Dietary bioactive compounds and possible health benefits of dragon fruit and star fruit

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Abstract

Novel plant-based products with a high content of phenolic compounds have been studied to assess their effect on health. This thesis has explored three different species of dragon fruit, *Hylocereus polyrhizus* (red flesh), *Hylocereus undatus* (white flesh), and *Hylocereus megalanthus* (yellow peel) as well as one species of star fruit (*Averrhoa carambola*) that may exert beneficial effects on health due to their bioactive potential.

A literature review was conducted followed by three experiments: 1) an *in vitro* study to analyse the total polyphenol content (TPC) and their antioxidant capacity in fresh, dehydrated, and frozen forms for all species, as well as their *in vitro* digestion. 2) A sensory evaluation and an *in vitro* digestion of red flesh dragon fruit and star fruit based beverages. 3) An *in vivo* trial in healthy individuals and those at risk of type two diabetes (T2D) to determine the effect of a beverage based on frozen red flesh dragon fruit on blood pressure, glycaemic and insulin response. The results highlighted the bioactive potential of red flesh dragon fruit and star fruit. Frozen for one-week red flesh dragon fruit (FRDF) showed higher TPC than other dragon fruit forms; star fruit showed the highest TPC for all fruits assessed. A correlation was found between TPC and antioxidant capacity. The highest release of polyphenols from all fruits and forms evaluated occurred during intestinal phase and there was a correlation between TPC and antioxidant capacity. Red flesh dragon fruit and star fruit were selected to prepare beverages based on fresh, frozen for one-week, and dehydrated forms; the FRDF based beverage was the more accepted and showed a high TPC during the intestinal phase of the *in vitro* digestion. The consumption of the FRDF based beverage during a 4-week period shown to reduce blood pressure and the insulin response-incremental area under the curve (IR-iAUC) in individuals at risk of T2D. To our knowledge, this is the first study to look at the bioaccessibility of dragon fruit and star fruit and the first to assess the bioactive potential of FRDF to be considered as a functional food.

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Conference Presentations

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List of Abbreviations

%	Percent
°C	Degree Celsius
ADH	Alcohol dehydrogenase
ALDH	Acetaldehyde dehydrogenase
ALP	Alkaline phosphatase
ALT	Alanine transferase
ANOVA	Analysis of variance
AST	Aspartate transaminase
BMI	Body mass index
BUN	Blood urea nitrogen
COPD	Chronic obstructive pulmonary disease
CRP	C reactive protein
dL	Decilitre
DNS	Dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EPOC	Effective practice and organisation of care
FBG	Fasting blood glucose
FF	Functional food
FPG	Fasting plasma glucose
FRAP	Ferric reducing antioxidant power
FRDF	Frozen red dragon fruit
FW	Fresh weight
g	Grams
GAE	Gallic acid equivalent
GR	Glycaemic response

HbA1c	Glycated haemoglobin
HCl	Hydrochloric acid
HDL	High-density lipoproteins
HDL-C	High-density lipoprotein-cholesterol
iAUC	Incremental area under the curve
IFRF	Insoluble fibre-rich fraction
IL-2	Interleukin 2
IL-23	Interleukin 23
IPAQ	International physical activity questionnaire
IR	Insulin response
Kg	Kilograms
L	Litre
LDL	Low-density lipoproteins
LDL-C	Low-density lipoprotein-cholesterol
M	Molar
m^2	Square metres
MDA	Malondialdehyde
mg	Milligram
mL	Millilitre
mmol	Millimol
mM	Millimolar
mm Hg	Millimetres of mercury
NHS	National Health System
nm	Nanometres
NO	Nitric oxide
OBU	Oxford Brookes University

OGTT	Oral glucose tolerance test
OxBCNH	Oxford Brookes Centre for Nutrition and Health
pH	Potential of hydrogen
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PrOOH	Protein hydroperoxide
PRODEP	*'Programa para el Desarrollo Profesional Docente*'
PROSPERO	International prospective register of systematic reviews database
RCTs	Randomised controlled trials
RFDF	Red flesh dragon fruit
RoB	Risk of bias
RoB2	Risk of bias 2
ROS	Reactive oxygen species
rpm	Revolutions per minute
RS	Reducing sugars
Scr(CREA)	Serum creatinine
SD	Standard deviation
SE	Standard error
SF	Star fruit
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TAC	Total antioxidant capacity
TAS	Total antioxidant status
TC	Total cholesterol

TG	Triglycerides
TNF-α	Tumour necrosis factor alpha
TPC	Total polyphenol content
TPTZ	Tripyridyl-s-triazine
v/v	Volume per volume
WD	Walking distance
WFDF	White flesh dragon fruit
WHO	World Health Organization
WWC	What Works Clearinghouse
x g	Times gravity
YPDF	Yellow peel dragon fruit
α	Alpha
β	Beta
μg	Microgram
μL	Microlitre
μM	Micromolar
μmol	Micromole
μU	Microunit

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Chapter 1: Literature review.

1.1 Introduction

The range of plant species that exist throughout the world provide a great opportunity to identify plants that could exert benefits to human health due to their chemical composition. Fruits, vegetables, and herbs have been widely recommended as part of a healthy diet, mostly due to their content of water, fibre, vitamins, minerals, and other phytochemicals (Park, 2021). In some countries, plants have been used as traditional medicines and alternative treatments; for example, the fruit of *Cicer arietinum* L. and the leaves of *Eucalyptus citirodora* have been used to help to control respiratory disorders, meanwhile the fruits known as noni and mangosteen have been reported to be used for the treatment of infectious diseases (Alamgeer *et al*., 2018; Benatrehina *et al*., 2018). The plant of *Dendrophthoe pentandra* L. has been used to treat high blood pressure and their leaves' extract has shown an anti-inflammatory and antioxidant effects (Hasan *et al*., 2018).

In order to understand the effects of different parts of plants as alternative medicines, the content of phytochemicals as well as their identification has been studied using diverse analytical methods (Infante *et al*., 2016; Xu, Zhang and Wang, 2016). The major bioactive compounds in plants are saccharides, organic acids, vitamins, minerals, and polyphenols; their quantity depends directly on the stage of maturity as well as on the species or variety (Zainudin *et al*., 2014; Denardin *et al*., 2015). The health effects are linked to the major phytochemicals found in plant products (Neri-Numa *et al*., 2018; Serrano, Ros and Nieto, 2018) for example, flowers and fruits, which are colourful and rich in vitamins and polyphenols, are used as antioxidants (chemical species capable of donating electrons to free radicals) and anti-inflammatory to reduce the oxidative stress linked to chronic non-communicable disease (Xiong *et al*., 2014; Santos-Sánchez *et al*.,

2019; Hayanga *et al*., 2016). Oxidative stress occurs when by-products from cellular metabolism known as reactive oxygen species (ROS), such as free radicals and reactive molecules, are produced in excess and the antioxidants in the body are not able to neutralize the excess (Betteridge, 2000). Oxidative stress has been related to diabetes and hypertension, two of the major non-communicable diseases in the world.

Type 2 diabetes (T2D), a non-communicable disease that is characterised by an increase in blood glucose level beyond normal levels due to insulin resistance, is a major public health concern. Worldwide, the number of people with T2D is increasing (World Health Organization, 2016). Prevalence of diabetes around the world rose from 422 million in 2014 (World Health Organization, 2023) to more than 520 million in 2021, from which 96 percent (%) suffered T2D (Ong *et al*., 2023). Tiredness, thirstiness, and the need to urinate more frequently are some of the mild symptoms of T2D; a high blood glucose level generates an increase in the production of ROS which can affect vision, blood vessels, and nerves in the long term, decreasing the life quality of those with T2D (World Health Organization, 2023; Caturano, *et al*., 2023, Pan American Health Organization, 2022). High blood pressure or hypertension is another non-communicable disease considered as a public health problem and a priority for the World Health Organization (WHO), who recommends having a healthy diet including high fruit and vegetable intake to control or prevent this condition (World Health Organization, 2015). According to the WHO (2023) more than 1.2 billion people suffer hypertension and less than 50 % are diagnosed. Blood pressure equal or higher than 140/90 millimetres of mercury (mm Hg) measured on two different days indicates hypertension. There are no symptoms unless the blood pressure is extremely high, when headaches, chest pain, or nausea can appear and cause heart disease if the hypertension is not controlled (World Health Organization, 2023_Hypertension). High blood pressure and oxidative stress

have been related since the last could cause endothelial damage and systemic inflammation (Griendling *et al*., 2021).

Many individuals with T2D or hypertension may use fruits as an alternative or complementary treatment. For example, in Central America, Algeria, Nigeria, India and Thailand, it has been documented that individuals with T2D report the use of fruits of native plants as an alternative treatment (Giovannini, Howes and Edwards, 2016; Telli, Esnault and Ould, 2016; Mohammed, Kumar and Rizvi, 2015; Chayarop *et al*., 2017). Hypertensive individuals have reported the use of fruits of traditional medicinal plants to treat the high blood pressure in Belize, Suriname, and different countries of Africa (Mphuthi and Husaini, 2022; Mans, Grant and Pinas, 2017; Lassale *et al*., 2022).

1.2 Traditional medicine

To date in several regions of the world such as America, Africa, and Asia, foods and beverages are consumed with the aim of reducing or controlling the symptoms of various non-communicable chronic disease (Giovannini, Howes and Edwards, 2016; Telli, Esnault and Ould, 2016; Mohammed, Kumar and Rizvi, 2015; Chayarop *et al*., 2017), using a practice known as alternative, complementary or traditional medicine (World Health Organization, 2000; Choudhury *et al*., 2018). Traditional medicine has been defined by the World Health Organization as '*the sum total of the knowledge, skill and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness*' (World Health Organization, 2019). Nowadays this form of medicine is practiced due to distinct reasons such as cultural, religious, or economic linked to the search of natural products (Mphuthi and Husaini, 2022; Kamyab *et al*., 2021). Natural products used as traditional medicine, contains oligosaccharides and polyphenolic compounds with a potential

positive effect on human health and have been suggested as ingredients to formulate functional foods (Eliaser *et al*., 2018; Chen, Wang and Liu, 2015; Chandrasekara and Shahidi, 2018). The concept of functional foods relates to edible products that, by regular consumption could enhance health or diminish the risk of a disease (Roberfroid, 2002; Ntrigiou *et al*., 2018).

Some researchers have explored the medicinal use of plants throughout the world and have found a wide diversity of species used to mitigate, control, or even prevent non-communicable chronic diseases; roots, stems, leaves, flowers, and fruits present different chemical compositions, so their contribution to traditional medicine is broad (Table 1.1). For example, the corm, an underground starchy stem (Diaz-Toribio and Putz, 2021), of '*camote de venado*' (*Psacalium paucicapitatum*) is traditionally used as an antidiabetic remedy in the South Region of Mexico and has been found an anti-inflammatory effect and hypoglycaemic activity probably due to the presence of fructooligosaccharides (De Rodríguez *et al*., 2017).

Stems and leaves of '*limoncillo*' (*Siparuna eggersii*) and '*teculen*' (*Otholobium mexicanum*) are used in Ecuador as antidiabetic plants and both exhibited a high antioxidant capacity and a high content of polyphenols (Armijos *et al*., 2018); methanolic extract of the latter has shown a moderate inhibition of alpha (α) amylase and a strong inhibition of α-glucosidase, hydrolytic enzymes related to the digestion of carbohydrates (Suárez *et al*., 2017).

Leaves of white mulberry (*Morus alba*) and leaves of holy basil (*Ocimum tenuiflorum*) are used in India to treat diabetes and has been reported that isopropanolic extracts inhibit pancreatic α-amylase (Sudha *et al*., 2011). The leaves of *Bidens cemua* and *Bidens frondosa*, two species from Türkiye used as traditional medicine, have been studied to determine their antidiabetic activity and their antioxidant capacity; the results

Table 1.1 Plants used as traditional medicine.

Reference / Plant	*Common Name*	*Parts of the Plant Used*	*Country*	*Health Effect*	*Compounds*
De Rodríguez et al., 2017					
Psacalium paucicapitatum	'*Camote de venado'*	Corm	Mexico	Ani-inflammatory, hypoglycaemic	Fructo-oligosaccharides
Armijos et al., 2018					
Siparuna eggersii	'*Limoncillo*'	Stems and leaves	Ecuador	Antidiabetic	Polyphenols
Otholobium mexicanum	'*Teculen*'	Stem and leaves	Ecuador	Antidiabetic	Polyphenols
Sudha et al., 2011					
Morus alba	White mulberry	Leaves	India	Antidiabetic	Polyphenols
Ocimum tenuiflorum	Holy basil	Leaves	India	Antidiabetic	Polyphenols
Icoz et al., 2017					
Bidens cemua	Nodding beggartick	Leaves	Türkiye	Hypoglycaemic	Phenolic compounds
Bidens frondosa	Devil's beggartick				
Seck et al., 2018					
Combretum micranthum	'*Kinkeliba*'	Leaves	Senegal	Anti-hypertensive	Flavonoids
Hibiscus sabdaridda	'*Bissap*'	Calyx	Senegal	Anti-hypertensive	Anthocyanins
Cui et al., 2018					
Malus halliana	Hall crabapple	Flowers	China	Antithrombotic	Flavonoids

Charehsaz et al., 2015					
Berberis crataegina	Barberry	Fruit	Türkiye	Cardiovascular protection	Polyphenols
Malta et al., 2013					
Pouteria guardneriana	*'Guapeva'*	Fruit	Brazil	Antiproliferative activity	
Byrsonoma verbascifolia	*'Murici'*	Fruit		on cancer cells	Polyphenols
Campomanesia cambessedeana	*'Gabiroba'*	Fruit			
Elfi-Susanti et al., 2012					
Hylocereus undatus	White dragon fruit	Fruit	Taiwan	Antidiabetic	Flavonoids
Saha, Guite and Das, 2018					
Averrhoa carambola	Star fruit	Fruit	India, China, Brazil	Hypotensive	Polyphenols

showed that flavonoids might be responsible for the hypoglycaemic effect (Icoz *et al*., 2017). In Senegal, the leaves of '*kinkeliba*' (*Combretum micranthum*) and the calyx of '*bissap*' (*Hibiscus sabdaridda*) are used as anti-hypertensive medication and has been documented that their positive effect reducing blood pressure may be because of the content of flavonoids and their anti-inflammatory effects in the former and the presence of anthocyanins with antioxidant and anti-inflammatory effects in the latter (Seck *et al*., 2018).

The flowers of hall crabapple (*Malus halliana*), a Chinese traditional medicine, have shown antithrombotic effects, possibly due to the flavonoid content (Cui *et al*., 2018).

The fruit of *Berberis crataegina*, is used in Türkiye to prevent cardiovascular disorders and has been determined their antioxidant capacity (Charehsaz *et al*., 2015). Three fruits from the region of '*Cerrado'* in Brazil, called '*guapeva*' (*Pouteria guardneriana*), '*murici*' (*Byrsonoma verbascifolia*) and '*gabiroba*' (*Campomanesia cambessedeana*), have been assessed to determine their content of flavonoids, their antioxidant capacity and their anti-proliferative activity on cancer cells; the results indicated a high antioxidant capacity and an inhibitory activity on the growth of the human liver cancer cells (Malta *et al*., 2013).

Other fruits that have been used in traditional medicine are dragon fruit (*Hylocereus* spp) and star fruit (*Averrhoa carambola*). Dragon fruit consumption by diabetic people in Taiwan and its use in Mexican traditional medicine has been reported (Elfi-Susanti *et al*., 2012; Gutiérrez *et al*., 2007); the medicinal use of star fruit in Brazil, China, India and Taiwan has been documented (Saha, Guite and Das, 2018). Both fruits have been linked to health benefits associated with antioxidant capacity, anti-inflammatory, and anti-diabetic activity, likely due to their content of bioactive compounds, such as oligosaccharides and polyphenols. The following sections will describe the findings in the literature regarding these tropical fruits that have been consumed in Europe as exotic

fruits during the recent years (Centre for the Promotion of Imports from Developing Countries, 2020).

1.3 Dragon fruit and star fruit

This section aims to highlight the chemical characteristics of dragon fruit (*Hylocereus* spp.) and star fruit (*Averrhoa carambola*), and to understand their potential health benefits. Both fruits have been studied due to their shape, colour, and flavour (Dembitsky *et al*., 2011). *In vitro* studies have highlighted the polyphenolic compounds as their most significant bioactive molecules (Elfi-Susanti *et al*., 2012; Saha, Guite and Das, 2018; Yan *et al*., 2013).

1.3.1 Dragon fruit and its antioxidant potential

Dragon fruit (*Hylocereus* spp.), also known as pitahaya, belongs to the *Cactaceae* family, is native to the American continent and has been introduced to other regions throughout the world (Ortiz-Hernandez and Carrillo-Salazar, 2012). There are three main species of dragon fruit (Figure 1.1): one with red-purple flesh, *Hylocereus polyrhizus*, that is called red dragon fruit; another known as white dragon fruit (*Hylocereus undatus)*, as it presents white flesh with pink peel, and finally *Hylocereus megalanthus*, that shows yellow peel and white flesh (Ibrahim *et al*., 2018; Temak *et al*., 2018).

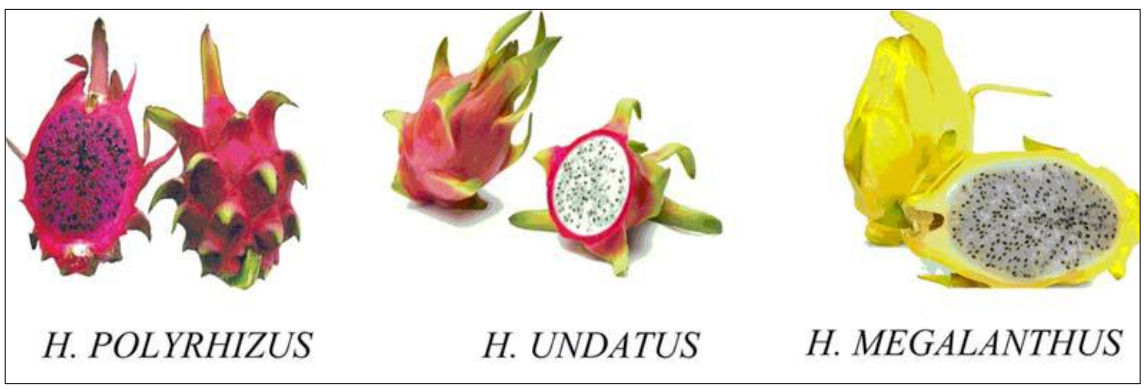


Figure 1.1 Species of *Hylocereus*. (Adapted from Ibrahim *et. al*., 2018, pp. 2). © 2018 Wiley Periodicals, Inc.

Some studies investigating the chemical composition of this exotic fruit report its proximal analysis as Table 1.2 shows. Because of the high content of moisture and the reduced shelf life exhibited by the pitahaya (Chaemsaint, Matan and Matan, 2018), studies have been performed to find the optimal conditions to preserve primarily, the compounds related to its colour. Betacyanins, the pigments in the red dragon fruit (*H. polyrhizus*) represent a natural source of colourants that is used in food industry and possesses antioxidant capacity (Rebecca, Boyce and Chandran, 2010; Stintzing, Schieber and Carle, 2002). The effect of the storage conditions on these pigments has been evaluated; the results proved that the content of betacyanins increased after six days when the fruit was kept at four degrees Celsius (°C) (Yong *et al*., 2018). The pigment obtained from red dragon fruit after pectinase treatment, was used to add colour to yoghurt; the addition of this pigment increases the free radical scavenging activity, probably because of the betacyanins presence (Gengatharan, Dykes and Choo, 2017). Some studies have focussed on the potential benefits of seeds from dragon fruit, either as a source of essential fatty acids because of the linoleic acid content or as a source of antioxidant compounds; findings showed the seeds from red and white dragon fruit (*H. polyrhizus* and *H. undatus,* respectively), contain approximately 49 % of essential fatty acids (Ariffin *et al*., 2009), meanwhile, catechin and quercetin were the major flavonoids found in the red dragon fruit seeds (Adnan, Osman and Abdul-Hamid, 2011). Some studies report that dragon fruit showed a high antioxidant capacity in addition to high polyphenol content (Ibrahim *et al*., 2018; Adnan, Osman and Abdul-Hamid, 2011). Some others suggest that dragon fruit could have hypoglycaemic effects and may be used to reduce the incidence of metabolic syndrome (Ramli *et al*., 2014). The total polyphenol content (TPC) has been quantified as well as the antioxidant capacity of dragon fruit species. The TPC of Malaysian red (*H. polyrhizus*) and white (*H. undatus*)

Table 1.2 Chemical composition of dragon fruit species (grams per 100 grams).

Dragon Fruit Species	*Moisture*	*Ash*	*Protein*	*Fat*	*Fibre*	*Reference*	*Country*
Hylocereus polyrhizus (Red flesh)							
	92.34	2.38	2.62	-	-	Alam *et al*., 2023	Bangladesh
	82.4-84.8	0.68-0.82	0.88-1.08	-	0.96-1.12	Arivalagan *et al*., 2021	India
Hylocereus undatus (White flesh)							
	93.91	2.40	2.52	-	-	Alam *et al*., 2023	Bangladesh
	84.3-84.8	0.79-0.83	0.93-1.11	-	0.80-0.82	Arivalagan *et al*., 2021	India
	84.15	0.57	0.12	0.72	4.30	Obregón-La Rosa *et al*., 2022	Peru
Hylocereus megalanthus (Yellow peel)							
	79.0	0.40	2.20	0.60	0.80	Lupuche *et al*., 2021	Peru
	84.4	0.56	0.22	0.41	1.20	Obregón-La Rosa *et al*., 2022	Peru

dragon fruit is reported to be 0.24 milligrams (mg) of Gallic acid equivalents (GAE) per gram (g), (mg GAE/g) and 0.28 mg GAE/g respectively (Choo and Yong, 2011). The red dragon fruit from Taiwan shows a TPC of its flesh's aqueous extract equal to 0.78 mg GAE/g (Tenore, Novellino and Basile, 2012). Red and white dragon fruit from Bangladesh showed a TPC of 1.48 mg GAE/g and 1.63 mg GAE/g respectively; meanwhile, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay reported an inhibition of the DPPH radical of 57.02 % for red and 89.75 % for white dragon fruit species (Alam *et al*., 2023). A study carried out in Korea reported 4.9 mg GAE/g in the flesh of red dragon fruit and 3.5 mg GAE/g in the flesh of white dragon fruit, regarding the antioxidant capacity, an inhibition of the DPPH radical was set at 33.25 % and 23.83 % respectively; the authors suggested that the TPC could be related to the antioxidant capacity found in both fruits as they reported a linear correlation coefficient between both parameters (Kim *et al*., 2011). A study investigating the characteristics of the yellow peel dragon fruit (*H. megalanthus*) in Peru, reported a TPC of 0.48 mg GAE/g and a 93.14 % of DPPH radical inhibition, both parameters were determined on a dry basis (Lupuche *et al*., 2021). Despite the TPC average for dragon fruit species is lower than the TPC found in products such as blackberries, 4.0 - 4.5 mg GAE/g (Liao *et al*., 2020) and spices such as turmeric, 45.2 - 76.8 mg GAE/g, the authors indicated the possibility of using dragon fruit as a functional food due to its content of bioactive compounds and its antioxidant potential.

In vivo studies have been undertaken to evaluate the effect of dragon fruit consumption in animal models. Wistar rats with induced metabolic syndrome were fed with corn starch in combination with the juice of red dragon fruit over eight weeks; results showed a positive effect on rats' health as the stiffness of the heart was reduced (Ramli *et al*., 2014). Furthermore, the juice of the white dragon fruit in mice fed with a high fat diet showed a positive effect as the levels of hepatic triglycerides and total cholesterol

decreased, and fasting glucose and insulin levels in serum were reduced significantly after fourteen weeks of consumption (Song *et al*., 2016).

Different edible products containing dragon fruit have been investigated using sensory tests to determine their acceptability. For example, a pasteurized red flesh dragon fruit juice obtained from a concentrate without seeds was preferred for sweetness and flavour when compared to juice sample and showed no difference for aroma and colour (Siow and Wong, 2017); the highest score for overall likeness for a bar from red dragon fruit flesh without seeds, was exhibited by the bar with 20 % of passion fruit juice and 1.65 % of pectin (Yen, *et al*., 2022). Based on a sensory test the best white dragon fruit leather was the one formulated using xanthan gum (Raj and Dash, 2022).

Clinical trials involving fresh and dried red dragon fruit have been performed. Target populations including those who are overweight/obese, and those with T2D were studied (Fadlilah *et al*., 2020; Wiardani, Moviana and Puryana, 2014); results showed positive effects on blood pressure and fasting blood glucose levels after treatment. Abd Hadi *et al*., (2012) found a significant reduction in blood glucose and triglyceride levels after four weeks consuming fresh red dragon fruit. Cheok *et al*., (2022) investigated the red dragon fruit powder and reported an improvement in the endothelial function after 14 days of consumption, meanwhile, Akhiruddin (2013) found that the plasma glucose levels were reduced by 22.9 % after four-week treatment. The juice obtained from the red dragon fruit also has been studied, Maharani and Saktiningsih (2022) found a reduction of 6.1 % in the levels of cholesterol after consuming it for 14 days and Girsang *et al*., (2020) reported a 1.6 millimol per litre (mmol/L) decrease in the blood glucose levels after 14 days.

1.3.2 Star fruit and its beneficial activity on health

Averrhoa carambola (Figure 1.2), also known as star fruit or carambola, is a small, juicy, and yellow tropical fruit native to Asia, which is usually consumed fresh (Muthu

et al., 2016; Dasgupta, Chakraborty and Bala, 2013; Hii and Ogugo, 2014). Its common name is due to the shape that presents when it is crosscut, similar to a five-peaked star (Ndukwe and Okhiku, 2018).

The chemical composition of star fruit is shown in Table 1.3 and its content of minerals is presented in Table 1.4. Star fruit is considered a rich source of bioactive compounds with therapeutic potential and the amount of minerals and phenolic compounds could be the basis for its use as a functional food (Xiaohui *et al*., 2014; Zainudin *et al*., 2014).

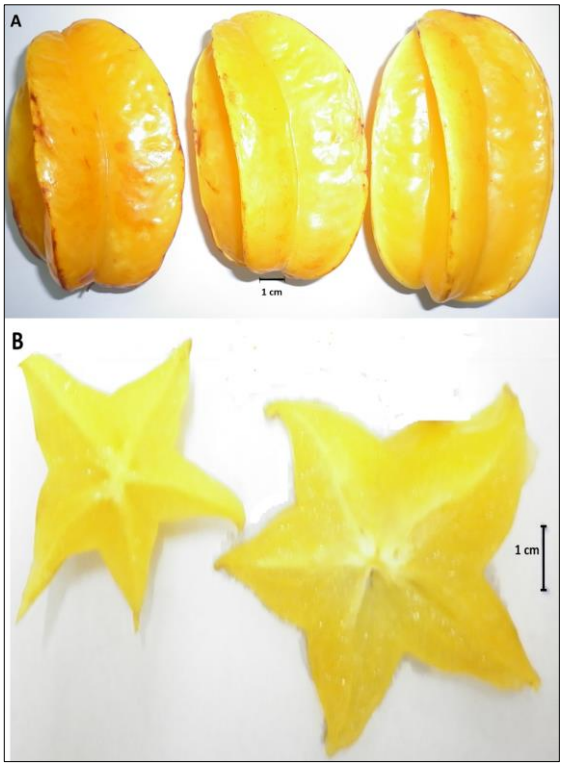


Figure 1.2 *Averrhoa carambola.* A: Ripen fruits, B: Crosscut (Taken from Muthu *et. al*., 2016, pp. 421).

Table 1.3 Chemical composition of star fruit (grams per 100 grams).

Star Fruit	*Moisture*	*Ash*	*Protein*	*Fat*	*Fibre*	*Reference*
Averrhoa carambola						
	92.0	0.4	0.7	0,1	1.8	Payal *et al*., 2012

Table 1.4 Mineral content in star fruit (milligrams per 100 grams).

Star Fruit	*Iron*	*Calcium*	*Potassium*	*Phosphorus*	*Reference*
Averrhoa carambola					
	0.40	4.52	125.00	19.00	Basena, Jamuna and Rafed, 2019

As a high content of water determines the stability of a product for a specific period, and star fruit exhibited a 92 % of moisture according to Payal *et al*., (2012), it has been studied to extend its shelf life. The use of edible coats based on alginate, chitosan and gum Arabic has been evaluated; after 12 days at 26 °C, a better visual appearance was observed in the star fruit coated with chitosan and gum Arabic, being the latter the treatment that retains the highest levels of polyphenols (Gol, Chaudhari and Rao, 2015). The content of phytochemicals in the star fruit is variable according to the ripening state (Benkeblia and Lopez, 2015). Because of the non-climacteric condition of star fruit, studies to establish the adequate harvest period to obtain the optimal amount of bioactive compounds have been performed (O'Hare, 1993; Zainudin *et al*., 2014). The content of polyphenols has been evaluated in commercial star fruit; the results stated that the higher quantity of phenolic compounds was shown at stage four of maturity with a concentration of 89.50 mg GAE/g (Noor-Asna and Noriham, 2014). A higher concentration of total polyphenols was found in ethanolic extracts (97.16 mg GAE/g) in comparison with aqueous extracts (77.00 mg GAE/g). It is suggested that star fruit could be consumed as a functional food, mostly due to its content of polyphenols (Khanam *et al*., 2015).

In vivo studies have investigated the effect of star fruit consumption in animal models such as rats, hamsters, and mice (Lakmal, *et al*., 2021). However, star fruit consumption should be done carefully as its content of oxalic acid and caramboxin has been reported to cause toxicity in those with renal disease (Wang *et al*., 2006; Stumpf *et al*., 2020).

As current evidence points to the polyphenols in dragon fruit and star fruit being of significance in their purported health benefits, an overview of the bioactive properties of these compounds and their link to health is necessary to provide further insight into the purported health benefits of dragon fruit and star fruit.

1.4 Polyphenols, sources, and health benefits

Secondary metabolites of plants are chemical compounds that allow the plants to survive under adverse environmental conditions (Ashraf *et al*., 2018). The structure of these molecules allows them to be grouped into alkaloids, terpenoids and phenolic compounds (Guerriero *et al*., 2018; Rodriguez-Mateos *et al*., 2014; Yang *et al*., 2018). The benefits that they provide to humans are well documented; terpenes and alkaloids are important to the pharmaceutical industry (Isah *et al*., 2018) meanwhile polyphenols are used by the food industry for flavouring or as colourants (Kallscheuer *et al*., 2019).

Polyphenolic compounds, also known as polyphenols, are the most abundant compounds found in fruits (Chiva-Blanch and Badimon, 2017, Vuong *et al*., 2014). Polyphenols present more than one hydroxyl group in one or more aromatic rings (benzene), and they are classified into phenolic acids, flavonoids, stilbenoids, and lignans (de Araújo *et al*., 2021; Šamec *et al*., 2021); their basic structures are presented in Figure 1.3. Phenolic acids present an aromatic ring with a carboxyl group, flavonoids and stilbenoids present two aromatic rings, however the former are linked by a pyranic ring and the latter by a methylene; lignans are structured by two phenylpropanoids joined between C8 and C8' (Šamec *et al*., 2021). Extraction of polyphenols has been linked to their hydrophilic nature given by their chemical structure, and organic solvents have been widely used to extract this type of compounds (Tsao, 2010). Different plant products such as prickly pears, blackberries, pomegranates, and some tropical fruits, are

good source of polyphenols as Table 1.5 shows (de Souza *et al*., 2015; Liao *et al*., 2020; Di Stefano *et al*., 2018; Jalal *et al*., 2015).

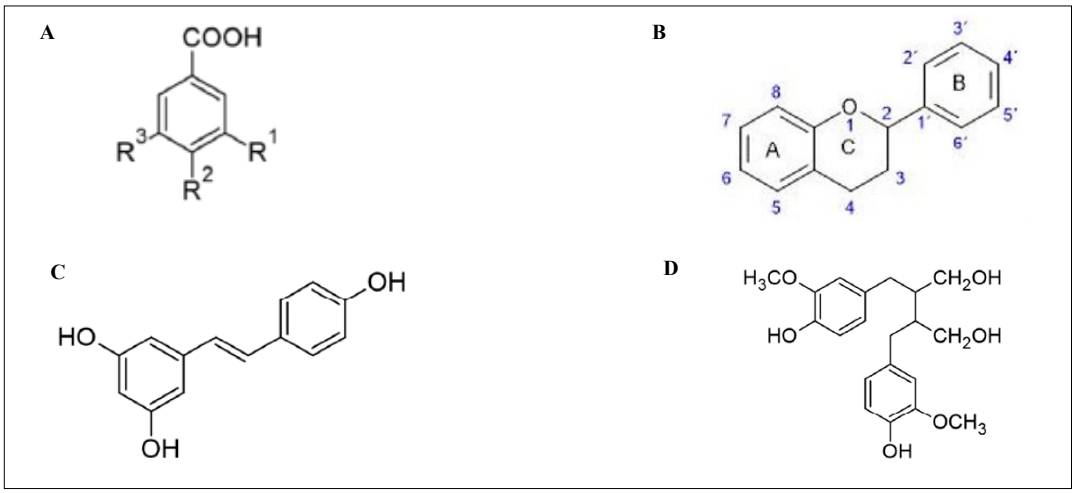


Figure 1.3 Polyphenol basic structures. A: Phenolic acids, B: Flavonoids, C: Stilbenoids, D: Lignans (Adapted from Šamec *et al*., 2021, pp. 2, 5, 13, 14).

Table 1.5 Total polyphenol content reported for different fruits.

Fruit	*Total Polyphenol Content*	*Reference*
Breadfruit (Artocarpus altilis)		
Methanol extract	781 mg GAE/g DB	Jalal *et al*., 2015
Blackberry (Rubus spp)		
Acetone-Water-Acetic acid extract	4.09 - 4.59 mg GAE/g	Liao *et al*., 2020
Pomegranate (Punica granatum)		
Juice	4.24 - 7.41 mg GAE/mL	Di Stefano *et al*., 2018
Prickly pear (Opuntia ficus indica)		
Water extract	0.12 mg GAE/g	De Souza *et al*., 2015
Red flesh dragon fruit (Hylocereus polyrhizus)		
Ethanol extract *Methanol extract*	0.24 - 4.90 mg GAE/g	Alam *et al*., 2023; Tenore, Novellino and Basile, 2012; Kim *et al*., 2011; Choo and Yong, 2011.
Star fruit (Averrhoa carambola)		
Water extract	89.50 mg GAE/g DB	Noor-Asna and Noriham, 2014
White flesh dragon fruit (Hylocereus undatus)		
Ethanol extract *Methanol extract*	0.28 - 3.5 mg GAE/g	Alam *et al*., 2023; Kim *et al*., 2011; Choo and Yong, 2011.
Yellow peel dragon fruit (Hylocereus megalanthus)		
Methanol extract	0.48 mg GAE/g DB	Lupuche *et al*., 2021

The total polyphenol content is expressed as milligrams of Gallic acid equivalent per gram or millilitre of sample (mg GAE/g, mg GAE/mL). DB: Dried basis

1.4.1 Health benefits

There is evidence to consider polyphenols as beneficial to prevent specific health conditions due to their biological activities such as antioxidant, anti-inflammatory, and antimicrobial capacity, among others (Chan *et al*., 2009).

Systematic reviews looking at the effects of polyphenols on health have been conducted in past years. Del Bo' *et al*., (2019) suggested that TPC and the source of these compounds be considered to determine the overall effects on human health. Amiot, Riva and Vinet (2016) systematically reviewed randomised controlled trials (RCTs) using polyphenol rich foods such as green tea, cocoa, soy, and cinnamon in individuals with metabolic syndrome and they found that the consumption of these products may improve the metabolic outcomes in people with metabolic syndrome such as lipid profile, blood pressure, and blood glucose. Trials on the relationships between polyphenols and effects on T2D have also been reviewed showing a relationship between overall polyphenol intake and reduced risk of T2D (Guasch-Ferré *et al*., 2017).

The concentration of compounds released from an edible product into the digestive fluids or bioaccessibility (Attri *et al*., 2017), has been linked to the effect of these compounds on health (Corona-Leo, Meza-Márquez and Hernández Martínez, 2021). Studies looking for the bioaccessibility of different compounds such as polyphenols and reducing sugars using *in vitro* gastrointestinal digestion have been carried out. Results showed that polyphenol bioaccessibility is linked to their chemical structure and stability (Nagar, Okun and Shpigelman, 2023; Ozkan, *et al*., 2023) and reducing sugars, monosaccharides which present a free ketone or aldehyde group and act as reducing agents (Jain, Goswami and Pandey, 2021), have been positively correlated to the TPC (Zeng *et al*., 2017; Khatri and Chhetri, 2020).

1.4.1.1 Polyphenols and glucose levels

A number of studies report the *in vitro* inhibition of key enzymes involved in the digestion of starch such as α-amylase and α-glucosidase (Li *et al*., 2018). For example, rich polyphenol blueberry and blackcurrant inhibited α-glucosidase, meanwhile, raspberry and strawberry showed an inhibition of α-amylase. (McDougall *et al*., 2005). Wild passion fruit in addition to purple potatoes contain phenolic compounds that are reported to inhibit α-amylase and α-glucosidase (Shanmugam, *et al*., 2018; Kalita *et al*., 2018). These reports revealed that it may be possible to use phenolic compounds as hypoglycaemic substances.

Animal studies have been conducted to determine the effect of different plant products such as juice, fruit skin, and extracts that contain polyphenols on blood glucose levels in laboratory species such as mice and rats. The results showed a lower concentration of glucose in blood after treatment. Grapefruit juice reduced blood glucose levels in Wistar rats probably due to its content of flavonoids (Hayanga *et al*., 2016). The consumption of peanut skin, rich in polyphenols such as catechins and procyanidins, has shown a decrease in the levels of plasma glucose in mice after four weeks of treatment (Toomer *et al*., 2019). An extract obtained from *Aguja nipponensis*, a species of bugle rich in flavonoids, was assessed in streptozotocin-induced diabetic mice and the results showed a decrease in the concentration of blood glucose (Hsieh *et al*., 2014).

1.4.1.2 Polyphenols and hypertension

Studies have indicated the positive effect of fruit polyphenols in the management of high blood pressure. *In vivo* studies have been designed to determine the relationship between phytochemicals in lab animals with induced cardiovascular disorders. For example, a mixture of berries, rich in TPC, was administered daily to rats fed a high salt diet and the results showed a normalised blood pressure after the treatment (Oudot *et al*., 2019). Mice with increased blood pressure levels induced by a high fructose-fat diet,

received polyphenolic extracts from apple peel and the results showed a cardio protective effect, probably due to the high content of TPC and total flavonoids found (Tian *et al*., 2018). Polyphenol-rich grapes and cacao have shown vascular protective effects, which have been linked to their content of flavonoids (Oak *et al*., 2018).

Other studies have been carried out to evaluate the effect of phenolic compounds on outcomes linked with high blood pressure in humans. Olive oil with different concentrations of phenolic compounds, 161.0 milligrams per kilogram (mg/Kg) (virgin oi1) and 4.7 mg/Kg (refined oil) was studied in a placebo-controlled crossover trial; the results showed that the olive oil with a higher content of polyphenols diminish oxidized low-density lipoproteins (LDL) and systolic blood pressure in participants with hypertension (Fitó *et al*., 2005). An intervention study that involved the consumption of 50 g/day of dark chocolate for a period of four weeks by individuals with hypertension with a body mass index (BMI) higher than 25 kilograms per square metres (Kg/m^2), reported a significant increase in endothelial function at the end of study (de Paula Nogueira *et al*., 2012).

The evidence discussed in this review of the literature clearly links the purported health benefits of dragon fruit and star fruit to their polyphenol constituents. However, to stablish more fully the significance of this relationship and the potential of dragon fruit and star fruit to confer benefits to health and thus be used as a functional food, an evaluation of the potential health effects of dragon fruit and star fruit both *in vitro* and *in vivo*, is required.

1.5 Aim of investigation

The aim of this investigation overall is to assess the effect of the polyphenol rich dragon fruit and star fruit on metabolic health outcomes.

