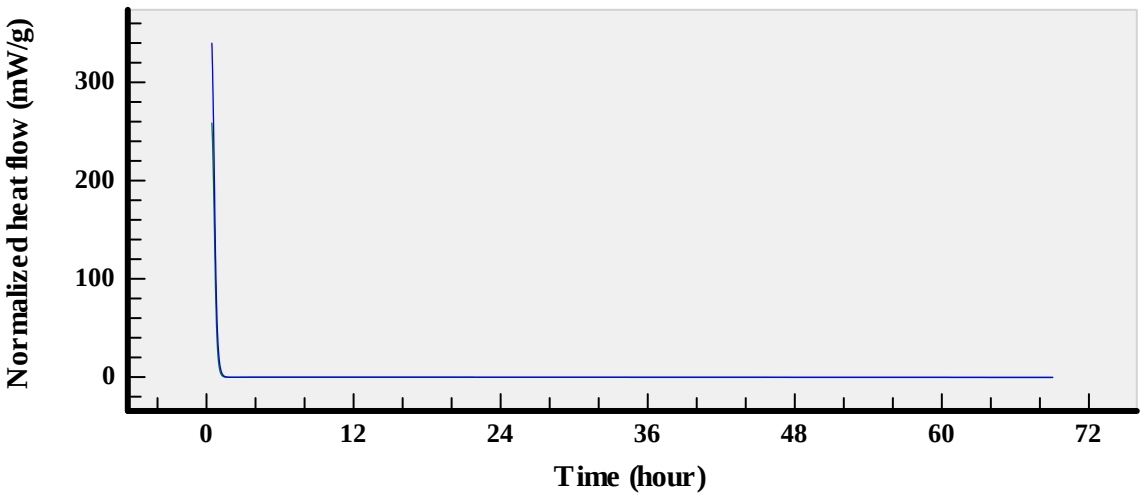

Figure A.10 Graph depicting the heat flow data of individual components of sample CS25, i.e. corn syrup solids (green) and pure *C. subternata* spray-dried extract (blue).

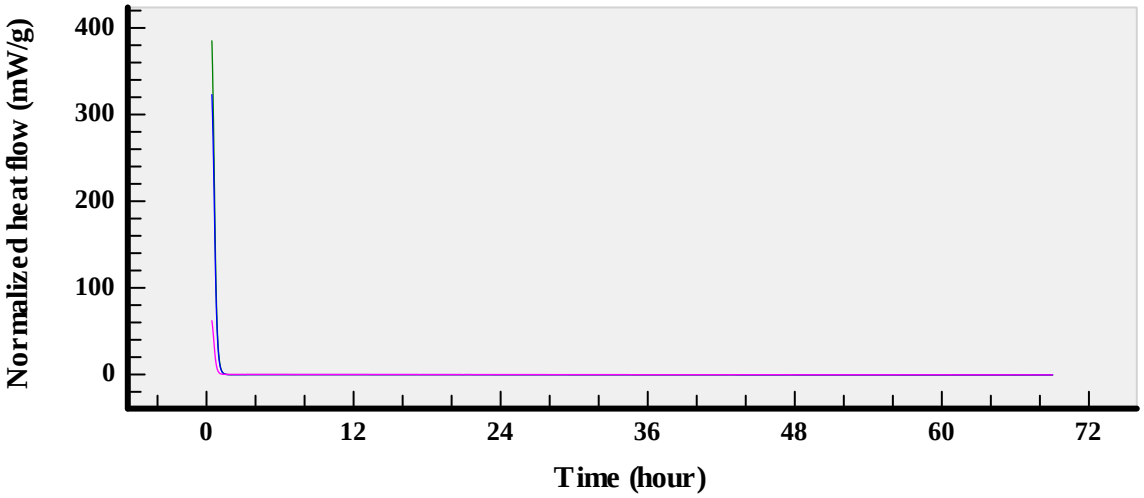

Figure A.11 Graph depicting the measured (green) and theoretical (blue) heat flow data for sample CS25 consisting of a 3:1 ratio (m/m) of spray-dried *C. subternata* extract and corn syrup solids, as well as the interaction (magenta) between the two components (difference between theoretical and measured heat flow).

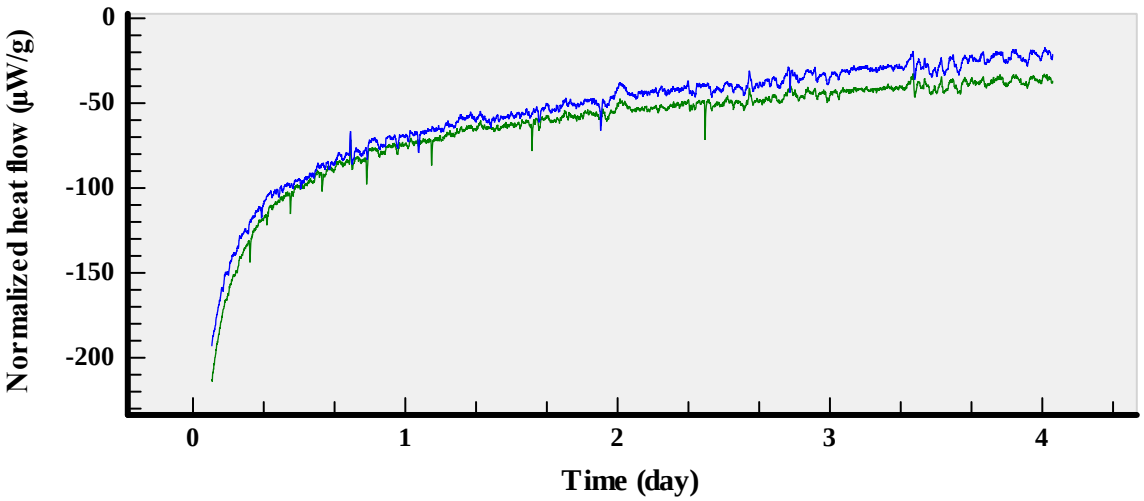

Figure A.12 Graph depicting the heat flow data of individual components of sample IN25, i.e. inulin (green) and pure *C. subternata* spray-dried extract (blue).

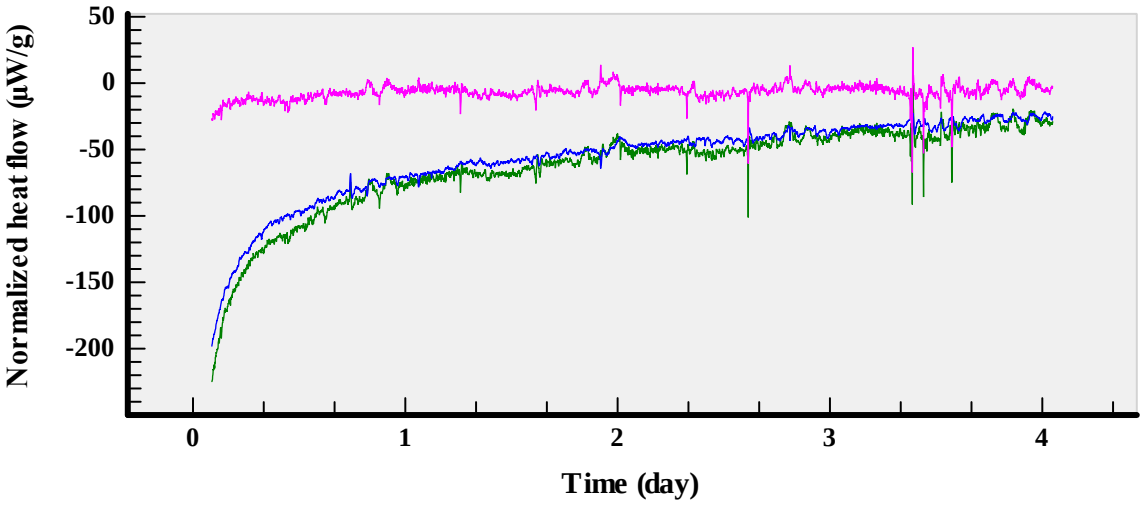

Figure A.13 Graph depicting the measured (green) and theoretical (blue) heat flow data for sample IN25 consisting of a 3:1 ratio (m/m) of spray-dried *C. subternata* extract and inulin, as well as the interaction (magenta) between the two components (difference between theoretical and measured heat flow).

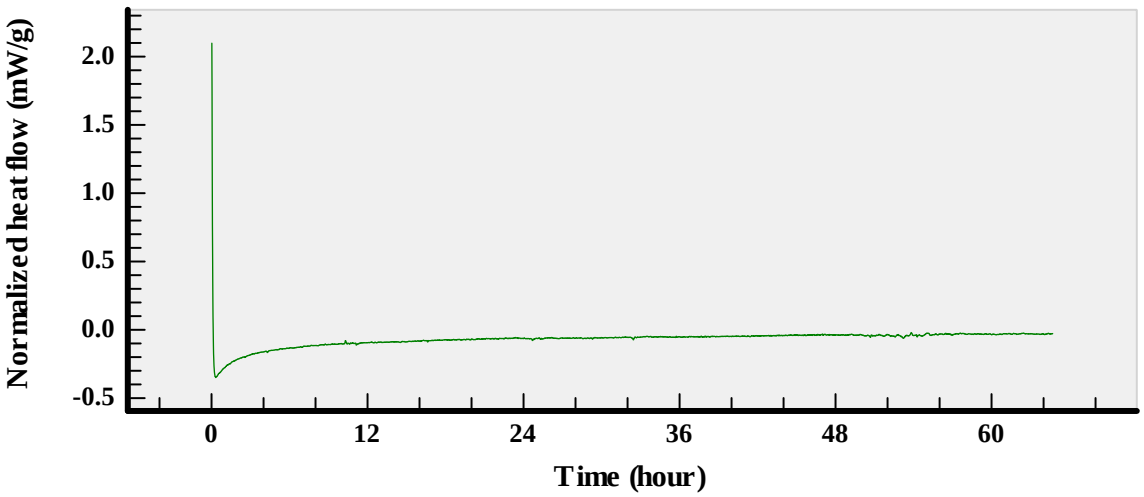

Figure A.14 Graph depicting the heat flow data of individual components of sample IN50, i.e. inulin (green) and pure *C. subternata* spray-dried extract (blue).

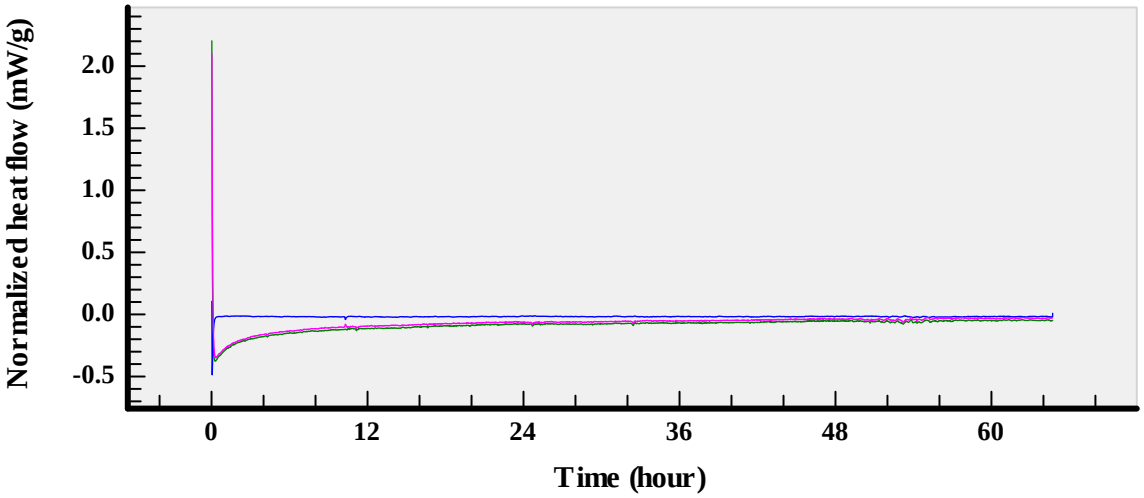

Figure A.15 Graph depicting the measured (green) and theoretical (theoretical) heat flow data for sample IN50 consisting of a 1:1 ratio (m/m) of pure *C. subternata* extract and inulin, as well as the interaction (magenta) between the two components (difference between theoretical and measured heat flow).