1.5.1 Objectives

- To analyse *in vitro* dragon fruit and star fruit to establish the content of bioactive compounds with possible effects on human metabolic health.
- To establish *in vitro* bioaccessibility of the polyphenols in dragon fruit and star fruit based products and determine their sensory acceptance in human participants, to select the product with the best outcomes.
- To determine the effects of the selected fruit-based product on glycaemic response, blood pressure, total antioxidant status (TAS), cholesterol, triglycerides, C reactive protein (CRP) and fasting plasma glucose (FPG) levels in human participants during a randomised controlled trial.

1.6 Thesis outline

After the literature review, conducted to state the background of the use and importance of both fruits, the first study was carried out to determine the content of polyphenols and the antioxidant capacity of three species of dragon fruit, *Hylocereus polyrhizus* (red flesh), *Hylocereus undatus* (white flesh), and *Hylocereus megalanthus* (yellow peel) as well as one species of star fruit (*Averrhoa carambola*). Fresh, dehydrated, and frozen forms were analysed for all species (Chapter 2). A second experiment, based on the results from the first study, evaluated the *in vitro* bioaccessibility of bioactive compounds and the sensory acceptance of red flesh dragon fruit and star fruit based products (Chapter 3). A systematic review was conducted on the effect of star fruit consumption on health due to its content of oxalic acid and caramboxin found in the literature (Chapter 4) and a randomised controlled trial was carried out to determine the

effect of the frozen red flesh dragon fruit based product in healthy individuals and those at risk of T2D (Chapter 5).

1.6.1 Novelty

These studies provided relevant information on the effect of processing and storage conditions on the content of bioactive compounds in dragon fruit and star fruit. The antioxidant characterization and bioaccessibility as well as sensory analysis of fresh, frozen, and dried dragon fruit and star fruit based products have not been done previously. To our knowledge, there are no studies showing the effects of processed frozen dragon fruit on blood pressure, glycaemic response, and biomarkers such as TAS, cholesterol, triglycerides, and CRP.

Chapter 2: Bioactive potential of dragon fruit and star fruit, an *in vitro* study.

2.1 Introduction

Dragon fruit (*Hylocereus* spp) and star fruit (*Averrhoa carambola*) are tropical fruits, with a high content of bioactive compounds which have been used in certain countries previously as traditional alternatives to control T2D and hypertension (Ibrahim e*t al*., 2018; Yan *et al*., 2013). Some studies suggest that phenolic compounds found in both fruits could be responsible for the beneficial effects on human health (Adnan, Osman and Abdul-Hamid, 2011; Choo and Yong, 2011; Khanam *et al*., 2015). Polyphenols are secondary metabolites produced by plants in opposition to adverse environmental conditions and are associated with the antioxidant activity that may contribute to protecting against the development of non-communicable chronic diseases (Pandey and Rizvi, 2009; Tian *et al.*, 2020). Antioxidants are defined as chemical species capable of donating electrons to free radicals and decreasing oxidative stress (Santos-Sánchez *et al*., 2019). Polyphenols show properties related to glucose metabolism such as starch digestibility, since flavonoids have demonstrated to inhibit α-amylase and α-glucoamylase, key enzymes on the starch breakdown; furthermore, polyphenols have been linked to glucose uptake, due to their role in the AMP-activated protein kinase pathway (Shahwan *et al*., 2022). However, the structural form or matrix of the fruit could affect the polyphenols bioactivity (Fitri *et al*., 2016); the National Agricultural Library of the United States Department of Agriculture defined the food matrix as '*the nutrient and non-nutrient components of foods and their molecular relationship to each other*'. Preservation methods, such as drying and freezing are needed to maintain the quality of produce. Some studies have been conducted to establish the effect of preservation methods in the content of bioactive compounds and their bioaccessibility in

these types of products (Olivas-Aguirre *et al*., 2017; Da Costa and Mercadante, 2018). Drying is a preservation method that reduces the content of water and the possibility of microbial damage, furthermore it changes the structure of the food, increases the chemical concentration and could modify the sensory attributes (Serna-Cock, Vargas-Muñoz and Aponte, 2015; Olivas-Aguirre *et al*., 2017); according to Olivas-Aguirre *et al*., (2017) the concentration of bioactive compounds from freeze drying mango and papaya was higher when compared to the fresh form of the fruits. Freezing preservation method, based in the effect of low temperatures in the water content, is used to slow down the rate of chemical changes and the microbial growth in plant products in order to maintain food quality (Neri *et al*., 2020); this is one of the most popular method for preserving fruits in countries such as Brazil (Da Costa and Mercadante, 2018) and has been determined that the effect on bioactive compounds may vary according to the type of fruit and the freezing temperatures (Khattab *et al*., 2015).

The biological activity of chemical species in the human body is related to changes that may occur during digestion (Zi-Chao *et al.,* 2019), as well as to the interaction between different chemical species, such as polyphenols and reducing sugars (Zeng *et al*., 2017). The bioaccessibility or amount of compounds released from an edible product into the digestive fluids (Attri *et al*., 2017) is crucial to understand in order to establish if the compound in question could be released into the bloodstream and contribute to a beneficial impact on human health. *In vitro* digestion is a laboratory method that simulates the conditions of *in vivo* digestion and has been previously used to evaluate the antioxidant capacity of diverse molecules after digestion (Lucas-González *et al*., 2018). So, it is necessary to elucidate the effect of digestion on the concentration of polyphenols and reducing sugars contained in dragon fruit and star fruit and on their potential bioactivity during the digestive process.

This study aimed to evaluate the bioactive potential of dried, fresh, and frozen forms of three species of dragon fruit and one species of star fruit by determining their polyphenol content and antioxidant capacity. In addition, the *in vitro* bioaccessibility of bio-compounds in three species of dragon fruit and one species of star fruit in dried, fresh, and frozen forms was determined.

2.2 Methods

All reagents were Sigma-Aldrich brand, unless specified in the procedure. One species of star fruit (SF), *Averrhoa carambola* and three different species of dragon fruit, *Hylocereus polyrhizus* [red flesh dragon fruit (RFDF)], *Hylocereus undatus* [white flesh dragon fruit (WFDF)], and *Hylocereus megalanthus* [yellow peel dragon fruit (YPDF)] were acquired from My Exotic Fruit, a United Kingdom online retailer.

Fresh, dehydrated, and frozen forms were analysed for all species. TPC and their antioxidant capacity were evaluated in aqueous, ethanolic, and acetone extracts. An *in vitro* digestion was carried out to determine the bioaccessibility of reducing sugars, polyphenols and antioxidant capacity during gastric and intestinal phases of digestion in all forms and species of fruits. Buccal phase was not included as it is recommended for rich-carbohydrates food (Wojtunik-Kulesza *et al*., 2020).

2.2.1 Determination of polyphenol content and antioxidant capacity of dragon fruit and star fruit

2.2.1.1 Sample preparation

RFDF, WFDF, and YPDF were washed with tap water, peeled, and chopped into small pieces (0.5 x 0.5 centimetres approximately). SF was washed with tap water, sliced to remove the seeds, and chopped in the same way as dragon fruits. One portion of each fruit was analysed freshly, meanwhile, a second one was frozen at -18 °C for four weeks, and a third one was dehydrated at 60 °C for 48 hours prior to analysis.

2.2.1.2 Extraction

Polyphenol extraction was carried out using three different solvents, 70 % acetone, 70 % ethanol, and water. Firstly, 200 mg of each sample was weighed in triplicate and put into glass amber bottles. Then, four millilitres (mL) of solvent was added and the lids were placed and closed. Bottles were put in an orbital shaker SK-300 (Lab Companion, Oxfordshire, United Kingdom) for two hours at 120 revolutions per minute (rpm) at room temperature (Figure 2.1). Finally, extracts were separated from solid samples by centrifugation for 15 minutes at 2500 times gravity (x g) in a centrifuge D-37520 (Heraus Instruments, Osterode, Germany) after being transferred into glass test tubes. Supernatants were stored at -20 ºC until analysis.

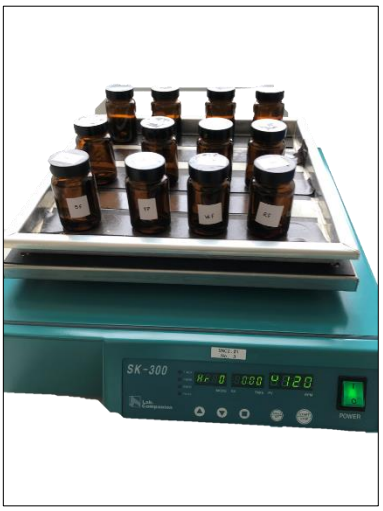


Figure 2.1 Polyphenol extraction in orbital shaker.

2.2.1.3 Determination of the total polyphenol content (TPC)

The TPC was determined by Folin-Ciocalteu's method to obtain results as micrograms (µg) GAE/g of sample (Agbor, Vinson and Donnelly, 2014). Firstly, a standard of gallic acid was made in each solvent used for the extraction, weighing 10 mg of gallic acid to make up to 10 mL with solvent. To start the analysis, 1.5 mL of diluted Folin-Ciocalteu reagent [1:10 volume per volume (v/v) with distilled water] was added to 200

microlitres (µL) of sample or standard in a glass test tube. After five minutes, 1.5 mL of sodium carbonate solution (60 g/L) was added. Then, incubation for 90 minutes at room temperature was carried out in the dark. Finally, the absorbance was read at 725 nanometres (nm) using the extraction solvent as the blank in a spectrophotometer UV-1800 (Shimadzu, Buckinghamshire, United Kingdom), Figure 2.2. Results for dehydrated samples were calculated based on fresh weight (FW) to be able to compare with fresh and frozen samples.

Figure 2.2 Spectrophotometer UV1800, Shimadzu.

2.2.1.4 Determination of the antioxidant capacity

To determine the antioxidant capacity, the ferric reducing antioxidant power (FRAP) and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity assays were carried out by spectrophotometric method. FW results for dehydrated samples were calculated.

The FRAP method determines the total antioxidant capacity in micromole per gram (µmol/g) of sample, using a standard of 1000 µmol ferrous sulphate (Benzie and Strain, 1996). Firstly, FRAP reagent was made using acetate buffer 300 millimolar (mM) potential of hydrogen (pH) 3.6, 10 mM tripyridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl) and 20 mM iron chloride in a ratio 10:1:1 v/v. Then, one

millilitre of distilled water was added into glass test tubes and incubated for five minutes at 37 ºC. After the incubation period, 25 µL of sample or standard was added and mixed in a vortex shaker Genius 3 (Ika, Oxfordshire, United Kingdom). Next, one millilitre of FRAP reagent was added, mixed in the vortex shaker, and incubated at 37 ºC for four minutes. Finally, absorbance was measured at 593 nm in a spectrophotometer UV-1800 (Shimadzu, Buckinghamshire, United Kingdom), using the extraction solvent as a blank.

DPPH radical scavenging activity assay involved the preparation of a 50 micromolar (µM) DPPH methanolic solution and a 1 mM ascorbic acid solution as standard. To start, 3.9 mL of DPPH solution was added to 0.1 mL of sample or solvent (used in the extraction) in a glass test tube. Then, an incubation period at 37 ºC was carried out for 30 minutes. Finally, using a spectrophotometer UV-1800 (Shimadzu, Buckinghamshire, United Kingdom), the absorbance was read at 517 nm. DPPH radical scavenging activity of the samples was calculated in percentage of the inhibition of the DPPH radical (Brand-Williams, Cuvelier and Berset, 1995).

2.2.2 *In vitro* digestion of dragon fruit and star fruit

Firstly, 2.5 g of each form (fresh, frozen, and dehydrated) of SF, RFDF, WFDF, and YPDF was weighed in triplicate, put into sample pots in the *in vitro* digestion unit with magnetic stirrers at 37 °C (Figure 2.3). Immediately, 30 mL of distilled water was added to each pot. 250 µL of sample was taken and added into test tubes with one millilitre of absolute ethanol to represent the baseline measurement for reducing sugars and a separate 1.5 mL of sample was taken and placed in glass test tubes in duplicate to represent the base line measurements for polyphenols and antioxidant capacity. Then the gastric phase was simulated by the addition of 0.1 mL of 10 % α-amylase, 0.8 mL of one molar (M) HCl and one mL of 10 % pepsin in 0.05 mL of HCl; after 30 minutes the gastric samples (in duplicate) were taken following a stablished method. Finally, to

simulate the intestinal phase, 2 mL of 1M sodium hydrocarbonate, 5 mL of 0.2 M malate buffer (pH 6), 5 mL of 10 % bile extract, 18 mL of water, one mL of 2 % pancreatin in 0.2 M malate buffer (pH 6), and 0.1 mL of amyloglucosidase were added; samples in duplicate were taken at 20, 60, and 120 minutes after starting the intestinal phase (Eriksen *et al*., 2017). Samples for the reducing sugars assay were stored overnight in the fridge at four ºC, samples for polyphenols and antioxidant capacity assays were placed in the freezer at -20 ºC until analysis. No blanks of the simulated digestion were assayed.

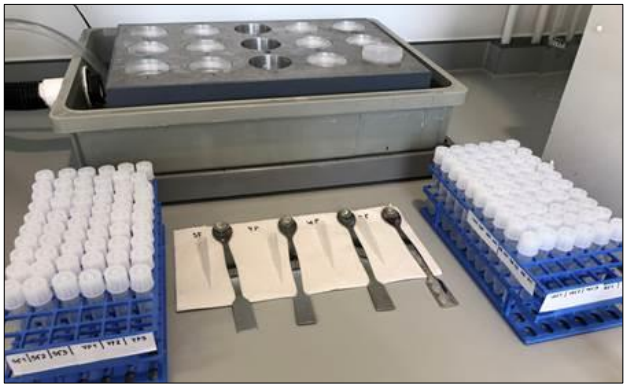


Figure 2.3 *In vitro* digestion unit and assay set up.

2.2.2.1 Determination of reducing sugars (RS)

Dinitrosalicylic acid (DNS) sugar analysis was carried out to measure the amount of reducing sugars (RS) released during the *in vitro* digestion phases, using distilled water as the blank and glucose (10 mg/mL) as the standard. Firstly, samples in ethanol were centrifuged for two minutes at 1000 x g. Then 50 µL of supernatant, blank or standard were placed into glass test tubes, and 250 µL of one percent amyloglucosidase in 0.1 M acetate buffer was added and mixed on the vortex shaker. After that, samples were incubated at room temperature for 10 minutes. Later, 750 µL of fresh DNS solution (0.5 mg/mL glucose, 4M sodium hydroxide, and DNS reagent in a ratio 1:1:5 v/v) were added and mixed on the vortex shaker Genius 3 (Ika, Oxfordshire, United Kingdom).

Next, supernatant, blanks and standards were incubated for 15 minutes in a water bath at 95 ºC. After incubation, sample tubes were placed in a cold-water bath for 15 seconds before adding four mL of distilled water. Finally, the absorbance was read at 530 nm using a spectrophotometer UV-1800 (Shimadzu, Buckinghamshire, United Kingdom). RS released were calculated in milligrams per gram of sample (mg/g).

2.2.2.2 Determination of the total polyphenol content (TPC)

The TPC was determined by Folin-Ciocalteu's method as mentioned in 2.2.1.3 to obtain results as μg GAE/g of sample. The results for dehydrated samples were calculated based on FW to compare with fresh and frozen samples.

2.2.2.3 Determination of the antioxidant capacity

To determine the antioxidant capacity during *in vitro* digestion phases, the FRAP and DPPH scavenging activity assays were carried out as described in 2.2.1.4 section. The results for dehydrated samples were calculated based on FW for the reasons given in section 2.2.2.2.

2.2.3 Statistical analysis

IBM Statistical Package for the Social Sciences (SPSS), version 28, was used to determine the normality of the data by the Shapiro-Wilk test. A One-Way Analysis of Variance (ANOVA) analysis and Tukey's post hoc test was performed when the data showed a normal distribution, and a Kruskal-Wallis non-Parametric analysis was used for the data that was not normally distributed. A Pearson's correlation analysis was carried out. Results for polyphenol content and antioxidant capacity are shown with a statistical significance set at $p < 0.05$, as mean of nine separate samples ± standard deviation (SD). Results for *in vitro* digestion are shown as mean of six separate samples ± SD with a statistical significance set at $p < 0.05$.

2.3 Results

2.3.1 Determination of polyphenol content and antioxidant capacity of dragon fruit and star fruit

2.3.1.1 Determination of the total polyphenol content (TPC)

The TPC in extracts obtained using acetone, ethanol, and water is shown in Table 2.1. A Kruskal-Wallis test showed that there was a statistically significant difference in the amount of polyphenols in acetone $\chi^2(23) = 188.545$, $p < 0.001$, ethanol $\chi^2(23) = 206.666$, $p < 0.001$, and water $\chi^2(23) = 198.294$, $p < 0.001$ between the different forms of dragon fruit and star fruit evaluated.

Regarding TPC in acetone extracts, there was no significant difference between fresh and frozen YPDF forms; meanwhile, there was found a significant difference between these forms and dehydrated form, which exhibited the highest TPC for YPDF, 773.40±60.16 μg GAE/g FW. In contrast, for WFDF, the fresh form showed the greatest TPC (570.12±29.07 μg GAE/g FW) and a significant difference when compared to frozen and dehydrated forms, between them no difference was found. Frozen for one-week RFDF showed the highest TPC (747.65±161.66 μg GAE/g FW) for this dragon fruit species, however there was no difference when compared to fresh and dehydrated. SF dehydrated form exhibited the highest TPC (3423.51±322.75 μg GAE/g FW) for all fruits and forms regarding acetone extracts, nevertheless, no difference between SF dehydrated, fresh and one-week frozen forms was found.

TPC in ethanol extracts exhibited similar results for YPDF as the dehydrated form showed the greatest content for this species of dragon fruit (492.09±37.60 μg GAE/g FW) and a significant difference between this form was found when compared to fresh and frozen for one, two, and four weeks. There was no significant difference between fresh and frozen WFDF, however, there is a difference between these forms and the dehydrated, which showed the highest TPC for WFDF (376.77±35.73 μg GAE/g FW).

Table 2.1 Total polyphenol content (μg GAE/g FW) extracted using different solvents from fresh, frozen, and dehydrated forms of three species of dragon fruit and one species of star fruit.

Fruit / Form	*70 % Acetone* ‡	*70 % Ethanol* ‡	*Water* ‡	*p-value*
Yellow Peel Dragon Fruit (Hylocereus megalanthus)				
Fresh ‡	485.36±21.42 ^{aC}	215.70±15.15 ^{aB}	115.15±28.43 ^{aA}	< 0.001
Frozen for one week †	471.27±15.35 ^{aB}	74.16±20.00 ^{aA}	447.36±37.22 ^{bB}	< 0.001
Frozen for two weeks ‡	416.67±102.63 ^{aB}	202.87±19.95 ^{aB}	66.63±10.23 ^{aA}	< 0.001
Frozen for three weeks ‡	499.39±36.21 ^{aB}	345.03±27.77 ^{bA}	328.76±63.94 ^{bA}	< 0.001
Frozen for four weeks ‡	487.73±62.88 ^{aB}	179.41±25.48 ^{aA}	183.40±53.81 ^{aA}	< 0.001
Dehydrated (FW) ‡	773.40±60.16 ^{cdC}	492.09±37.60 ^{bcB}	207.70±36.60 ^{abA}	< 0.001
White Flesh Dragon Fruit (Hylocereus undatus)				
Fresh †	570.12±29.07 ^{bC}	259.67±10.76 ^{aB}	146.37±32.96 ^{aA}	< 0.001
Frozen for one week ‡	365.84±32.10 ^{aB}	50.08±15.17 ^{aA}	456.33±21.52 ^{bC}	< 0.001
Frozen for two weeks ‡	225.62±26.44 ^{aB}	58.26±10.26 ^{aA}	275.14±14.63 ^{bB}	< 0.001
Frozen for three weeks ‡	236.81±27.11 ^{aB}	256.03±7.29 ^{aB}	172.31±30.48 ^{aA}	< 0.001
Frozen for four weeks ‡	264.44±27.54 ^{aC}	128.41±24.74 ^{aA}	125.19±22.45 ^{aA}	< 0.001
Dehydrated (FW) †	409.64±36.96 ^{aB}	376.77±35.73 ^{bB}	159.93±19.48 ^{aA}	< 0.001
Red Flesh Dragon Fruit (Hylocereus polyrhizus)				
Fresh ‡	704.11±67.98 ^{cC}	414.67±31.67 ^{bB}	152.93±32.71 ^{aA}	< 0.001
Frozen for one week ‡	747.65±161.66 ^{cB}	133.14±36.20 ^{aA}	861.57±41.01 ^{bcdB}	< 0.001
Frozen for two weeks ‡	513.50±37.68 ^{aB}	293.79±18.45 ^{bA}	329.94±75.67 ^{bA}	< 0.001
Frozen for three weeks †	487.61±21.83 ^{aC}	451.40±19.27 ^{bB}	323.45±15.46 ^{bA}	< 0.001
Frozen for four weeks ‡	569.65±43.66 ^{bB}	265.15±35.12 ^{aA}	173.75±35.27 ^{aA}	< 0.001
Dehydrated (FW) †	648.09±107.61 ^{cB}	594.85±42.56 ^{bcdeB}	490.61±21.00 ^{bA}	< 0.001
Star Fruit (Averrhoa carambola)				
Fresh †	1043.61±133.39 ^{deC}	394.99±69.37 ^{bB}	126.75±10.84 ^{aA}	< 0.001
Frozen for one week ‡	1301.77±262.69 ^{eB}	547.55±60.74 ^{bcdA}	560.73±45.27 ^{bcdA}	< 0.001
Frozen for two weeks ‡	412.13±64.43 ^{aB}	105.91±5.94 ^{aA}	124.54±24.47 ^{aA}	< 0.001
Frozen for three weeks ‡	428.97±272.68 ^{aB}	244.42±28.25 ^{aB}	116.98±7.07 ^{aA}	< 0.001
Frozen for four weeks ‡	357.62±30.76 ^{aB}	251.49±81.12 ^{aB}	91.44±74.48 ^{aA}	< 0.001
Dehydrated (FW) ‡	3423.51±322.75 ^{eC}	781.01±74.48 ^{bcdeA}	530.92±73.29 ^{bcA}	< 0.001
p-value	< 0.001	< 0.001	< 0.001	

The total polyphenol content is expressed as micrograms of Gallic acid equivalent per gram of sample based on fresh weight (μg GAE/g FW). FW: Fresh weight. †Normal distribution, One Way ANOVA and Tukey´s post hoc test. ‡Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=9) ± standard deviation. Different lower-case letter in the same column represents a significant difference between mean values ($p < 0.05$). Different capital letter in the same row represents a significant difference between mean values ($p < 0.05$).

The greatest TPC for RFDF was found in the dehydrated form (594.85±42.56 μg GAE/g FW), nevertheless, there was no significant difference between dehydrated, fresh, and frozen for two and three weeks. Similar to acetone extracts, dehydrated SF showed the highest TPC (781.01±74.48 μg GAE/g FW) for all fruits and forms in ethanol extracts, however, no difference between SF dehydrated, fresh and one-week frozen forms was found.

Water extracts obtained from one-week frozen form exhibited the greatest TPC for YPDF (447.36±37.22 μg GAE/g FW), WFDF (456.33±21.52 μg GAE/g FW), RFDF (861.57±41.01 μg GAE/g FW), and SF (560.73±45.27 μg GAE/g FW). Despite one-week frozen YPDF showed the greatest TPC for YPDF forms, there was no difference when compared to the frozen for three weeks and dehydrated forms. TPC exhibited by frozen for one-week and frozen for two weeks WFDF were not significantly different, however, there was a significant difference between both forms and fresh, dehydrated, and frozen for three and four-week forms. There was no significant difference between RFDF water extracts obtained from fresh and four-week frozen forms, nevertheless, there was a significant difference between these forms when comparted to dehydrated, one-week, two-week, and three-week frozen forms. Frozen for one-week and dehydrated SF forms were not significantly different, however, there was a significant difference when compared both forms to fresh, two, three, and four-week frozen forms.

According to Table 2.1, there was a statistically significant difference in the TPC between solvents for dragon fruit and star fruit forms ($p < 0.001$). YPDF in all forms showed the highest TPC when extracted with 70% acetone. WFDF in fresh (570.12±29.07 μg GAE/g FW), frozen for four weeks (264.44±27.54 μg GAE/g FW), and dehydrated (409.64±36.96 μg GAE/g FW) forms exhibited the highest TPC also in 70% acetone; meanwhile, water extracted the highest TPC for WFDF frozen for one-week (456.33±21.52 μg GAE/g FW) and frozen for two weeks (275.14±14.63 μg

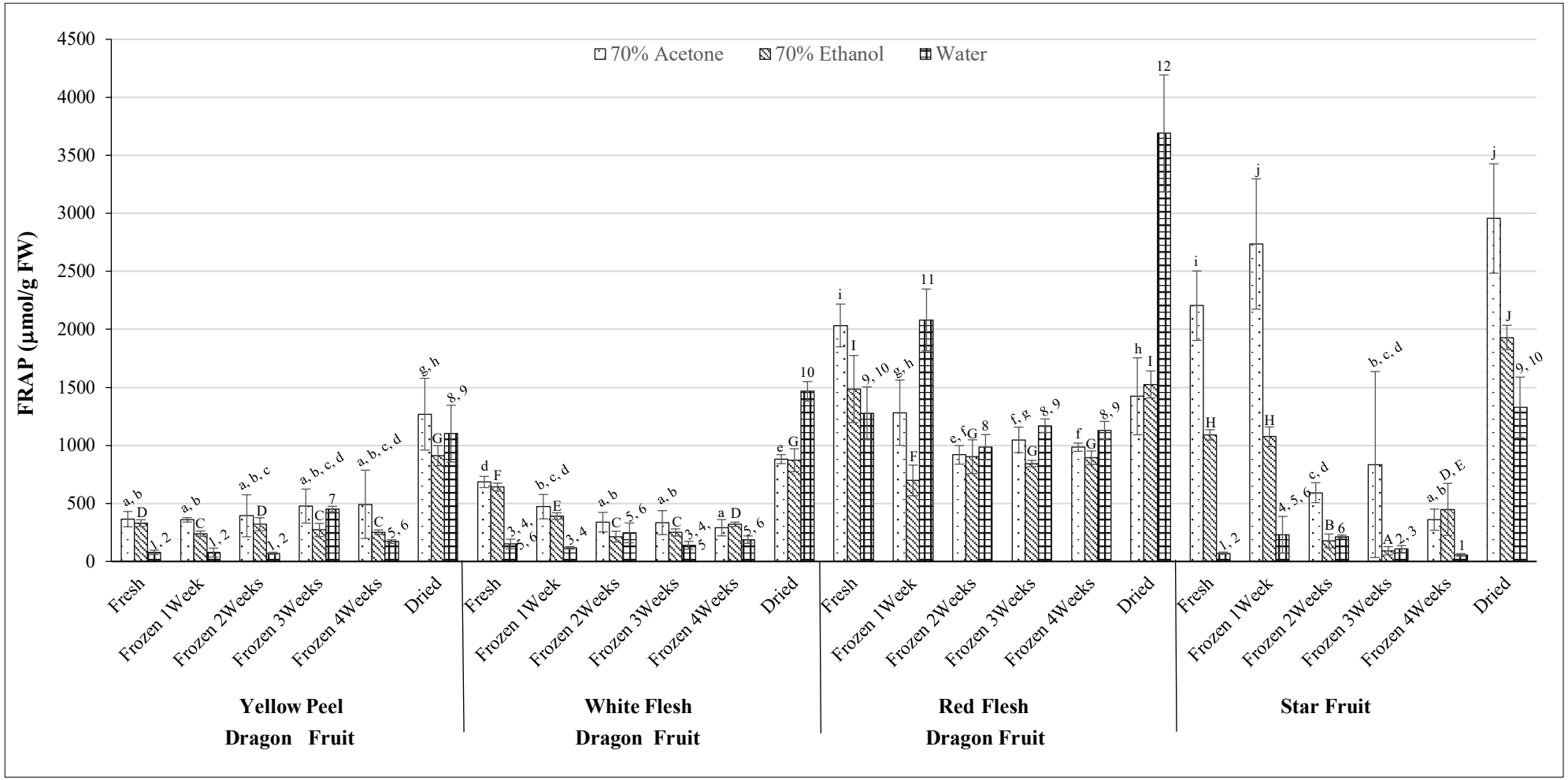
GAE/g FW). Most of the RFDF forms showed the highest TPC in 70% acetone extracts, but also in water (RFDF frozen for one-week, 861.57±41.01 μg GAE/g FW). All SF forms exhibited the highest TPC in the 70% acetone extracts when compared to ethanol and water, being the dehydrated form which showed the highest TPC, 3423.51±322.75 μg GAE/g FW.

Comparing forms for dragon fruit species, Table 2.1 shows that fresh RFDF (704.11±67.98 μg GAE/g FW) extracted with 70% acetone showed the highest TPC when compared to other fresh species; regarding frozen forms, RFDF frozen for one-week (861.57±41.01 μg GAE/g FW) water extract exhibited the highest TPC; finally, 70% acetone extract form dehydrated YPDF (773.40±60.16 μg GAE/g FW) showed the highest TPC when compared to dehydrated WFDF and dehydrated RFDF.

2.3.1.2 Determination of the antioxidant capacity

Figure 2.4 shows the average FRAP in the extracts ranged from 55 to 3689 μmol/g. A significant difference between fruit forms was established for acetone extracts $\chi^2(23) = 185.207$, $p < 0.001$, ethanol extracts $\chi^2(23) = 198.032$, $p < 0.001$, and water extracts $\chi^2(23) = 198.906$, $p < 0.001$.

FRAP in acetone extracts, showed no significant difference between fresh and frozen YPDF forms; meanwhile, there was found a significant difference between these forms and dehydrated form, which exhibited the highest FRAP for YPDF, 1269.37±309.20 μmol/g FW. For WFDF, there was a significant difference when compared fresh, frozen, and dehydrated forms; the highest FRAP was exhibited by dehydrated WFDF (881.66±38.82 μmol/g FW). Fresh RFDF showed the highest FRAP (2034.02±184.13 μmol/g FW) for this dragon fruit species, and there was a significant difference when compared it to fresh and dehydrated forms. Dehydrated SF exhibited the highest FRAP (2955±471.31 μmol/g FW) for all fruits and forms, followed by SF frozen for one-week (2736±561.83 μmol/g FW); there was a significant difference



FRAP: ferric reducing antioxidant power. µmol/g FW: micromole per gram of sample based on fresh weight. FW: fresh weight. Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=9), vertical bars represent standard deviation. Different lower-case letters represent a significant difference between acetone extracts mean values ($p < 0.05$). Different capital letters represent a significant difference between ethanol extracts mean values ($p < 0.05$). Different numbers represent a significant difference between water extracts mean values ($p < 0.05$).

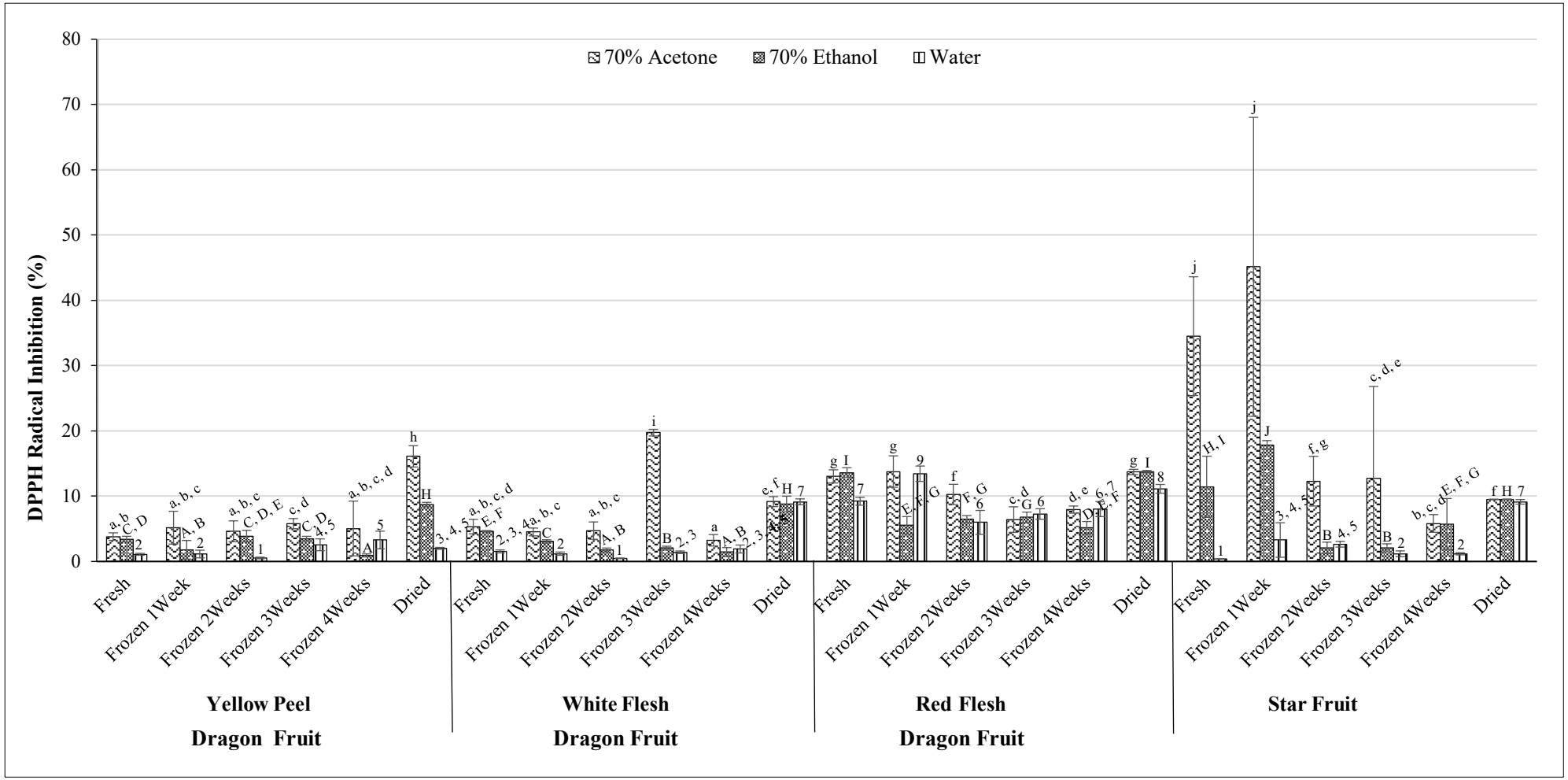
Figure 2.4 FRAP from extracts obtained from three species of dragon fruit and one species of star fruit, using acetone, ethanol, and water.

in FRAP when compared these forms to fresh and frozen for two, three, and four weeks. FRAP in ethanol extracts exhibited that dehydrated YPDF showed the greatest FRAP for this species of dragon fruit (911.50±86.25 μmol/g FW) and a significant difference when compared it to fresh and frozen forms. A significant difference was found between WFDF forms; frozen for four weeks WFDF showed the lowest FRAP (213.85±46.36 μmol/g FW), meanwhile the greatest was showed by the dehydrated form (872.48±98.88 μmol/g FW). The greatest FRAP for RFDF was found in the dehydrated form (1525.10±116.55 μmol/g FW); results showed a significant difference between dehydrated and frozen forms, nevertheless, no difference between dehydrated and fresh RFDF (1485.85±289.05 μmol/g FW) was found. Similar to acetone extracts, dehydrated SF showed the highest FRAP (1929.51±105.28 μmol/g FW) for all fruits and forms in ethanol extracts.

Water extracts obtained from dehydrated form exhibited the greatest FRAP for YPDF (1101.67±244.70 μmol/g FW), WFDF (1465.86±84.43 μmol/g FW), RFDF (3689.84±503.54 μmol/g FW), and SF (1327.04±261.54 μmol/g FW). The greatest FRAP for all fruits and forms was showed by dehydrated RFDF.

The inhibition of DPPH radical by the acetone, ethanol, and water extracts is presented in Figure 2.5. There was a statistically significant difference between fruits in acetone, ethanol, and water extracts, $\chi^2(23) = 174.285$, $p < 0.001$, $\chi^2(23) = 193.992$, $p < 0.001$, and $\chi^2(23) = 189.815$, $p < 0.001$, respectively.

Acetone extracts from YPDF showed that there was a statistical difference between fruit forms, being the dehydrated YPDF (16.10±1.63 %) the one with the highest DPPH radical inhibition. For WFDF a significant difference between frozen for three weeks (19.73±0.50 %), which showed the highest DPPH radical inhibition, and dehydrated, fresh, frozen for one, two, and four weeks was found. Regarding RFDF, there was no difference between fresh (13.03±1.00 %), dehydrated (13.70±0.40 %), and frozen for



DPP: 2,2-diphenyl-1-picrylhydrazyl. %: percentage. Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=9), vertical bars represent standard deviation. Different lower-case letters represent a significant difference between acetone extracts mean values ($p < 0.05$). Different capital letters represent a significant difference between ethanol extracts mean values ($p < 0.05$). Different numbers represent a significant difference between water extracts mean values ($p < 0.05$).

Figure 2.5 DPPH radical inhibition of three species of dragon fruit and one species of star fruit extracts obtained using acetone, ethanol, and water.

one-week (13.77±2.40 %) forms; however, there was a significant difference between these forms and frozen for two, three, and four weeks. There was no significant difference between SF extracts obtained from fresh (34.52±9.09 %) and frozen for one-week (45.14±22.90 %) SF; nevertheless, the latter showed the highest DPPH radical inhibition.

DPPH radical inhibition in ethanol extracts from dragon fruit species showed a significant difference between fruit forms, being the dehydrated, the one which exhibited the highest percentage of DPPH radical inhibition for YPDF (8.72±0.32 %), WFDF (8.81±0.76 %), and RFDF (13.76±0.17 %). Regarding SF, the extract obtained from SF frozen for one-week showed 17.85±0.65 % of DPPH radical inhibition, being the highest for all fruits and forms.

The water extracts which exhibited the greatest DPPH radical inhibition where those obtained from YPDF frozen for four weeks (3.29±1.35 %), dehydrated WFDF (9.14±0.44 %), RFDF frozen for one-week (13.44±1.17 %) and dehydrated SF (9.13±0.34 %).

Pearson's correlation to determine the relationship between TPC and antioxidant capacity (FRAP and DPPH radical inhibition) is shown in Table 2.2.

Results showed there was a significant strong, positive correlation between TPC and FRAP for acetone extracts $r(216) = 0.792$, $p < 0.001$ (two tailed) and ethanol extracts $r(216) = 0.892$, $p < 0.001$ (two tailed); furthermore, a moderate positive correlation was found for water extracts $r(2016) = 0.483$, $p < 0.001$ (two tailed). A strong and moderate correlation was showed between TPC and DPPH radical inhibition for ethanol $r(216) = 0.762$, $p < 0.001$ (two tailed) and water extracts $r(216) = 0.537$, $p < 0.001$ (two tailed), respectively.

Table 2.2 Correlation of total polyphenol content and antioxidant capacity of different extracts from three species of dragon fruit and one species of star fruit.

Extract / Solvent	*TPC Vs FRAP*		*TPC Vs DPPH*	
	r	***p***	***r***	***p***
70 % Acetone	0.792**	<0.001	0.288**	<0.001
70 % Ethanol	0.829**	<0.001	0.762**	<0.001
Water	0.483**	<0.001	0.537**	<0.001

TPC: Total polyphenol content. FRAP: Ferric reducing antioxidant power. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical inhibition. r: Pearson´s correlation coefficient. Asterisks (**): Statistically significant correlation, $p < 0.01$, two-tailed

2.3.2 *In vitro* digestion of dragon fruit and star fruit

2.3.2.1 Determination of reducing sugars (RS)

The amount of RS released from YPDF, WFDF, RFDF, and SF in all forms, during *in vitro* digestion is shown in Table 2.3. There was a significant difference between *in vitro* digestion phases for the RS released ($p < 0.001$).

The highest release of RS from fresh and frozen forms of dragon fruit occurred during the intestinal phase mainly at 20 minutes, nevertheless, fresh YPDF (202.18±53.24 mg/g FW) and RFDF frozen for two weeks (185.26±8.07 mg/g FW) showed the highest release at 120 minutes. Dehydrated YPDF and WFDF exhibited the greatest release of RS during gastric phase (32.65±5.16 mg/g FW and 80.96±6.38 mg/g FW, respectively); meanwhile, for dehydrated RFDF the intestinal phase at 60 minutes (66.30±19.92 mg/g FW) showed the highest release.