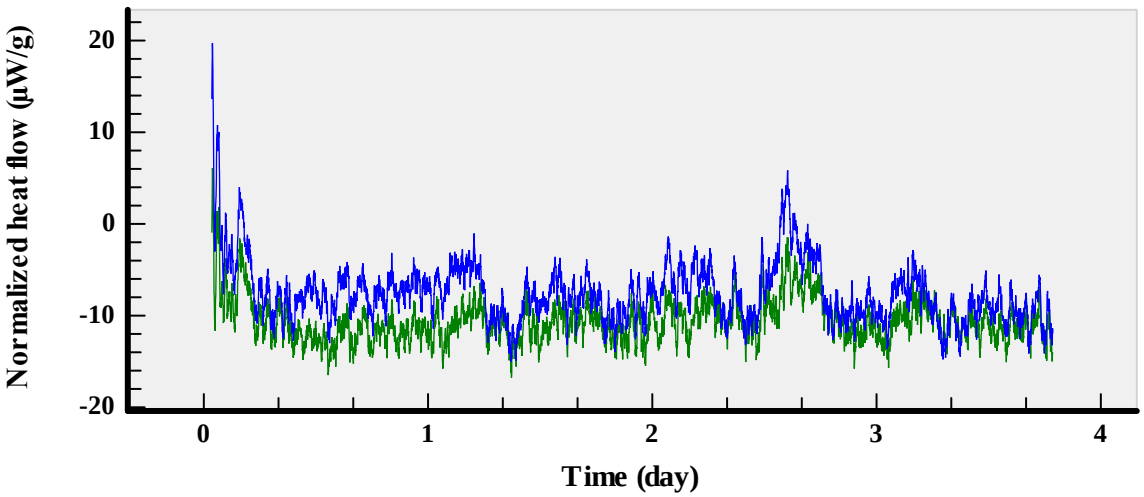

Figure A.16 Graph depicting the heat flow data of individual components of sample IN75, i.e. inulin (green) and pure *C. subternata* spray-dried extract (blue).

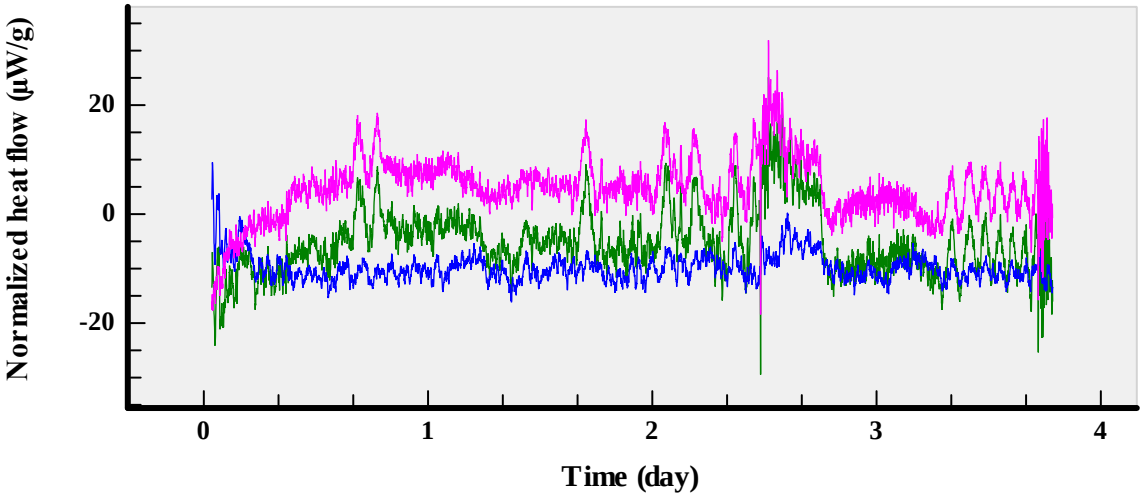

Figure A.17 Graph depicting the measured (green) and theoretical (blue) heat flow data for sample IN75 consisting of a 1:3 ratio (m/m) of spray-dried *C. subternata* extract and inulin, as well as the interaction (magenta) between the two components (difference between theoretical and measured heat flow).

Addendum B

Supplementary results pertaining to Chapter 4

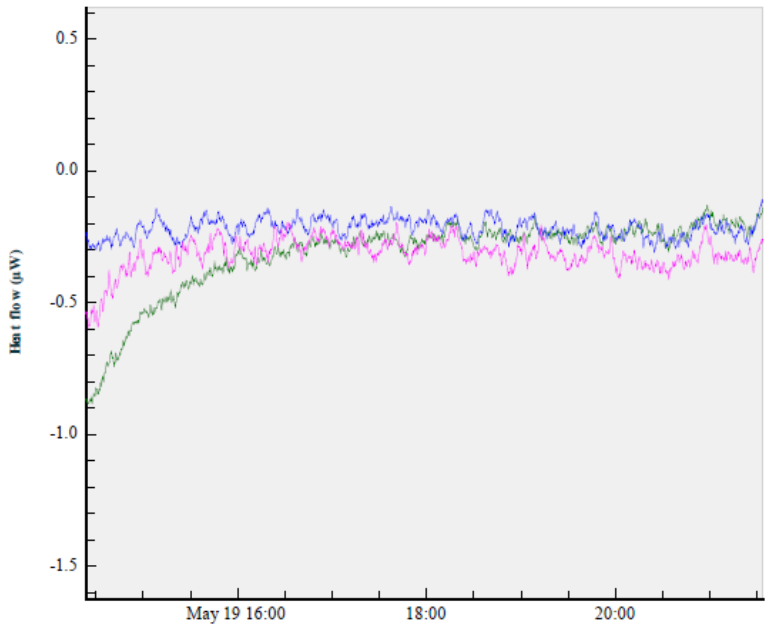

Figure B.1 Graph depicting the heat flow data of T2 (magenta; extract spray-dried with inulin in a ratio of 3:1) at 25 °C, as well as the individual components from which it is constituted individual, i.e. inulin (blue) and *C. subternata* extract (green).