Fresh (115.40±13.99 mg/g FW) and dehydrated (36.86±2.08 mg/g FW) forms of SF showed the highest release of RS during the intestinal phase at 20 minutes. SF frozen for one-week 57.80±7.35 mg/g FW, frozen for two weeks 169.74±25.10 mg/g FW, and frozen for four weeks 199.82±11.13 mg/g FW, exhibited the highest release during the intestinal phase at 60 minutes.

Table 2.3 Reducing sugars (mg/g FW) released from fresh, frozen, and dehydrated dragon fruit species and star fruit during *in vitro* digestion.

Fruit / Form	*Base Line*	*Gastric Phase*	*Intestinal Phase (20 minutes)*	*Intestinal Phase (60 minutes)*	*Intestinal Phase (120 minutes)*	*p-value*
Yellow Peel Dragon Fruit (Hylocereus megalanthus)						
Fresh ‡	16.83 ± 3.60^{a}	71.15 ± 7.64^{ab}	142.40 ± 40.79^{b}	136.43 ± 35.86^{b}	202.18 ± 53.24^{bc}	<0.001
Frozen for one week †	1.79 ± 1.71^{a}	68.77 ± 14.92^{b}	95.53 ± 19.02^{b}	84.27 ± 23.00^{b}	84.90 ± 14.12^{b}	<0.001
Frozen for two weeks †	62.06 ± 0.72^{a}	89.97 ± 5.23^{b}	149.64 ± 7.41^{d}	87.48 ± 10.29^{b}	134.41 ± 12.51^{c}	<0.001
Frozen for four weeks †	97.31 ± 9.12^{a}	104.53 ± 14.82^{a}	220.31 ± 45.48^{c}	182.15 ± 33.11^{bc}	166.02 ± 19.26^{b}	<0.001
Dehydrated (FW) †	4.73 ± 1.92^{a}	32.65 ± 5.16^{b}	25.09 ± 6.99^{b}	23.48 ± 5.05^{b}	25.85 ± 6.48^{b}	<0.001
White Flesh Dragon Fruit (Hylocereus undatus)						
Fresh †	97.75 ± 11.14^{a}	140.61 ± 26.52^{b}	221.82 ± 45.34c	198.56 ± 9.82^{c}	145.58 ± 13.55^{b}	<0.001
Frozen for one week ‡	50.52 ± 10.^{63a}	135.76 ± 4.73^{ab}	175.63 ± 14.03^{b}	167.83 ± 7.09^{b}	140.96 ± 14.02^{ab}	<0.001
Frozen for two weeks †	97.05 ± 10.37^{a}	126.91 ± 4.98^{b}	189.66 ± 16.65^{c}	169.7 7± 19.69^{c}	185.16 ± 26.35^{c}	<0.001
Frozen for four weeks †	39.37 ± 13.08^{a}	98.49 ± 8.12^{b}	143.80 ± 17.49^{c}	100.29 ± 26.80^{b}	110.26 ± 4.09^{b}	<0.001
Dehydrated (FW) †	5.48 ± 2.37^{a}	80.96 ± 6.38^{c}	72.29 ± 6.07^{bc}	63.93 ± 7.40^{b}	64.50 ± 1.92^{b}	<0.001
Red Flesh Dragon Fruit (Hylocereus polyrhizus)						
Fresh †	57.89 ± 7.76^{a}	105.50 ± 16.61^{b}	172.72 ± 31.73^{d}	156.39 ± 28.47^{cd}	131.34 ± 24.36^{bc}	<0.001
Frozen for one week †	49.10 ± 26.57^{a}	111.86 ± 6.63^{b}	150.27 ± 21.14^{c}	143.38 ± 17.57^{c}	138.86 ± 13.63^{bc}	<0.001
Frozen for two weeks ‡	92.20 ± 4.34^{a}	130.85 ± 23.03^{ab}	164.03 ± 47.35^{bc}	147.47 ± 6.96^{abc}	185.26 ± 8.07^{bc}	<0.001
Frozen for four weeks ‡	149.25 ± 12.41^{a}	200.97 ± 12.99^{ab}	332.00 ± 30.47^{c}	270.06 ± 21.89^{bc}	228.01 ± 9.07^{ac}	<0.001
Dehydrated (FW) †	8.09 ± 1.18^{a}	51.39 ± 11.87^{b}	57.75 ± 13.58^{b}	66.30 ± 19.92^{b}	63.92 ± 9.37^{b}	<0.001

Star Fruit (Averrhoa carambola)						
Fresh †	36.25 ± 9.52^{a}	97.44 ± 10.07^{bc}	115.40 ± 13.99^{c}	81.17 ± 10.89^{b}	94.21 ± 23.35^{bc}	<0.001
Frozen for one week ‡	1.19 ± 1.44^{a}	37.09 ± 9.96^{ac}	49.38 ± 13.84b^{c}	57.80 ± 7.35^{bc}	40.33 ± 4.48^{ac}	<0.001
Frozen for two weeks †	53.35 ± 10.00^{a}	84.43 ± 2.87^{b}	114.59 ± 11.66^{c}	169.74 ± 25.10^{d}	122.07 ± 23.10^{c}	<0.001
Frozen for four weeks ‡	72.92 ± 13.60^{a}	111.47 ± 13.76^{ab}	178.49 ± 42.35^{b}	199.82 ± 11.13^{bc}	160.94 ± 21.00^{b}	<0.001
Dehydrated (FW) ‡	9.23 ± 4.27^{a}	32.24 ± 9.26^{b}	36.86 ± 2.08^{b}	30.84 ± 3.58^{ab}	29.97 ± 4.60^{ab}	0.001

Reducing sugars are expressed as milligrams per gram of sample based on fresh weight. FW: Fresh weight. †Normal distribution, One Way ANOVA and Tukey´s post hoc test. ‡Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=6) ± standard deviation. Different letters in the same row represent a significant difference between mean values ($p < 0.05$).

2.3.2.2 Determination of the total polyphenol content (TPC)

Table 2.4 shows the TPC released from different forms of YPDF, WFDF, RFDF, and SF during the *in vitro* digestion process. The results showed a significant difference in the TPC released between the phases of digestion ($p < 0.001$).

The highest release of polyphenols from all forms of dragon fruit occurred during the intestinal phase mainly at 20 minutes but also at 60 and 120 minutes. For YPDF frozen for one-week at 60 minutes (2161.36±230.77 μg GAE/g FW); for WFDF forms at 60 minutes, the frozen for two weeks (1436.18±169.04μg GAE/g FW) and the dehydrated (578.27±17.09μg GAE/g FW); for RFDF frozen for one-week, 120 minutes (1767.47±206.56 μg GAE/g FW), although this was only slightly higher than at 60 minutes (1762.88±169.43 μg GAE/g FW); for RFDF frozen by two weeks, 120 minutes (1512.86±209.02 μg GAE/g FW), and finally the dehydrated RFDF at 60 minutes (788.54±172.94 μg GAE/g FW) only slightly higher than at 120 minutes (786.70±82.02 μg GAE/g FW).

SF showed the highest release of polyphenols during the intestinal phase. Fresh (2571.33±175.19 μg GAE/g FW) and frozen for one-week (1884.61±170.58 μg GAE/g FW) at 60 minutes. Frozen for two weeks (2019.06±186.23 μg GAE/g FW), frozen for four weeks (2440.08±82.48 μg GAE/g FW), and dehydrated SF (795.25±22.18 μg GAE/g FW) showed the highest polyphenol release at 20 minutes.

Table 2.4 Total polyphenol content (μg GAE/g FW) released from fresh, frozen, and dehydrated dragon fruit species and star fruit during *in vitro* digestion.

Fruit / Form	*Base Line*	*Gastric Phase*	*Intestinal Phase (20 minutes)*	*Intestinal Phase (60 minutes)*	*Intestinal Phase (120 minutes)*	*p-value*
Yellow Peel Dragon Fruit (Hylocereus megalanthus)						
Fresh ‡	65.13 ± 3.49^{a}	800.90 ± 57.53^{ab}	2323.47 ± 91.26^{c}	2230.50 ± 121.12^{bc}	2101.68 ± 225.79^{bc}	<0.001
Frozen for one week ‡	17.13 ± 1.73^{a}	358.85 ± 149.69^{ab}	1909.44 ± 142.81^{bc}	2161.36 ± 230.77^{c}	1899.44 ± 157.10^{bc}	<0.001
Frozen for two weeks †	164.50 ± 4.57^{a}	628.22 ± 149.57^{b}	1954.90 ± 238.74^{c}	1904.96 ± 190.53^{c}	1879.75 ± 54.23^{c}	<0.001
Frozen for four weeks ‡	125.37 ± 18.76^{a}	537.18 ± 94.36^{ab}	2239.65 ± 303.23^{c}	2206.83 ± 429.93^{bc}	1676.35 ± 361.32^{ac}	<0.001
Dehydrated (FW) †	217.69 ± 39.32^{a}	600.43 ± 8.32^{b}	744.77 ± 39.90^{c}	695.34 ± 41.91^{c}	683.07 ± 47.88^{c}	<0.001
White Flesh Dragon Fruit (Hylocereus undatus)						
Fresh ‡	74.54 ± 14.91^{a}	800.79 ± 127.50^{ab}	2389.18 ± 599.67^{c}	2018.06 ± 109.73^{bc}	1930.95 ± 171.60^{bc}	<0.001
Frozen for one week ‡	2.81 ± 3.95^{a}	338.78 ± 53.53^{ab}	1623.42 ± 217.77^{b}	1560.36 ± 188.91^{b}	1484.21 ± 265.33^{b}	<0.001
Frozen for two weeks ‡	40.37 1.04^{a}	458.13 ± 94.90^{ab}	1402.20 ± 371.75^{bc}	1436.18 ± 169.04^{c}	1323.59 ± 124.69^{bc}	<0.001
Frozen for four weeks ‡	129.20 ± 13.61^{a}	697.50 ± 120.22^{ab}	2259.88 ± 505.16^{c}	2033.41 ± 75.97^{bc}	2166.61 ± 453.68^{bc}	<0.001
Dehydrated (FW) †	26.48 ± 13.22^{a}	441.09 ± 28.56^{c}	538.54 ± 64.60^{c}	578.27 ± 17.09^{c}	540.34 ± 36.33^{c}	<0.001
Red Flesh Dragon Fruit (Hylocereus polyrhizus)						
Fresh †	118.95 ± 5.83^{a}	932.85 ± 171.50^{b}	2846.75 ± 333.10^{d}	2448.27 ±175.79^{c}	2268.26 ± 221.36^{c}	<0.001
Frozen for one week ‡	2.15 ± 2.36^{a}	364.70 ± 105.99^{ab}	1551.63 ± 50.76^{ac}	1762.88 ± 169.43^{bc}	1767.47 ± 206.56^{c}	<0.001
Frozen for two weeks ‡	41.30 ± 0.69^{a}	358.74 ± 109.05^{ab}	1440.05 ± 201.27^{bc}	1460.14 ± 282.68^{bc}	1512.86 ± 209.02^{c}	<0.001
Frozen for four weeks †	177.50 ± 30.05^{a}	922.93 ± 99.29^{b}	2737.19 ± 126.88^{d}	2633.39 ± 96.61^{c}	2430.44 ± 245.18^{cd}	<0.001
Dehydrated (FW) †	34.43 ± 5.09^{a}	416.61 ± 171.34^{b}	653.28 ± 151.79^{c}	788.54 ± 172.94^{c}	786.70 ± 82.02^{c}	<0.001

Star Fruit (Averrhoa carambola)						
Fresh †	146.11 ± 9.53[a]	726.30 ± 88.74[b]	2489.82 ± 140.95[c]	2571.33 ± 175.19[c]	2438.33 ± 160.56[c]	<0.001
Frozen for one week †	32.85 ± 6.22[a]	379.01 ± 71.68[b]	1680.86 ± 273.57[c]	1884.61 ± 170.58[c]	1664.84 ± 210.61[c]	<0.001
Frozen for two weeks ‡	152.75 ± 1.04[a]	636.58 ± 54.03[ab]	2019.06 ± 186.23[c]	1798.77 ± 236.15[bc]	1678.07 ± 258.54[bc]	<0.001
Frozen for four weeks ‡	152.66 ± 21.59[a]	784.46 ± 93.55[ab]	2440.08 ± 82.48[c]	2425.07 ± 305.55[bc]	2134.85 ± 78.58[ac]	<0.001
Dehydrated (FW) †	428.38 ± 101.88[a]	621.97 ± 94.32[b]	795.25 ± 22.18[c]	748.38 ± 40.93[c]	756.18 ± 42.71[c]	<0.001

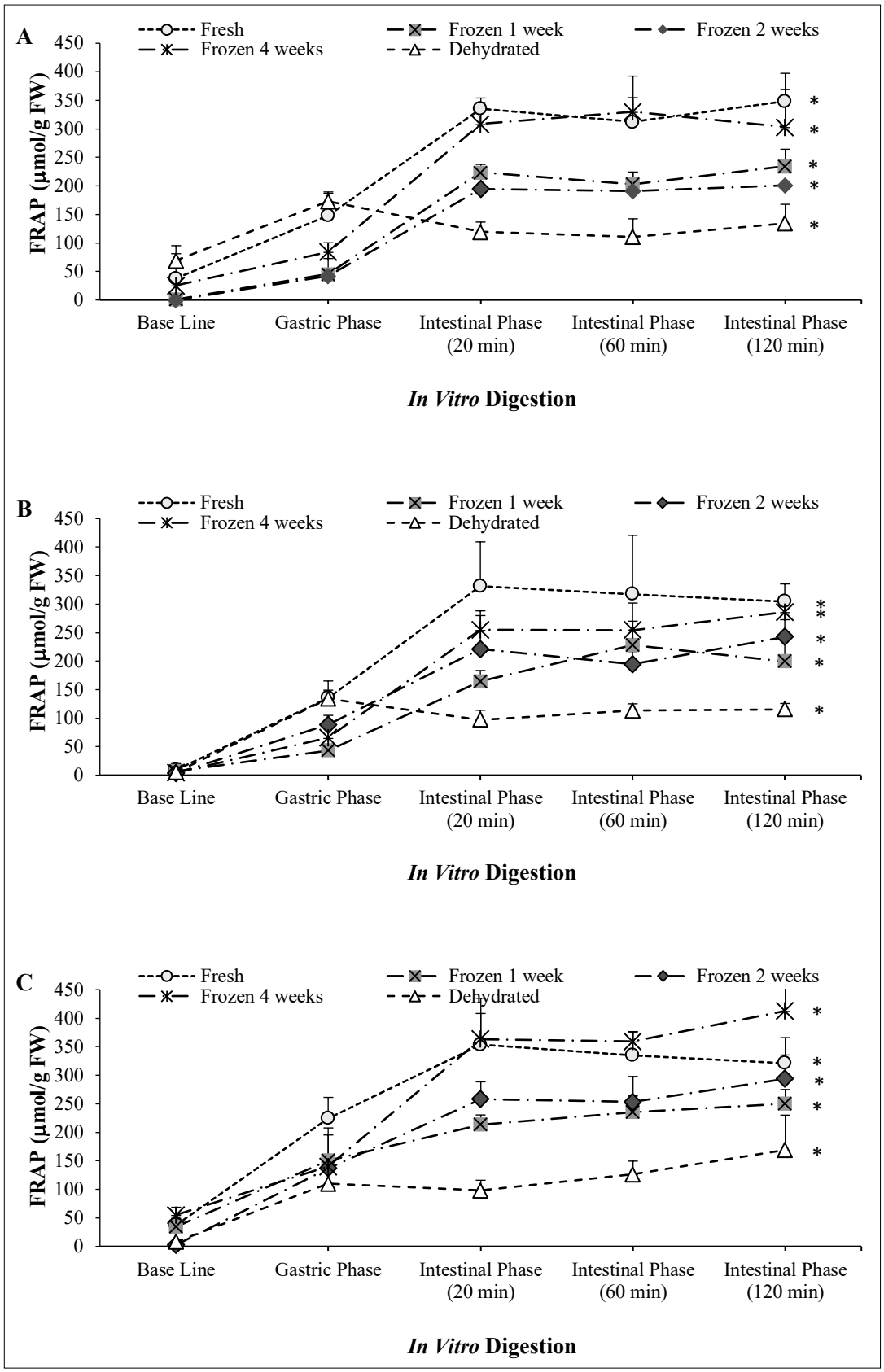
The total polyphenol content is expressed as micrograms of Gallic acid equivalent per gram of sample based on fresh weight. FW: Fresh weight. †Normal distribution, One Way ANOVA and Tukey´s post hoc test. ‡Not normal distribution, Kruskal Wallis and post hoc test. Values represented as mean (n=6) ± standard deviation. Data with different letters in the same row show significant difference ($p < 0.05$).

2.3.2.3 Determination of the antioxidant capacity

FRAP values during *in vitro* digestion for different forms of dragon fruit species and star fruit are presented in Figure 2.6 and 2.7, respectively. Significant difference was found between digestion phases ($p < 0.001$).

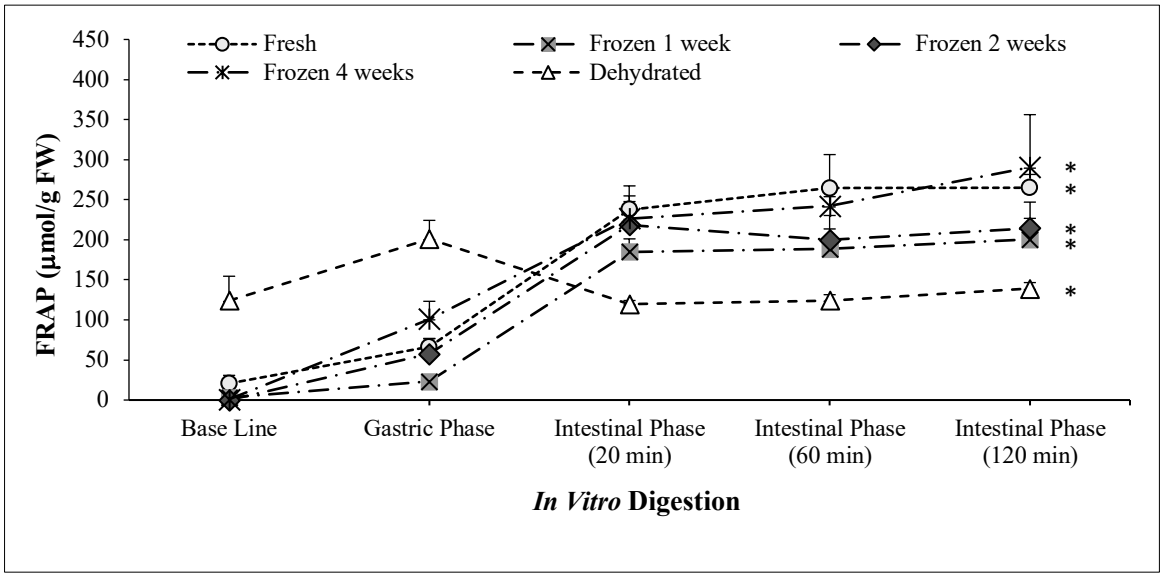
Fresh and frozen forms of dragon fruit exhibited the highest FRAP mainly at intestinal phase 120 minutes but also at 60 and 20 minutes. YPDF frozen for four weeks (329.88±62.51 μmol/g FW) and WFDF frozen for one-week (228.18±73.79 μmol/g FW) at 60 minutes; meanwhile, the highest FRAP for fresh WFDF and RFDF was found at 20 minutes, 331.62±77.58 μmol/g FW and 353.72±54.63 μmol/g FW, respectively. Dehydrated YPDF (172. 95±16.34 μmol/g FW) and dehydrated WFDF (134.06±15.13 μmol/g FW), showed the highest FRAP at gastric phase; meanwhile, dehydrated RFDF (169.01±61.13 μmol/g FW) at intestinal phase 120 minutes.

The highest FRAP for fresh, frozen for one-week, and frozen for four weeks SF occurred during intestinal phase at 120 minutes; intestinal phase at 20 minutes showed the highest FRAP for SF frozen for two weeks, 218.43±48.78 μmol/g FW. The dehydrated form of SF exhibited the highest FRAP during gastric phase, 200.96±23.28 μmol/g FW.



FRAP: ferric reducing antioxidant power. µmol/g FW: micromole per gram of sample based on fresh weight. FW: fresh weight. min: minutes. Values represented as mean (n=6); vertical bars represent standard deviation. Asterisk (*) indicates significant differences between phases $p < 0.05$.

Figure 2.6 Ferric Reducing Antioxidant Power (FRAP) from five forms of three species of dragon fruit during *in vitro* digestion. A: Yellow peel, B: White flesh, C: Red flesh.



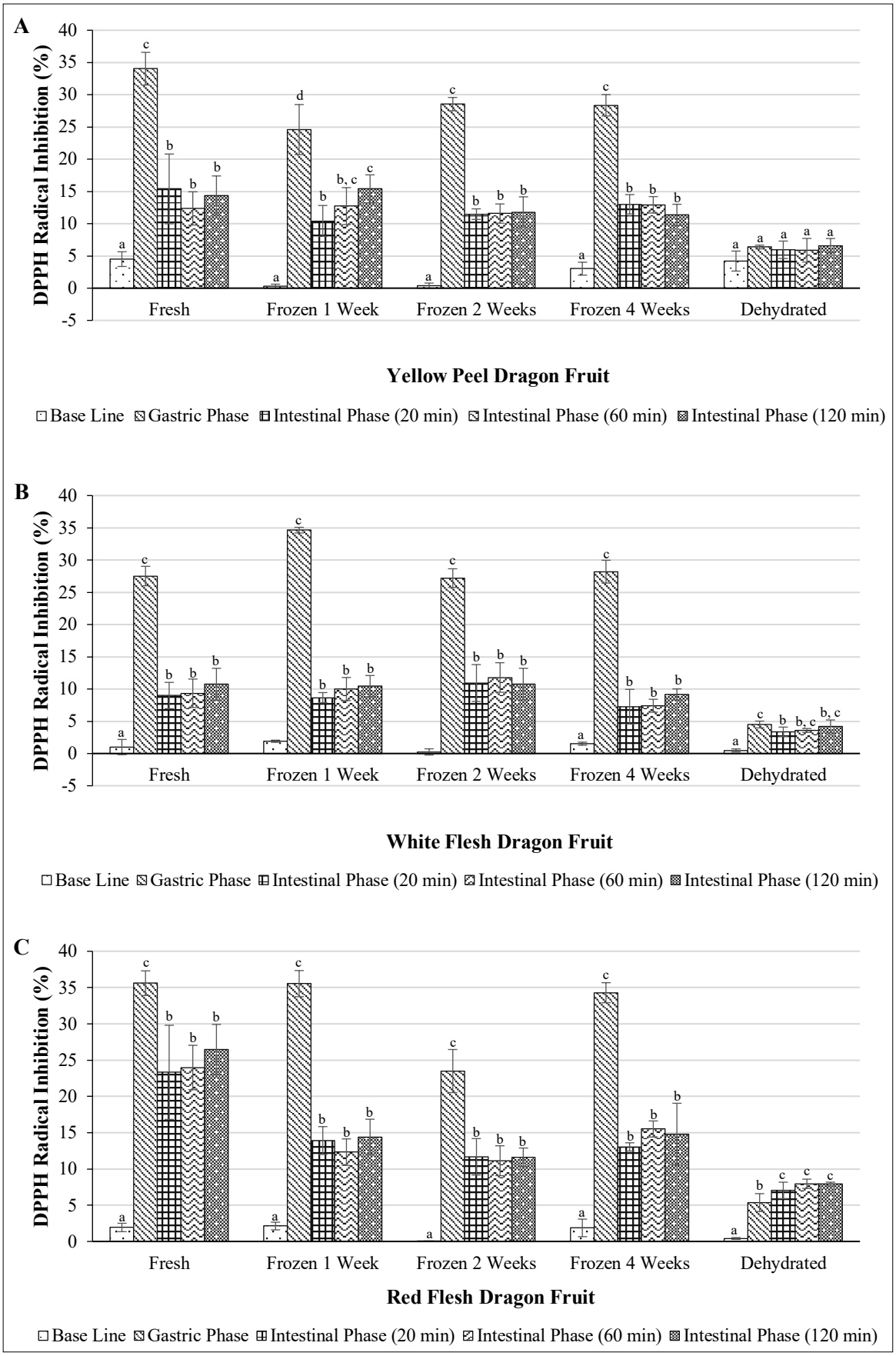
FRAP: ferric reducing antioxidant power. µmol/g: micromole per gram of sample based on fresh weight. FW: fresh weight. min: minutes. Values represented as mean (n=6); vertical bars represent standard deviation. Asterisk (*) indicates significant differences between phases $p < 0.05$.

Figure 2.7 Ferric Reducing Antioxidant Power (FRAP) from star fruit forms during *in vitro* digestion.

Figure 2.8 shows the inhibition of the DPPH radical by fresh, frozen, and dehydrated forms of YPDF, WFDF, and RFDF, meanwhile, Figure 2.9 shows the results obtained by SF forms at the phases of the *in vitro* digestion. There was a significant difference in the DPPH radical inhibition between digestion phases for each fruit evaluated ($p < 0.001$), except for the dehydrated YPDF.

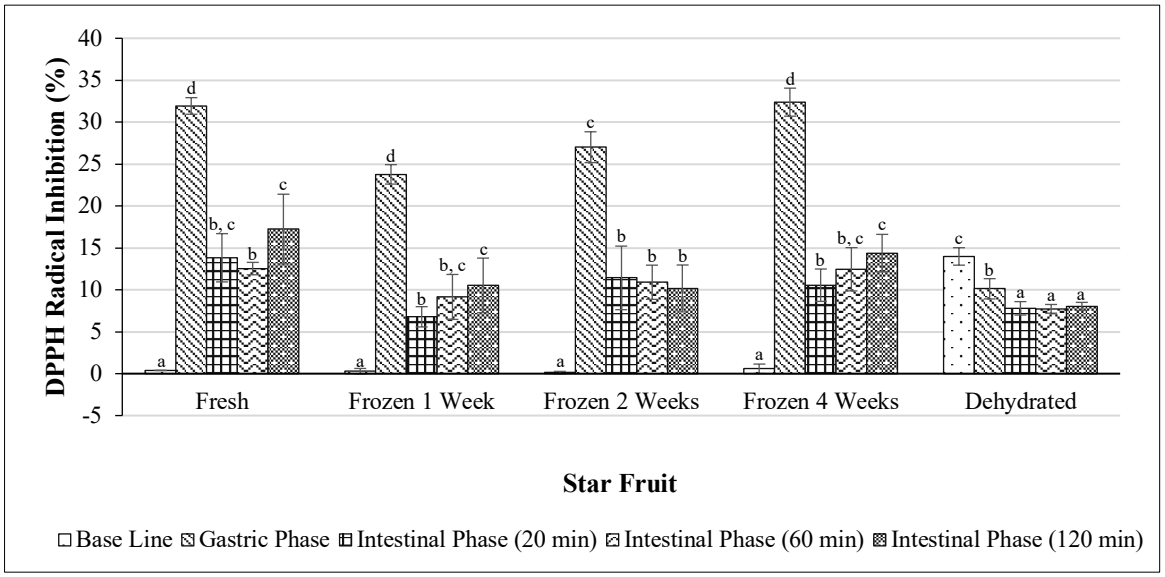
The highest DPPH radical inhibition from all forms of YPDF and WFDF occurred during the gastric phase as well as for fresh and frozen forms of RFDF; dehydrated RFDF (7.95±0.64 %) showed the highest DPPH radical inhibition during the intestinal phase at 60 minutes.

SF exhibited the highest DPPH radical inhibition at gastric phase for fresh (31.93±0.99 %), frozen for one-week (23.77±1.16 %), frozen for two weeks (27.01±1.84 %), and frozen for four weeks (32.38±1.67 %); meanwhile, dehydrated SF showed the highest DPPH radical inhibition at baseline (13.98±1.04 %), following by gastric phase (10.12±1.21 %).



DPPH: 2,2-diphenyl-1-picrylhydrazyl. %: percentage. min: minutes. Values represented as mean (n=6); vertical bars represent standard deviation. Different letters in the same form fruit represent a significant difference between phases mean values ($p < 0.05$).

Figure 2.8 DPPH radical inhibition of three species of dragon fruit forms during *in vitro* digestion. A: Yellow peel, B: White flesh, C: Red flesh.



DPPH: 2,2-diphenyl-1-picrylhydrazyl. %: percentage. min: minutes. Values represented as mean (n=6); vertical bars represent standard deviation. Different letters in the same form fruit represent a significant difference between phases mean values ($p < 0.05$).

Figure 2.9 DPPH radical inhibition of star fruit forms during *in vitro* digestion.

Pearson's correlation between RS and antioxidant capacity (FRAP and DPPH radical inhibition) during *in vitro* digestion is shown in Table 2.5. There was a statistically significant strong positive correlation between RS and FRAP for all YPDF, WFDF, and RFDF extracts ($p < 0.001$); furthermore, a moderate positive correlation was found for fresh SF $r(30) = 0.558$, $p < 0.001$, and a strong positive correlation for these variables was showed by the SF frozen forms; there was a weak positive correlation between RS and FRAP for dehydrated SF $r(30) = 0.282$, $p = 0.131$.

The relationship between RS and DPPH radical inhibition showed a significant moderate correlation for fresh RFDF $r(30) = 0.442$, $p = 0.015$, frozen for one-week YPDF $r(30) = 0.584$, $p < 0.001$, dehydrated YPDF $r(30) = 0.535$, $p = 0.002$, and fresh SF $r(30) = 0.618$, $p < 0.001$; meanwhile, there was a strong positive correlation between variables for dehydrated forms of WFDF $r(30) = 0.905$, $p < 0.001$ and RFDF $r(30) = 0.881$, $p < 0.001$. A statistically strong, negative correlation was found for dehydrated SF, $r(30) = -0.717$, $p < 0.001$.

Table 2.5 Correlation between reducing sugars and antioxidant capacity from fresh, frozen, and dehydrated dragon fruit species and star fruit during *in vitro* digestion.

Fruit / Form	*RS Vs FRAP*		*RS Vs DPPH*	
Yellow Peel Dragon Fruit (Hylocereus megalanthus)	***r***	***p***	***r***	***p***
Fresh ‡	0.846**	<0.001	0.047	0.806
Frozen for one week †	0.801**	<0.001	0.584**	<0.001
Frozen for two weeks †	0.716**	<0.001	0.142	0.454
Frozen for four weeks †	0.721**	<0.001	-0.107	0.684
Dehydrated (FW) †	0.716**	<0.001	0.535**	0.002
White Flesh Dragon Fruit (Hylocereus undatus)				
Fresh †	0.636**	<0.001	0.042	0.826
Frozen for one week ‡	0.727**	<0.001	0.262	0.162
Frozen for two weeks †	0.837**	<0.001	0.092	0.628
Frozen for four weeks †	0.714**	<0.001	0.207	0.273
Dehydrated (FW) †	0.949**	<0.001	0.905**	<0.001
Red Flesh Dragon Fruit (Hylocereus polyrhizus)				
Fresh †	0.852**	<0.001	0.442*	0.015
Frozen for one week †	0.807**	<0.001	0.238	0.130
Frozen for two weeks ‡	0.742**	<0.001	0.305	0.101
Frozen for four weeks ‡	0.742**	<0.001	0.076	0.690
Dehydrated (FW) †	0.830**	<0.001	0.881*	<0.001
Star Fruit (Averrhoa carambola)				
Fresh ‡	0.558**	0.001	0.618**	<0.001
Frozen for one week ‡	0.712**	<0.001	0.340	0.066
Frozen for two weeks †	0.757**	<0.001	0.172	0.364
Frozen for four weeks †	0.800**	<0.001	0.055	0.772
Dehydrated (FW) ‡	0.282	0.131	-0.717**	<0.001

RS: Reducing sugars. FRAP: Ferric reducing antioxidant power. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical inhibition. r: Pearson´s correlation coefficient. Two Asterisks (**): Statistically significant correlation, $p < 0.01$, two-tailed. One asterisk (*): Statistically significant correlation, $p < 0.05$, two-tailed.

Table 2.6 shows the Pearson's correlation to determine the relationship between TPC and antioxidant capacity (FRAP and DPPH radical inhibition) during *in vitro* digestion. Results showed there was a strong, positive correlation between TPC and FRAP, which was statistically significant for all fruit forms ($p < 0.001$).

Table 2.6 Correlation between total polyphenol content and antioxidant capacity from fresh, frozen, and dehydrated dragon fruit species and star fruit during *in vitro* digestion.

Fruit / Form	*TPC Vs FRAP*		*TPC Vs DPPH*	
Yellow Peel Dragon Fruit (Hylocereus megalanthus)	***r***	***p***	***r***	***p***
Fresh ‡	0.937**	<0.001	-0.007	0.972
Frozen for one week †	0.964**	<0.001	0.139	0.464
Frozen for two weeks †	0.953**	<0.001	0.041	0.831
Frozen for four weeks †	0.920**	<0.001	0.007	0.972
Dehydrated (FW) †	0.525**	<0.001	0.521**	0.003
White Flesh Dragon Fruit (Hylocereus undatus)				
Fresh †	0.921**	<0.001	0.024	0.898
Frozen for one week ‡	0.845**	<0.001	-0.235	0.211
Frozen for two weeks †	0.855**	<0.001	0.112	0.544
Frozen for four weeks †	0.950**	<0.001	-0.134	0.482
Dehydrated (FW) †	0.891**	<0.001	0.853**	<0.001
Red Flesh Dragon Fruit (Hylocereus polyrhizus)				
Fresh †	0.941**	<0.001	0.461*	0.010
Frozen for one week †	0.885**	<0.001	-0.080	0.674
Frozen for two weeks ‡	0.874**	<0.001	0.144	0.449
Frozen for four weeks ‡	0.956**	<0.001	0.062	0.745
Dehydrated (FW) †	0.829**	<0.001	0.942**	<0.001
Star Fruit (Averrhoa carambola)				
Fresh ‡	0.987**	<0.001	0.101	0.596
Frozen for one week ‡	0.968**	<0.001	-0.034	0.858
Frozen for two weeks †	0.931**	<0.001	0.095	0.619
Frozen for four weeks †	0.910**	<0.001	0.019	0.919
Dehydrated (FW) ‡	0.033	0.864	-0.770**	<0.001

TPC: Total polyphenol content. FRAP: Ferric reducing antioxidant power. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical inhibition. r: Pearson´s correlation coefficient. Two Asterisks (**): Statistically significant correlation, $p < 0.01$, two-tailed. One asterisk (*): Statistically significant correlation, $p < 0.05$, two-tailed.

The relationship between TPC and DPPH radical inhibition showed a significant moderate correlation for frozen for one-week YPDF $r(30) = 0.521$, $p = 0.003$ and fresh RFDF $r(30) = 0.461$, $p = 0.010$; meanwhile, there was a strong positive correlation between variables for dehydrated forms of WFDF $r(30) = 0.853$, $p < 0.001$ and RFDF

$r(30) = 0.942$, $p < 0.001$. A statistically strong, negative correlation was found for dehydrated SF, $r(30) = -0.770$, $p < 0.001$.

2.4 Discussion

This study aimed to determine the TPC and the antioxidant capacity of fresh, frozen, and dehydrated forms of three species of dragon fruit and one species of star fruit and to evaluate their bioaccessibility.

The results of this study corroborate previous findings, which found difference in the TPC between dragon fruit species (Al-Mekhlafi *et al*., 2021; Arivalagan *et al*., 2021). Extracts obtained from fresh, frozen, and dehydrated YPDF, WFDF, and RFDF, using acetone exhibited the highest TPC when compared to those using ethanol and water. Acetone extracts from SF also showed higher TPC than ethanol and water extracts. Polarity of the solvents and the polyphenol structure play an essential role when extraction occurs (Złotek *et al*., 2016); water, ethanol and acetone have been widely used to extract different phytochemical such as polyphenols (Alara, Abdurahman and Ukaegbu, 2021). Some studies found that acetone extracted higher TPC than ethanol and other showed that water exhibited the highest TPC extraction (Liu *et al*., 2009; Sulaiman *et al*., 2011); differences probably due to food matrix and type of polyphenol.

Preservation methods, such as drying and freezing affect the natural fruit matrix, causing changes in the TPC (Mojzer *et al*., 2016; Fitri *et al*., 2016). This study found that dragon fruit showed significant differences when compared TPC between forms (fresh, frozen, and dehydrated). The highest TPC in acetone extracts was found in dehydrated YPDF, frozen for one-week RFDF, and fresh WFDF; for ethanol extracts, all dehydrated dragon fruit species exhibited the highest TPC; meanwhile, frozen for one-week form showed the highest TPC in water extracts. Regarding SF, dehydrated form exhibited the highest TPC in acetone and ethanol extracts; water extract from

frozen for one-week SF showed the highest TPC. The current results indicate an effect of the fruit matrix in TPC, as dehydration concentrates the chemical compounds in fruit and freezing could change the cell structure, modifying the food matrix.

FRAP assay is a widely used method to determine the antioxidant capacity of polyphenols (Brainina, Stozhko and Vidrevich, 2019), results in this study showed that the greatest FRAP was found mainly in the dehydrated dragon fruit species, as well as in the dehydrated SF, independently of the solvent used; nevertheless, acetone extracts were those with the overall highest FRAP. These findings are similar to the obtained by other studies comparing solvents to evaluate the antioxidant capacity of plant raw materials, as they found a greatest antioxidant capacity in acetone extracts (Zhang *et al*., 2023). Current results indicate that the highest DPPH radical inhibition was exhibited by acetone extracts, followed by ethanol extracts; however, the percentage of DPPH radical inhibition was lower than the reported for YPDF (Lupuche *et al*., 2021), RFDF, and WFDF (Alam *et al*., 2023; Kim *et al*., 2011), using methanol as an extraction solvent.

The positive, strong correlation between TPC and FRAP found in this study for acetone and ethanol extracts support previous studies that relate the antioxidant capacity of dragon fruit to their TPC and suggested that polyphenols play an important role in the antioxidant capacity exhibited by dragon fruit species (Abd-Manan, *et al*., 2019, Luu *et al*., 2021). Nevertheless, the content of other chemical compounds such as ascorbic acid has been related to the antioxidant capacity of dragon fruit species (Choo and Young, 2011).

The link between the positive effect of polyphenols on health and their bioaccessibility has been reported (Corona-Leo, Meza-Márquez and Hernández-Martínez, 2021). As digestion is a complicated process affected by diverse factors and its study is expensive and difficult, different methodologies for an *in vitro* digestion has been proposed to

evaluate the bioaccessibility of different compounds, including polyphenols (Mihaylova *et al*., 2021). Results from this study, which is, to our knowledge, the first study to carry out an *in vitro* digestion for dragon fruit species and star fruit, showed differences between digestion phases in the release of RS and polyphenols. The highest RS release had mainly occurred during intestinal phase, mainly at 20 minutes for all dragon fruit and star fruit forms, supporting previous studies that reported an increase in the RS released during gastrointestinal *in vitro* digestion of the jackfruit pulp, another tropical fruit (Zhu, *et al*., 2019). Similar behaviour was showed by polyphenols as the greatest release was found, mainly during intestinal phase at 20 minutes' time point for all dragon fruit forms; however, SF showed the greatest TPC at 60 minutes' time point of the intestinal phase. Parada and Aguilera (2007) reported the influence of the solubilisation properties of polyphenols as well as their interactions with other molecules on the polyphenols bioaccessibility; furthermore, the effect of the preservation methods may influence the structure of polyphenols affecting their bioaccessibility. The effect of polyphenols on human health is linked to the type of polyphenol and its stability; for example, flavonoids cannot be absorbed, but are sensitive to pH changes and digestive enzymes that break down their structure, whereas phenolic acids can be absorbed into the blood when they reach the stomach (Chen *et al.,* 2020).

Regarding to antioxidant capacity, the highest FRAP for fresh, frozen, and dehydrated RFDF and SF was exhibited during the intestinal phase; meanwhile, the highest DPPH radical inhibition was found at gastric phase, probably due to acidic pH conditions, as reported by studies for pomegranate-based products, grapefruit and mango (Fawole and Opara, 2016; Chen *et al*., 2014); the proposed mechanism indicates an hydrolysis of the polyphenol glycosides occurring during the gastric phase of the digestion (Tagliazucchi *et al*., 2010) and a subsequent increase in the antioxidant capacity.

The strong, positive correlation exhibited between RS and FRAP, as well as between TPC and FRAP, corroborate previous findings as some *in vitro* studies have reported a positive relationship between the antioxidant capacity and the TPC. However, there are other studies that have found a decrease in the antioxidant capacity of polyphenols as the digestion progressed (Laya and Koubala, 2020; Aguillón-Osma *et al*., 2019); this behaviour might denote a degradation of the polyphenols, as studies found low molecular weight polyphenols after *in vitro* digestion and subsequent fermentation (Rocchetti *et al*., 2020). There are few studies reporting the individual polyphenols in dragon fruit and star fruit, providing the opportunity for further research to understand the polyphenols bioaccessibility; quercetin was found as the main polyphenol in SF and two species of dragon fruit, RFDF and WFDF (Khanam *et al*., 2015; Attar *et al*., 2022).

This study has shown novel findings regarding the effect of *in vitro* digestion on dragon fruit and star fruit under fresh, frozen, and dehydrated conditions.

2.5 Conclusion

Polyphenol content and the antioxidant capacity of dragon fruits are dependent on the species and the form of preservation. The release of polyphenols found in dragon fruit (*Hylocereus* spp) and star fruit (*A. carambola*), changes between *in vitro* digestion phases in fresh, frozen, and dehydrated samples. To understand the significance of these findings, work on the bioavailability of the polyphenols and what these means with regards to health is needed.

Chapter 3: *In vitro* digestion and sensory evaluation of products based on red flesh dragon fruit and star fruit.

3.1 Introduction

Polyphenols have been extensively studied due to their antioxidant capacity through which they neutralize active species known as free radicals generated by oxidative processes (El Gharras, 2009). The effect of polyphenols on human health is linked to the type and stability of polyphenol, for example flavonoids are sensitive to digestive enzymes and pH changes whereas phenolic acids can be absorbed when they reach the stomach (Chen *et al*., 2020). Some food properties could be conferred or modified by polyphenol content, such as colour, astringency, and bitterness; the quantity of polyphenols in foods could also be affected by storage conditions and processing (Pandey and Rizvi, 2009).

Different plant-based products have been formulated to determine their potential impact on health as functional foods because of their antioxidant capacity and polyphenol content (Coman *et al*., 2018; Yu and Ahmedna, 2013). Fruits, including tropical species such as red flesh dragon fruit (*Hylocereus polyrhizus*) and star fruit (*Averrhoa carambola*) possess antioxidant capacity and have been used as part of traditional alternatives in various countries to manage T2D and hypertension (Acham *et al*., 2018; Ibrahim *et al*., 2018; Yan *et al*., 2013). Studies have shown that both fruits contain polyphenols and based on these findings have suggested that these compounds may be linked to their positive effects on health (Adnan, Osman and Abdul-Hamid, 2011; Choo and Yong, 2011; Khanam *et al*., 2015).

Factors that are said to influence the health benefits of polyphenols include in addition to their chemical structure, the food matrices within which they are found; the latter being a factor associated with their bioavailability (Zi-Chao *et al*., 2019; Fitri *et al*.,

2016). Bioavailability could be assessed by *in vivo* procedures; however, due to the high cost of these studies, some *in vitro* assays involving cells culture have been developed (Yun *et. al*., 2004). Bioaccessibility involves *in vitro* methodologies under laboratory conditions, through liberation from the food matrix, via gastrointestinal digestion and assimilation by the intestinal epithelium. *In vitro* digestion is used to mimic the process of digestion and has been used to investigate different edible products (Attri *et al*., 2017; Lucas-González *et al*., 2018).

Novel food is defined by the European Commission as '*food that had not been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force*' (European Commission, 2023); plants that have been used as traditional food in countries outside the European Union is considered as a novel food. Sensory attributes are considered a key factor to determine the acceptance of novel foods (Civille and Oftedal, 2012).

Sensory evaluation is a method to determine the human response to food and it has been a valuable tool to establish the quality and acceptance of an edible product by consumers (Ross, 2009; Van Trijp and Schifferstein, 1995). This evaluation, as its name suggests, involves the human senses and has been approved as a scientific method by different organizations around the world, such as by the Institute of Food Technologists and the Institute of Food Science and Technology. There are different tests to establish the sensory attributes that make an acceptable product; the evaluation could be discriminative, descriptive, or affective. Affective evaluation is also known as a hedonic test that does not require trained evaluators and is used to determine the preference between various products, using, generally a 9-point scale (Kemp, Hollowood and Hort, 2009; Howes, 2015). To find out if a product is liked or disliked and to what extent, usually an acceptance study is carried out, in which the samples to evaluate will be given to participants simultaneously with written instructions and an evaluation form.

Currently, consumers around the world are looking for new sensory experiences and health benefits in foods. Major tropical fruits such as mango, mangosteen, guava, papaya and pineapple have been leading the global market (Food and Agriculture Organization of the United Nations, 2023), however, exotic fruits such as passion fruit, lychees, pitahaya, and carambola have a potential market in Europe to enhance the new trend (Centre for the Promotion of Imports from Developing Countries, 2020). Pitahaya also known as dragon fruit and carambola well known as star fruit are exotic tropical fruits that have been studied to determine their potential health benefits in fresh and preserved forms. However, bioaccessibility and sensory acceptance has not been evaluated on beverages formulated from RFDF and SF (fresh, frozen, and dehydrated).

Based on the results from Chapter 2 in this thesis, the fruits that exhibited the highest TPC, RFDF (*H. polyrhizus*) and SF (*A. carambola*), were selected to design and formulate beverages based on fresh, dehydrated, and one-week frozen fruits, as the latter form exhibited the highest TPC in water extracts. Therefore, this study aimed to evaluate the *in vitro* bioaccessibility of polyphenols and sensory acceptability in six different beverages based on fresh, frozen, and dehydrated RFDF and SF; reducing sugar assay was not carried out. To date, there are no reports or reviews describing the *in vitro* bioaccessibility of polyphenols from edible products based on RFDF or SF.

3.2 Methods

Fruits used for product preparation were acquired at My Exotic Fruit, a United Kingdom online retailer; meanwhile all reagents for *in vitro* digestion were Sigma-Aldrich brand, unless specified in the procedure.

3.2.1 Product preparation

Beverages were formulated to obtain fresh, frozen, and dehydrated fruit based products being selected those with 290 μg GAE/mL for RFDF and 490 μg GAE/mL for SF (Figure 3.1), due to their appearance (colour and consistency) (Jain and Gupta, 2005).

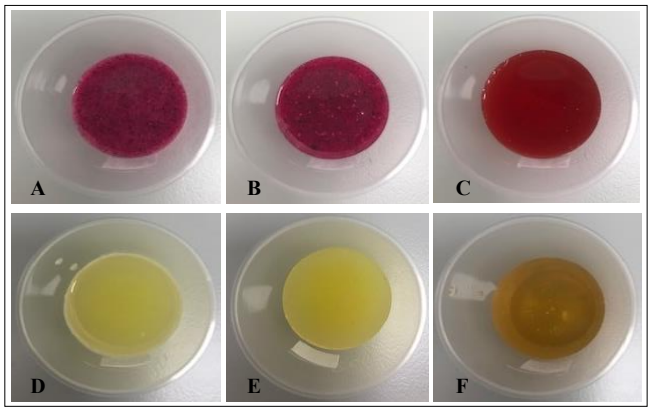


Figure 3.1 Fruit-based products. A: Fresh dragon fruit, B: Frozen dragon fruit, C: Dehydrated dragon fruit, D: Fresh star fruit, E: Frozen star fruit, F: Dehydrated star fruit.

The following procedure was carried out in the Oxford Brookes Centre for Nutrition and Health (OxBCNH) kitchen to prepare the fruit-based products.

Dragon fruit was washed with tap water, peeled, and chopped. One portion was used to prepare the fresh fruit product, meanwhile, a second portion was frozen at -18 °C for one week to make frozen fruit product, and a third portion was dehydrated at 60 °C for 48 hours prior to create a dehydrated fruit product. Seeds from SF were removed after which the same procedure used for RFDF described above was used. The fruit beverages were prepared an hour before the *in vitro* digestion and sensory evaluation as given below:

3.2.1.1 Fresh and frozen fruit-based products

The fruit was ground in a blender (Nutribullet 600 series) for one minute and then the amount required was measured (fresh RFDF: 84 mL, frozen RFDF: 70 mL, fresh SF: 75 mL, frozen SF: 60 mL) and water was added to

prepare 100 mL of the product. SF products were used after filtering to remove the fibrous part.

3.2.1.2 Dehydrated fruit-based products

Dried fruit was put into a container with water for 30 minutes and then the mixture was filtered twice.

Finally, fresh, frozen, and dehydrated fruit based products were added with 6.25 % of lime juice as a final step to add a familiar flavour.

3.2.2 *In vitro* digestion of fruit-based products

Firstly, 2.5 g of each beverage was weighed in triplicate into sample pots with magnetic stirrers at 37 °C in the *in vitro* digestion unit. Immediately, 30 mL of water was added to each pot and a 1.5 mL of sample was taken in duplicate (baseline measurement). Then to simulate the gastric phase, 0.1 mL of 10 % α-amylase, 0.8 mL of 1M hydrochloric acid and 1 mL of 10 % pepsin solution were added; after 30 minutes, the gastric samples were taken in duplicate. Finally, the intestinal phase was simulated by the addition of 2 mL of sodium hydrocarbonate (1M), 5 mL of 0.2 M sodium malate buffer (pH 6), 5 mL of bile extract (10 %), 17 mL of water, one mL of pancreatin (2 %) and 0.1 mL of amyloglucosidase; samples in duplicate were taken at 20, 60, and 120 minutes after starting this phase (Eriksen *et al*., 2017). All samples were stored in a freezer at -20 °C until analysis. No blanks of the simulated digestion were assayed.

3.2.2.1 Determination of the total polyphenol content (TPC)

Folin-Ciocalteu's method was used to determine the TPC released during each phase of digestion as described on Chapter 2, section 2.2.1.3 (Agbor, Vinson and Donnelly, 2014). Results were obtained as μg GAE/mL of sample.

3.2.2.2 Determination of the antioxidant capacity

FRAP and DPPH scavenging activity assays were carried out as mentioned previously in Chapter 2, section 2.2.1.4 to determine the antioxidant capacity during *in vitro*

digestion phases. The total antioxidant capacity is reported as μmol/g of sample for FRAP, using a standard of 1000 μmol ferrous sulphate; meanwhile, DPPH radical scavenging activity of the samples was calculated in percentage of DPPH radical inhibition (Benzie and Strain, 1996; Brand-Williams, Cuvelier and Berset, 1995).

3.2.3 Sensory evaluation of fruit-based products

The University Research Ethics Committee approved this study with the Registration Number: UREC 201379 (Appendix 3).

3.2.3.1 Recruitment

Participants between 18 and 50 years were recruited for a one-hour session at OxBCNH, located at Sinclair Building, third floor in Headington Campus, Oxford. Different means such as posters (Appendix 4) on Oxford Brookes University (OBU) notice boards, dissemination of emails with a brief description of the study to the Research Activity Google Group database, and short advertisement in local online newspaper (Daily Info) were used to disseminate the recruitment requirements as much as possible.

After volunteers indicated their interest, the participant information sheet (Appendix 5) was given to them. Once they accepted to take part, the consent form (Appendix 6) was signed, and a health-screening questionnaire (Appendix 7) was completed.

Volunteers with no allergies to fruit, non-smokers, and without renal disease were selected to take part in the sensory evaluation. Exclusion criteria were:

- Taking medication that may affect the sense of taste,
- Being pregnant or breastfeeding, or
- Having a cold or Covid-19 related symptoms seven days before the testing session.

3.2.3.2 Testing session

Participants were asked to use hand sanitiser and to wear a face-covering while indoors when attending the session to reduce the risk acquiring Covid-19. The test was carried out in an individual sensory booth under controlled environmental conditions (Figure 3.2).

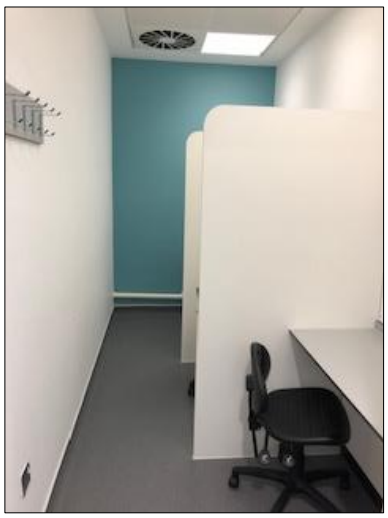


Figure 3.2 Sensory booths at OxBCNH.

Six samples (10 mL each) in randomized order and randomly coded with three-digit numbers (Figure 3.3) were given to each participant to evaluate six different attributes [appearance (colour and consistency), colour, taste, viscosity, mouthfeel, and overall acceptance]. A 9-point hedonic scale, where 9 = 'like extremely', 8 = 'like very much', 7 = 'like moderately', 6 = 'like slightly', 5 = 'neither like or dislike', 4 = 'dislike slightly', 3 = 'dislike moderately', 2 = 'dislike very much', and 1 = 'dislike extremely', was used to evaluate the attributes in an online webpage designed using Compusense software. Participants logged into Compusense on a personal device, such as a mobile phone, tablet, or laptop with the username and password given previously by email and followed the instructions. Between samples, the participants cleaned the palate with a sip of water and a bite of a cracker to avoid any interference.

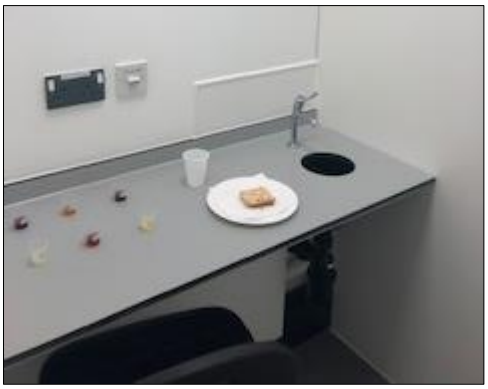


Figure 3.3 Individual sensory booth set up for hedonic test.

3.2.4 Statistical analysis

SPSS software, version 28, was used to conduct the statistical analysis of data.

3.2.4.1 In vitro digestion

For polyphenol content and antioxidant capacity, normality of the data was determined by the Shapiro-Wilk test. One Way ANOVA analysis and Tukey's post hoc test were used to compare the results obtained between *in vitro* digestion phases when the data showed a normal distribution and Kruskal-Wallis non-Parametric analysis was performed for the data not normally distributed. A Pearson's correlation analysis was carried out. The results are shown as the mean of six determinations ± SD with a statistical significance set at $p < 0.05$.

3.2.4.2 Sensory evaluation

For sensory attributes, a Friedman non-parametric test and Wilcoxon signed-rank post hoc test with Bonferroni adjustment were carried out, resulting in a significance level set at $p < 0.0033$. The results are shown as median to identify the central tendency of the liking scores (Sader *et al*., 2020).

3.3 Results

3.3.1 *In vitro* digestion of fruit-based products

3.3.1.1 Determination of the total polyphenol content (TPC)

The amount of TPC released from dehydrated, fresh, and frozen fruits-based beverages during *in vitro* digestion is shown in Table 3.1. There was a statistically significant difference between digestion phases for TPC released ($p < 0.001$).

For RFDF beverages the highest TPC released occurred during intestinal phase at different time point for each form of fruit; dehydrated based beverage at 20 minutes (2185.06±256.84 μg GAE/mL), fresh based beverage at 120 minutes (1544.38±226.32 μg GAE/mL), and frozen based beverage at 60 minutes 2041.03±153.20 μg GAE/mL). Nevertheless, no significant difference was found between time points of the intestinal phase for each form of fruit.

SF beverages exhibited the highest TPC released also at intestinal phase; dehydrated based beverage and frozen based beverage at 60 minutes (2880.71±815.62 μg GAE/mL and 1615.53±214.81 μg GAE/mL, respectively), meanwhile, fresh based beverage showed the highest released at 120 minutes, 1669.12±220.41 μg GAE/mL.

Table 3.1 Total polyphenol content (µg GAE/mL) released from red flesh dragon fruit and star fruit beverages during *in vitro* digestion.

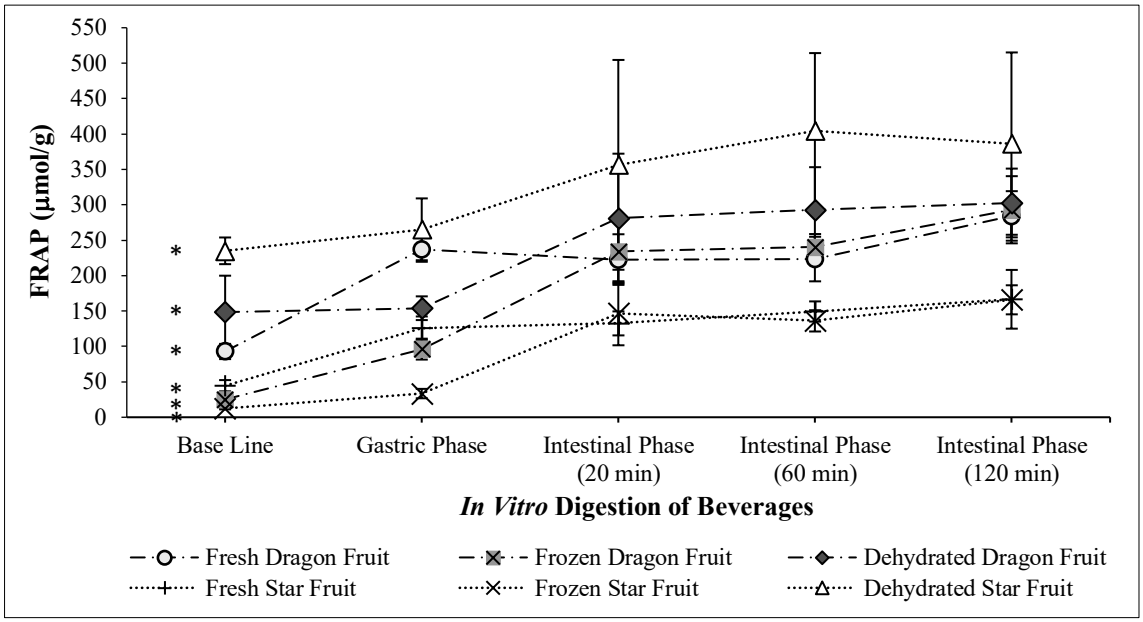
Product	*Base Line*	*Gastric Phase*	*Intestinal Phase (20 minutes)*	*Intestinal Phase (60 minutes)*	*Intestinal Phase (120 minutes)*	*p-value*
Red Flesh Dragon Fruit Beverage						
Dehydrated†	256.45±10.56[a]	759.08±28.34[b]	2185.06±256.84[c]	2092.74±270.92[c]	1879.07±243.60[c]	<0.001
Fresh†	78.67±22.47[a]	499.32±48.33[b]	1325.44±106.50[c]	1454.44±166.10[c]	1544.38±226.32[c]	<0.001
Frozen‡	38.09±18.28[a]	468.30±59.76[ab]	1987.40±188.85[c]	2041.03±153.20[c]	1967.23±161.97[c]	<0.001
Star Fruit Beverage						
Dehydrated‡	646.53±23.54[a]	1215.74±283.17[ab]	2858.65±900.36[b]	2880.71±815.62[b]	2762.13±745.93[b]	<0.001
Fresh†	97.08±10.94[a]	362.46±29.94[b]	1259.84±152.83[c]	1521.13±89.74[d]	1669.12±220.41[d]	<0.001
Frozen‡	62.29±35.31[a]	319.83±57.92[ab]	1395.67±78.51b[c]	1615.53±214.81[c]	1493.35±113.94b[c]	<0.001

The total polyphenol content is expressed as micrograms of Gallic acid equivalent per millilitre of sample. †Normal distribution, One Way ANOVA and Tukey´s post hoc test. ‡Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=6) ± standard deviation. Different letters in the same row represent a significant difference between mean values ($p < 0.05$).

3.3.1.2 Determination of the antioxidant capacity

Figure 3.4 illustrates the average of FRAP during *in vitro* digestion of RFDF and SF based beverages. A significant difference between phases was established for all beverages. Fresh and frozen RFDF and SF based products at $p < 0.001$, dehydrated dragon fruit based product at $p = 0.002$, and dehydrated SF based product at $p = 0.003$. The highest FRAP for all dragon fruit based products was showed at intestinal phase 120 minutes; dehydrated (302.59±48.53 μmol/g), fresh (284.29±34.96 μmol/g), and frozen (293.00±47.41 μmol/g). However, there was no difference between intestinal phase time points.

SF based products showed the highest FRAP during the intestinal phase mainly at 120 minutes (fresh 166.60±41.47 μmol/g and frozen 165.85±20.52 μmol/g), but also at 60 (dehydrated 404.4±109.82 μmol/g). SF dehydrated based beverage exhibited the highest FRAP when compared to other SF forms.

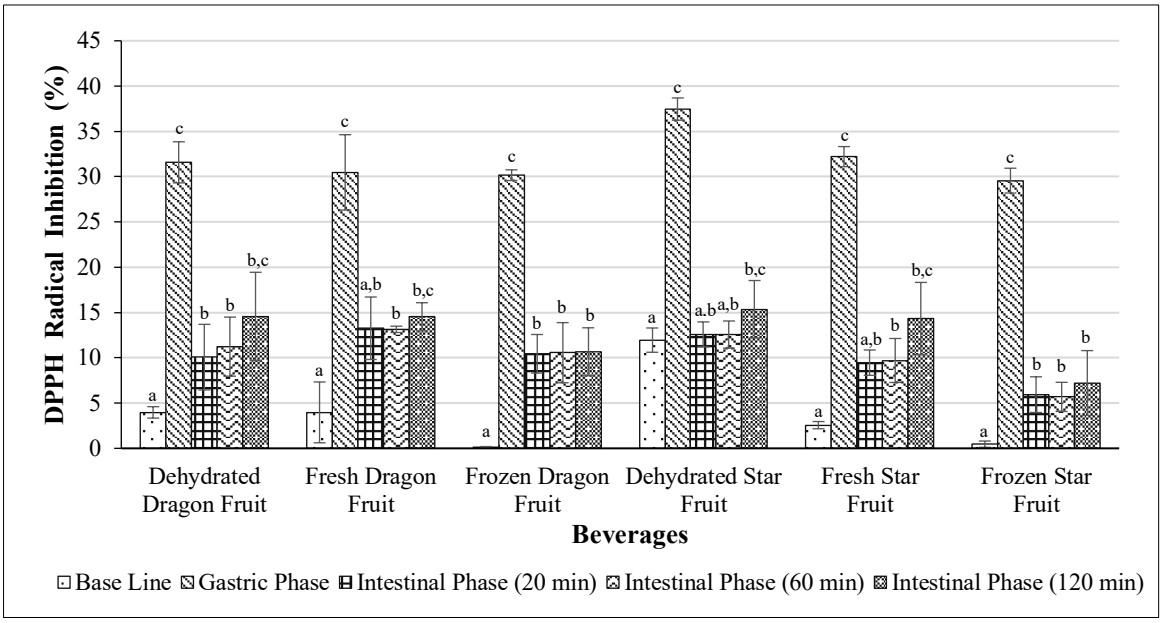


FRAP: ferric reducing antioxidant power. μmol/g: micromole per gram. min: minutes. †Normal distribution, One Way ANOVA and Tukey´s post hoc test. ‡Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=6) ± standard deviation. (*) indicates significant differences between phases $p < 0.05$.

Figure 3.4 Ferric Reducing Antioxidant Power (FRAP) from red flesh dragon fruit and star fruit based products during *in vitro* digestion.

Figure 3.5 shows the inhibition of the DPPH radical by fresh, frozen, and dehydrated RFDF and SF based beverages during *in vitro* digestion. There was a significant difference in the DPPH radical inhibition between the phases of digestion for each product evaluated ($p < 0.05$).

The highest DPPH radical inhibition for all RFDF and SF based beverages occurred during the gastric phase and was statistically different when compared to other phases of digestion. Dehydrated RFDF based beverage (31.58±2.27 %) showed the highest DPPH radical inhibition when compared to other forms of RFDF, as well as the dehydrated SF based beverage (37.46±1.23 %) when compared to fresh and frozen SF based beverages.



DPPH: 2,2-diphenyl-1-picrylhydrazyl. %: percentage. min: minutes. Not normal distribution, Kruskal-Wallis and post hoc test. Values represented as mean (n=6), vertical bars represent standard deviation. Different letters in the same beverage represent a significant difference between phases mean values ($p < 0.05$).

Figure 3.5 DPPH radical inhibition of red flesh dragon fruit and star fruit based products during *in vitro* digestion.

Table 3.2 shows the Pearson's correlation carried out to determine the relationship between TPC and antioxidant capacity (FRAP and DPPH radical inhibition) during *in vitro* digestion for RFDF and SF based beverages. Results showed there was a strong, positive correlation between TPC and FRAP, which was statistically significant for all

fruit forms ($p < 0.001$). The strongest correlation for RFDF based beverages was exhibited by frozen RFDF based product $r(30) = 0.939$, $p < 0.001$ when compared to dehydrated and fresh based products; also frozen SF based product showed the strongest correlation $r(30) = 0.926$, $p < 0.001$ for SF based products. There was no correlation between TPC and DPPH radical inhibition for all beverages.

Table 3.2 Correlation between total polyphenol content and antioxidant capacity from red flesh dragon fruit and star fruit beverages during *in vitro* digestion.

Product	*TPC Vs FRAP*		*TPC Vs DPPH*	
Red Flesh Dragon Fruit Beverage	***r***	***p***	***r***	***p***
Dehydrated	0.837**	<0.001	0.090	0.636
Fresh	0.744**	<0.001	0.027	0.886
Frozen	0.939**	<0.001	-0.080	0.673
Star Fruit Beverage				
Dehydrated	0.902**	<0.001	-0.323	0.081
Fresh	0.763**	<0.001	-0.119	0.533
Frozen	0.926**	<0.001	-0.296	0.112

TPC: Total polyphenol content. FRAP: Ferric reducing antioxidant power. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical inhibition. r: Pearson´s correlation coefficient. Two Asterisks (**): Statistically significant correlation, $p < 0.01$, two-tailed. One asterisk (*): Statistically significant correlation, $p < 0.05$, two-tailed.

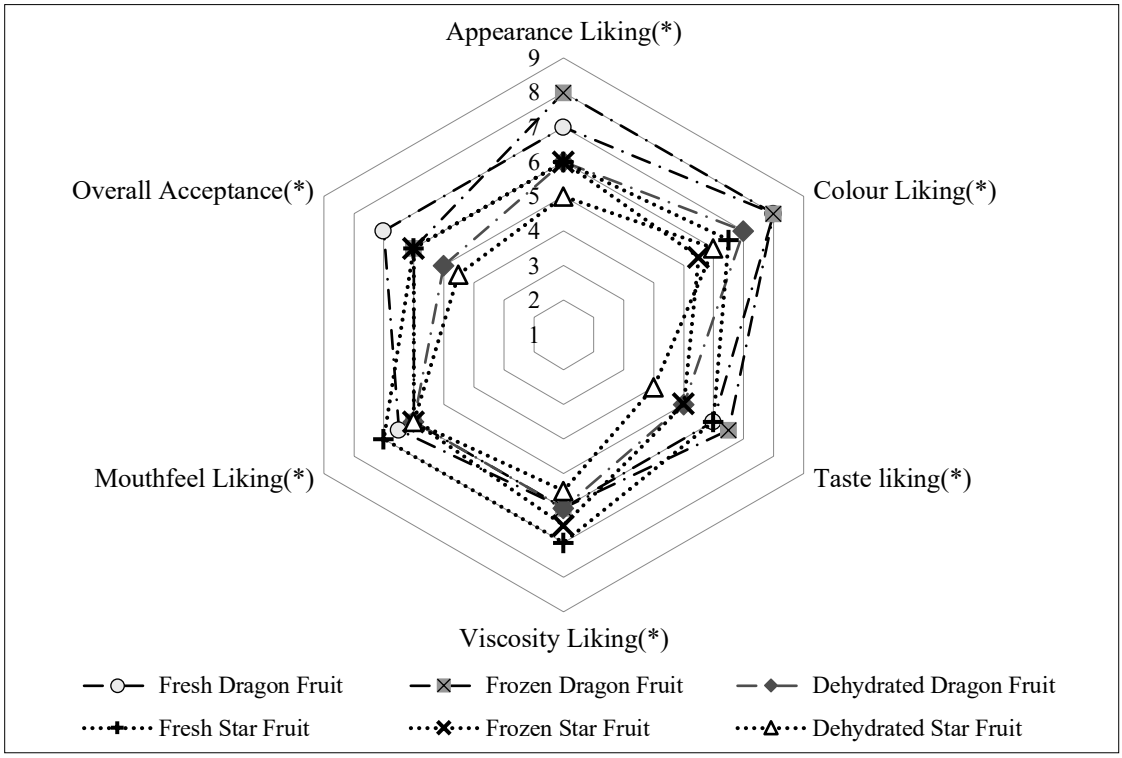
3.3.2. Sensory evaluation of fruit-based products

Fifty volunteers between 18 and 50 years were recruited to participate in the sensory evaluation and forty-seven completed the study, three were excluded as one was a smoker (an exclusion criterion), and two more did not complete essential information in the health questionnaire. There were 30 females and 17 males with an average age of 31.1 years ± 9.2, with most participants between 21 and 30 years; demographic information of participants obtained from health questionnaires is shown on Table 3.3.

Table 3.3 Characteristics of the participants of the sensory evaluation of products based on red flesh dragon fruit and star fruit, n=47.

Characteristic	*Frequency*	*Percentage*
Gender		
Female	30	63.8
Male	17	36.2
Age group (years)		
18-20	4	8.5
21-30	23	48.9
31-40	10	21.3
41-50	10	21.3

Medians for sensory attributes, including appearance, colour, taste, viscosity, mouthfeel, and overall acceptance are shown in Figure 3.6. Significant differences were shown between products for all attributes evaluated ($p < 0.001$). The RFDF based products were the most preferred for four sensory attributes; frozen based beverage for appearance (8, 'like very much'), colour (8, 'like very much´), and taste (6.5, 'like slightly); meanwhile, fresh based beverage obtained the most preferred score for overall acceptance (7, 'like moderately'). Fresh SF based beverage was the most preferred for viscosity (7, 'like moderately') and mouthfeel (7, 'like moderately') attributes.



Values represented as median, n=47. Friedman test, asterisk (*) represents a significant difference between median values ($p < 0.001$). 9-point scale: 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely.

Figure 3.6 Radar graph for sensory attributes of red flesh dragon fruit and star fruit based products.

3.3.2.1 Sensory attributes of dragon fruit and star fruit products

Regarding appearance, according to the Friedman test, there was a statistically significant difference between products, χ^2 (5) = 65.832, $p < 0.001$. After a Wilcoxon signed rank test a Bonferroni correction was performed, and significant differences were found between RFDF based products, ($p < 0.001$). There were significant differences between fresh (7, 'like moderately') and dehydrated (6, 'like slightly'), and between frozen (8, 'like very much') and dehydrated (6, 'like slightly') RFDF based beverages; the frozen fruit based product showed the highest appearance liking score, 8 ('like very much') on a 9-point hedonic scale. Significant differences were found between fresh RFDF based beverage and the dehydrated SF based product ($p < 0.001$) for appearance; it was 8 ('like very much') for the frozen RFDF which was significantly different to the scores, which ranged from 5.5-6.0 ('neither like or dislike' to 'like slightly') for fresh, frozen, and dehydrated SF based beverages ($p < 0.001$). A significant difference was

observed between frozen (8, ‘like very much’) and dehydrated (5, ‘neither like or dislike’) SF based beverages for this attribute ($p < 0.001$).

A significant difference for colour was found between products, χ^2 (5) = 87.467, $p < 0.001$. RFDF based beverages and SF based beverages were statistically different for colour. Fresh and frozen RFDF based beverages with scores in the 8, ‘like very much’ range, were preferred more than the beverage prepared with dehydrated RFDF (7, ‘like moderately’), and fresh (6.5, ‘like slightly’ range), frozen (5.5, ‘neither like or dislike’), and dehydrated (6, ‘like slightly’) SF based beverages ($p < 0.001$). A significant difference between fresh (6.5, ‘like slightly’ range) and dehydrated (6, ‘like slightly’) SF based beverages was found ($p = 0.003$) as the last one showed a darker colour. The most accepted beverages based on colour as a sensory attribute were fresh (8, ‘like very much’) and frozen (8, ‘like very much’) RFDF based beverages. Meanwhile, the less accepted was the frozen SF beverage which scored as 5 (‘neither like or dislike’).

In terms of taste, a statistically significant difference between tested beverages was shown, χ^2 (5) = 49.587, $p < 0.001$. Fresh and frozen RFDF based beverages were scored as 6 (‘like slightly’) which was significantly different to dehydrated RFDF (5, ‘neither like or dislike’) and dehydrated SF based beverage which was ranked in the 4, ‘dislike slightly’ score ($p < 0.001$). Fresh and frozen SF based beverage were significantly different to the dehydrated SF based beverage ($p < 0.001$) with regards to taste. The highest score for this attribute was exhibited by fresh and frozen RFDF based beverages in the 6 (‘like slightly’) range. The dehydrated SF based beverage, showed the lowest score, being allocated in the 4 (‘dislike slightly’) scale.

Viscosity liking results showed a statistically significant difference between beverages, χ^2 (5) = 29.231, $p < 0.001$. After the post-hoc test, fresh, frozen, and dehydrated RFDF based beverages presented lower preference than fresh SF based beverage (7, ‘like moderately’) at $p < 0.001$, $p = 0.001$ and $p < 0.001$, respectively. Fresh and frozen SF

based beverages scored as 6 and 7 (‘like slightly’ and ‘like moderately’, respectively) and were the most accepted beverages according to this attribute, both were statistically significant different to dehydrated SF based beverage ($p < 0.001$).

According to the Friedman test, there was a statistically significant difference between beverages’ mouthfeel, $\chi 2$ (5) = 18.790, p = 0.002 showing the frozen SF based product the highest score, being classified as 7, ‘like moderately’. Fresh, frozen, and dehydrated SF and RFDF based beverages were scored at the ‘like slightly’ range (6.0-6.5).

3.3.2.2 Overall acceptance

There was a statistically significant difference in the overall acceptance score between the tested beverages, χ^2 (5) = 46.324, $p < 0.001$. Fresh RFDF based beverage received the highest score at 7 (‘like moderately’) for the overall acceptance, followed by frozen RFDF, fresh SF, and frozen SF, ranked into the ‘like slightly’ range (6). The less preferred beverage was the based on dehydrated SF (4.5, ‘dislike slightly’ scale).

3.4 Discussion

The aim of this study was to evaluate the bioaccessibility of polyphenols and the sensory acceptance of six beverages based on fresh, frozen, and dehydrated RFDF and SF. One of the main findings in this study is that the beverages based on frozen and dried forms of RFDF and SF showed higher polyphenol release and antioxidant capacity than fresh forms during *in vitro* digestion. According to Sęczyk *et al*., (2021) bioaccessibility of phenolic compounds relies on the type of compound, its interaction with the food matrix, and processing. Food processing involves operations to transform raw materials in an aim to extend product shelf life, maintain or improve sensorial characteristics and nutritional status, reduce microorganisms and/or enzyme activity. Heat as a preservation method has adverse effects on food characteristics such as colour, and content of nutrients, however, the use of elevated temperatures for short times has

been used to retain properties in some food products. Studies found that thermal preservation methods increase the bioaccessibility of polyphenols due to its effect in the glycosidic links between polysaccharides and polyphenols (Cilla *et al*., 2018, Aherne *et al*., 2010). Freezing is a preservation method used to maintain the characteristics of the food as close as possible to the fresh form of the product. (Fellows, 2022). Low temperatures may change the food matrix structure allowing the release of polyphenols. During this study RFDF and SF were subjected to drying and freezing process before they were used to prepare the fruit-based products tested as both are tropical fruits with a short shelf life due to their high content of water, 82.40-92.34 % for RFDF and 92 % for SF (Alam *et al*., 2023; Arivalagan *et al*., 2021; Payal *et al*., 2012).

Current results showed a significant difference between the phases of the *in vitro* digestion for TPC released and their antioxidant capacity. The highest TPC was found during the intestinal phase for all RFDF and SF based beverages; dehydrated forms exhibited higher TPC than fresh and frozen forms. Findings showed an effect of the preservation method on the food matrix and then in the bioaccessibility of polyphenols. The bioaccessibility of bioactive compounds is dependent on the type of preservation method, the type of compound, and the food matrix; furthermore, the bioaccessibility of the polyphenols is also influenced by their interactions with each other and their solubilisation during digestion (Cilla *et al*., 2018; Parada and Aguilera, 2007).

The increase in TPC after gastric digestion shown during this study has been reported for different fruit-based products such as grape molasses, apricot and plum leathers and vegetable juices (Kamiloglu and Capanoglu, 2014; Wootton-Beard, Moran and Ryan, 2011). The results obtained from all the forms of fruits evaluated in this study were higher than those found in grape molasses and tomato juice and similar to the amount reported for the apricot leather (Kamiloglu and Capanoglu, 2014; Wootton-Beard, Moran and Ryan, 2011). The intestinal phase involves a marked increase in pH from a

pH of 1.5-3.0 in the gastric phase to 8.0 in the intestinal phase that allows the release of phenolic compounds from the remaining food matrix (Desseva and Mihaylova, 2019).

In this study, the highest FRAP for all beverages was found during the intestinal phase at 120 minutes, meanwhile DPPH radical inhibition occurred during the gastric phase for fresh, frozen, and dehydrated RFDF and SF based beverages. A strong, positive correlation was found between TPC and FRAP concurring with previous *in vitro* studies that have reported a positive relation between the antioxidant capacity and the TPC released (Laya and Koubala, 2020; Aguillón-Osma *et al*., 2019). Free radical scavenging has been related to pH changes, so each digestion phase within their particular pH could determine the antioxidant capacity from the released phenolic compounds (Ghosh, Chakraborty and Raychaudhuri, 2015).

This study showed that the polyphenol release and the antioxidant capacity of red flesh dragon fruit (*Hylocereus polyrhizus*) and star fruit (*Averrhoa carambola*) based beverages, change between phases of *in vitro* digestion for dehydrated, fresh, and frozen fruit based beverages. Bioaccessibility of phenolic compounds differ in accordance with the fruit and the food matrix used to prepare the products; frozen dragon fruit based beverage and dehydrated star fruit based product exhibited the highest accessible amount of polyphenol at the end of the *in vitro* digestion, providing them a potential use as functional foods.

The main findings during sensory evaluation in this study, were that appearance, colour, and taste were the main attributes to contribute to the overall acceptance of fresh and frozen fruit based beverages. Although the dehydrated fruit based beverages were the products with highest release of polyphenols, they were ranked as ‘dislike slightly’ for overall acceptance. These results could be due to polyphenols associated with bitterness, such as (-)-epicatechin or procyanidin that activate the bitter taste receptors, TAS2Rs (Jaeger *et al*., 2009; Soares *et al*., 2013). Functional foods are reported to have lower

acceptability among consumers when the acceptance test is carried out without informing them of the potential benefits that are related to the product tested (Vidigal *et al*., 2011). Therefore, if participants had known the potential positive effects, this may have increased the liking of the dehydrated fruit-based beverages.

Sensory evaluation showed a food matrix effect on sensory attributes. Appearance, colour, taste, and viscosity are key attributes that influenced the sensory acceptance of beverages based on dragon fruit and star fruit. Higher liking scores were found in frozen dragon fruit based beverage for appearance, colour, taste, and overall acceptance. Fresh and frozen star fruit beverages reached the highest scores for viscosity and mouthfeel.

This is the first study to date to investigate the effect of *in vitro* digestion on polyphenols and antioxidant capacity of red flesh dragon fruit and star fruit based products using fresh, frozen, and dehydrated fruit. This study is also the first to explore the sensory attributes of these products. In light of the findings of this study, further research is needed to determine the bioavailability of phenolic compounds in these beverages and their effect in human studies.