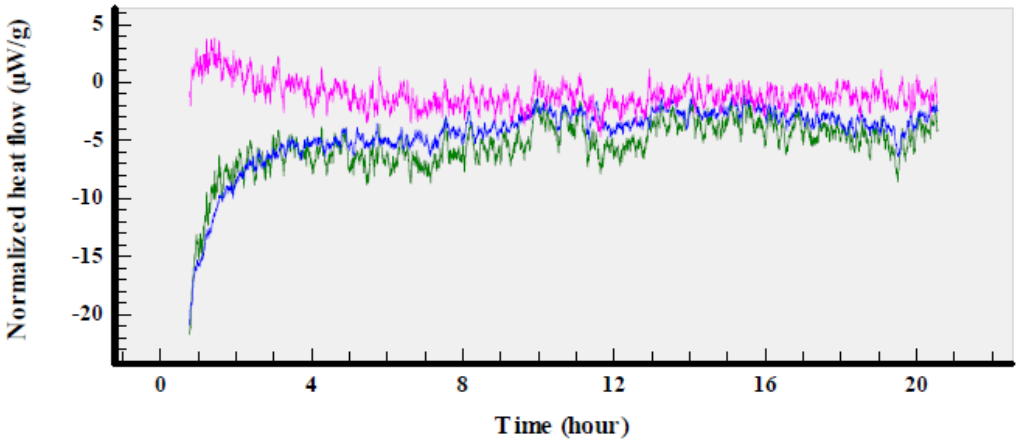

Figure B2 Graph depicting the measured (green) and theoretical (blue) heat flow data for T2 consisting of a 3:1 ratio (m/m) of pure *C. subternata* extract spray-dried with inulin, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 25 °C.

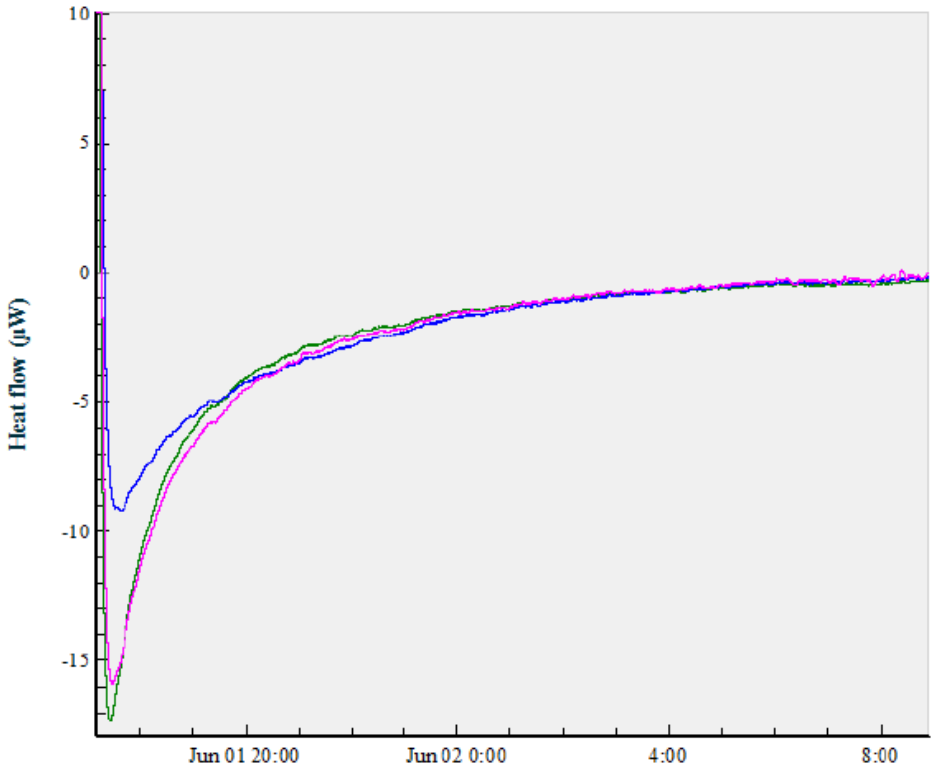

Figure B.3 Graph depicting the heat flow data of T2 (magenta; extract spray-dried with inulin in a ratio of 3:1) at 40 °C, as well as the individual components from which it is constituted, i.e. inulin (blue) and green *C. subternata* spray-dried extract (green).

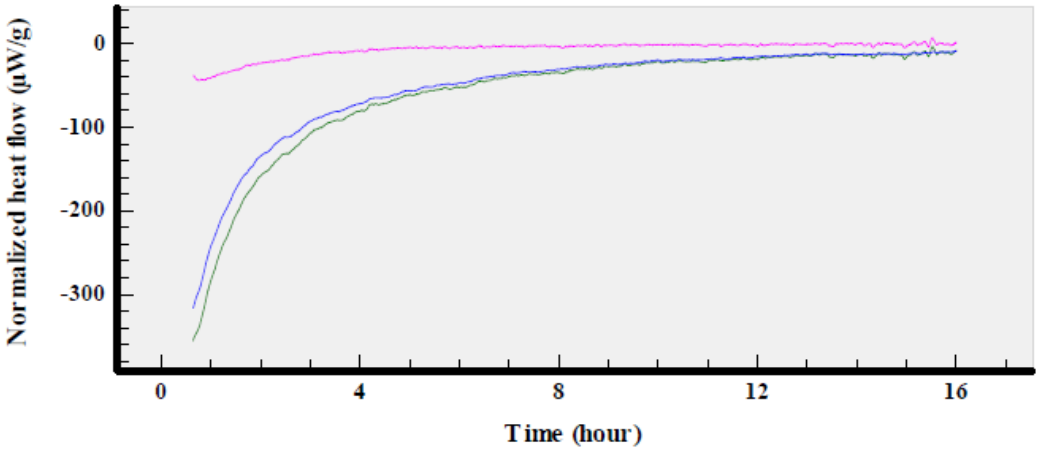

Figure B.4 Graph depicting the measured (green) and theoretical (blue) heat flow data for T2 consisting of a 3:1 ratio (m/m) of pure *C. subternata* extract spray-dried with inulin, as well as the interaction (magenta) between these components (difference between theoretical and measured heat flow) at 40 °C.