3.5 Limitations

A limitation for sensory evaluation was that the recruited participants were not trained to conduct an acceptance study, and that those at risk of T2D were not considered to take part in the trial. The key limitation in this study is the lack of power.

3.6 Conclusion

This study suggests, based on the amount of TPC released from frozen RFDF and dehydrated SF based beverages, post *in vitro* digestion, that these beverages may have potential health benefits.

Chapter 4: Star fruit and health, a systematic review of *in vivo* studies.

4.1 Introduction

Averrhoa carambola, a tropical fruit known as carambola or star fruit (SF) due to its five-point star shape has been reported as a folklore or traditional medicine used in tropical and subtropical countries to manage certain health conditions (Saha, Guite and Das, 2018). The use of *Averrhoa carambola*'s fruits, leaves and roots has been stated as part of communities' traditions to treat diabetes, and hypertension, among other conditions (Saghir *et al*., 2013). SF is shown to be a rich source of vitamins and minerals as well as different bioactive compounds including some polyphenols and fibre linked to its beneficial properties on human health (Muthu *et al*., 2016). Some studies reported antioxidant capacity as well as antihyperglycemic, antihyperlipidemic and antihypertensive properties. Yang *et al*., (2015) linked its polyphenol content to possible positive effects on health. Fibre also has a beneficial role on glucose metabolism, as soluble dietary fibre, such as inulin and pectin could control the levels of blood glucose probably due to the formation of viscous solutions (Goof *et al*., 2018).

Despite the above-mentioned health benefits, intoxication after SF consumption has been stated in different case reports linked with the content of oxalic acid and caramboxin (Wang *et al*., 2006; Stumpf *et al*., 2020). Nausea and vomiting are some of the common symptoms showed by patients after SF or SF-based products such as juice consumption (Ruhan *et al*., 2018; Chang and Yeh, 2004; Abeysekera *et al*., 2015). Acute kidney injury has been reported in individuals with normal renal function after SF consumption on an empty stomach (Ruhan *et al*., 2018). Those with T2D have been diagnosed with induced oxalate nephropathy and acute kidney injury after half SF consumed the day before and the consumption of 200 mL of SF juice obtained from six pieces of fruit, respectively (Chang and Yeh, 2004; Abeysekera *et al*., 2015).

Haemodialysis has been used to treat SF toxicity with successful recovery of the patients according to Barman *et al*., (2016) and Stumpf *et al.*, (2020). The risk of mortality from SF consumption has been reported as high in individuals with kidney disease such as nephropathy due to the severity of the symptoms (Chua *et al*., 2017).

Different reviews have been published on SF in recent years. Lakmal *et al.*, (2021) reviewed case reports, animal, and *in vitro* studies to describe the nutritional, phytochemical, and medicinal characteristics of various parts of *Averrhoa carambola* including fruit, leaves, and roots. Original reports and case series were selected to review the symptoms related to SF intoxication as well as treatment strategies (Aranguren, Vergara and Rosselli, 2017). The mechanisms of the toxicity exhibited by SF have also been reported (Yasawardene *et al*., 2020). However, a systematic review on the benefits of SF on health has not been done; furthermore, the results obtained from the sensory evaluation in this thesis (Chapter 3), showed that SF based beverages were the least preferred, so SF was selected to be investigated in this Chapter. The aim of this review was to identify and evaluate human and animal studies carried out *in vivo* to determine the evidence on health effects of SF and SF-based products.

4.2 Methods

A protocol was designed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page *et al*., 2021) and registered on International Prospective Register of Systematic Reviews Database (PROSPERO), CRD42021272234 for human studies and CRD42021272263 for animal studies. The search, study selection, data collection processes, and the quality of studies selected were assessed independently by two reviewers. Any discrepancy was discussed by a third reviewer before a decision was made.

4.2.1 Eligibility criteria

4.2.1.1 Inclusion

SF and SF-based products.

4.2.1.1.1 Human studies

- Randomized controlled trials and experimental design, such as crossover or repeated measures studies in adults.
- Studies that include baseline (prior treatment), studies that had another intervention, or group without intervention were accepted.

4.2.1.1.2 Animal studies

- *In vivo* experimental and controlled studies in all animal species.
- Studies that include baseline measurements, studies that had another intervention and/or used vehicle treated animals and non-exposed control group were selected.

4.2.1.2 Exclusion

Studies written in languages other than English and studies including SF leaves, roots, stems, and products not based on fruit were excluded. Observational studies, reviews, conference abstracts, *in vitro*, *ex vivo*, silico models, and pre-clinical studies were not included, since there are reviews including these criteria. Studies involving sham-treated animals were excluded.

4.2.2 Search strategy

Academic Search Complete, PubMed, and Web of Science databases were used by two independent reviewers to search for the following search terms to retrieve results in English from 1970 to August 2021: "*Averrhoa carambola*" OR "star fruit" OR starfruit OR carambol*; health; favourable OR benefi* OR positiv*; unfavourable OR negative OR toxi*; diabet* OR glyce*; hypertens* OR "blood pressure". The complete search strategy is presented in Table 4.1.

Table 4.1 Search strategy, terms and expanders (*).

Search Number	*Terms and expanders (*)*
1	"*Averrhoa carambola*" OR "star fruit" OR starfruit OR carambol*
2	Health
3	Favourable OR benefi* OR positiv*
4	Unfavourable OR negative OR toxi*
5	3 OR 4
6	2 AND 5
7	Diabet* OR glyce*
8	Hypertens* OR "blood pressure"
9	7 OR 8
10	6 OR 9
11	1 AND 10

4.2.3 Study selection, data collection process and data items

Titles and abstracts were screened before retrieving full texts to assess the inclusion criteria. For human studies, author, study title, year of publication and country, study design and sample size, characteristics of participants such as age and gender as well as the intervention and outcomes were extracted. Animal model, age and sex, number of groups and animals per group, intervention, and outcomes were obtained for animal studies.

4.2.4 Study risk of bias assessment

For human studies the Cochrane Effective Practice and Organisation of Care (EPOC) (2017) risk of bias (RoB) criteria was used to evaluate the quality of before-after studies assessing eight domains (random sequence generation, allocation concealment, baseline outcome measurements similar, baseline characteristics similar, incomplete outcome data, knowledge of the allocated interventions adequately prevented during study, protection against contamination, and selective outcome reporting), meanwhile RCTs were evaluated for six different domains (randomization process, deviation from intended interventions, missing outcome data, measurement of the outcome, selection of

the reported result, and overall bias) by the Cochrane risk of bias 2 (RoB2) tool; single-case studies also known as repeated measures studies were assessed to determine if they meet the What Works Clearinghouse (WWC) standards (What Works Clearinghouse, 2020). Animal studies were assessed by the Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias (SYRCLE RoB) tool (Hooijmans *et al.*, 2014).

4.3 Results

From 785 titles retrieved, 771 were excluded after the screening process and 14 reports were retrieved for full text screening. A total of 10 studies were selected to be included in the review, four human studies and six animal studies (Figure 4.1). The findings were separated into two groups: one for the effect of SF or SF-based products on human health and the other for the effect on different animal models.

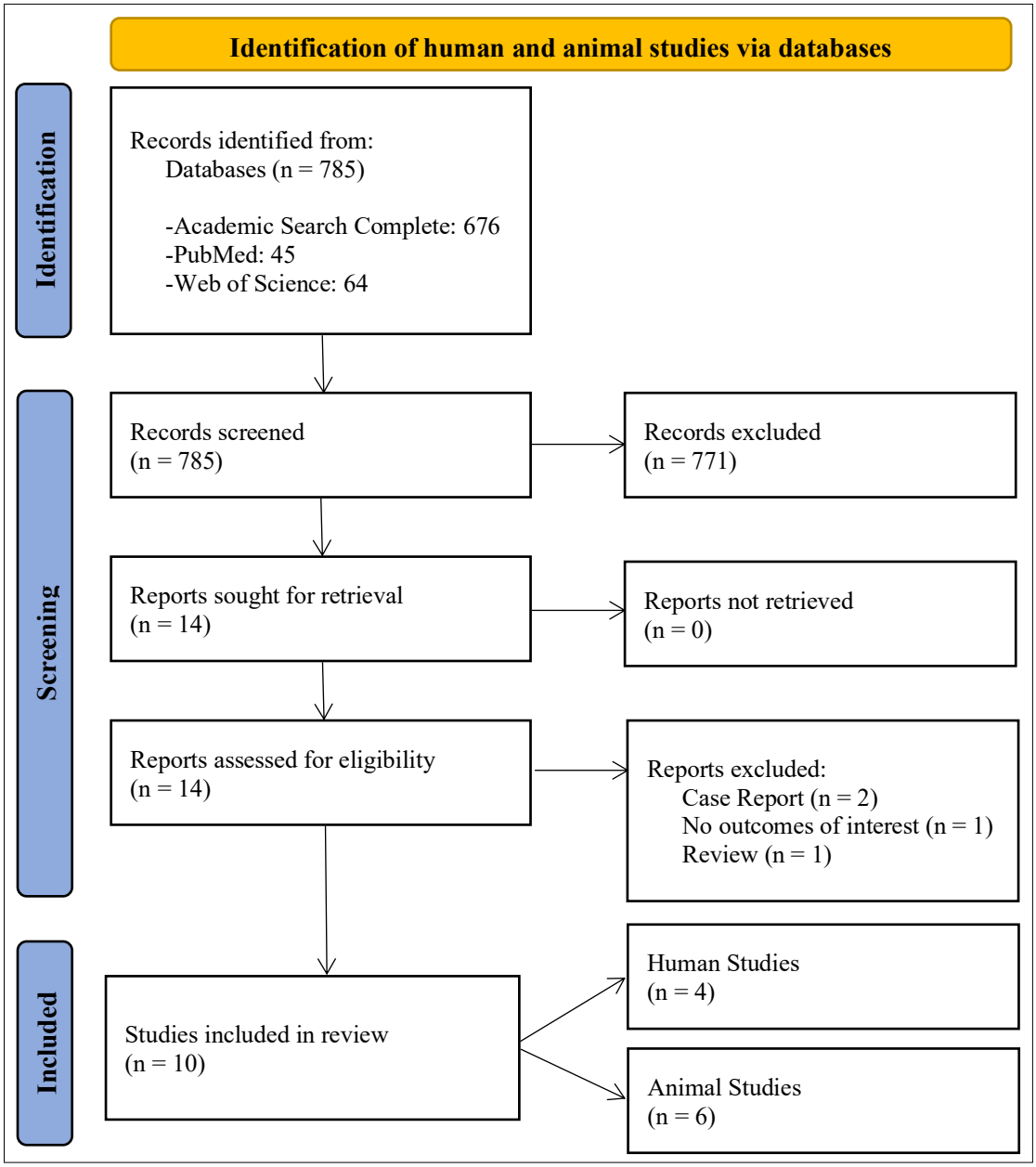


Figure 4.1 PRISMA flow diagram for identification of studies (Source: Page *et al.*, 2021).

4.3.1 Human studies

Four studies evaluating SF based products in adult participants published between 2015 and 2020 met the inclusion criteria (Table 4.2). One study (Artana *et al.*, 2020) was carried out in Indonesia as a controlled before-after study. The remaining three were conducted in Thailand, one as a randomized control trial (Pothasak *et al.*, 2020) and two more as repeated measures studies (Leelarungrayub *et al.*, 2016; Leelarungrayub *et al.*,

2015). A total of 149 participants from 18 to 82 years were evaluated to determine changes in different health outcomes such as blood pressure, oxidative stress, or inflammatory biomarkers, and walking distance (WD) as measurement of physical capacity, after consuming SF juice or a SF-based product.

Intervention description, population, and the outcomes obtained from the studies included in this review are shown in Table 4.2. A single study (Pothasak *et al*., 2020) determined the effects of a SF-based product, meanwhile the other three studies evaluated SF juice effects (Artana *et al*., 2020; Leelarungrayub *et al*., 2016; Leelarungrayub *et al*., 2015). Participants were subjects with hypertension (Artana *et al*., 2020), stable chronic obstructive pulmonary disease (COPD) (Pothasak *et al*., 2020), and healthy older participants (Leelarungrayub *et al*., 2016; Leelarungrayub *et al*., 2015). Blood pressure was determined in a short-term study, one hour after consumption (Artana *et al*., 2020). Total antioxidant capacity (TAC), plasma vitamins, and oxidative stress biomarkers such as malondialdehyde (MDA) and protein hydroperoxide (PrOOH) were determined after four-week consumption of a SF-honey product and SF juice (Pothasak *et al*., 2020; Leelarungrayub *et al*., 2016). Two studies (Pothasak *et al*., 2020; Leelarungrayub *et al*., 2015) evaluated the concentration of different inflammatory markers, such as tumour necrosis factor alpha (TNF-α) or interleukin-23 (IL-23), after four-week interventions with the SF products. Lipid profile was determined in a four-week study (Leelarungrayub *et al*., 2016) and the effect of SF juice and SF-honey on WD in elderly and participants with COPD was evaluated after four weeks (Pothasak *et al*., 2020; Leelarungrayub *et al*., 2015).

Artana *et al*., (2020) found that participants with hypertension that consumed 200 mL of SF juice or 200 mL of watermelon juice reduced systolic and diastolic pressure significantly after one hour of consumption; however, when SF juice was mixed with an equal volume of watermelon juice the blood pressure had reverted to normal levels. A

SF-honey product was studied on participants with stable COPD to evaluate its effect combined with and without exercise (walking daily for 30 minutes) and to compare it with an only-exercise group and a group without treatment (Pothasak *et al*., 2020). After four weeks of consuming 150 mL of warm water containing 10 g of the SF-honey product twice daily, the amount of vitamin C in plasma increased similar to the group that combined the SF-honey consumption with exercise. The TAC in plasma increased in both groups receiving the SF-honey product, however, the values where higher in the group that combined exercise and SF-honey. The levels of TNF-α, an inflammatory cytokine, as well as the oxidative stress biomarker MDA, were significantly reduced in the groups that received the SF-honey product with and without exercise when compared to the only-exercise group. WD rose significantly in the SF-based product with exercise group when compared to the group without treatment.

SF juice prepared from 100 g of fruit and taken twice a day for four weeks significantly increased the TAC and the content of vitamins A and C in plasma of healthy elderly participants (Leelarungrayub *et al*., 2016). The levels of oxidative stress markers such as MDA and PrOOH decreased significantly, as well as the low-density lipoprotein-cholesterol (LDL-C) after four weeks. However, there was no difference in triglycerides and total cholesterol levels when compared to baseline. Finally, a study involving older participants (Leelarungrayub *et al*., 2015) showed a significant increase in the WD and a significant decrease for TNF-α, IL-23, and nitric oxide (NO) after four weeks of intervention with 100 g of SF juice ingested twice a day.

Table 4.2 Human studies design, participants' characteristics, and outcomes.

Author and Year of Publication	*Title*	*Country*	*Study Design*	*Intervention*	*Population*	*Sample Size*	*Age and Gender of Participants*	*Outcomes*
Artana et al.,								
2020	Traditional therapy of watermelon-starfruit juice for reducing blood pressure of hypertension.	Indonesia	Controlled before-after study	200 mL -Watermelon juice (n=11): 100 g +100 mL of water, once. -Starfruit juice (n=11):100 g + 100 mL of water, once. -Watermelon-starfruit juice (n=11): 100 g watermelon + 100 g starfruit + 100 mL of water, once.	Hypertensive (systolic ≥ 140 mm Hg, diastolic ≥ 90 mm Hg)	33	Over 18 years Males and females	↓ Systolic and diastolic pressure.
Pothasak et al.,								
2020	Prototype star fruit-honey product and effectiveness on antioxidants, inflammation and walking distance in participants with stable chronic obstructive pulmonary disease (COPD).	Thailand	Randomised trial	-Control group (n=10). -Supplement group (n=20): 10g in 150 mL of warm water twice daily, 4 weeks. -Exercise group (n=15): walking 30 min daily, 4 weeks. -Supplement + Exercise group (n=15), 4 weeks.	Stable COPD	60	49 – 82 years 31 males, 29 females	↑ Total antioxidant capacity. ↑ Vitamin C in plasma. ↓ MDA. ↓ TNF-α. ↑ 6-minutes WD.

Leelarungrayub et al., 2016	A preliminary study of the effects of star fruit consumption on antioxidant and lipid status in elderly Thai individuals.	Thailand	Repeated measures study	100 g Star fruit juice twice daily, 4 weeks.	Healthy elderly	27	69.5 ± 5.3 years 19 males, 8 females	↑ Total antioxidant capacity. ↓ MDA and PrOOH. ↑ Vitamin A, C, and E in plasma. Cholesterol, triglycerides, ↑ HDL-C ↓ LDL-C.
Leelarungrayub et al., 2015	Consumption of star fruit juice on pro-inflammatory markers and walking distance in the community dwelling elderly.	Thailand	Repeated measures study	100 g Star fruit juice twice per day after meals, 4 weeks.	Healthy elderly	29	72.4 ± 8.3 years 20 males, 9 females	↓ TNF-α, IL-2, ↓ IL-23. ↓ Nitric oxide. ↑ 6-minutes WD.

↓: reduced; ↑: increased; mm Hg: millimetres of mercury; COPD: chronic obstructive pulmonary disease; MDA: malondialdehyde; TNF-α: tumour necrosis factor alpha; WD: walking distance; PrOOH: protein hydroperoxide; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; IL-2: interleukin 2; IL-23: interleukin-23.

4.3.2 Animal studies

Six studies testing SF in animal models, which were published between 2005 and 2021, met the inclusion criteria (Table 4.3). Two studies were conducted in America, one in Brazil (Rodrigues da Silva *et al*., 2021) and one in Mexico (Herman-Lara *et al*., 2014), meanwhile, four more studies were carried out in Asia, two in China (Pham *et al*., 2017; Zhang *et al*., 2016), one in Malaysia (Khoo *et al*., 2010), and one in Taiwan (Chau, Chien and Chen, 2005). A total of 401 animals including rats (Rodrigues da Silva *et al*., 2021; Khoo *et al*., 2010), mice (Pham *et al*., 2017; Zhang *et al*., 2016; Herman-Lara *et al*., 2014) and hamsters (Chau, Chien and Chen, 2005) were studied. A nephropathy induced model was used to evaluate SF toxic effects (Rodrigues da Silva *et al*., 2021), meanwhile, streptozotocin induced diabetic and hypercholesteraemic animals were used to determine star fruit´s effects on lipid profile (Pham *et al*., 2017; Herman-Lara *et al*., 2014). Ethanol metabolism was evaluated in animals with induced acute alcohol intoxication (Zhang *et al*., 2016), meanwhile, healthy animals were studied to assess the toxic and intestinal health effects of SF-based products (Khoo *et al*., 2010; Chau, Chien and Chen, 2005), respectively.

Rodrigues da Silva *et al*., (2021) evaluated the effect of SF in rats with and without induced nephropathy by surgery; one millilitre of SF juice was administered four times during the first day, and three times during the second day. Creatinine and urea were determined in plasma after two days of a gavage procedure and the nephropathic groups showed a significant increase in both parameters; nevertheless, adverse effects were showed by rats with induced nephropathy as epileptic seizures were noticed.

Three doses (100, 50, and 25 g/Kg of body weight) of SF juice were administered via gavage for 21 days to streptozotocin induced diabetic mice and compared to the normal (healthy) and the model (diabetic) groups (Pham *et al*., 2017). A significant decrease was found in different parameters such as fasting blood glucose, triglycerides, and total

cholesterol in the groups given high (100 g/kg body weight) and moderate (50 g/kg body weight) dosages of SF juice, when compared to the model group (diabetic plus distilled water). A reduction of the oxidative stress was found, as the liver tissue MDA, an indicator of free radical injury (Atiba *et al*., 2016), was significantly reduced in the group receiving the high dose of SF juice and the hepatic superoxide dismutase (SOD) showed a significant increase in the groups receiving the high and the moderate dosages of SF juice when compared to the model group.

Zhang *et al*., (2016) determined the effect of 20 different fruits in mice with induced ethanol intoxication, including SF in two independent studies. The first study showed a significant decrease in the concentration of ethanol in the blood of the mice after two hours of the consumption of 10 mL/kg body weight of SF juice and a significant increase in the acetaldehyde concentration, showing that the ethanol has been metabolized. Hepatic activity of alcohol dehydrogenase (ADH) decreased slightly, meanwhile, acetaldehyde dehydrogenase (ALDH) decreased significantly. The second study investigated the effect of the SF juice on alanine transferase (ALT) and aspartate transaminase (AST) activities in serum as well as hepatic MDA and SOD. The results found that a dose of 12 mL/kg of body weight of the SF juice had a positive effect as it resulted in the decrease in AST activity, as well as in the level of MDA after six hours of consumption.

A cookie with five percent of the micronized insoluble fibre fraction (IFF) of SF and a diet containing 50 g of the IFF of SF per kilogram, were investigated in mice; both formulations included cholesterol and were compared with a positive diet (cholesterol) and a negative diet (no cholesterol, no fibre) (Herman-Lara *et al*., 2014). After 30 days, there was a significant decrease in serum levels of triacylglycerols, total cholesterol, high-density lipoproteins (HDL), and low-density lipoproteins (LDL) in both groups with SF treatment comparing them with the positive diet control group.

A study to evaluate the relationship between the storage period on the properties of SF juice and its toxicity in female rats was carried out (Khoo *et al*., 2010). After 14 days of treatment, a significant rise of ALT levels in animals treated with 10 mL of SF juice per kilogram of body weight stored for three hours at room temperature showed toxic effect on liver when compared to those that received distilled water as a treatment.

Finally, four diets comparing the effect of a fibre free diet, versus a diet with cellulose (5 %), a diet with the IFF from SF (5.79 %) and a diet with the IFF from carrot (5.69 %) were studied in healthy hamsters (Chau, Chien and Chen, 2005). After 30 days of consumption, different intestinal enzymes were analysed. Ileum enzymes such as maltase, lactase and sucrose were evaluated without significant difference between groups. Diets with fibre reduced cecal pH significantly, meanwhile diets with insoluble fibre from SF and carrot diminished the cecal and fecal ammonia. Activity of beta (β)-glucoronidase, β-glucosidase and urease (faecal bacterial enzymes) was significantly reduced by diets including SF and carrot insoluble fibre, indicating an improvement in the intestinal health.

Table 4.3 Animal studies design and animal's characteristics.

Author and Year of Publication	***Title***	***Country***	***Study Design*** *(Number and type of experimental groups / Number of animals per group)*	***Animal Model*** *(Species/Age/Sex)*	***Intervention*** *(Type/Dose/Duration)*	***Primary Outcomes*** *(Body weight / Blood, Urine or Faeces)*
Rodrigues da Silva et al.,						
2021	Standardization of experimental model regarding star fruit intoxication in Wistar rats suffering with nephropathy	Brazil	1. Shaw (n=5). 2. Water gavage (n=5). 3. Star fruit juice gavage (n=5). 4. Nephropathic group with water gavage (n=5). 5. Nephropathic group with star fruit juice gavage (n=5).	25 Wistar rats / adult / male	1. Shaw. 2. Water gavage on day 9 and 10. 3. Star fruit juice gavage on day 9 (1 mL, 4 times a day) and 10 (1 mL, 3 times a day). 4. Nephropathic group (surgery on day 8) with water gavage on day 9 and 10. 5. Nephropathic group (surgery on day 8) with star fruit juice gavage on day 9 (1 mL, 4 times a day) and 10 (1 mL, 3 times a day). Electrode implant on day 1	↑ Urea and creatinine. Electroencephalographic records.
Pham et al.,						
2017	Effects of *Averrhoa carambola* L. (*Oxalidaceae*) juice mediated on hyeprglycemia,	China	1. Normal control (n=10). 2. Model control (n=10). 3. Metformin control (n=10).	60 Kunming mice / male	1. Normal control: healthy and distilled water. 2. Model control: diabetic and distilled water. 3. Metformin control: diabetic and metformin	Day 0, 7, and 21: ↓ FBG and body weight. Day 21: ↓ TC and TG.

	hyperlipidemia, and its influence on regulatory protein expression in the injured kidneys of streptozotocin-induced diabetic mice		4. High extract *Averrhoa carambola* juice, 100g/Kg body weight (n=10). 5. Moderate extract *Averrhoa carambola* juice, 50g/Kg body weight (n=10). 6. Low extract *Averrhoa carambola* juice, 25g/Kg body weight (n=10).		(320 mg/Kg body weight) for 21 days. 4. High: diabetic and EACJ (100g/Kg body weight) for 21 days. 5. Moderate: diabetic and EACJ (50g/Kg body weight) for 21 days. 6. Low: diabetic and EACJ (25g/Kg body weight) for 21 days.	HDL-C, LDL-C. ↓ Scr (CREA) and BUN. ↓ MDA, ↑ SOD in liver. ↑ Fasting blood insulin. Kidney and pancreatic tissues.
***Zhang et al.*,** 2016	Effects of 20 selected fruits on ethanol metabolism: potential health benefits and harmful impacts	China	<u>Study 1:</u> 1. Control (n=6). 2 - 21. 20 fruit groups (n=6). <u>Study 2:</u> 1. Blank control (n=5). 2. Model (n=5).	<u>Study 1:</u> 126 Kunming mice / 7 week / male <u>Study 2:</u> 110	<u>Study 1:</u> All groups: 52 % ethanol (4g/Kg body weight). 1. Control: water (10ml/Kg body weight). 2 - 21. Fruit groups: juice (100g fruit for 60 Kg body weight). <u>Study 2:</u> 1. Blank control: water (0.2 mL) Group 2 - 22: 52 % Ethanol (6g/Kg body	<u>Study 1:</u> Two hours after: ↓ Ethanol in blood. ↑ Acetaldehyde in blood. ↓ ADH and ALDH in liver. <u>Study 2:</u> Six hours after: ↓ ALT and AST in serum. SOD and ↓ MDA in liver.

			3 - 22: 20 fruit groups (n=5).		weight). After 30 minutes: 2. Model: water (12 mL/Kg body weight). 3-22: Fruit groups: juice (12 mL/Kg body weight).	
Herman-Lara et al.,						
2014	Impact of micronized starfruit (*Averrhoa carambola* L) fibre concentrate on lipid metabolism in mice	Mexico	1. Negative control (n=7). 2. Positive control (n=7). 3. IFRF (n=7). 4. FF (cookie) (n=7).	28 Mice strain C57BL/6 / 6-7 weeks / male	1. Negative control diet *ad libitum* for 30 days. 2. Positive control diet with 1 % cholesterol *ad libitum* for 30 days. 3. IFRF diet with 1 % cholesterol and 50g IFRF/Kg *ad libitum* for 30 days. 4. FF diet with 1 % cholesterol and 128g FF/Kg with 5 % of IFRF *ad libitum* for 30 days.	Body weight weekly ↓ Cholesterol and triglycerides. ↓ HDL and LDL. Liver histological analysis RESULTS
Khoo et al.,						
2010	Evaluation of the toxic effect of star fruit on serum biochemical parameters in rats	Malaysia	1. Control (n=5). 2. Fresh *Averrhoa carambola* juice (n =5). 3. *Averrhoa carambola* juice at 25 ± C for 1 hour.	20 Sprague Dawley rats / 14 weeks / female	1. Control: water 2. Fresh *Averrhoa carambola* juice, 2 mL once a day for 14 days. 3. *Averrhoa carambola* juice at 25 ± C for 1 hour, 2 mL once a day for 14 days.	Day 0, 3, 7, and 14: Body weight, food consumption and water intake. Day 15:

			4. *Averrhoa carambola* juice at 25 ± C for 3 hours.		4. *Averrhoa carambola* juice at 25 ± C for 3 hours, 2 mL once a day for 14 days.	↑ ALT. AST, ALP. ↓ Urea. Creatinine in serum.
Chau, Chien and Chen,						
2005	Influence of insoluble fibre fractions from carambola and carrot on intestinal enzymes and faecal bacterial enzymes in hamsters	Taiwan	1. Fibre-free (n=8). 2. Cellulose (n=8). 3. Carambola (n=8). 4. Carrot (n=8).	32 Golden Syrian hamsters /6 weeks old / male	1. Fibre-free diet *ad libitum* for 30 days. 2. Diet with 5 % of cellulose *ad libitum* for 30 days. 3. Diet with 5.79 % of carambola insoluble fibre fraction *ad libitum* for 30 days. 4. Diet with 5.69 % of carrot insoluble fibre fraction *ad libitum* for 30 days.	Body weight every 48 hours. Intestinal disaccharidase. ↓ Cecal pH and ammonia. ↓ ALP in serum. ↓ Faecal bacterial enzymes. ↓ Faecal ammonia.

↓: reduced; ↑: increased; FBG: fasting blood glucose; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; Scr(CREA): serum creatinine; BUN: blood urea nitrogen; ADH: alcohol dehydrogenase; ALDH: acetaldehyde dehydrogenase; ALT: alanine aminotransferase; AST: aspartate transaminase; SOD: superoxide dismutase; MDA: malondialdehyde; IFRF: insoluble fibre-rich fraction; FF: functional food; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALP: alkaline phosphatase.

4.3.3 Risk of bias

The quality of human studies found a high risk of bias for Artana *et al*., (2020) before-after study in two of eight different domains due to the study design characteristics according to the Cochrane EPOC RoB tool; random sequence generation and allocation concealment were the domains with high risk. The Pothasak *et al*., (2020) trial showed a high risk of bias in the following domains: randomization process, deviation from intended interventions and selection of the reported result, generating a high risk overall. Two studies (Leelarungrayub *et al*., 2016; Leelarungrayub *et al*., 2015) showed a high risk of bias since two domains (the inter-assessor agreement and the attempts to demonstrate effect over time) indicated reservations to meet the WWC standards.

The risk of bias for animal studies was assessed by the SYRCLE RoB's tool, evaluating ten different entries within six types of bias (Table 4.4). Two studies, Rodrigues da Silva *et al*., (2021) and Pham *et al*., (2017), showed high risk in five entries regarding sequence generation, allocation concealment, blinding, random outcome assessment, and outcome assessor blinding, as expected. Baseline characteristics were unclear for five studies (Rodrigues da Silva *et al*., 2021; Pham *et al*., 2017; Zhang *et al*., 2016; Herman-Lara *et al*., 2014; Chau, Chien and Chen, 2005), meanwhile, the studies developed by Zhang *et al*., (2016), Herman-Lara *et al*., (2014), Khoo *et al*., (2010), and Chau, Chien and Chen (2005), exhibited a low risk of bias for sequence generation, incomplete outcome data, selective outcome reporting, and other sources of bias. Blinding was the entry with a high risk for all animal studies.

Table 4.4 SYRCLE's risk of bias for animal studies.

Study	*Selection bias*			*Performance bias*		*Detection bias*		*Attrition bias*	*Reporting bias*	*Other*
	Sequence generation	*Baseline characteristics*	*Allocation concealment*	*Random housing*	*Blinding*	*Random outcome assessment*	*Outcome assessor blinding*	*Incomplete outcome data*	*Selective outcome reporting*	*Other sources of bias*
Rodrigues da Silva et al., *2021*	H	U	H	U	H	H	H	U	U	L
Pham et al., *2017*	H	U	H	U	H	H	H	U	L	L
Zhang et al., *2016*	L	U	H	U	H	U	H	L	L	L
Herman-Lara et al., *2014*	L	U	H	L	H	H	H	L	L	L
Khoo et al., *2010*	L	L	H	U	H	H	H	L	L	L
Chau, Chien and Chen, *2005*	L	U	L	H	H	U	H	U	L	L

H: high risk of bias; L: low risk of bias; U: unclear.

4.4 Discussion

This systematic review was conducted to evaluate the effects of SF intake on health outcomes in human and animal studies. Overall, this systematic review has demonstrated a beneficial health effect on adults and animal models after consumption of SF based products, as well as adverse effects in animal models.

According to the WHO, hypertension is a health condition where blood pressure values are higher than 90 mm Hg (diastolic) and 120 mm Hg (systolic), with its prevalence increasing worldwide in recent years (World Health Organization, 2023_Hypertension). SF juice consumption by a hypertensive population showed a decrease in systolic and diastolic blood pressure one-hour post consumption (Artana *et al*., 2020). Authors suggested that the results obtained may be due to the potassium concentration, which has been reported to be 167-168 mg/100 g in SF (Muthu *et al*., 2016). Potassium, an essential mineral that is also known as an electrolyte because of its positive charge when in a solution, plays a role in the balance of fluids in the body alongside sodium; the former inside and the latter outside the cells. Potassium rich food has shown to have a hypotensive effect, and this is because of the role that potassium plays in regulating blood pressure (Whelton, 2018; Castañeda-Bueno, Ellison and Gamba, 2022); the intake of high levels of potassium and low levels of sodium has been linked to low blood pressure (Jackson *et al*., 2018). The non-balance produced between potassium-sodium concentration will cause a greater amount of sodium excreted in the urine (Koo *et al*., 2018) which also increases its volume contributing to reduce blood pressure. Filippini *et al*., (2020) suggested that potassium supplementation in individuals with hypertension may reduce blood pressure; the above should be done following the recommendations made by the WHO and other organizations such as the United Kingdom National Health System (NHS) considering an adult intake of potassium of 90 mmol per day to reduce blood pressure.

Dietary fibre intake has shown a beneficial effect on adults with hypertension as it helps to reduce high blood pressure; different mechanisms have been linked to this effect, such as the relationship of fibre with improving insulin sensitivity and the elasticity of blood vessels, as well as the role of nitric oxide produced after fibre intake in vasodilation (Reynolds *et al*., 2022). SF is rich in water-insoluble fibre fractions (46.0-58.2 %), mainly pectin and hemicellulose with greater cation exchange and water uptake capacity than cellulose (Saghir *et al*., 2013; Chau, Chien and Chen, 2005), this type of fibre is also known as non-fermentable as it is not-used by the intestinal microbiota (Aleixandre and Miguel, 2008); insoluble fibre has been related to the reduction of total-cholesterol and LDL-cholesterol, probably due to the fibre link to the bile and cholesterol in the intestine, also it is suggested that the presence of polyphenols may contribute to this activity (Zunft, Koebnick, and Haber, 2004).

SF juice intake and SF-honey consumption exhibited an increase in the TAC and the vitamin C levels, and a decrease in the concentration of the oxidative stress marker, MDA, in plasma, after four-week trials in two different populations, older people and individuals with stable COPD (Leelarungrayub *et al*., 2016; Pothasak *et al*., 2020). Both authors suggested that antioxidants present in star fruit, such as polyphenols and vitamin C, might exert the positive effect on oxidative stress and provided anti-inflammatory capacity. Findings showed the benefits of consumption of SF by elderly individuals and the use of SF as a functional ingredient to contribute to reduce chronic inflammation and levels of oxidative stress that characterizes older and COPD individuals (Shing *et al.,* 2017).

Animal trials used hamsters, rats and mice as models to assess the effects of SF juice consumption and the insoluble fibre fraction from SF intake. Favourable effects of dietary fibre in health are well known, some types can influence the microflora (Shiau and Chang, 1983; Gill *et al*., 2021) and others may show benefits on blood pressure and

lipid profiles (Streppel *et al*., 2005). Fibre consumption has been used as an adjuvant in the management of chronic conditions such as T2D and hypertension (Kendall, Esfahani and Jenkins, 2010). Chau, Chien and Chen (2005) found positive effects on intestinal function in hamsters after receiving the insoluble fibre fraction (pectic substances and hemicellulose) from SF for a 30-day period. Fecal bacterial enzymes, β-glucoronidase and β-glucosidase, are related to the production of toxic metabolites (Walsh *et al*., 2020), thus, the reduction caused by the diets containing the SF insoluble fibre fraction, indicates an improvement in the intestinal health. Insoluble fibre fractions from SF have been shown to reduce the total cholesterol, triglycerides, HDL and LDL in mice when consumed for 30 days (Herman-Lara *et al*., 2014). These results could be due to interactions between fibre and lipids, preventing intestinal absorption. Diverse mechanisms have been proposed for the above, from a reduction in the transit time, to lipid-drops coating, to an increase in the bile production directly linked to the cholesterol metabolism (Chen, Jiao and Ma, 2008; Mun *et al*., 2006; Herman-Lara *et al*., 2014).

SF juice consumption has been found to improve the fasting blood glucose and lipid profile in diabetic mice after 21 days (Pham *et al*., 2017). The hypoglycaemic effect may be due to the high fibre concentration in the fruit and/or the presence of polyphenols; meanwhile, the hypocholesterolaemic activity could be mediated by micronized fibre (Lakmal *et al*., 2021). Zhang *et al*., (2016) found a positive effect in mice when assessing the intake of SF fruit juice on ethanol metabolism. Findings showed a significant decrease in the concentration of ethanol in blood, and a significant increase in the blood acetaldehyde levels; however, there were no correlation between ethanol concentration and ALDH, so, the results exhibited in this study may be due to changes in the absorption of ethanol. Despite the above, authors suggested the potential of SF to be used to formulate functional foods due their bioactive compounds. The

presence of polyphenols in star fruit has been documented (Muthu *et al*., 2016), as well as the positive effect of polyphenols on the damage caused by chronic alcohol consumption (Fiore *et al*., 2020); so, further research is needed to elucidate the mechanism of action of star fruit juice on the reduction of ethanol in blood.

ALT levels were increased after 14 days of consumption of SF juice stored for three hours at room temperature (Khoo *et al*., 2010). The presence of the ALT in blood indicates liver injury or disease (Lu and Kacew, 2002); therefore, the period of juice storage influenced the toxicity effects of SF on rats. Findings were related to the rise in the content of methanol in the SF juice under the study conditions as the production of methanol in juices may occurred due to the hydrolysis of pectin by the enzyme known as pectinesterase (Possner *et al*., 2014). Khoo *et al*., (2010) proposed the mechanism of action mentioned above as SF is rich in pectin.

Findings in both -human and animal studies- suggest that SF possesses hypoglycaemic, antioxidant and anti-inflammatory capacities; nevertheless, further research is needed to elucidate mechanisms of action and to determine safe doses.

4.5 Conclusion

The small number of human studies in which the effects of SF consumption was assessed limits the interpretation of the outcomes of this systematic review. Positive effect on metabolic outcomes from human studies, as well as positive and negative effects on metabolic outcomes from animal studies, granted further research to be conducted.

Chapter 5: Effects of red dragon fruit consumption on insulin response and blood pressure, in healthy individuals and those at risk of type 2 diabetes.

5.1 Introduction

According to the World Health Organization, more than 420 million people worldwide have diabetes. Diabetes is defined as a chronic condition linked to abnormally high levels of glucose in the bloodstream. There are two main types of diabetes: insulin-dependent diabetes also known as type 1 (T1D) and non-insulin dependent diabetes or type 2 (T2D) among others (World Health Organization, 2021). T2D is a condition that has been linked to lifestyle, which includes eating habits and physical activity, in addition to hereditary factors (Dietrich *et al*., 2019; Gingras, Hivert and Oken, 2018). A family history of T2D, being overweight, and age, are some of the other main factors that can put a person at risk of developing this type of diabetes. Moreover, sedentary lifestyle, sleeping disorders, and other conditions such as high blood pressure are also considered as risk factors for T2D (Evans *et al*., 2021; Carbone *et al*., 2019; Chattu *et al*., 2019).

Fasting plasma glucose (FPG), the oral glucose tolerance test (OGTT), and glycated haemoglobin (HbA1c) quantification are laboratory tests that can be used to determine if someone is at risk of developing T2D. When FPG levels are higher than 7 mmol/L for two separate tests, the WHO's Global Health Observatory states that this is a diagnosis for T2D (Global Health Observatory, 2023). Normal FPG values are between 3.9-5.6 mmol/L and people with higher plasma levels than normal but lower than diabetes levels are considered at risk of developing T2D (Echouffo-Tcheugui and Selvin, 2021).

The interest for novel food to help to reduce the risks of developing chronic diseases such as T2D has been rising. Most have been based on natural food and drink sources that have been used in a traditional way to manage conditions by different cultures over time. For example, a Himalayan tar-like exudate known as Shilajit, rich in fulvic acid as well as tropical fruits rich in bioactive compounds such as watermelon and guava (Winkler and Ghosh, 2018; Acham *et al*., 2018). Dragon fruit (*Hylocereus* spp) has been shown to have a high content of bioactive compounds including polyphenols that are linked to positive health outcomes in conditions such as diabetes and hypertension (Ibrahim *et al*., 2018). Thus, clinical trials to investigate the effects of dragon fruit in individuals at risk of these health conditions should be performed.

The effect of red flesh dragon fruit (*Hylocereus polyrhizus*) on health has been studied in a small number of clinical trials. Fresh red dragon fruit has been tested in participants who are overweight/obese and in individuals with T2D as part of seven day and ten-day trials, respectively (Fadlilah *et al*., 2020; Wiardani, Moviana and Puryana, 2014); showing a reduction in blood sugar levels. The effect of consuming red dragon fruit juice for14 days was investigated in women (as the high prevalence of high cholesterol) and in subjects with T2D (Maharani and Saktiningsih, 2022; Girsang *et al*., 2020); findings demonstrated a reduction in cholesterol and glucose levels, respectively. Powder obtained from dried red dragon fruit was investigated in both, healthy individuals in a 14-day trial (Cheok *et al.*, 2022), and individuals at risk of T2D in a four-week study (Akhiruddin, 2013); results exhibited an endothelial function improved for the former and a reduction in plasma blood glucose for the latter. One further clinical trial was conducted in participants with T2D for a period of four weeks, without stopping their medication, showing a significant decrease in blood glucose and triglycerides (Abd Hadi *et al*., 2012). Authors linked their positive findings to the presence of bioactive compounds, such as polyphenols.

Results in this thesis from Chapter 2 showed that frozen for one-week red flesh dragon fruit exhibited a high content of polyphenols and a greater release of these compounds during the intestinal phase of an *in vitro* digestion, when compared with other dragon fruit species. In the sensory evaluation study (Chapter 3), frozen red dragon fruit beverage scored a higher liking for overall acceptance when compared to fresh and dried red dragon fruit based beverages. In the light of the reported beneficial effect of red dragon fruit and the high content and release of polyphenols in and from frozen red dragon fruit (FRDF) beverage, and its high likeability, the aim of this short-term study was to investigate the effect of this beverage on FPG, glycaemic response (GR), insulin response (IR) and blood pressure after four weeks of consumption using a parallel study design using healthy subjects and those at risk of T2D.

5.2 Methods

5.2.1 Study registration

This study was registered on ClinicalTrials.gov with the identifier NCT05199636 in January 2022.

5.2.2 Study design

A parallel study was designed, in which the effect of two treatments (general health guidelines and FRDF based beverage) on blood glucose, blood pressure, insulin response, and biomarkers was investigated in individuals at risk of T2D and in healthy individuals. Each participant was randomly assigned to one testing group using Research Randomizer (https://www.random.org). The study lasted four weeks and involved three testing sessions, as shown in Figure 5.1.

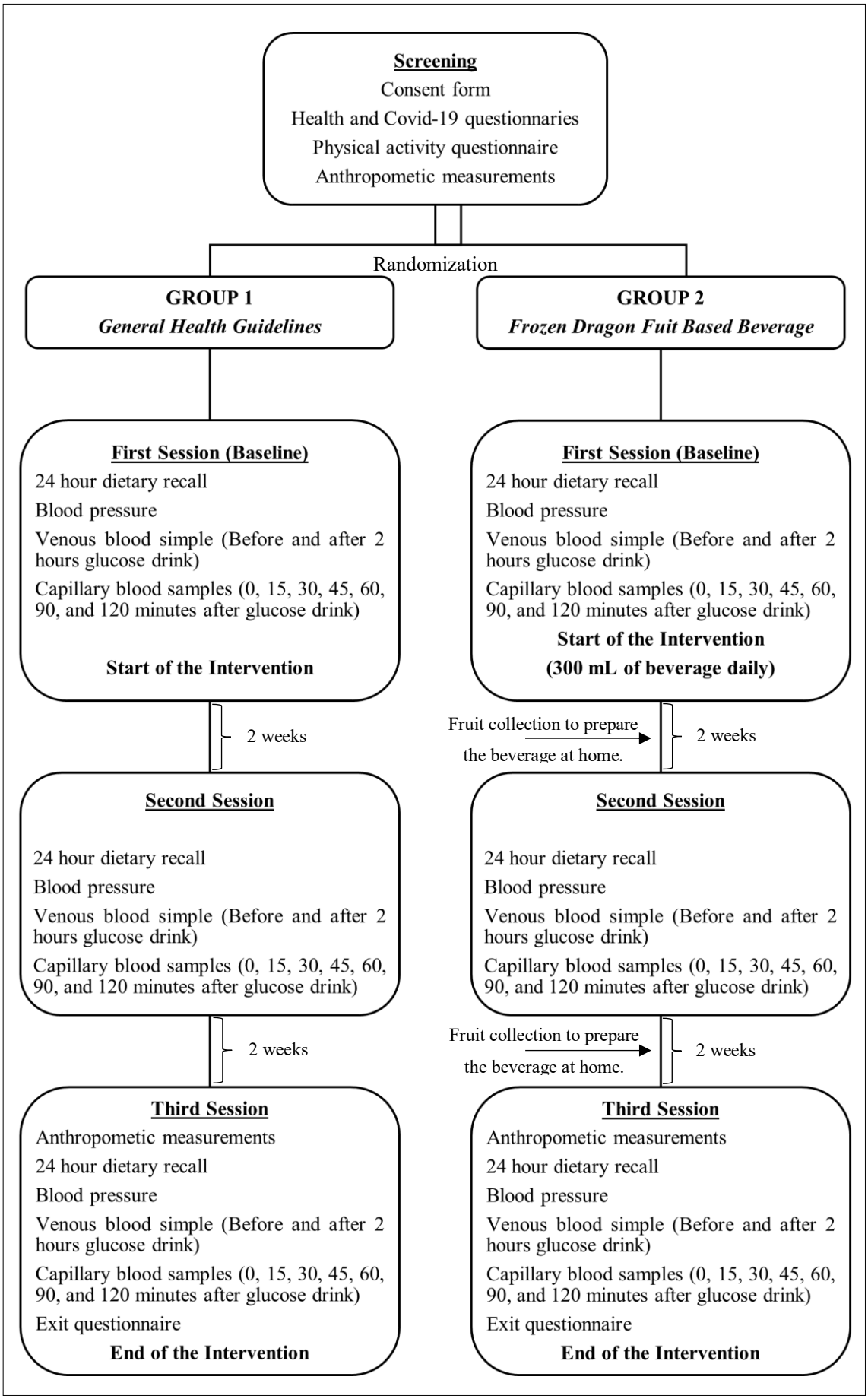


Figure 5.1 Summary of the study protocol.

A sample size of 32 participants was calculated to have 90 % of power at 0.05 level of significance (two-sided) to detect a difference of 17.46 ± 14.11 milligrams per decilitre (mg/dL) (1.0 ± 0.8 mmol/L) for FPG (p=0006). The participants were randomly divided into two groups and asked either to consume 330 mL of frozen dragon fruit based beverage once a day or to follow general health guidelines for four weeks. A 24-hour recall was recorded at the beginning, halfway through and at the end of the intervention (week-0, week-2, and week-4). This study with the Registration Number UREC 211527 was approved by the University Research Ethics Committee (Appendix 8).

5.2.3 Recruitment

Participants between 18 and 65 years were recruited by various means such as posters (Appendix 9) on Oxford Brookes University and Oxfordshire notice boards including those in groceries stores and community centres, emails to the Research Activity Google Group database, and an advertisement posted in Daily Info, a local online newspaper.

Interested volunteers received two participant information sheets by email, one for individuals at risk of T2D and other one for healthy individuals (Appendices 10 and 11). If potential participants accepted to take part and were deemed eligible, three sessions were arranged to visit the OxBCNH, located at the Headington Campus, Oxford.

Volunteers who met at least two of the following criteria were selected to take part in the study as individuals at risk of T2D:

- Have a parent or sibling with T2D.
- Have a BMI greater or equal to 25.0 Kg/m^2
- Have a sedentary lifestyle (low/moderate physical activity).

Volunteers with a BMI between 18.5 and 24.9 Kg/m^2, without diabetes, hypertension or kidney disease were eligible as healthy participants. Exclusion criteria included Covid-

19 related symptoms, allergies to dragon fruit, taking medication that requires prescription, being pregnant or breastfeeding.

5.2.4 Testing sessions

5.2.4.1 First visit

5.2.4.1.1 Screening

In a fasted state, screening was carried out in the OxBCNH consulting room. Each volunteer provided written consent (Appendix 12) and completed two screening questionnaires (health and Covid-19) and the International Physical Activity Questionnaire (IPAQ) (Appendices 13, 14, and 15). Participants were given the option to give both venous and finger prick blood samples or just finger prick blood samples. BMI data was collected; height was measured barefoot using a free-standing digital stadiometer Seca274 (Seca LTD, Birmingham, UK), and weight was measured using a body composition analyser MC-980MA plus (Tanita, Amsterdam, The Netherlands).

5.2.4.1.2 Testing session

Each testing session started by asking participants to take part in a self-administered 24-hour dietary recall. Systolic and diastolic blood pressure was taken after 10 minutes of rest with a digital blood pressure monitor UA-767 plus (A&D Medical, Tokyo, Japan), two more measurements were taken at five-minute intervals. Of those who agreed, nine mL of venous blood sample was collected by venepuncture from fasted participants with a BD-Vacutainer Eclipse blood collection needle and holder into serum and plasma separation tubes (BD Vacutainer SST Advance and BD Vacutainer Grey Cap) to determine total antioxidant status (TAS), cholesterol, triglycerides, C-reactive protein (CRP) and FPG. Baseline capillary blood samples were taken by finger-prick using a Unistick-3 lancet and analysed on the Glucose 201 DM system. Following this, a standard (Jagannathan *et al*., 2020) glucose drink [75g of glucose (Myvegan, Myprotein, England, UK) in 250 mL of water] was consumed by participants to

determine glucose tolerance for the oral glucose test (OGTT), and a four mL venous sample was taken after two hours. During the two hours, finger prick blood samples were taken with a single use Unistick-3 lancet (Owen Mumford, UK) at 0, 15, 30, 45, 60, 90, and 120 minutes. This was collected to evaluate the glycaemic and insulin response profile, using a blood glucose analyser Glucose 201 DM System (HemoCue, Sweden) and an electro-chemi-luminescence immunoassay analyser Cobas e411 (Roche Diagnostics, UK), respectively (section 5.2.6.1 and 5.2.6.2). The treatment described in the section 5.2.5 below was given to the participant to start the intervention.

5.2.4.2 Second visit

The second testing session was held two weeks after the treatment began and the protocol described in section 5.2.4.1.2 for the first testing session was followed.

5.2.4.3 Third visit

After four weeks (± 1 day) of treatment the third session was conducted, as mentioned in 5.2.4.1.2 section. Anthropometric measurements were recorded, and an exit questionnaire was applied. The participants were asked to return the follow up log (a hardcopy record) to the researcher.

5.2.5 Treatments

5.2.5.1 Treatment for testing group 1

Group 1 received advice to follow general health guidelines based on the Eatwell Guide daily for four weeks and also received guidelines for T2D prevention from the National Institute for Health and Care Excellence (NICE). As supporting material an Eatwell Guide and a treatment follow up log, to monitor if the participant followed the advice, were given (Appendix 16).

5.2.5.2 Treatment for testing group 2

The treatment for group 2 involved drinking 330 mL of a FRDF based beverage daily for four weeks. The components of the product were frozen dragon fruit pulp (My Exotic Fruit, Ingatestone, UK), water, and lime juice (Tesco Plc, England, UK) as stated in Section 3.2.1 of this thesis. To prepare the beverage with a concentration of polyphenols of 303.04 μg GAE/mL, dragon fruit pulp was frozen at -18 °C for a week to be ground in a blender (Nutribullet 600 series) for one minute, then water and lime juice were added and stirred before consumption. At the beginning of the intervention, the product was made by the researcher, after that the beverage was prepared by the participants following a standard procedure. Frozen fruit, lime juice, and the standard procedure were given to take away after the first and second sessions in order to prepare the treatments at home. Paper logs for their treatment follow up as well as comments or side effects were recorded daily (Appendix 17).

5.2.6 Blood analysis

5.2.6.1 Glycaemic response (GR)

Capillary blood samples obtained by a finger prick with a single use Unistick-3 lancet (Owen Mumford, UK) were collected, after discarding the first two drops, using a 5 μL disposable micro-cuvette (HemoCue, Sweden). These were analysed immediately in the Glucose 201 DM System (HemoCue, Sweden) to record the blood glucose level in mmol/L. A quality control test was performed prior to each testing session with the GlucoTrol-NG, Level 2 (HemoCue, Sweden) to assure the precision of results.

5.2.6.2 Insulin response (IR)

After the capillary blood samples for GR were taken, a pre-chilled micro tube containing dipotassium ethylendiaminetetraacetic acid (K_2EDTA) (BD Microtainer, UK) was used to collect approximately 375 μL of blood sample for insulin analysis. Samples were placed immediately on ice until centrifugation at 4000 rpm for 10

minutes (Sigma 1-14, UK.) to separate the plasma which was transferred into 1.5 mL Eppendorf tubes and placed at -20 °C until analysis in the automated analyser Cobas e411 (Roche Diagnostics, UK).

5.2.6.3 Fasting glucose plasma and biomarkers

Venous blood samples taken into serum and plasma separation tubes (BD Vacutainer SST Advance and BD Vacutainer Grey Cap) were centrifuged in a Biofuge Primo (Heraeus Instruments) at 1300 x g for 10 minutes. Serum and plasma were separated and placed into 1.5 mL labelled Eppendorf tubes and transferred into a freezer at -20 °C until photometric analysis in the fully automated analyser Daytona Plus (Randox, UK) for cholesterol, triglycerides, TAS, CRP, and FPG.

5.2.7 Statistical analysis

SPSS, version 28, was used to conduct the statistical analysis of the data. The incremental area under the curve (iAUC) values for GR and IR for healthy individuals and those at risk of T2D were calculated by the trapezoidal rule (International Organization for Standardization, 2010). The normality of the data was tested prior to the statistical analysis using a Shapiro-Wilks test, a natural log transformation was made for data with a non-normal distribution. The homogeneity of variances assumption was checked using Levene's test. Sphericity was determined by Mauchly´s test and when the assumption was not met a Greenhouse-Geisser adjustment was made. A mixed ANOVA was used to examine interactions between three time points ("week-0", "week-2" and "week-4", within the participant groups) and treatments ("general health advice" and "dragon fruit beverage", between participant groups) on all variables. A repeated measure ANOVA with post hoc analysis with a Bonferroni correction was used to determine differences between time points for all variables within each treatment group. The results are shown as mean ± SD with a significance accepted at the alpha level of $p < 0.05$.

5.3 Results

Thirty-two volunteers aged between 16 and 60 years were recruited to participate in the screening session. Four volunteers did not fulfil the inclusion criteria, four more did not arrive due to personal circumstances, one was taking blood pressure medication, and one was unable to fast. Therefore, twenty-two participants consented to start the study, one dropped out after the first testing session, another one dropped out during the second session, and two more missed one testing session. Eighteen participants (twelve females and six males) completed the study, nine healthy and nine at risk of T2D, as Table 5.1 shows.

The participants at risk belonged to five different ethnicities (Asian, Black, Mixed, White and Other), while the healthy participants were either of Asian or White ethnicities. There were statistically significant differences for weight and BMI between healthy and at-risk participants (Table 5.1).

Table 5.1 Baseline characteristics of the participants.

Participants	*Condition*	*Age (years)*	*Height (cm)*	*Weight (Kg)*	*BMI (Kg/m²)*
9 *(7F, 2M)* *(2A, 2B, 1Mi, 3W, 1O)*	*At risk of T2D*	31.78 ± 12.11	168.79 ± 9.16	82.61 ± 16.69	29.04 ± 5.69
9 *(5F, 4M)* *(3A, 6W)*	*Healthy*	28.44 ± 5.20	169.72 ± 5.31	62.43 ± 13.00	22.75 ± 1.93
p-value		*0.459*	*0.795*	*0.011**	*0.006**

cm: centimetres; Kg: kilograms; BMI: body mass index; Kg/m^2: kilograms per square metre; F: female; M: male; A: Asian; B: Black; Mi: Mixed; W: White; O: Other. T2D: type 2 diabetes. Values are presented as mean ± standard deviation. Asterisk (*) denotes mean values in the same column were significantly different at $p < 0.05$.

5.3.1 Blood pressure

Systolic and diastolic blood pressure results are shown in Table 5.2. There was no evidence of a main effect of time points on treatments $F(2,14) = 0.122$, $p = 0.886$, $\eta_p^2 = 0.017$ for systolic blood pressure evaluated in the participants at risk of T2D and there was no significant effect of treatments $F(1,7) = 0.064$ $p = 0.808$, $\eta_p^2 = 0.009$. The

interaction between time and the treatment was not significant F(2,14) = 0.338, p = 0.719, $\eta_p^2 = 0.046$.

Systolic blood pressure for healthy participants showed no significant difference in time F(2,14) = 2.022, p = 0.169, $\eta_p^2 = 0.224$ and between treatment F(1,7) = 0.167, p = 0.695, $\eta_p^2 = 0.023$. There was no significant difference between time and treatment F(2,14) = 0.373, p = 0.695, $\eta_p^2 = 0.051$.

Table 5.2 Blood pressure for groups that received general health advice or FRDF based beverage at week-0, week-2, and week-4 after treatment.

Blood Pressure (mm Hg)	***General Health Advice***		***FRDF Based Beverage***	
	At risk of T2D	***Healthy***	***At risk of T2D***	***Healthy***
Systolic				
Week 0	110 ± 6	108 ± 10	113 ± 4	104 ± 14
Week 2	110 ± 8	103 ± 5	111 ± 5	102 ± 12
Week 4	110 ± 10	108 ± 6	109 ± 8	104 ± 9
Diastolic				
Week 0	79 ± 9	67 ± 6	81 ± 8	69 ± 6
Week 2	77 ± 11	65 ± 4	80 ± 2	67 ± 7
Week 4	77 ± 14	68 ± 5	77 ± 11	68 ± 2

FRDF: frozen red dragon fruit; mm Hg: millimetres of mercury; T2D: type 2 diabetes.
Values represented as mean (n=18) ± standard deviation.

There was a trend for lower diastolic blood pressure in those at risk of T2D after four weeks of treatment F(2,14) = 0.991, p = 0.396, $\eta_p^2 = 0.124$. The difference between treatment was not significant F(1,7) = 0.049, p = 0.831, $\eta_p^2 = 0.007$ and there was no significant interaction between three time points and treatment F(2,14) = 0.272, p = 0.766, $\eta_p^2 = 0.037$.

A no significant difference between three time points was found for diastolic pressure in healthy participants F(2,14) = 0.595, p = 0.565, $\eta_p^2 = 0.078$. Treatment differences were

not significant F(1,7) = 0.160, p = 0.701, η_p^2 = 0.022 and there were no interactions between time and treatments F(2,14) = 0.156, p = 0.857, η_p^2 = 0.022.

5.3.2 Blood glucose

Table 5.3 shows the fasting blood glucose and blood glucose concentration after two hours of drinking a standard glucose solution in groups receiving the general health advice or the FRDF beverage at three time points.

There was no significant difference between time points for subjects at risk of T2D for fasting blood glucose F(2,14) = 0.301, p = 0.745, η_p^2 = 0.041 and there was no evidence of a main effect of treatments F(1,7) = 1.278 p = 0.296, η_p^2 = 0.154. Interaction between time and treatment was not significant F(2,14) = 0.093, p = 0.911, η_p^2 = 0.013.

Fasting blood glucose for healthy participants exhibited a non-significant difference between time points F(2,14) = 0.879, p = 0.437, η_p^2 = 0.112, and a significant difference between treatment F(1,7) = 6.705 p = 0.036, η_p^2 = 0.489. There was no interaction between time and treatment F(2,14) = 0.265, p = 0.771, η_p^2 = 0.037.

Glucose levels after two hours did not show a significant difference between time points F(2,14) = 0.682, p = 0.521, η_p^2 = 0.089 for individuals at risk of T2D. There was a significant difference between treatments F(1,7) = 6.499 p = 0.038, η_p^2 = 0.481 and no significant difference between time and treatment F(2,14) = 2.171, p = 0.151, η_p^2 = 0.237.

A non-significant difference between time or treatments was found for healthy participants and there was no interaction between them, at F(2,14) = 1.198, p = 0.331, η_p^2 = 0.146, F(1,7) = 0.693 p = 0.433, η_p^2 = 0.090 and F(2,14) = 1.855, p = 0.193, η_p^2 = 0.209, respectively.

Table 5.3 Fasting blood glucose and 2-hour glucose after a standard glucose drink in groups that received general health advice or FRDF based beverage at week-0, week-2, and week-4 after treatment.

Blood Glucose (mmol/L)	*General Health Advice*		*FRDF Based Beverage*		*p-value*
	At risk of T2D	*Healthy*	*At risk of T2D*	*Healthy*	
Fasting					
Week 0	4.80 ± 0.97	4.30 ± 0.41*	5.15 ± 0.56	4.82 ± 0.62*	< 0.05
Week 2	4.68 ± 0.40	4.33 ± 0.39*	5.18 ± 0.30	5.12 ± 0.53*	< 0.05
Week 4	4.92 ± 0.80	4.18 ± 0.49*	5.25 ± 0.17	4.80 ± 0.42*	< 0.05
2-hour after					
Week 0	5.26 ± 1.16*	5.23 ± 0.67	7.35 ± 0.95*	5.26 ± 1.13	< 0.05
Week 2	5.62 ± 0.74*	4.70 ± 0.50	6.32 ± 0.70*	5.98 ± 1.61	< 0.05
Week 4	5.28 ± 1.40*	4.70 ± 1.41	6.65 ± 0.62*	4.98 ± 0.83	< 0.05

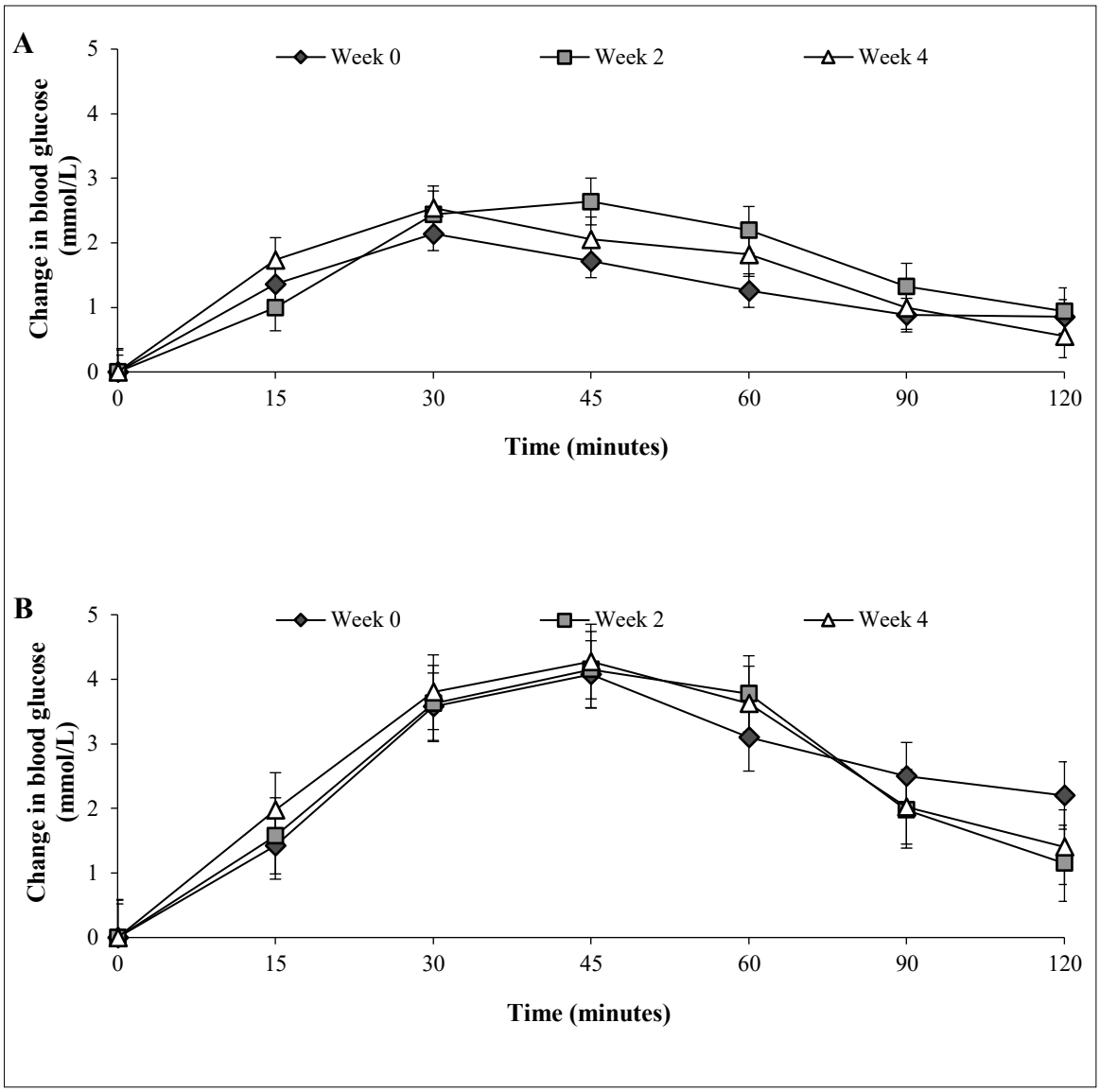
FRDF: frozen red dragon fruit; mmol/L: millimol per litre; T2D: type 2 diabetes. Values represented as mean (n=18) ± standard deviation. Asterisk (*) in the same row denotes significant difference between treatments at $p < 0.05$.

5.3.3 Glycaemic response (GR) during the oral glucose tolerance test (OGTT)

Changes in blood glucose over two hours after consumption of a standard glucose drink in groups receiving the general health advice or the FRDF beverage at three time points are shown in Figure 5.2 and 5.3.

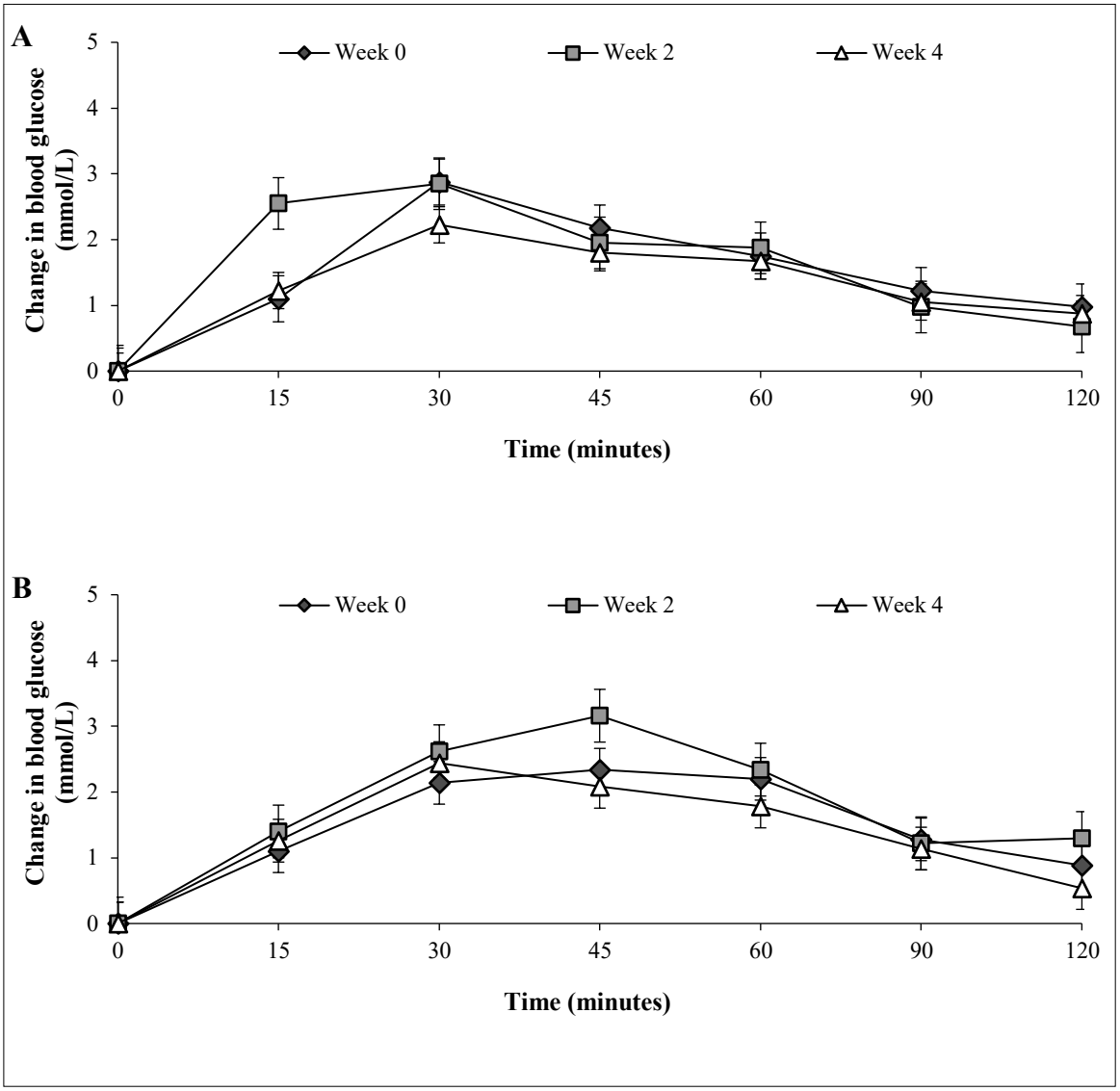
Blood glucose values for participants at risk of T2D showed no significant difference over time. $F(2,36) = 0.889$, $p = 0.420$, $\eta_p^2 = 0.047$ for participants drinking the FRDF beverage, and $F(1.57,44) = 2.135$, $p = 0.143$, $\eta_p^2 = 0.088$ for those receiving the general health advice after a Grenhouse-Geisser correction (Figure 5.2).

No significant difference was found between the three time points evaluated for blood glucose in healthy participants following general health advice or receiving the FRDF beverage, $F(2,36) = 0.184$, $p = 0.833$, $\eta_p^2 = 0.010$ and $F(2,48) = 2.028$, $p = 0.143$, $\eta_p^2 = 0.078$, respectively (Figure 5.3).



mmol/L: millimol per litre. Values are presented as means; vertical error bars represent standard error.

Figure 5.2 Changes in blood glucose response over 120 minutes after a standard glucose drink in participants at risk of T2D at three time points. A: General health advice, B: Frozen red dragon fruit based beverage.



mmol/L: millimol per litre. Values are presented as means; vertical error bars represent standard error.

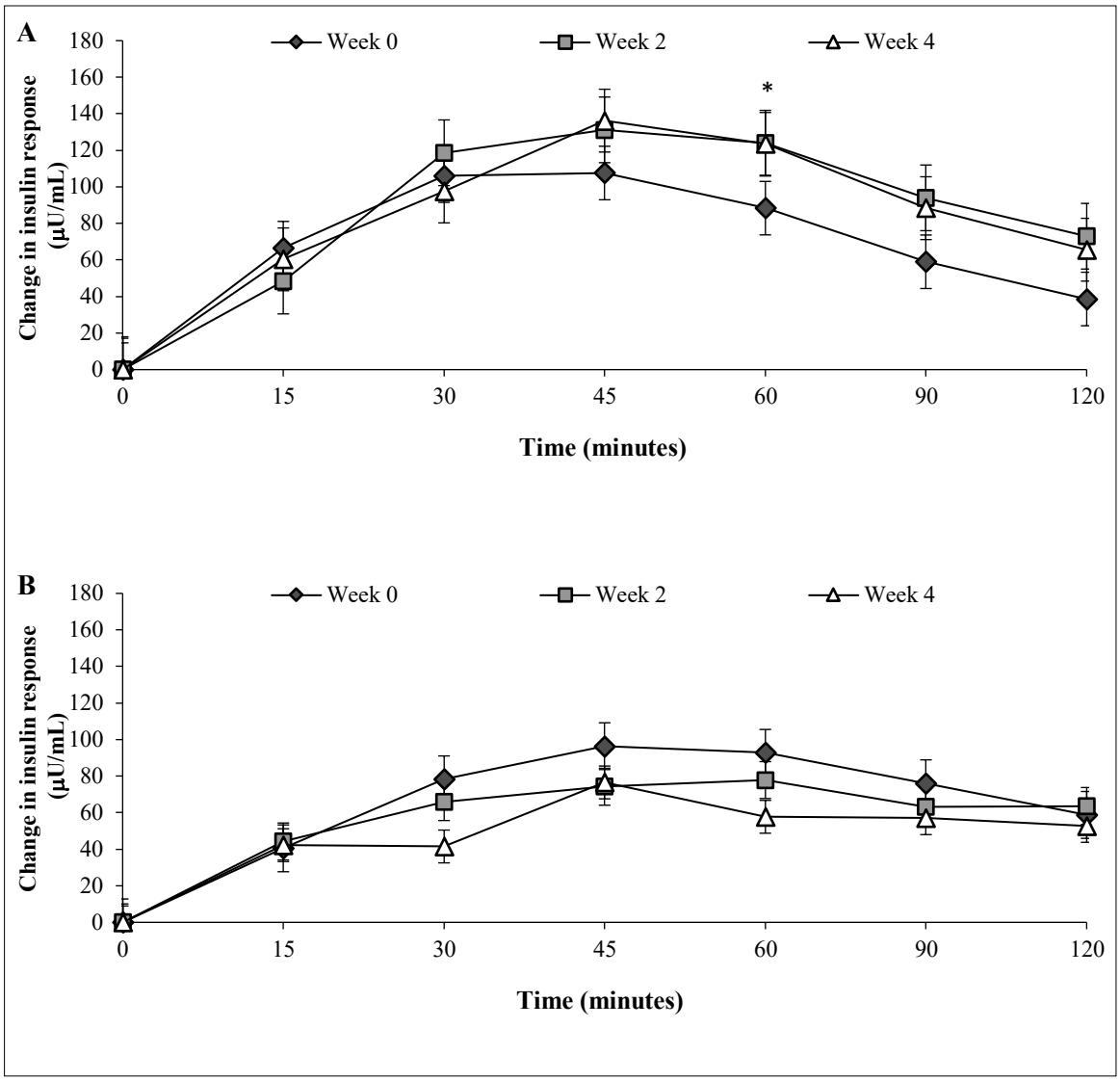
Figure 5.3 Changes in blood glucose response over 120 minutes after a standard glucose drink in healthy participants at three time points. A: General health advice, B: Frozen red dragon fruit based beverage.

5.3.4 Insulin response (IR) during the oral glucose tolerance test (OGTT)

Figures 5.4 and 5.5 show the change in insulin response in participants at risk of T2D and healthy participants, respectively.

There was evidence of a main effect of time points (week-0, week-2, week-4) on insulin concentration after consumption of standard glucose drink in participants at risk of T2D receiving general health advice $F(2,48) = 3.350$, $p = 0.043$, $\eta_p^2 = 0.122$. The change in

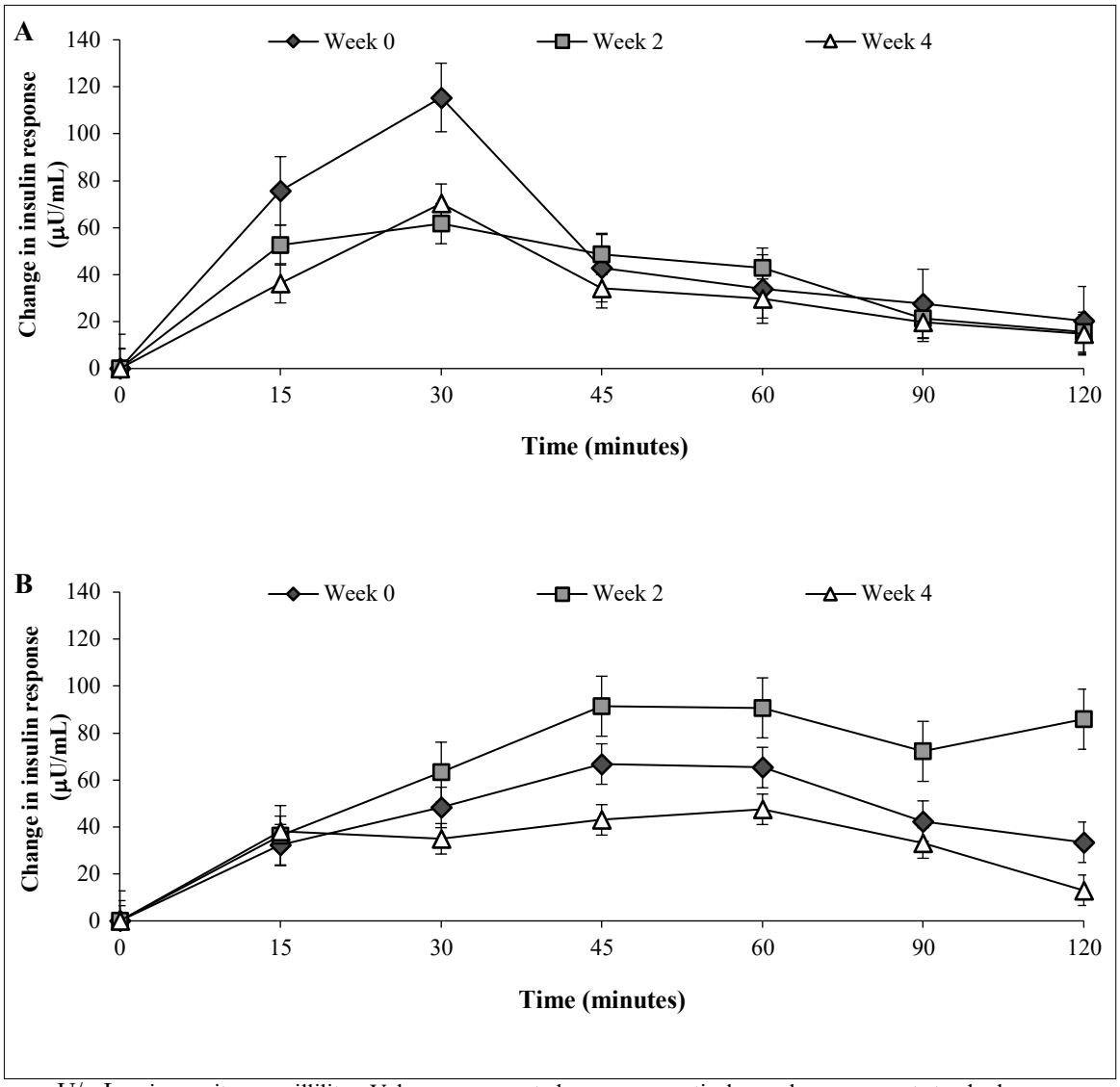
insulin response in those at risk of T2D who consumed the FRDF based beverage was no significant between times $F(2,36) = 2.980$, $p = 0.063$, $\eta_p^2 = 0.142$ (Figure 5.4).



µU/mL: micro units per millilitre. Values are presented as means; vertical error bars represent standard error. Asterisk (*) denotes significant difference at $p < 0.05$.

Figure 5.4 Changes in insulin response over 120 minutes in participants at risk of T2D at three time points. A: General health advice, B: Frozen red dragon fruit based beverage.

There was no effect of time points (week-0, week-2 and week-4) on insulin concentration after the consumption of the standard glucose drink in healthy participants after Greenhouse-Geisser correction, $F(1.15,36) = 0.187$, $p = 0.705$, $\eta_p^2 = 0.010$ for those receiving the general health advice, and $F(1.61,48) = 2.858$, $p = 0.080$, $\eta_p^2 = 0.106$ for those who consumed the FRDF based beverage (Figure 5.5).



µU/mL: micro units per millilitre. Values are presented as means; vertical error bars represent standard error.

Figure 5.5 Changes in the insulin response over 120 minutes in healthy participants at three time points. A: General health advice, B: Frozen red dragon fruit based beverage.