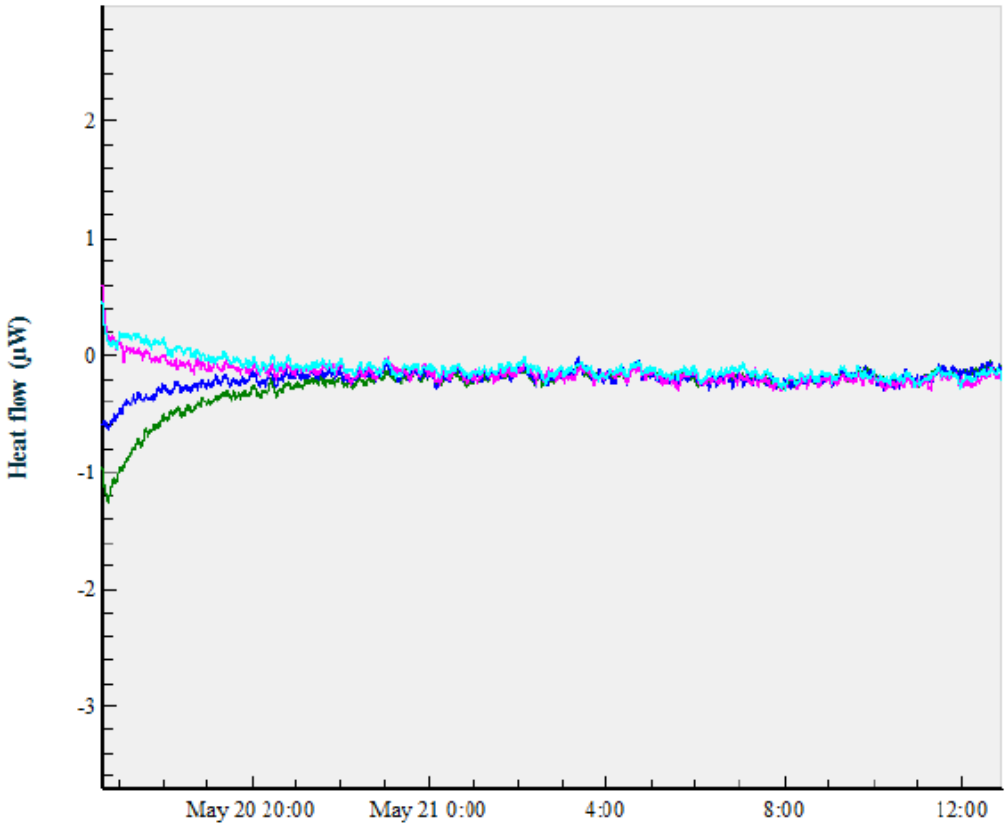

Figure B.5 Graph depicting the heat flow data of T3 (turquoise), at 25 °C, as well as the individual components from which it is constituted, i.e. inulin (blue) and green *C. subternata* extract (green) and sugar (magenta).

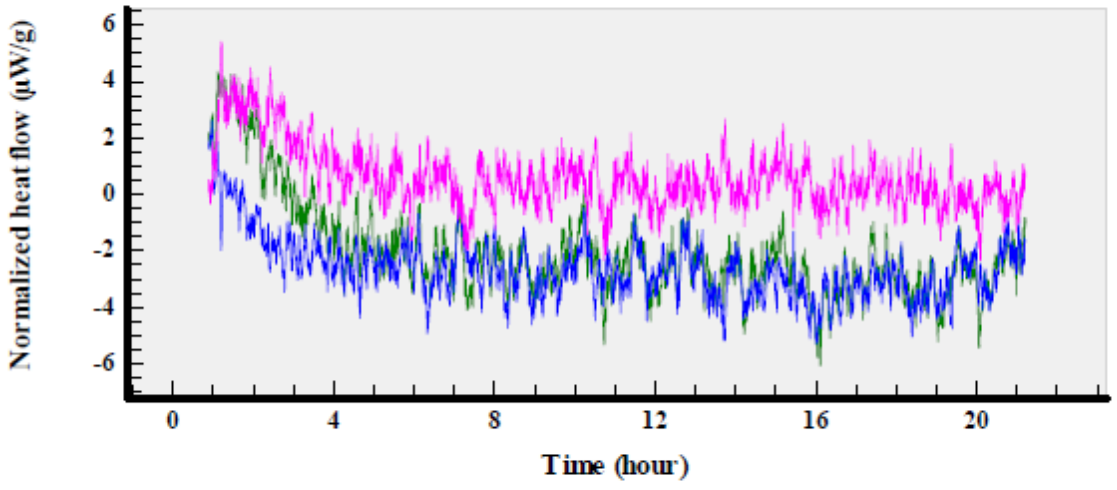

Figure B.6 Graph depicting the measured (green) and theoretical (blue) heat flow data for T3 consisting of a mixture of pure *C. subternata* extract spray-dried with inulin and mixed with sugar, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 25 °C.

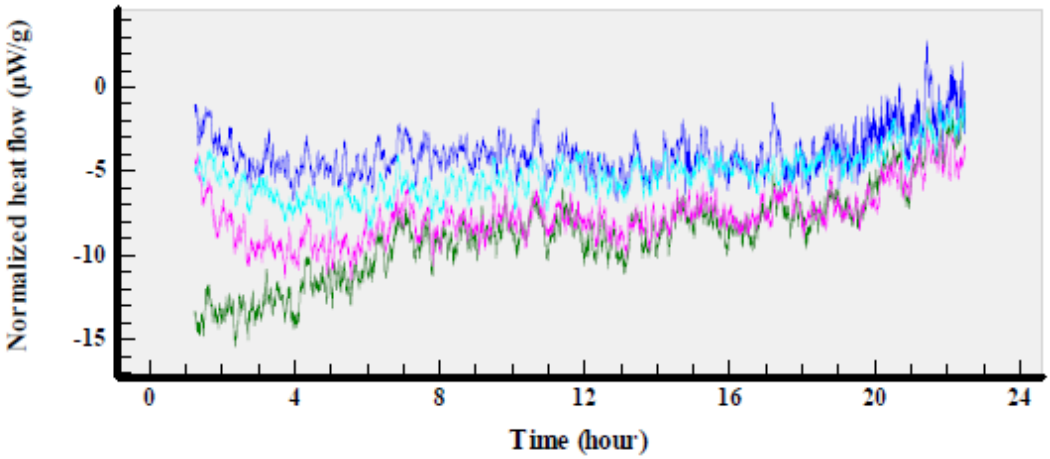

Figure B.7 Graph depicting the heat flow data obtained for the individual components which constitute T4 at 25 °C, i.e. inulin (blue), green *C. subternata* extract (green), xylitol (magenta) and stevia (turquoise).