5.3.5 Incremental area under the curve for glycaemic response (GR-iAUC) and incremental area under the curve for insulin response (IR-iAUC)

GR-iAUC and IR-iAUC results are shown in Table 5.4. There was no significant effect of time points on the GR-iAUC in participants at risk of T2D $F(2,14) = 0.184$, $p = 0.834$, $\eta_p^2 = 0.026$, however, a significant main effect of treatments was found $F(1,7) = 8.702$, $p = 0.021$, $\eta_p^2 = 0.554$. No significant interactions between time and treatments were found $F(2,14) = 0.454$, $p = 0.644$, $\eta_p^2 = 0.061$.

No significant effect of time $F(2,14) = 0.345$, $p = 0.714$, $\eta_p^2 = 0.047$ or between treatments $F(1,7) = 0.011$, $p = 0.918$, $\eta_p^2 = 0.002$ was found in healthy participants.

There were no significant interactions between time and treatments F(2,14) = 0.299, p = 0.746, $\eta_p^2 = 0.041$.

Table 5.4 iAUC for blood glucose and insulin in groups that received general health advice or FRDF based beverage at week-0, week-2 and week-4 after treatment.

iAUC	*General Health Advice*		*FRDF Based Beverage*	
	At risk of T2D	*Healthy*	*At risk of T2D*	*Healthy*
Glucose				
Week 0	140.54 ± 80.05*	182.31 ± 81.42	313.87 ± 43.10*	175.60 ± 87.95
Week 2	192.70 ± 92.65*	189.24 ± 147.73	301.69 ± 132.24*	211.42 ± 122.89
Week 4	168.54 ± 62.61*	156.65 ± 72.99	314.06 ± 124.89*	188.09 ± 106.98
Insulin				
Week 0	8486.74 ± 10230.53	5387.54 ± 2383.02	7777.26 ± 6004.99	5457.26 ± 5102.95
Week 2	11135.48 ± 9249.90	4238.14 ± 1452.62	7292.31 ± 3880.00	8361.57 ± 10758.78
Week 4	10798.70 ± 11489.98	4044.01 ± 3356.44	5311. 84 ± 4400.41	4001.24 ±984.98

iAUC: incremental area under the curve; FRDF: frozen red dragon fruit; T2D: type 2 diabetes. Values are presented as mean ± standard deviation. Asterisk (*) in the same row denotes a significant difference between treatments at $p < 0.05$.

After a repeated measures ANOVA with a Greenhouse-Geisser correction, the IR-iAUC for participants at risk of T2D were non-significant across the three time points F(1.066,7.459) = 1.269, p = 0.299, $\eta_p^2 = 0.154$ and there was a non-significant difference between treatments F(1,7) = 0.512, p = 0.497, $\eta_p^2 = 0.068$. Interactions were not significant F(1.066,7.459) = 0.496, p = 0.514, $\eta_p^2 = 0.066$.

Healthy participants showed no significant main effects of time points F(2,14) = 1,533, p = 0.250, $\eta_p^2 = 0.180$. Between the group that received the general health advice and the group that consumed the FRDF based beverages no significant effect of treatment was found F(1,7) = 0.156, p = 0.704, $\eta_p^2 = 0.022$. There was also no significant interaction between time and treatments F(2,14) = 0.814, p = 0.463, $\eta_p^2 = 0.104$.

5.3.6 Biomarkers

Tables 5.5 and 5.6 show values for cholesterol, triglycerides, TAS and CRP in serum obtained from participants who agreed to give venous blood samples. Table 5.6 shows only the biomarkers values for those healthy individuals who received the general health advice as none of the participants that consumed the FRDF based beverage gave venous blood samples.

After a mixed ANOVA analysis, the results showed that there was no significant difference between time points for cholesterol, triglycerides, and TAS in participants at risk of T2D, $F(2,6) = 0.685$, $p = 0.539$, $\eta_p^2 = 0.186$; $F(2,6) = 0.099$, $p = 0.907$, $\eta_p^2 = 0.032$; and $F(2,6) = 1.184$, $p = 0.369$, $\eta_p^2 = 0.283$ respectively. There was evidence of main effect of treatments on CRP levels, $F(1,3) = 36.036$, $p = 0.009$, $\eta_p^2 = 0.923$.

Table 5.5 Biomarkers in serum for individuals at risk of T2D.

Participants at risk of T2D	*Cholesterol (mmol/L)*	*Triglycerides (mmol/L)*	*TAS (mmol/L)*	*CRP (mg/L)*
General Health Advice				*
Week 0	0.61 ± 0.08	0.78 ± 0.29	0.28 ± 0.01	10.23 ± 5.92
Week 2	0.62 ± 0.05	0.57 ± 0.22	0.29 ± 0.02	8.83 ± 2.02
Week 4	0.68 ± 0.03	0.68 ± 0.16	0.30 ± 0.04	12.54 ± 12.98
FRDF Based Beverage				*
Week 0	0.72 ± 0.59	0.37 ± 0.27	0.28± 0.02	0.83 ± 0.45
Week 2	0.66 ± 0.08	0.48 ± 0.37	0.31 ± 0.07	0.58 ± 0.22
Week 4	0.71 ± 0.20	0.29 ± 0.15	0.31 ± 0.08	0.53 ± 0.20

T2D: type 2 diabetes; mmol/L: millimol per litre; TAS: total antioxidant status; CRP: C-reactive protein; mg/L: milligrams per litre; FRDF: frozen red dragon fruit. Values are presented as mean ± standard deviation. Mean values in the same column with an asterisk (*) were significantly different at $p < 0.05$.

Table 5.6 Biomarkers in serum for healthy individuals.

Healthy Participants	*Cholesterol (mmol/L)*	*Triglycerides (mmol/L)*	*TAS (mmol/L)*	*CRP (mg/L)*
General Health Advice				
Week 0	0.54 ± 0.02	0.24 ± 0.07	0.41 ± 0.00	0.38 ± 0.05
Week 2	0.52 ± 0.00	0.34 ± 0.11	1.35 ± 0.80	0.37 ± 0.06
Week 4	0.52 ± 0.04	0.33 ± 0.18	0.45 ± 0.05	MD

mmol/L: millimol per litre; TAS: total antioxidant status; CRP: C-reactive protein; mg/L: milligrams per litre; MD: missed data. Values are presented as mean ± standard deviation.

5.4 Discussion

The aim of this study was to determine the effects of consuming FRDF beverage during a four-week period in healthy individuals and those at risk of T2D, to our knowledge this is the first study to explore the health effects of a frozen form of red flesh dragon fruit. The ethnicity of the participants at risk included those mentioned by NHS as risk factors to develop T2D, such as Asian and Black origin. Systolic and diastolic pressure showed a decrease (~ 4 mm Hg units) in individuals at risk of T2D as the FRDF product consumption time passed. Due to their bioactive compounds and their association with reducing the risk of developing chronic non-communicable diseases including cardiovascular disease and T2D, fruit intake is encouraged as part of a healthy diet (Zhan *et al.,* 2017). Short term studies in which the effect of consumption of red dragon fruit on blood pressure on healthy and individuals who are overweight or obese have been investigated and reported a decrease in blood pressure. The intake of dehydrated red flesh dragon fruit for 14 days resulted in a decrease in both, systolic and diastolic blood pressure when compared to a placebo in healthy individuals; however, there was no significant difference between treatments (Cheok *et al*., 2022). Fresh red flesh dragon fruit consumption for seven days by individuals who were obese or overweight decreased systolic blood pressure, there was a significant difference compared to control group without treatment (Fadlilah *et al*., 2020). Authors linked their results to the

presence of polyphenolic compounds and vitamin E and C found in the dragon fruit. In the present study, the effect of FRDF based beverage on blood pressure, exhibited similar relation with blood pressure levels; the presence of tocotrienol in red dragon fruit, a compound related to the cholesterol pathway, has been linked to the effect of red flesh dragon fruit consumption on blood pressure (Fadlilah *et al*., 2020; Hernawati *et al*., 2018; Chen, *et al*., 2011)

Research into polyphenol compounds has increased the last decade, and this is likely due to their presence in diverse edible plants around the world and the current interest in novel food associated with health benefits (Adebooye, Alashi and Aluko, 2018). Some studies suggest that the antioxidant properties of polyphenol rich foods are responsible for their role in glucose metabolism (Fraga *et al*., 2019; Giacco *et al*., 2020). A few clinical trials have been carried out in which the effect of red flesh dragon fruit on glucose metabolism was investigated in target populations such as those with a BMI higher than 25.0 Kg/m^2 and those with T2D. When thirty-two overweight/obese individuals were selected to eat 180 g per day of red dragon fruit flesh for seven days, results showed a non-significant difference between fasting blood glucose at baseline and after the intervention (Fadlilah *et al*., 2020). Similar results were found in this study as there was no difference in fasting blood glucose between time points in healthy individuals, and those at risk of T2D, who consumed 330 mL of FRDF beverage. Studies carried out to investigate other high-polyphenol fruits such as strawberries and cranberries have found similar results in terms of fasting glucose in participants with metabolic syndrome (Basu *et al*., 2010; Basu *et al*., 2011). Contrary to the above, interventions investigating the effects of red dragon fruit consumption in participants with T2D found a significant difference in FPG between baseline and after the treatment. Girsang *et al*., (2020), found a significant decrease in FPG from 19.9 to 18.0 mmol/L in 30 subjects with T2D after 14 days' consumption of 250 mL of red dragon

fruit juice. Findings from a four-week trial evaluating the consumption of 400 g per day by 22 individuals with T2D who were taking medication to control glucose levels showed a significant reduction in fasting blood glucose after a four-week treatment (Abd Hadi *et al*., 2012). Researchers attributed their results to the amount of bioactive compounds, such as polyphenols (flavonoids), vitamin C, and fibre. Antioxidants has been linked to the reduction of blood glucose levels increasing the secretion of insulin (Ihara *et al*., 2000), and reducing the oxidative stress (Girsang *et al*., 2020; Park and Park, 2021); meanwhile, dietary fibre has been reported to have a positive impact to reduce blood glucose due to its capacity to absorb water, reduce the digestion and gastric emptying, and therefore slowing the absorption of glucose (Fadlilah *et al*., 2020; Abd Hadi *et al*., 2012; Venn and Mann, 2004). Polyphenol rich diets have been reported to have an effect reducing the presence of glucose in the blood (Tresserra-Rimbau *et al*., 2016) by diverse mechanisms such as the inhibition of glucose absorption (Scalbert *et al*., 2005) or stimulating insulin secretion (Guasch-Ferré *et al*., 2017).

Discrepancies between results from the studies mentioned above and those found in this thesis may be due to the type of groups studied, the sample size, concentration of polyphenols, as well as the form of the fruit investigated, the latter suggesting that the food matrix within the polyphenols are present may influence their impact of their bioactive properties.

To our knowledge, this is the first study investigating the effect of FRDF on biomarkers. Results for total cholesterol showed a numerical reduction after two weeks of intervention in subjects at risk of T2D receiving the FRDF beverage. Similarly, Maharani and Saktiningsih (2022) evaluated the consumption of red dragon fruit juice in 18 healthy women between 30 and 49 years and results showed a significant cholesterol-lowering effect after 14 days. According to the authors, findings could be due to the content of antioxidants and fibre in red dragon fruit; furthermore, they linked

the reduction in cholesterol to the presence of tocotrienol and anthocyanins, molecules involved in the cholesterol metabolism, suppressing the synthesis or reducing its production, respectively (Maharani and Saktiningsih, 2022; Wallace, Slavin and Frankenfeld, 2016). In this study, triglycerides and CRP (an inflammation biomarker) levels were on a downward trend after the four-week intervention with FRDF beverage in individuals at risk of T2D but remained within their normal range (< 1.7 mmol/L for triglycerides and < 3.0 mg/L for CRP). Polyphenol effect on CRP levels has been linked to food matrix, as grapes ($p = 0.004$), raisins, and grapes juice ($p = 0.038$) showed a significant decrease in CRP levels, meanwhile grape powder ($p=0.152$) did not after a meta-analysis performed by Sarkhosh-Khorasani and Hosseinzadeh (2021); furthermore, authors stated that polyphenol effect on CRP leans mainly on the dose and the period of receiving the rich-polyphenol food; however, the type of preservation method and storage conditions may influence the polyphenol effect. More than one mechanism has been proposed for the anti-inflammatory effect of polyphenols, such as gene expression and the increase of the intestinal microbiota, which reduce the production of CRP (Kris-Etherton *et al.*, 2004; Singh *et al.*, 2019). Based on the above, further research is needed to compare dragon fruit matrix effect in biomarkers.

The lack of RCTs performed evaluating red flesh dragon fruit, suggests that further research is needed comparing different matrices for dragon fruit polyphenols in healthy individuals and those at risk of chronic conditions.

5.5 Limitations

This study was limited by a few factors including: the period of recruitment which took place shortly after security measures were lifted due to the Covid-19 pandemic and the small sample size, due to the lack of consent from many participants to having

venepuncture. Therefore, further research is warranted including studies with a larger, full powered sample size and for a longer period of treatment. The population under study was those at risk of T2D or a healthy control sample, so results cannot be extrapolated to other target population.

5.6 Conclusion

In conclusion, a FRDF based beverage may have the potential to reduce blood pressure and IR-iAUC in individuals at risk of T2D, in addition to improving lipid profile and CRP levels in this population. However, due to the limitations of the study, further research is needed to elucidate the mechanisms involved in reducing these metabolic outcomes and the influence of individual polyphenols contained in frozen FRDF to further understand their role in health outcomes.

Chapter 6: Overview and future prospects.

The aim of this PhD thesis was to investigate dragon fruit and star fruit and the effect of dragon fruit on metabolic health. The novel work conducted as part of this thesis provided evidence of the potential health benefits of dragon fruit and star fruit. An *in vitro* study examined the content of polyphenols and the antioxidant capacity in three species of dragon fruit and one species of star fruit, analysing fresh, frozen, and dehydrated forms; the release of polyphenolic compounds during an *in vitro* digestion was examined (Chapter 2). The results showed that frozen for one-week red flesh dragon fruit was the dragon fruit species with the highest content of polyphenols. To assess the significance of the polyphenols in these different forms of fruit an *in vitro* bioaccessibility study of polyphenols and a sensory evaluation were conducted in products based on fresh, frozen, and dehydrated forms of red flesh dragon fruit and star fruit (Chapter 3). The results showed that the beverage based on frozen for one-week red flesh dragon fruit received the highest scores for overall acceptance. A systematic review (Chapter 4) was conducted on star fruit consumption and its *in vivo* health effects due to the low acceptance observed during the sensory evaluation in the study of Chapter 3. Based on the results of Chapter 3, an *in vivo* study (Chapter 5) was designed to find out the effect of the most preferred RFDF based beverage (FRDF) on blood pressure, GR, IR and other biomarkers in healthy individuals and in those at risk of T2D. The purpose of this chapter is to present an overview of this thesis.

6.1 Overview

This thesis examined dragon fruit and star fruit, with the aim of identifying the potential use and effects of these tropical fruits on human health. This was addressed through a literature review to identify evidence that suggest that the high content of polyphenol in

dragon fruit and star fruit could improve risk factors of chronic disease such as T2D and hypertension. Authors like Ibrahim *et al*., (2018), Adnan, Osman and Abdul-Hamid (2011), and Ramli *et al*., (2014) pointed out the potential benefits of dragon fruit species, meanwhile O'Hare (1993) and Zainudin *et al*., (2014) reported the content of polyphenols in star fruit and the possibility to use star fruit as a functional ingredient. Most of the studies to quantify the content of phenolic compounds were undertaken on fresh form of white flesh and red flesh dragon fruit (Choo and Yong, 2011; Alam *et al.*, 2023; Kim *et al*., 2011) and just one study was looked at the composition of yellow peel dragon fruit (Lupuche *et al*., 2021). As not all species and forms of fruit have been investigated, this thesis assessed some forms that have not been reported in the literature; furthermore, no study to date has explored the bioaccessibility of polyphenols during an *in vitro* digestion. For this reason, the study in Chapter 2 was conducted aiming to determine the bioactive potential looking at TPC and antioxidant capacity of dragon fruit and star fruit, as well as the bioaccessibility of their polyphenols. To the best of our knowledge, this study is the first to investigate the *in vitro* digestion release of polyphenols from fresh, frozen and dehydrated forms of star fruit, red flesh, white flesh, and yellow peel dragon fruit. It was found that 70% acetone extracted the highest TPC from star fruit and the most of the dragon fruit species, as previously studies reported (Liu *et al*., 2009; Sulaiman *et al*., 2011); nevertheless, the water extract obtained from the red flesh dragon fruit, frozen for one-week, was significantly higher in TPC than the extracts from white flesh and yellow peel species, showing similar results to those obtained in previous studies that compared different species of dragon fruit (Choo and Yong, 2011; Alam *et al.*, 2023; Kim *et al*., 2011). Meanwhile, star fruit exhibited the highest TPC for all fruits tested. Studies had reported a linear correlation between TPC and antioxidant capacity (Abd-Manan *et al*., 2019, Luu *et al*., 2021) similar to the findings in this study between TPC and FRAP. As food matrix has

showed an effect on TPC and antioxidant capacity, further research is needed to identify the changes on polyphenol structures. Novel findings regarding bioaccessibility of polyphenols extracted from fresh, frozen, and dehydrated forms of dragon fruit and star fruit were provided. There were differences between the phases of digestion for the release of RS and polyphenols, being the intestinal phase at 20 minutes which exhibited the highest release of compounds, and a strong correlation was found between polyphenols and FRAP during *in vitro* digestion. According to the results, red dragon fruit species and star fruit were used to prepare beverages based on fresh, frozen, and dehydrated forms for the analysis of the *in vitro* digestion and the sensory acceptance trial presented in Chapter 3. The frozen dragon fruit based beverage was the most accepted and exhibited the highest amount of polyphenols released during the intestinal phase of *in vitro* digestion. No previous study has reported these outcomes from dragon fruit and star fruit based beverages (Chapter 3). Findings allow us to strongly recommend further research exploring changes in polyphenols during the digestion process to understand their bioaccessibility; sensory trials looking for target population acceptance is also recommended. Then, the systematic review conducted for Chapter 4, was designed to compile the previous research looking for the effect of star fruit consumption due to the results obtained in Chapter 3. Positive health effects in animal models, such as a reduction in fasting blood glucose, body weight, cholesterol, triglycerides, and MDA were found (Rodrigues da Silva *et al*., 2021; Herman-Lara *et al*., 2014; Pham *et al*., 2017; Zhang *et al*., 2016; Khoo *et al*., 2010; Chau, Chien and Chen, 2005); also positive effects were found in human populations with hypertension, COPD and elderly despite the toxic and therefore negative effect of star fruit consumption reported in individuals with renal disease. Further research is encouraged to conduct RCTs to evaluate the effect of the SF consumption in healthy individuals. Finally, Chapter 5 shows the effect of a FRDF based beverage consumption by healthy

individuals and those at risk of T2D. There was a decrease in the IR and iAUC for those at risk of T2D receiving the beverage for a four-week period; however, there was a non-significant difference between time points evaluated. This short-term study highlighted the potential health benefit of the consumption of FRDF, allowing us to propose long-term RCTs involving larger number of participants and using different doses of the FRDF based beverage. Also, the further design of products based on this form of dragon fruit, such as shots based on the beverage investigated during this study, smoothies, and ice lollies, among others is encouraged.

The findings from this thesis showed the potential impact of consuming a FRDF based beverage on metabolic outcomes in a target population. Thus, regular consumption of frozen red dragon fruit, as part of the five a day fruit and vegetable recommendations, could be beneficial to those at risk of T2D.

6.3 Future prospects

This PhD thesis provided novel findings for the use of dragon fruit as functional food, and its impact on blood pressure, GR and IR for individuals at risk of T2D. Further research is warranted to design powerful RCTs to evaluate, compare and elucidate the mechanism of the effect of polyphenols contained in red dragon fruit using fresh, frozen, and dehydrated matrices in metabolic outcomes. Future trials with a larger number of participants and long-term interventions are strongly recommended. Further RCTs to assess the polyphenol dose as well as the frequency of consumption are needed; RCTs to evaluate the effect of red dragon fruit polyphenols in oxidative stress and inflammatory biomarkers are recommended.

References

- Abd Hadi, N., Mohamad, M., Rohin, M. A. K. and Yusof, R. M. (2012) 'Effects of red pitaya fruit (*Hylocereus polyrhizus*) consumption on blood glucose level and lipid profile in type 2 diabetic subjects', *Borneo Science*, 31, pp. 127-142.
- Abd-Manan, E., Abd Gani, S.S., Zaidan, U. H. and Halmi, M. I. E. (2019) 'Characterization of antioxidant activities in red dragon fruit (*Hylocereus polyrhizus*) pulp water-based extract', *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences,* 61(2), pp. 170-180.
- Abeysekera, R. A., Wijetunge, S., Nanayakkara, N., Wazil1, A. W. M., Ratnatunga, N. V. I., Jayalath, T. and Medagama, A. (2015) 'Star fruit toxicity: A cause of both acute kidney injury and chronic kidney disease: A report of two cases', *BMC Research Notes*, 8:796. Available at: https://doi.org/10.1186/s13104-015-1640-8.
- Acham, I. O., Ahemen, S., Ukeyima, M. T. and Girgih, A. T. (2018) 'Tropical fruits: Bioactive properties and health promoting benefits in chronic disease prevention and management', *Asian Food Science Journal*, 3(1), pp. 1-13.
- Adebooye, O. C., Alashi, A. M. and Aluko, R. E. (2018) 'A brief review on emerging trends in global polyphenol research', *Journal of Food Biochemistry*, 42. Available at: https://doi.org/10.1111/jfbc.12519.
- Adnan, L., Osman, A. and Abdul-Hamid, A. (2011) 'Antioxidant activity of different extracts of red pitaya (*Hylocereus polyrhizus*) seed', *International Journal of Food Properties*, 14(6), pp. 1171-1181. Available at: https://doi.org/10.1080/10942911003592787.
- Agbor, G., Vinson, J. and Donnelly, P. (2014) 'Folin-Ciocalteau reagent for polyphenolic assay', *International Journal of Food Science, Nutrition and Dietetics*, 3(801). Available at: https://doi.org/10.19070/2326-3350-1400028.

- Aguillón-Osma, J., Luzardo-Ocampo, I., Cuellar-Nuñez, M. L., Maldonado-Celis, M. E., Loango-Chamorro, N. and Campos-Vega, R. (2019) 'Impact of *in vitro* gastrointestinal digestion on the bioaccessibility and antioxidant capacity of bioactive compounds from passion fruit (*Passiflora edulis*) leaves and juice extracts', *Journal of Food Biochemistry*, 43(7). Available at: https://doi.org/10.1111/jfbc.12879.
- Aherne, S. A., Daly, T., Jiwan, M. A., O'Sullivan, L. and O'Brien, N. M. (2010) 'Bioavailability of carotene isomers from raw and cooked carrots using an *in vitro* digestion model coupled with a human intestinal Caco-2 cell model', *Food Research International*, 43, pp. 1449-1454.
- Akhiruddin, M. A.-S. B. (2013) 'Nutritional composition, antioxidant properties of *Hylocereus polyrhizus* powder and their effects on plasma glucose level and lipid profiles in diabetic rats and pre-diabetic subjects', MSc Thesis, University of Putra, Malaysia.
- Alam, M., Biswas, M., Ahmed, J., Hosain, M. A., Alam, A., Khan, M. H. H. and Molla, M. M. (2023) 'Physico-chemical properties, antioxidant activity and bioactive compounds in edible and non-edible portions of dragon fruit cultivars native to Bangladesh', *Food Research*, 7(4), pp. 194-203. Available at: https://doi.org/10.26656/fr.2017.7(4).243.
- Alamgeer, Younis, W., Asif, H., Sharif, A., Riaz, H., Bukhari, I. A. and Assiri, A. M. (2018) 'Traditional medicinal plants used for respiratory disorders in Pakistan: A review of the ethno-medicinal and pharmacological evidence', *Chinese Medicine*, 13(1). Available at: https://doi.org/10.1186/s13020-018-0204-y.
- Alara, O. R., Abdurahman, N. H. and Ukaegbu, C. I. (2021) 'Extraction of phenolic compounds: A review', *Current Research in Food Science*, 4, pp. 200-214. Available at: https://doi.org/10.1016/j.crfs.2021.03.011.

- Aleixandre, A. and Miguel, M. (2008) 'Dietary fiber in the prevention and treatment of metabolic syndrome: A review', *Critical Reviews in Food Science and Nutrition*, 48(10), pp. 905-912. Available at: htpps://doi.org/10.1080/10408390701761886.
- Al-Mekhlafi, N. A., Mediani, H., Ismail, N. H., Abas, F., Dymerski, T., Lubinska-Szczygeł, M., Vearasilp, S. and Gorinstein, S. (2021) 'Metabolomic and antioxidant properties of different varieties and origins of dragon fruit', *Microchemical Journal*, 160(B). Available at: https://doi.org/10.1016/j.microc.2020.105687.
- Amiot, M. J., Riva, C. and Vinet, A. (2016) 'Effects of dietary polyphenols on metabolic syndrome features in humans: A systematic review'. [Systematic review]. *Obesity Reviews*, 17(7) pp. 573-86. Available at: https://doi.org/10.1111/obr.12409.
- Aranguren, C., Vergara, C. and Rosselli, D. (2017) 'Toxicity of star fruit (*Averrhoa carambola*) in renal patients: A systematic review of the literature'. [Systematic review]. *Saudi Journal of Kidney Diseases and Transplantation,* 28(4), pp. 709-715.
- Ariffin, A. A., Bakar, J., Tan, C. P., Rahman, R. A., Karim, R. and Loi, C. C. (2009) 'Essential fatty acids of pitaya (dragon fruit) seed oil', *Food Chemistry*, 114(2), pp. 561-564. Available at: https://doi.org/10.1016/j.foodchem.2008.09.108.
- Arivalagan, M., Karunakaran, G., Roy, T. K., Dinsha, M., Sindhu, B. C., Shilpashree, V. M., Satisha, G. C. and Shivashankara, K. S. (2021) 'Biochemical and nutritional characterization of dragon fruit (*Hylocereus* species)', *Food Chemistry*, 353. Available at: https://doi.org/10.1016/j.foodchem.2021.129426.
- Armijos, C. P., Meneses, M. A., Guamán-Balcázar, M. C., Cuenca, M. and Suarez, A. I. (2018) 'Antioxidant properties of medicinal plants used in the Southern Ecuador', *Journal of Pharmacognosy and Phytochemistry*, 7(1), pp. 2803-2818.
- Artana, W., Rosinata, N. P. Y., Putra, I. P. A. G. and Putri, I. L. (2020) 'Traditional therapy of watermelon-starfruit juice for reducing blood pressure of hypertension',

Systematic Reviews in Pharmacy, 11(3), pp. 566-574. Available at: https://doi.org/10.31838/srp.2020.3.74.

- Ashraf, M. A., Iqbal, M., Rasheed, R., Hussain, I., Riaz, M. and Arif M. S. (2018) 'Environmental stress and secondary metabolites in plants: An overview', in *Plant Metabolites and Regulation under Environmental Stress*, pp. 153-167. Available at: https://doi.org/10.1016/B978-0-12-812689-9.00008-X.
- Atiba, A. S., Abbiyesuku, F. M., Oparinde, D. P., 'Niran-Atiba, T. A. and Akindele, R. A. (2016) 'Plasma malondialdehyde (MDA): An indication of liver damage in women with pre-eclamsia', *Ethiopian Journal of Health Sciences*, 26(5), pp. 479-486. Available at: htteps://doi.org/10.4314/ejhs.v26i5.10.
- Attar, Ş. H., Gündeşli, M-A., Urün, I., Kafkas, S., Kafkas, N. E., Ercisli, S., Ge, C., Mlcek, J. and Adamkova, A. (2022) 'Nutritional analysis of red-purple and white-fleshed pitaya (*Hylocereus*) species', *Molecules*, 27. Available at: https://doi.org/10.3390/molecules27030808.
- Attri, S., Singh, N., Singh, T. R. and Goel, G. (2017) 'Effect of *in vitro* gastric and pancreatic digestion on antioxidant potential of fruit juices', *Food Bioscience*, 17, pp. 1-6. Available at: https://doi.org/10.1016/j.fbio.2016.10.003.
- Barman, A. K., Goel, R., Sharma, M. and Mahanta, P. J. (2016) 'Acute kidney injury associated with ingestion of star fruit: Acute oxalate nephropathy,' *Indian Journal of Nephrology*, 26(6), pp. 446-448. Available at: https://doi.org/10.4103/0971-4065.175978.
- Basena, K. K., Jamuna, K. V. and Rafed, R. (2019) 'Physico-chemical properties of starfruit (*Averrhoa carambola*)', *International Journal of Chemical Studies*, 7(5), pp. 1258-1269.
- Basu, A., Betts, N. M., Ortiz, J., Simmons, B., Wu, M. and Lyons, T. J. (2011) 'Low energy cranberry juice decreases lipid oxidation and increases plasma antioxidant

capacity in women with metabolic syndrome', *Nutrition Research*, 31, pp. 190-196. Available at: https://doi.org/10.1016/j.nutres.2011.02.003.

- Basu, A., Fu, D. X., Wilkinson, M., Simmons, B., Wu, M., Betts, N. M., Du, M. and Lyons, T. J. (2010) 'Strawberries decrease atherosclerotic markers in subjects with metabolic syndrome', *Nutrition Research*, 30, pp. 462-469. Available at: https://doi.org/10.1016/j.nutres.2010.06.016.
- Benatrehina, P. A., Pan, L., Naman, C. B., Li, J. and Kinghorn, A. D. (2018) 'Usage, biological activity, and safety of selected botanical dietary supplements consumed in the United States', *Journal of Traditional and Complementary Medicine*, 8(2), pp. 267-277. Available at: https://doi.org/10.1016/j.jtcme. 2018.01.006.
- Benkeblia, N. and Lopez, M. G. (2015) 'Saccharides and fructooligosaccharides composition of green and ripe *Averrhoa carambola*, *Blighia sapida* and *Spondias dulcis* fruits', *Food Chemistry*, 176, pp. 314-318. Available at: https://doi.org/ 10.1016/j.foodchem.2014.12.080.
- Benzie, I. F. and Strain, J. J. (1996) 'The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay', *Analytical Biochemistry*, 239, pp. 70-76.
- Betteridge, D. J. (2000) 'What is oxidative stress?' *Metabolism: Clinical and Experimental*, 49(2 Suppl 1), pp. 3-8.
- Brainina, K., Stozhko, N. and Vidrevich, M. (2019) 'Antioxidants: terminology, methods, and future considerations', *Antioxidants*, 8(297). Available at: https://doi.org/10.3390/antiox8080297.
- Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995) 'Use of a free radical method to evaluate antioxidant activity', *LWT-Food Science and Technology*, 28(1), pp. 25-30. Available at: https://doi.org/10.1016/S0023-6438(95)80008-5.

- Carbone, S., Del Buono, M. G., Ozemek, C. and Lavie, C. J. (2019) 'Obesity, risk of diabetes and role of physical activity, exercise training and cardiorespiratory fitness', *Progress in Cardiovascular Diseases,* 62(4), pp. 327-333. Available at: https://doi.org/10.1016/j.pcad.2019.08.004.
- Castañeda-Bueno, M., Ellison, D. H. and Gamba, G. (2022) 'Molecular mechanisms for the modulation of blood pressure and potassium homeostasis by the distal convoluted tubule', *EMBO Molecular Medicine*, 14(2). Available at: https://doi.org/10.15252/emmm.202114273.
- Caturano, A., D'Angelo, M., Mormone, A., Russo, V., Mollica, M. P., Salvatore, T., Galiero, R., Rinaldi, L., Vetrano, E., Marfella, R., Monda, M., Giordano, A. and Sasso, F. C. (2023) 'Oxidative stress in type 2 diabetes: Impacts from pathogenesis to lifestyle modifications' *Current Issues in Molecular Biology*, 45, pp. 6651-6666. Available at: https://doi.org/10.3390/cimb45080420.
- Centre for the Promotion of Imports from Developing Countries (CBI) The Netherlands (2020) '*The European market potential for exotic tropical fruit'*, Available at: https://www.cbi.eu/market-information/fresh-fruit-vegetables /exotic-tropical-fruit/market-potential (Accessed: 11 September 2023).
- Chaemsaint, S., Matan, N. and Matan N. (2018) 'Effect of peppermint oil on the shelf-life of dragon fruit during storage', *Food Control*, 90, pp. 172-179. Available at: https://doi.org/10.1016/j.foodcont.2018.03.001.
- Chan, S.G., Murphy, P. A., Ho, S. C., Kreiger, N., Darlington, G., So, E. K. F. and Chong, P. Y. Y. (2009) 'Isoflavonoid content of Hong Kong soy foods', *Journal of Agricultural and Food Chemistry.* 57(12), pp. 5386-5390. Available at: https://doi.org/10.1021/jf803870k.
- Chandrasekara, A. and Shahidi, F. (2018) 'Herbal beverages: Bioactive compounds and their role in disease risk reduction – A review', *Journal of Traditional and*

Complementary Medicine, 8(4), pp. 451-458. Available at: https://doi.org/10.1016/j.jtcme.2017.08.006.

- Chang, C.-H. and Yeh, J.-H. (2004) 'Non-convulsive status epilepticus and consciousness disturbance after star fruit (*Averrhoa carambola*) ingestion in a dialysis patient', *Nephrology*, 9, pp. 362-365.
- Charehsaz, M., Sipahi, H., Celep, E., Üstündağ, A., Cemiloğlu Ülker, Ö., Duydu, Y., Aydin, A. and Yesilada, E. (2015). 'The fruit extract of *Berberis crataegina* DC: Exerts potent antioxidant activity and protects DNA integrity', *DARU Journal of Faculty of Pharmacy,* 23(24). Available at: https://doi.org/10.1186/s40199-015-0108-7.
- Chattu, V. K., Chattu, S. K., Burman, D., Spence, D. W. and Pandi-Perumal, S. R. (2019) 'The interlinked rising epidemic of insufficient sleep and diabetes mellitus', *Healthcare*, 7 (37). Available at: https://doi.org/10.3390/healthcare7010037.
- Chau, C.-F., Chien, P.-J. and Chen, C.-H. (2005) 'Influence of insoluble fiber fractions from carambola and carrot on intestinal enzymes and fecal bacterial enzymes in hamsters', *Nutrition Research*, 25(10), pp. 947-957. Available at: https://doi.org/10.1016/j.nutres.2005.09.021.
- Chayarop, K., Peungvicha, P., Temsiririrkkul, R., Wongkrajang, Y., Chuakul, W. and Rojsanga, P. (2017) 'Hypoglycaemic activity of *Mathurameha*, a Thai traditional herbal formula aqueous extract, and its effect on biochemical profiles of streptozotocin-nicotinamide-induced diabetic rats', *BMC Complementary and Alternative Medicine*, 17(1). Available at: https://doi.org/10.1186/s12906-017-1851-8.
- Chen, J., Yang, J., Ma, L., Shahzad, N. and Kim, C. K. (2020) 'Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid

groups of phenolic acids', *Scientific Reports*, 10(1). Available at: https://doi.org/10.1038/s41598-020-59451-z.

- Chen, X.-Y., Wang, R.-F. and Liu, B. (2015) 'An update on oligosaccharides and their esters from traditional Chinese medicines: Chemical structures and biological activities', *Evidence-Based Complementary and Alternative Medicine*, 2015, pp. 1-23. Available at: https://doi.org/10.1155/2015/512675.
- Chen, G.-L., Chen, S.-G., Zhao, Y.-Y., Luo, C.-X., Lib, J. and Gao, Y.-Q. (2014) 'Total phenolic contents of 33 fruits and their antioxidant capacities before and after *in vitro* digestion', *Industrial Crops and Products*, 57, pp. 150-157. Available at: https://doi.org/10.1016/j.indcrop.2014.03.018.
- Chen, Z.-Y., Ma, K. Y., Liang, Y., Peng, C. and Zuo, Y. (2011) 'Role and classification of cholesterol-lowering functional foods', *Journal of Functional Foods*, 3, pp. 61-69. Available at: https//doi.org/10.1016/j.jff.2011.02003.
- Chen, Z.-Y., Jiao, R. and Ma, K. Y. (2008) 'Cholesterol-lowering nutraceuticals and functional foods', *Journal of Agricultural and Food Chemistry*, 56(19), pp. 8761-8773. Available at: https://doi.org/10.1021/jf801566r.
- Cheok, A., Xu, Y., Zhang, Z., Caton, P. W. and Rodriguez-Mateos, A. (2022) 'Betalain-rich dragon fruit (pitaya) consumption improves vascular function in men and women: a double-blind, randomized controlled crossover trial', *The American Journal of Clinical Nutrition*, 115, pp. 1418-1431.
- Chiva-Blanch, G. and Badimon, L. (2017) 'Effects of polyphenol intake on metabolic syndrome: Current evidence from human trials', *Oxidative Medicine and Cellular Longevity*. Available at: https://doi.org/10.1155/2017/5812401.
- Choo, W.-S. and Yong, W. K. (2011) 'Antioxidant properties of two species of *Hylocereus* fruits', *Advances in Applied Science Research*, 2(3), pp. 418-425.

- Choudhury, H., Pandey, M., Hua, C. K., Mun, C. S., Jing, J. K., Kong, L., Ern, L. Y., Ashraf, N. A., Kit, S. W., Yee, T. S., Pichika, M. R., Gorain, B. and Kesharwani, P. (2018) 'An update on natural compounds in the remedy of diabetes mellitus: A systematic review'. [Systematic review]. *Journal of Traditional and Complementary Medicine*, 8(3). Available at: https://doi.org/10.1016/j.jtcme.2017.08.012.
- Chua, C.-B., Sun, C.-K., Tsuib, H.-W., Yang, P.-J., Lee, K.-H., Hsu, C.-W. and Tsai, I.-T. (2017) 'Association of renal function and symptoms with mortality in star fruit (*Averrhoa carambola*) intoxication', *Clinical Toxicology*, 255(7), pp. 624-628. Available at: http://dx.doi.org/10.1080/15563650.2017.1314490.
- Cilla, A., Bosch, L., Barberá, R. and Alegría, A. (2018) 'Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols', *Journal of Food Composition and Analysis*, 68, pp. 3-15, Available at: https://doi.org/10.1016/j.jfca.2017.01.009.
- Civille, G. V. and Oftedal, K. N. (2012) 'Sensory evaluation techniques-make "good for you" taste "good"', *Physiology and Behaviour*, 107(4), pp. 598-605. Available at: https://doi.org/10.1016/j.physbeh.2012.04.015.
- Cochrane Effective Practice and Organisation of Care (EPOC) (2017) *Suggested risk of bias criteria for EPOC reviews.* EPOC Resources for Review Authors. Available at: epoc.cochrane.org/epoc-resources-review-authors (Accessed: 10 May 2022).
- Coman, M. M., Oancea, A. M., Verdenelli, M. C., Cecchini, C., Bahrim, G. E., Orpianesi, C., Cresci, A. and Silvi, S. (2018) 'Polyphenol content and *in vitro* evaluation of antioxidant, antimicrobial and prebiotic properties of red fruit extracts', *European Food Research and Technology*, 244, pp. 735-745. Available at: https://doi.org/10.1007/s00217-017-2997-9.