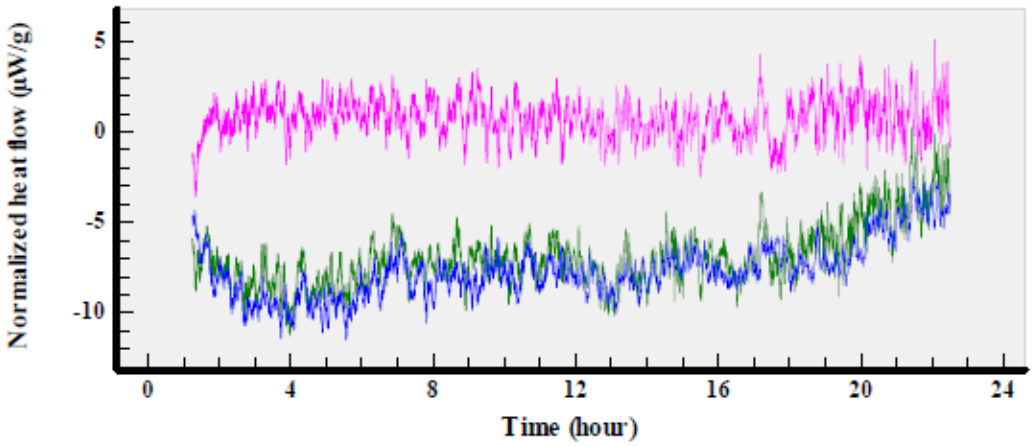

Figure B.8 Graph depicting the measured (green) and theoretical (blue) heat flow data for T4, consisting of a mixture of pure *C. subternata* extract spray-dried with inulin and mixed with xylitol and stevia, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 25 °C.

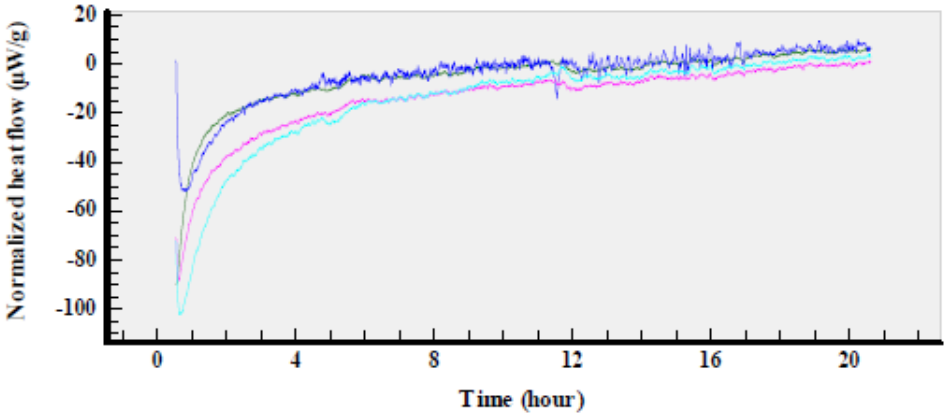

Figure B.9 Graph depicting the heat flow data obtained for individual components which make up T4, at 40 °C, i.e. inulin (blue) and pure *C. subternata* spray-dried extract (green), xylitol (magenta) and stevia (turquoise).

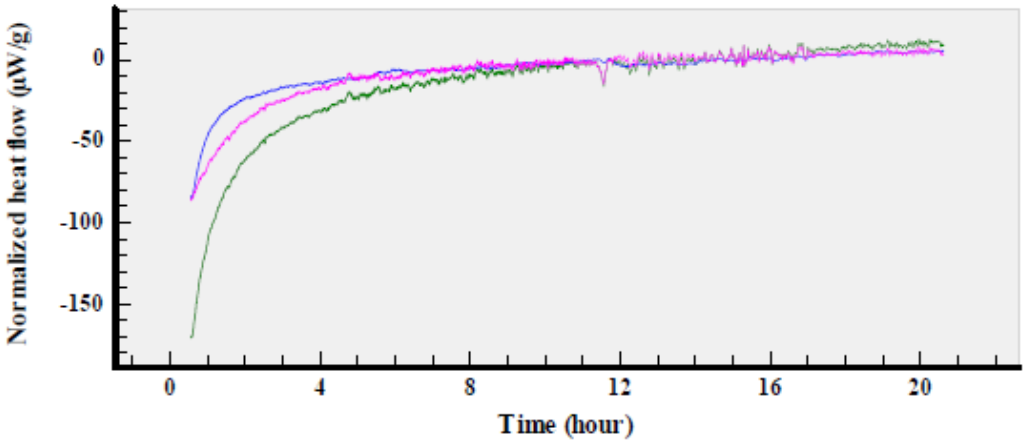

Figure B.10 Graph depicting the measured (green) and theoretical (blue) heat flow data for T4 consisting of a mixture of pure *C. subternata* extract spray-dried with inulin and mixed with xylitol and stevia, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 40 °C.

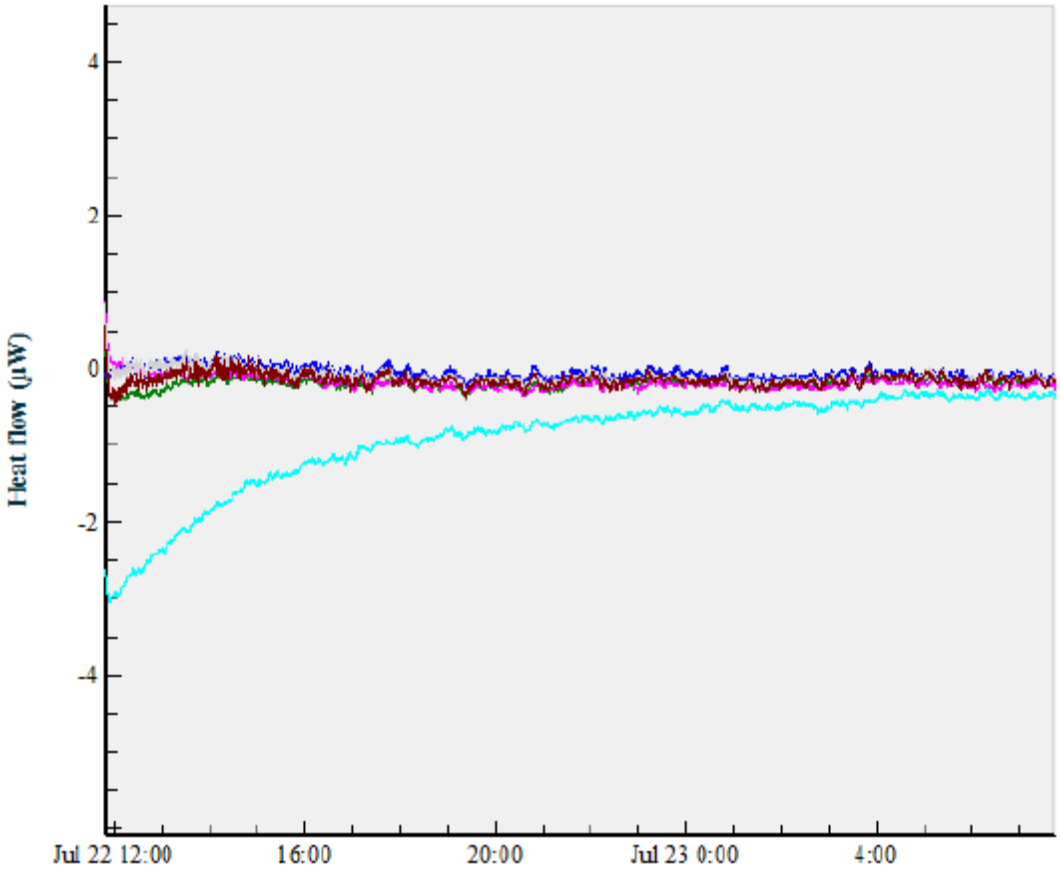

Figure B.11 Graph depicting the heat flow data of T5 (maroon) at 25 °C, as well as the individual components from which it is constituted, i.e. inulin (blue), green *C. subternata* extract (green), sugar (magenta), citric acid (turquoise) and ascorbic acid (grey).

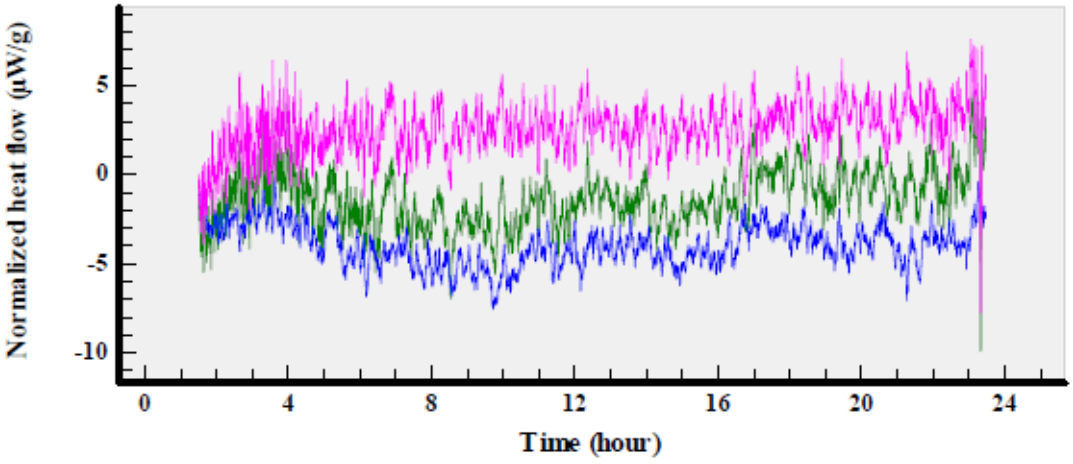

Figure B.12 Graph depicting the measured (green) and theoretical (blue) heat flow data for T5 consisting of a mixture of pure *C. subternata* extract spray-dried with inulin and mixed with sugar, citric acid and ascorbic acid, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 25 °C.

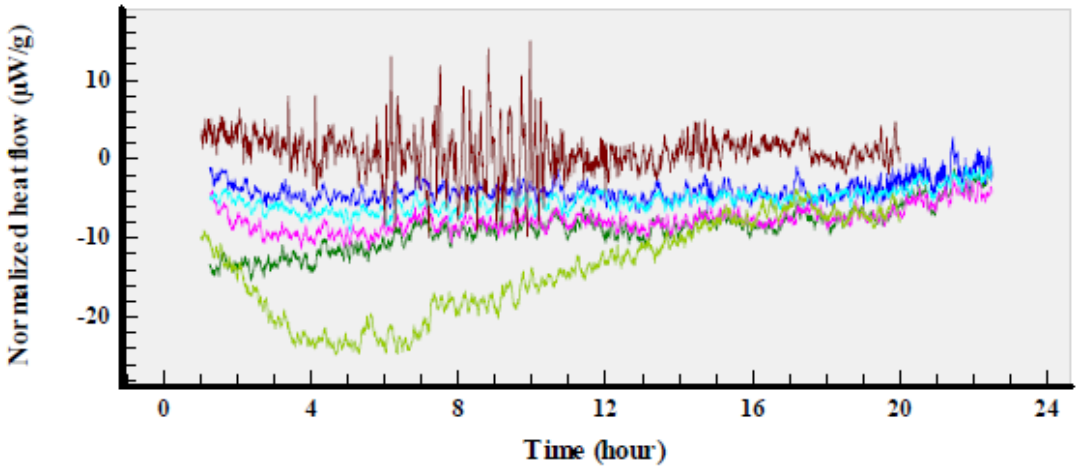

Figure B.13 Graph depicting the heat flow data of individual components which make up T6 at 25 °C, i.e. inulin (blue), green *C. subternata* extract (dark green), xylitol (magenta), stevia (turquoise), citric acid (light green) and ascorbic acid (maroon).

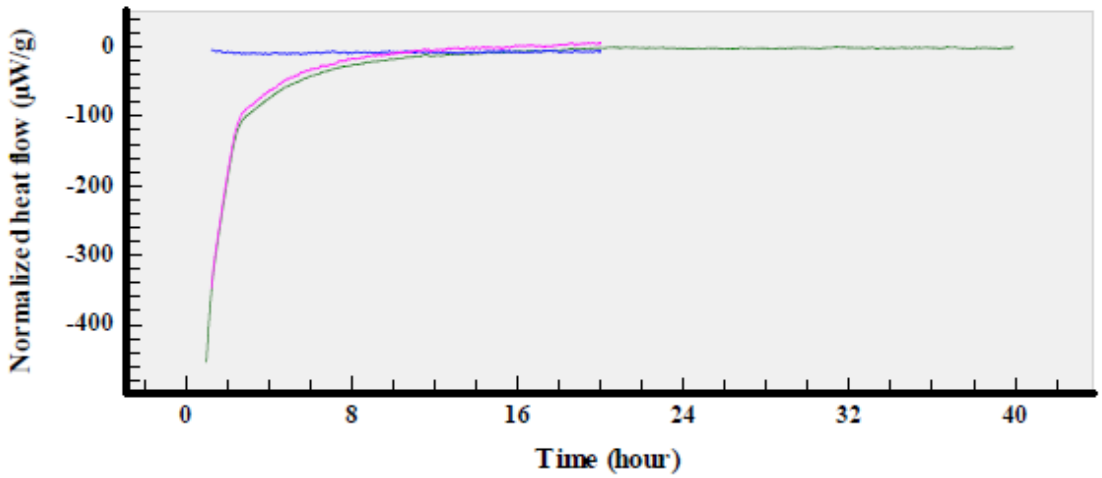

Figure B.14 Graph depicting the measured (green) and theoretical (blue)heat flow data for T6 consisting of a mixture of pure *C. subternata* extract spray-dried with inulin and mixed with xylitol, stevia, citric acid and ascorbic acid, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 25 °C.

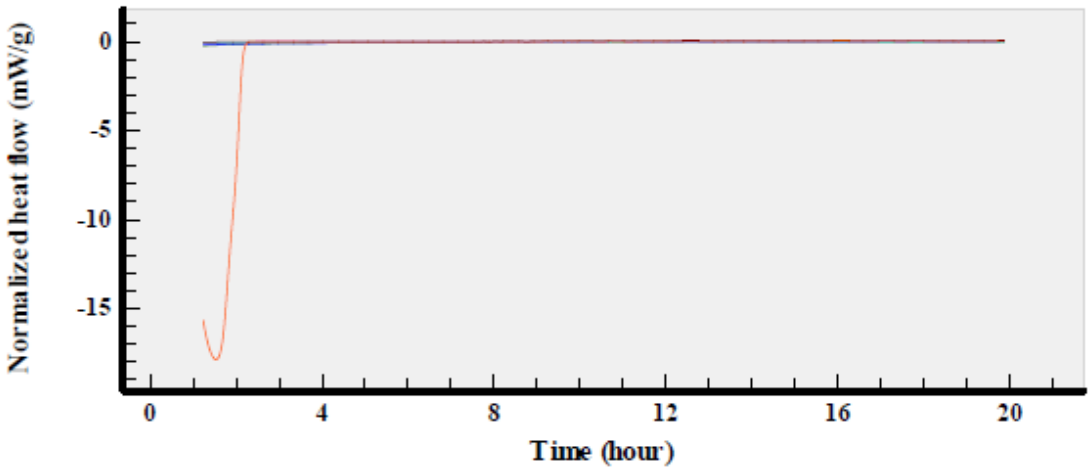

Figure B.15 Graph depicting the heat flow data of individual components which make up T6, at 40 °C, i.e. inulin (blue), green *C. subternata* extract (green), xylitol (magenta), stevia (turquoise), citric acid (orange) and ascorbic acid (maroon).

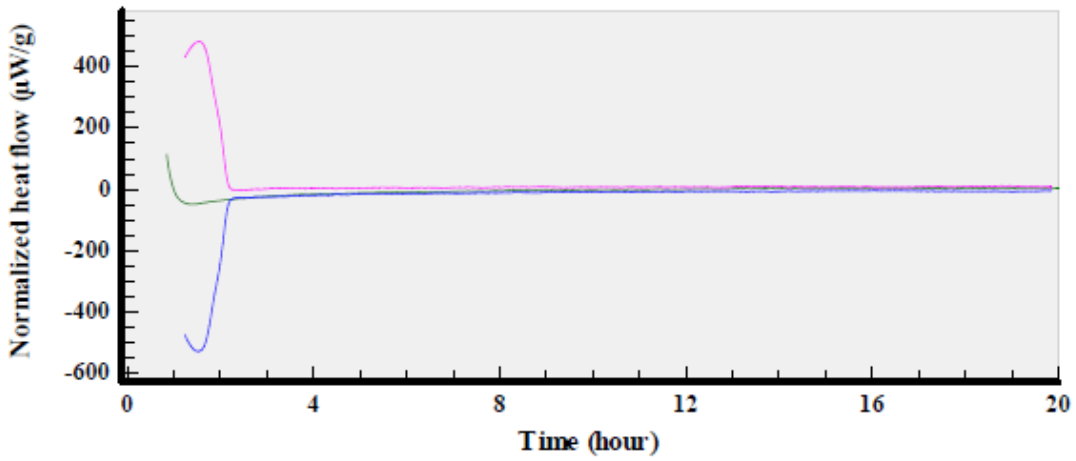

Figure B.16 Graph depicting the measured (green) and theoretical (blue) heat flow data for T6 consisting of a mixture of pure *C. subternata* extract spray-dried with inulin and mixed with xylitol, stevia, citric acid and ascorbic acid, as well as the interaction (magenta) between the components (difference between theoretical and measured heat flow) at 40 °C.