- Corona-Leo, L. S., Meza-Márquez, O. G. and Hernández-Martínez, D. M. (2021) 'Effect of *in vitro* digestion on phenolic compounds and antioxidant capacity of different apple (*Malus domestica*) varieties harvested in Mexico', *Food Bioscience*, 43. Available at: https://doi.org/10.1016/j.fbio.2021.101311.
- Cui, L., Xing, M., Xu, L., Wang, J., Zhang, X., Ma, C. and Kang, W. (2018) 'Antithrombotic components of *Malus halliana* Koehne flowers', *Food and Chemical Toxicology*, 119, pp. 326-333. Available at: https://doi.org/10.1016/j.fct.2018.02.049.
- Da Costa, G. A. and Mercadante, A. Z. (2018) '*In vitro* bioaccessibility of free and esterified carotenoids in cajá frozen pulp-based beverages', *Journal of Food Composition and Analysis*, 68, pp. 53-59. Available at: https://doi.org/10.1016/j.jfca.2017.02.012.
- Dasgupta, P., Chakraborty, P. and Bala, N. N. (2013). '*Averrhoa carambola*: An updated review', *International Journal of Pharma Research and Review*, 2(7), pp. 54-63.
- De Araújo, F. F., de Paulo Farias, D., Neri-Numa, I. A. and Pastore, G. M. (2021) 'Polyphenols and their applications: An approach in food chemistry and innovation potential', *Food Chemistry*, *338*. Available at https://doi.org/10.1016/j.foodchem.2020.127535.
- De Paula Nogueira, L., Paranhos Knibel, M., Simas Gonçalves Torres, M. R., Nogueira Neto, J. F. and Sanjuliani, A. F. (2012) 'Consumption of high-polyphenol dark chocolate improves endothelial function in individuals with stage 1 hypertension and excess body weight', *International Journal of Hypertension*. Available at: https://doi.org/10.1155/2012/147321.
- De Rodríguez D. J., García-Hernández L. C., Rocha-Guzmán N. E., Moreno-Jiménez M. R., Rodríguez-García R., Díaz-Jiménez M. L. V., Flores-López M. L.,

Villarreal-Quintanilla, J. A., Peña-Ramos F. M. and Carrillo-Lomelí D. A. (2017) 'Hypoglycemic and anti-inflammatory effects of *Psacalium paucicapitatum* corms infusions', *Industrial Crops & Products*, 107, pp. 482-488. Available at: https://doi.org/10.1016/j.indcrop.2017.04.022.

- De Souza, R. L. A., Santana, M. F. S., de Macedo, E. M. S., de Brito, E. S. and Correia, R. T. P. (2015) 'Physicochemical, bioactive and functional evaluation of the exotic fruits *Opuntia ficus-indica* and *Pilosocereus pachycladus* Ritter from the Brazilian caatinga', *Journal of Food Science and Technology*, 52(11), pp. 7329-7336. Available at: https://doi.org/10.1007/s13197-015-1821-4.
- Del Bo', C., Bernardi, S., Marino, M., Porrini, M., Tucci, M., Guglielmetti, S., Cherubini, A., Carrieri, B., Kirkup, B., Kroon, P., Zamora-Ros, R., Liberona, N. H., Andres-Lacueva, C. and Riso, P. (2019) 'Systematic review on polyphenol intake and health outcomes: Is there sufficient evidence to define a health-promoting polyphenol-rich dietary pattern?'. [Systematic Review]. *Nutrients*, 11(6):1355. Available at: https://doi.org/10.3390/nu11061355.
- Dembitsky, V. M., Poovarodom, S., Leontowicz, H., Leontowicz, M., Vearasilp, S., Trakhtenberg, S. and Gorinstein, S. (2011) 'The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites', *Food Research International*, 44(7), pp. 1671-1701. Available at: https://doi.org/10.1016/j.foodres.2011.03.003.
- Denardin, C. C., Hirsch, G. E., da Rocha, R. F., Vizzotto, M., Henriques, A. T., Moreira, J. C. F., Guma, F. T. C. R. and Emanuelli, T. (2015) 'Antioxidant capacity and bioactive compounds of four Brazilian native fruits', *Journal of Food and Drug Analysis*, 23(3), pp. 387-398. Available at: https://doi.org/10.1016/j.jfda.2015.01.006.

- Desseva, I. and Mihaylova, D. (2019) 'Influence of *in vitro* gastrointestinal digestion on phytochemicals in pomegranate juice', *Food Science and Technology*. Available at: https://doi.org/10.1590/fst.07219.
- Di Stefano, V., Pitonzo, R., Novara, M. E., Bongiorno, D., Indelicato, S., Gentile, C., Avellone, G., Bognanni, R., Scandurra, S. and Melilli, M. G. (2018) 'Antioxidant activity and phenolic composition in pomegranate (*Punica granatum* L.) genotypes from south Italy by UHPLC/Orbitrap-MS approach', *Journal of the Science of Food and Agriculture*. Available at: https://doi.org/10.1002/jsfa.9270.
- Diaz-Toribio, M. H. and Putz, F. E. (2021) 'Underground carbohydrate stores and storage organs in fire-maintained longleaf pine savannas in Florida, USA', *American Journal of Botany*, 108(3), pp. 432-442.
- Dietrich, S., Jacobs, S., Zheng, J. S., Meidtner, K., Schwingshackl, L. and Schulze, M. B. (2019) 'Gene-lifestyle interaction on risk of type 2 diabetes: A systematic review'. [Systematic Review]. *Obesity Reviews*, 20, pp. 1557-1571. Available at: https://doi.org/10.1111/obr.12921.
- Echouffo-Tcheugui, J. B. and Selvin, E. (2021) 'Prediabetes and what it means: The epidemiological evidence', *Annual Review of Public Health*, 42(1), pp. 59-77. Available at: https://doi.org/10.1146/annurev-publhealth-090419-102644.
- El Gharras, H. (2009) 'Polyphenols, food sources, properties and applications – A review', *International Journal of Food Science and Technology*, 44, pp. 2512-2518. Available at: https://doi.org/10.1111/j.1365-2621.2009.02077.x.
- Elfi-Susanti, V. H., Budi-Utomo, S., Syukri, Y. and Redjeki, T. (2012) 'Phytochemical screening and analysis polyphenolic antioxidant activity of methanolic extract of white dragon fruit (*Hylocereus undatus*)', *Indonesian Journal of Pharmacy*, 23(1), pp. 60-64. Available at: https://doi.org/10.14499/ indonesianjpharm0iss0pp60-64.

- Eliaser, E. M., Ho, J. H., Hashim, N. M., Rukayadi, Y., Ee, G. C. L. and Razis, A. F. A. (2018) 'Phytochemical constituents and biological activities of *Melicope lunu-ankenda'*, *Molecules*, 23(10). Available at: https://doi.org/10.3390/molecules 23102708.
- Eriksen, J. N., Luu, A. Y., Dragsted, L. O. and Arrigoni, E. (2017) 'Adaption of an *in vitro* digestion method to screen carotenoid liberation and *in vitro* accessibility from differently processed spinach preparations', *Food Chemistry*, 224, pp. 407-413.
- European Commission (2023) *Novel Food*. Available at: https://food.ec.europa.eu/ safety/novel-food_en (Accessed: 27 September 2023).
- Evans, M., Morgan, A. R., Patel, D., Dhatariya, K., Greenwood, S., Newland-Jones, P., Hicks, D., Yousef, Z., Moore, J., Kelly, B., Davies S. and Dashora, U. (2021) 'Risk prediction of diabetes missing million: Identifying individuals at high risk of diabetes and related complications', *Diabetes Therapy,* 12, pp. 87-105. Available at: https://doi.org/10.1007/s13300-020-00963-2.
- Fadlilah, S., Sucipto, A., Khasanah, F., Setiawan, D. and Rahil, N. H. (2020) 'Dragon fruit (*Hylocereus polyrhizus*) effectively reduces fasting blood sugar levels and blood pressure on excessive nutritional status', *Pakistan Journal of Medical and Health Sciences,* 14(2), pp. 1405-1412.
- Fawole, O. A. and Opara, U. L. (2016) 'Stability of total phenolic concentration and antioxidant capacity of extracts from pomegranate co-products subjected to *in vitro* digestion', *BMC Complementary and Alternative Medicine*, 16(358). Available at: https://doi.org/10.1186/s12906-016-1343-2.
- Fellows, P. J. (2022) *Food processing technology. Principles and practice*. 5th edition. United Kingdom:Elsevier.

- Filippini, T., Naska, A., Kasdagli, M. I., Torres, D., Lopes, C., Carvalho, C., Moreira, P., Malavolti, M., Orsini, N., Whelton, P. K. and Vinceti, M. (2020) 'Potassium intake and blood pressure: A dose-response meta-analysis of randomized controlled trials'. [Systematic review]. *Journal of the American Heart Association*, 9(12). Available at: https://doi.org/10.1161/JAHA.119.015719.
- Fiore, M., Messina, M. P., Petrella, C., D'Angelo, A., Greco, A., Ralli, M., Ferraguti, G., Tarani, L., Vitali, M. and Ceccanti M. (2020) 'Antioxidant properties of plant polyphenols in the counteraction of alcohol-abuse induced damage: Impact on the Mediterranean diet', *Journal of Functional Foods*, 71. Available at: https://doi.org/10.1016/j.jff.2020.104012.
- Fitó, M., Cladellas, M., de la Torre, R., Martí, J., Alcántara, M., Pujadas-Bastardes, M., Marrugat, J., Bruguera, J., López-Sabater, M. C., Vila, J. and Covas, M. I. (2005) 'Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: A randomized, crossover, controlled, clinical trial', *Atherosclerosis*, 181(1), pp. 149-158. Available at: https://doi.org/10.1016/j.atherosclerosis.2004.12.036.
- Fitri, A., Andriani, M., Sudarman, A., Toharmat, T., Yonekura, L., Tamura, H. and Ramli, N. (2016) 'Screening of antioxidant activities and their bioavailability of tropical fruit by-products from Indonesia', *International Journal of Pharmacy and Pharmaceutical Sciences*. 8 (6), pp. 96-100.
- Food and Agriculture Organization of the United Nations (FAO) (2023) *Major tropical fruits market review – Preliminary results 2022*, Rome Italy:FAO.
- Fraga, C. G., Croft, K. D., Kennedy, D. O. and Tomás-Barberán, F. A. (2019) 'The effects of polyphenols and other bioactives on human health', *Food and Function*, 10, pp. 514-528. Available at: https://doi.org/10.1039/c8fo01997e.
- Gengatharan, A., Dykes, G. A. and Choo, W.-S. (2017) 'The effect of pH treatment and refrigerated storage on natural colourant preparations (betacyanins) from red

pitahaya and their potential application in yoghurt', *LWT-Food Science and Technology*, 80, pp. 437-445. Available at: https://doi.org/10.1016/j.lwt.2017.03.014.

- Ghosh, S., Chakraborty, R. and Raychaudhuri, U. (2015) 'Determination of pH-dependent antioxidant activity of palm (*Borassus flabellifer*) polyphenol compounds by photoluminol and DPPH methods: A comparison of Redox reaction sensitivity', *3 Biotech*, 5(5), pp. 633-640. Available at: https://doi.org/10.1007/s13205-014-0260-7.
- Giacco, R., Costabile, G., Fatati, G., Frittitta, L., Maiorino, M. I., Marelli, G., Parillo, M., Pistis, D., Tubili, C., Vetrani, C. and Vitale, M. (2020) 'Effects of polyphenols on cardio-metabolic risk factors and risk of type 2 diabetes. A joint position statement of the Diabetes and Nutrition Study Group of the Italian Society of Diabetology (SID), the Italian Association of Dietetics and Clinical Nutrition (ADI) and the Italian Association of Medical Diabetologists (AMD)', *Nutrition, Metabolism and Cardiovascular Diseases*, 30, pp. 355-367.
- Gill, S. K., Rossi, M., Bajka, B. and Whelan, K. (2021) 'Dietary fibre in gastrointestinal health and disease', *Nature Reviews, Gastroenterology & Hepatology*, 18 (2), pp. 101-116. Available at: https://doi.org/10.1038/s41575-020-00375-4.
- Gingras, V., Hivert, M.-F. and Oken, E. (2018) 'Early life exposures and risk of diabetes mellitus and obesity', *Current Diabetes Reports* 18(89) pp. 88-89.
- Giovannini, P., Howes, M.-J. R. and Edwards, S. E. (2016) 'Medicinal plants used in the traditional management of diabetes and its sequelae in Central America: A review', *Journal of Ethnopharmacology*, 184, pp. 58-71. Available at: https://doi.org/10.1016/j.jep.2016.02.034.

- Girsang, R., Purba, R., Barus, D., Sari, N. and Purba, T. (2020) 'The effect of red dragon fruit toward blood glucose levels decreased', *Proceedings of the International Conference on Health Informatics and Medical Application Technology*, pp. 66-73. Available at: https://doi.org/10.5220/0009463600660073.
- Global Health Observatory (GHO) (2023) *Raised blood glucose/diabetes*. Available at: https://www.who.int/data/gho/indicator-metadata-registry/imr-details/3156 (Accessed: 12 September 2023).
- Gol, N. B., Chaudhari, M. L. and Rao, T. V. R. (2015) 'Effect of edible coatings on quality and shelf life of carambola (*Averrhoa carambola* L.) fruit during storage', *Journal of Food Science and Technology*, 52(1), pp. 78-91. Available at: https://doi.org/10.1007/s13197-013-0988-9.
- Goof, H. D., Repin, N., Fabek, H., Khoury, D. E. and Gidley, M. J. (2018) 'Dietary fibre for glycaemia control: Towards a mechanistic understanding', *Bioactive Carbohydrates and Dietary Fibre*, 14, pp. 39-53. Available at: http://dx.doi.org/10.1016/j.bcdf.2017.07.005.
- Griendling, K. K., Camargo, L. L., Rios, F. J., Alves-Lopes, R., Montezano, A. C. and Touyz, R. M. (2021) 'Oxidative stress and hypertension', *Circulation Research*, 128(7), pp. 993-1020. Available at: https://doi.org/10.1161/CIRCRESAHA.121.318063.
- Guasch-Ferré, M., Merino, J., Sun, Q., Fitó, M. and Salas-Salvadó, J. (2017) 'Dietary polyphenols, Mediterranean diet, prediabetes, and type 2 diabetes: A narrative review of the evidence', *Oxidative Medicine and Cellular Longevity*, 2017:6723931. Available at: https://doi.org/10.1155/2017/6723931.
- Guerriero, G., Berni, R., Muñoz-Sanchez, J. A., Apone, F., Abdel-Salam, E. M., Qahtan, A. A., Alatar, A. A., Cantini, C., Cai, G., Hausman, J. F., Siddiqui, K. S., Hernández-Sotomayor, S. M. T. and Faisal, M. (2018) 'Production of plant

secondary metabolites: Examples, tips and suggestions for biotechnologists', *Genes*, 9(6). Available at: https://doi.org/10.3390/genes9060309.

- Gutiérrez, R. M. P., Solís, R. V., Baez, E. G. and Flores, J. M. M. (2007) 'Microvascular protective activity in rabbits of triterpenes from *Hylocereus undatus*', *Journal of Natural Medicines*, *61*(3), pp. 296-301. Available at: https://doi.org/10.1007/s11418-007-0150-y.
- Hasan, M., Ali, M. T., Khan, R., Palit, P., Islam, A., Seidel, V., Akter, R. and Nahar, L. (2018) 'Hepatoprotective, antihyperglycemic and antidiabetic effects of *Dendrophthoe pentandra* leaf extract in rats', *Clinical Phytoscience: International Journal of Phytomedicine and Phytotherapy*, 4(1). Available at: https://doi.org/ 10.1186/s40816-018-0076-9.
- Hayanga, J. A., Ngubane, S. P., Murunga, A. N. and Owira, P. M. O. (2016) 'Grapefruit juice improves glucose intolerance in streptozotocin-induced diabetes by suppressing hepatic gluconeogenesis', *European Journal of Nutrition*, 55(2), pp. 631-638. Available at: https://doi.org/10.1007/s00394-015-0883-4.
- Herman-Lara, E., Elvira-Torales, L. I., Rodriguez-Miranda, J., Torruco-Uco, J. G., Carmona-García, R., Mendoza-García, P. G., García, H. S., Soto-Rodríguez, I., Sánchez-Valdivieso, E. and Martínez-Sánchez, C. E. (2014) 'Impact of micronized starfruit (*Averrhoa carambola* L.) fiber concentrate on lipid metabolism in mice', *International Journal of Food Sciences and Nutrition*, 65(7), pp. 862-867. Available at: https://doi.org/10.3109/09637486.2014.918590.
- Hernawati, Setiawan, N. A., Shintawati, R. and Priyandoko, D. (2018) 'The role of red dragon fruit peel (*Hylocereus polyrhizus*) to improvement blood lipid levels of hyperlipidaemia male mice', *Journal of Physics: Conference Series*, 1013. Available at: https://doi.org/10.1088/1742-6596/1013/1/012167.

- Hii, C. L. and Ogugo, J. F. (2014) 'Effect of pre-treatment on the drying kinetics and product quality of star fruit slices', *Journal of Engineering Science and Technology*, 9(1), pp. 123-135.
- Hooijmans, C. R., Rovers, M. M., de Vries, R. B., Leenaars, M., Rirskes-Hoitinga, M. and Langendam, M. W. (2014) 'SYRCLE's risk of bias tool for animal studies', *BMC Medical Research Methodology*, 14. Available at: https://doi.org/10.1186/1471-2288-14-43
- Howes, D. (2015) 'The science of sensory evaluation: An ethnographic critique' in Drazin, A. and Kuechler, S. (eds.) *The Social Life of Materials*. Bloomsbury Academic, pp. 81-97. Available at: http://centreforsensorystudies.org/occasional-papers/the-science-of-sensory-evaluation/
- Hsieh, C.-W., Cheng, J.-Y., Ho, W.-J., Wang, T.-H. and Wang, H.-J. (2014) 'Hypoglycaemic effects of *Ajuga* extract *in vitro* and *in vivo*', *Journal of Functional Foods*, 6(1), pp. 224-230. Available at: https://doi.org/10.1016/j.jff.2013.10.010.
- Ibrahim, S. R. M., Mohamed, G. A., Khedr, A. I. M., Zayed, M. F. and El-Kholy, A. A.-E. S. (2018) 'Genus *Hylocereus*: Beneficial phytochemicals, nutritional importance, and biological relevance – A review', *Journal of Food Biochemistry,* 42(2). Available at: https://doi.org/10.1111/jfbc.12491.
- Icoz, U. G., Orhan, N., Aslan, M. and Altun, L. (2017) '*In vitro* and *in vivo* antioxidant and antidiabetic activity studies on standardized extracts of two *Bidens* species', *Journal of Food Biochemistry*, 41(6). Available at: https://doi.org/10.1111/jfbc.12429.
- Ihara, Y., Yamada, Y., Toyokuni, S., Miyawaki, K., Ban, N., Adachi, T., Kuroe, A., Iwakura, T., Kubota, A., Hiai, H. and Seino, Y. (2000) 'Antioxidant α-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes', *Federation of European Biochemical Societies Letters*, 473, pp. 24-26.

- Infante, J., Rosalen, P. L., Lazarini, J. G., Franchin, M., de Alencar, S. M. and Chamani, J. (2016) 'Antioxidant and anti-inflammatory activities of unexplored Brazilian native fruits', *PloS One*, 11(4). Available at: https://doi.org/10.1371/journal.pone.0152974.
- International Organization for Standardization [IS0] (2010) *Food Products – Determination of the glycaemic index (GI) and recommendation for food classification, ISO 26642:2010*, Geneva Switzerland : International Organization for Standardization. Available at: https://www.iso.org/obp/ui/#iso:std:iso:26642:ed-1:v1:en.
- Isah, T., Umar, S., Mujib, A., Sharma, M. P., Rajasekharan, P. E., Zafar, N. and Frukj, A. (2018) 'Secondary metabolism of pharmaceuticals in the plant *in vitro* cultures: Strategies, approaches, and limitations to achieving higher yield', *Plant Cell Tissue and Organ Culture*, 132, pp. 239-265. Available at: https://doi.org/10.1007/s11240-017-1332-2.
- Jackson, S. L., Cogswell, M. E., Zhao, L., Terry, A. L., Wang, C.-Y., Wright, J., Coleman, S. M., King, M. D., Bowman, B., Chen, T.-C., Merrit, R. and Loria, C. M. (2018) 'Association between urinary sodium and potassium excretion and blood pressure among adults in the United States. National health and nutrition examination survey, 2014', *Circulation*, 137, pp. 237-246. Available at: htpps://doi.org/10.1161/CIRCULATIONAHA.117.029193.
- Jaeger, S. R., Axten, L. G., Wohlers, M. W. and Sun-Waterhouse, D. (2009) 'Polyphenol-rich beverages: Insights from sensory and consumer science', *Journal of the Science of Food and Agriculture*, 89(14), pp. 2356–2363. Available at: https://doi.org/10.1002/jsfa.3721.
- Jagannathan, R., Neves, J. S., Dorcely, B., Chung, S. T., Tamura, K., Rhee, M. and Bergman, M. (2020) 'The oral glucose tolerance test: 100 years later', *Diabetes,*

Metabolic Syndrome and Obesity: Targets and Therapy. 13, pp. 3787-3805. Available at: https://doi.org/10.2147/DMSO.S246062.

- Jain, B. P., Goswami, S. K. and Pandey S. (2021) 'Carbohydrate', in Jaim, B. P. (ed.) *Protocols in Biochemistry and Clinical Biochemistry*. Academic Press. Available at: http://doi.org/10.016/B978-0-12-822007-8.00007-6.
- Jain, V. and Gupta, K. (2005) 'Food and nutritional analysis: Overview' in Worsfold, P., Townshend, A. and Poole, C. (ed.) *Encyclopedia of Analytical Science* Elsevier.
- Jalal, T. K., Ahmed, I. A., Mikail, M., Momand, L., Draman, S., Isa, M. L. M., Abdull-Rasad, M. S. B., Nor-Omar, M., Ibrahim, M. and Abdul-Wahab, R. (2015) 'Evaluation of antioxidant, total phenol and flavonoid content and antimicrobial activities of *Artocarpus altilis* (breadfruit) of underutilized tropical fruit extracts', *Applied Biochemistry and Biotechnology*, 175(7), pp. 3231-3243. Available at: https://doi.org/10.1007/s12010-015-1499-0.
- Kalita, D., Holm, D. G., LaBarbera, D. V., Petrash, J. M. and Jayanty, S. S. (2018) 'Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds', *PloS One*, 13(1). Available at: https://doi.org/10.1371/journal.pone.0191025.
- Kallscheuer, N., Classen, T., Drepper, T. and Marienhagen, J. (2019) 'Production of plant metabolites with applications in the food industry using engineered microorganisms', *Current Opinion in Biotechnology*, 56, pp. 7-17. Available at: https://doi.org/10.1016/j.copbio.2018.07.008.
- Kamiloglu, S. and Capanoglu, E. (2014) '*In vitro* gastrointestinal digestion of polyphenols from different molasses (pekmez) and leather (pestil) varieties', *International Journal of Food Science & Technology*, 49(4), pp. 1027-1039. Available at: https://doi.org/10.1111/ijfs.12396.

- Kamyab, R., Namdar, H., Torbati, M., Ghojazadeh, M., Araj-Khodaei, M. and Bagher Fazljou, S. M. (2021) 'Medicinal plants in the treatment of hypertension: A review', *Advanced Pharmaceutical Bulletin*, 11(4), pp. 601-617. Available at https://doi.org/10.34172/apb.2021.090.
- Kemp, S. E., Hollowood, T. and Hort, J. (2009) *Sensory evaluation: A practical handbook*. Chichester, United Kingdom:Ames; Iowa, United States:Wiley-Blackwell. Available at: https://www-dawsonera-com.oxfordbrookes.idm.oclc.org/readonline/9781444322637 (Accessed: 7 February 2020).
- Kendall, C. W. C., Esfahani, A. and Jenkins, D. J. A. (2010) 'The link between dietary fibre and human health', *Food Hydrocolloids*, 24(1), pp 42-48.
- Khanam, Z., Sam, K. H., Zakaria, N. H. B. M., Ching, C. H. and Bhat, I. U. H. (2015) 'Determination of polyphenolic content, HPLC analyses and DNA cleavage activity of Malaysian *Averrhoa carambola* L. fruit extracts', *Journal of King Saud University-Science*, 27(4), pp. 331-337.
- Khatri, D. and Chhetri, S. B. B. (2020) 'Reducing sugar, total phenolic content, and antioxidant potential of Nepalese plants', *BioMed Research International*. Available at: https://doi.org/10.1155/2020/7296859.
- Khattab, R., Celli, G. B., Ghanem, A. and Brooks, M. S.-L. (2015) 'Effect of frozen storage on polyphenol content and antioxidant activity of haskap berries (*Lonicera caerulea* L.)', *Journal of Berry Research*, 5, pp. 231-242. Available at: http://doi.org/10.3233/JBR-150105.
- Khoo, Z. Y., The, C. C., Rao, N. K. and Chin, J. H. (2010) 'Evaluation of the toxic effect of star fruit on serum biochemical parameters in rats', *Pharmacognosy Magazine*, 6(22), pp. 120-124. Available at: https://doi.org/10.4103/0973-1296.62899.

- Kim, H., Choi, H. K., Moon, J. Y., Kim, Y. S., Mosaddik, A. and Cho, S. K. (2011) 'Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content', *Journal of Food Science*, 76(1), pp. 38-45. Available at: https://doi.org/10.1111/j.1750-3841.2010.01908.x.
- Koo, H., Hwang, S., Kim, T. H., Kang, S. W., Oh, K.-H., Ahn, C. and Kim, Y. O. (2018) 'The ratio of urinary sodium and potassium and chronic kidney disease progression. Results from the Korean cohort study for outcomes in patients with chronic kidney disease (KNOW-CKD)', *Medicine*, 97(44). Available at: https://doi.org/10.1097/MD.0000000000012820.
- Kris-Etherton, P. M., Lefevre, M., Beecher, G. R., Gross, M. D., Keen, C. L. and Etherton, T. D. (2004) 'Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis', *Annual Review of Nutrition*, 24, pp. 511-538. Available at: https://doi.org/10.1146/annurev.nutr.23.011702.073237.
- Lakmal, K., Yasawardene, P., Jayarajah, U. and Seneviratne, S. L. (2021) 'Nutritional and medicinal properties of star fruit (*Averrhoa carambola*): A review', *Food Science and Nutrition*, 9(3), pp. 1810-1823. Available at: https://doi.org/10.1002/fsn3.2135.
- Lassale, C., Gaye, B., Ibrahima, B. D., Jean, B. M., Kouadio, E. K., Charles, K. K., Méo, S. I., Jean, L. T., Damorou, J. M., Ibrahim, A. T., Balde, D. M., Dzudie, A., Houenassi, M., Kane, A., Kimbally-Kaki, S. G., Kingue, S., Limbole, E., Kuate, L. M., Ferreira, B., Nhavoto, C., Abdallahi, S. A., Azizi, M., N'Guetta, R., Antignac, M. and Jouven, X. (2022) 'Use of traditional medicine and control of hypertension

in 12 African countries', *BMJ Global Health,* 7(6), Available at: http://dx.doi.org/10. 1136/bmjgh-2021-008138.

- Laya, A. and Koubala, B. B. (2020) 'Polyphenols in cassava leaves (*Manihot esculenta* Crantz) and their stability in antioxidant potential after *in vitro* gastrointestinal digestion', *Heliyon*, 6. Available at: https://doi.org/10.1016/ j.heliyon.2020.e03567.
- Leelarungrayub, J., Yankai, A., Pinkaew, D., Puntumetakul, R., Laskin, J. J. and Bloomer, R. J. (2016) 'A preliminary study on the effects of star fruit consumption on antioxidant and lipid status in elderly Thai individuals', *Clinical Interventions in Aging*, 11, pp. 1183-1192.
- Leelarungrayub, J., Laskin, J. J., Bloomer, R. J. and Pinkaew, D. (2015) 'Consumption of star fruit juice on pro-inflammatory markers and walking distance in the community dwelling elderly', *Archives of Gerontology and Geriatrics*, 64, pp. 6-12. Available at: https://doi.org/10.1016/j.archger. 2015.12001.
- Li, K., Yao, F., Xue, Q., Yang, L., Li, X., Sun, L. and Liu, Y. (2018) 'Inhibitory effects against α-glucosidase and α-amylase of the flavonoids-rich extract from *Scutellaria baicalensis* shoots and interpretation of structure-activity relationship of its eight flavonoids by a refined assign-score method', *Chemistry Central Journal*, 12. Available at: https://doi.org/10.1186/s13065-018-0445-y.
- Liao, X., Greenspan, P., Srivastava, A. and Pegg, R. B. (2020) 'Characterizing the phenolic constituents of U. S. south-eastern blackberry cultivars', *Journal of Berry Research*, 10, pp. 311-327. Available at: https://doi.org/10.3233/JBR-190475.
- Liu, S.-C., Lin, J.-T., Wang, C.-K., Chen, H.-Y. and Yang, D.-J. (2009) 'Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers', *Food Chemistry*, 114(2), pp. 577-581. Available at: https://doi.org/ 10.1016/j.foodchem.2008.09.088.

- Lu, F. C. and Kacew, S. (2002) *Lu's basic toxicology: Fundamentals, target organs, and risk assessment*. 4th ed. London;NewYork:Taylor & Francis.
- Lucas-González, R., Viuda-Martos, M., Pérez-Alvarez, J. A. and Fernández-López, J. (2018) '*In vitro* digestion models suitable for foods: Opportunities for new fields of application and challenges', *Food Research International*, 107, pp. 423-436. Available at: https://doi.org/10.1016/j.foodres.2018.02.055.
- Lupuche, E. Q., Chávez, P. J. A., Medina-Pizzali, M. L., Loayza G. L. and Apumayta, S. E. (2021) 'Chemical characterization, polyphenol content and antioxidant capacity of two pitahaya ecotypes (*Hylocereus* spp.)', *Revista Facultad Nacional de Agronomía Medellín*, 74(3), pp. 9723-9734. Available at: https://doi.org/10.15446/rfnam.v74n3.92821.
- Luu, T., Le, T., Huynh, N. and Quintela-Alonso, P. (2021) 'Dragon fruit: A review of health benefits and nutrients and its sustainable development under climate changes in Vietnam'. *Czech Journal of Food Sciences*, 39(2), pp. 71-94. Available at: https://doi.org/10.17221/139/2020-CJFS
- Maharani, A. D. and Saktiningsih, H. (2022) 'The effect of red dragon fruit (*Hylocereus polyrhizus*) juice on total cholesterol level in women', *Journal Biologi Lingkungan, Industri dan Kesehatan*, 9(1). Available at: https://doi.org/10.31289/ biolink.v9i1.7495.
- Malta, L. G., Tessaro, E. P., Eberlin, M., Pastore, G. M. and Liu, R. H. (2013) 'Assessment of antioxidant and antiproliferative activities and the identification of phenolic compounds of exotic Brazilian fruits', *Food Research International*, 53(1), pp. 417-425. Available at: https://doi.org/10.1016/j.foodres.2013.04.024.
- Mans, R. A. D., Grant, A. and Pinas, N. (2017) 'Plant-based ethnopharmacological remedies for hypertension in Suriname', *Herbal Medicine*, Edited by: Phillip F. Builders, IntechOpen. Available at: http://dx.doi.org/10.5772/intechopen.72106.

- McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A. and Stewart, D. (2005) 'Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase', *Journal of Agricultural and Food Chemistry*, 53(7), pp. 2760-2766. Available at: https://doi.org/10.1021/jf0489926.
- Mihaylova, D., Desseva, I., Stoyanova, M., Petkova, N., Terzyika, M. and Lante A. (2021) 'Impact of *in vitro* gastrointestinal digestion on the bioaccessibility of phytochemical compounds from eight fruit juices', *Molecules*, 26. Available at: https://doi.org/10.3390/molecules26041187.
- Mohammed, A., Kumar, D. and Rizvi, S. I. (2015) 'Antidiabetic potential of some less commonly used plants in traditional medicinal systems of India and Nigeria', *Journal of Intercultural Ethnopharmacology*, 4(1), pp. 78-85. Available at: https://doi.org/10.5455/jice.20141030015241.
- Mojzer, E. B., Hrnčič, M. K., Škerget, M., Knez, Ž. and Bren, U. (2016) 'Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects', *Molecules*, 21. Available at: https://doi.org/10.3390/molecules21070901.
- Mphuthi, D. D. and Husaini, D. C. (2022) 'Traditional medicinal plants used by hypertensive patients in Belize: A qualitative evaluation of beliefs and practices', *Bulletin of the National Research Centre*, 46(1), pp. 1-11. Available at: https://doi.org/10.1186/s42269-022-00789-x.
- Mun, S., Decker, E. A., Park, Y., Weiss, J. and McClements, D. J. (2006) 'Influence of interfacial composition on *in vitro* digestibility of emulsified lipids: Potential mechanism for chitosan's ability to inhibit fat digestion', *Food Biophysics*, 1(1), pp. 21-29. Available at: https://doi.org/10.1007/s11483-005-9001-0.
- Muthu, N., Lee, S. Y., Phua, K. K. and Bhore, S. J. (2016) 'Nutritional, medicinal and toxicological attributes of star-fruits (*Averrhoa carambola* L.): A review',

Bioinformation. 12(12), pp. 420-424. Available at: https://doi.org/10.6026/97320630012420.

- Nagar, E. E., Okun, Z. and Shpigelman, A. (2023) '*In vitro* bioaccessibility of polyphenolic compounds: The effect of dissolved oxygen and bile', *Food Chemistry*, 404. Available at: https://doi.org/10-1016/j.foodchem.2022.134490.
- Ndukwe, G. I. and Okhiku J. O. (2018) 'Chemical composition and antimicrobial activity of the volatile oils of *Averrhoa carambola* L. (star fruit) grown in Nigeria´, *Journal of Chemical Society of Nigeria*, 43(2), pp. 141-150.
- Neri, L., Faieta, M., Mattia, C. D., Sacchetti, G., Mastrocola, D. and Pottia, P. (2020) 'Antioxidant activity in frozen plant foods: Effect of cryoprotectants, freezing process and frozen storage', *Foods*, 9(12). Available at: https://doi.org/10.3390/foods9121886.
- Neri-Numa, I. A., Soriano-Sancho, R. A., Pereira, A. P. A. and Pastore, G. M. (2018) 'Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest', *Food Research International*, 103, pp. 345-360. Available at: https://doi.org/10.1016/j.foodres.2017.10.053.
- Noor-Asna, A. and Noriham, A. (2014) 'Antioxidant activity and bioactive components of *Oxalidaceae* fruit extracts', *The Malaysian Journal of Analytical Sciences'*, 18(1), pp. 116-126.
- Ntrigiou, V., Ntrigios, I., Rigopoulos, N., Dimou, C. and Koutelidakis, A. E. (2018) 'Functional food consumption correlates with anthropometric characteristics and body composition in healthy adults', *Current Topics in Nutraceuticals Research*, 16(4), pp. 279-288.
- Oak, M. H., Auger, C., Belcastro, E., Park, S.-H., Lee, H.-H. and Schini-Kerth, V. B. (2018) 'Potential mechanisms underlying cardiovascular protection by

polyphenols: Role of the endothelium', *Free Radical Biology and Medicine*, 122, pp. 161-170. Available at: https://doi.org/10.1016/j.freeradbiomed. 2018.03.018.

- Obregón-La Rosa, A. J., Contreras-López, E., Elías-Peñafiel, C., Muñoz-Jauregui, A. M., Yuli-Posadas, R. A. and Cóndor-Salvatierra, E. J. (2022) 'Nutritional and physicochemical profile of the pitahaya cultivated in the central coast of Peru', *Revista de la Facultad de Agronomía*, 39(1). Available at: https://doi.org/10.47280/ RevFacAgron(LUZ).v39.n1.11.
- O'Hare, T. J. (1993) 'Postharvest physiology and storage of carambola (starfruit): A review', *Postharvest Biology and Technology*, 2(4), pp. 257-267. Available at: https://doi.org/10.1016/09255214(93) 90031-W.
- Olivas-Aguirre, F. J., Gonzalez-Aguilar, G. A., Velderrain-Rodrıguez, G. R., Torres-Moreno, H., Robles-Zepeda, R. E., Vazquez-Flores, A. A., de la Rosa, L. A. and Wall-Medrano, A. (2017) 'Radical scavenging and anti-proliferative capacity of three freeze-dried tropical fruits', *International Journal of Food Science and Technology*, 52(7), pp. 1699-1709. Available at: https://doi.org/10.1111/ijfs.13408.
- Ong, K. L., Stafford, L. K., McLaughlin, S. A., Boyko, E. J., Vollset, S. E., Smith, A. E., Dalton, B. E., Duprey, J., Cruz, J. A., Hagins, H., Lindstedt, P. A., Aali, A., Abate, Y. H., Abate, M. D., Abbasian, M., Abbasi-Kangevari, Z., Abbasi-Kangevari, M., Abd ElHafeez, S., Abd-Rabu, R., et al. (2023). 'Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021'. [Systematic review]. *Lancet (London, England)*, *402*(10397), pp. 203-234. https://doi.org/10.1016/ S0140-6736(23)01301-6.
- Ortiz-Hernandez, Y. D. and Carrillo-Salazar, J. A. (2012) 'Pitahaya (*Hylocereus spp*.): A short review', *Comunicata Scientiae*, 3(4), pp. 220-237.

- Oudot, C., Gomes, A., Nicolas, V., Le Gall, M., Chaffey, P., Broussard, C., Calamita, G., Mastrodonato, M., Gena, P., Perfettini, J. L., Hamelin, J., Lemoine, A., Fischmeister, R., Vieira, H. L. A., Santos, C. N. and Brenner, C. (2019) 'CSRP3 mediates polyphenols-induced cardioprotection in hypertension', *The Journal of Nutritional Biochemistry*, 66, pp. 29-42. Available at: https://doi.org/10.1016/j.jnutbio.2019.01.001.
- Ozkan, G., Sakarya, F. B., Tas, D., Yurt, B., Ercisli, S. and Capanoglu, E. (2023) 'Effect of *in vitro* digestion of the phenolic content of herbs collected from Eastern Anatolia', *ACS Omega*, 8(14), pp. 12730-12738. Available at: https://doi.org/10.1021/acsomega.2c07881.
- Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., Stewart, L. A., Thomas, J., Tricco, A. C., Welch, V. A., Whiting, P. and Moher, D. (2021) 'The PRISMA 2020 statement: An updated guideline for reporting systematic reviews', *British Medical Journal*, 372(71). Available at: https://doi.org/10.1136/bmj.n71.
- Pan American Health Organization (PAHO) (2022) *Panorama of Diabetes in the Americas*. Washington, DC. Available at: https://doi.org/10.37774/9789275126332.
- Pandey, K. B. and Rizvi, S. I. (2009) 'Plant polyphenols as dietary antioxidants in human health and disease', *Oxidative Medicine and Cellular Longevity,* 2(5), pp. 270-278. Available at: https://doi.org/10.4161/oxim.2.5.9498.
- Parada, J. and Aguilera, J. M. (2007) 'Food microstructure affects the bioavailability of several nutrients', *Journal of Food Science*, 72(2), pp. 21-32. Available at: https://doi.org/10.1111/j.1750-3841.2007.00274.x.

- Park, H. A. (2021) ‘Fruit intake to prevent and control hypertension and diabetes’, *Korean Journal of Family Medicine*, 42(1), pp. 9-16. Available at: https://doi.org/10.4082/kjfm.20.0225.
- Park, S. and Park S.-Y. (2021) ‘Can antioxidants be effective therapeutics for type 2 diabetes?’, *Yeungnam University Journal of Medicine*, 38(2), pp. 83-94. Available at: https://doi.org/10.12701/yujm.2020.00563.
- Payal, G., Pankti, K., Manodeep, C. and Kamath, J. (2012) ‘Phytochemical and pharmacological profile of *Averrhoa carambola* Linn: An overview’, *International Research Journal of Pharmacy*, 3(1), pp. 88-92.
- Pham, H. T. T., Huang, W., Han, C., Li, J., Xie, Q., Wei, J., Xu, X., Lai, Z., Huang, X., Huang, R. and Wen, Q. (2017) ‘Effects of *Averrhoa carambola* L. (*Oxalidaceae*) juice mediated on hyperglycemia, hyperlipidemia, and its influence on regulatory protein expression in the injured kidneys of streptozotocin-induced diabetic mice’, *American Journal of Translational Research*, 9(1), pp. 36-49.
- Possner, D., Zimmer, T., Kürbel, P. and Dietrich H. (2014) ‘Methanol contents of fruit juices and smoothies in comparison to fruits and a simple method for the determination thereof’, *Deutsche Lebensmittel-Rundschau: Zeitschrift für Lebensmittelkunde und Lebensmittelrecht*, pp. 66-69.
- Pothasak, Y., Leelarungrayub, J., Natakankitkul, S. and Singhatong, S. (2020) ‘Prototype star fruit-honey product and effectiveness on antioxidants, inflammation and walking distance in participants with stable chronic obstructive pulmonary disease (COPD)’, *Pharmacognosy Journal*, 12(5), pp. 1121-1134.
- Raj, G. V. S. B. and Dash, K. K. (2022) ‘Development of hydrocolloids incorporated dragon fruit leather by conductive hydro drying: Characterization and sensory evaluation’, *Food Hydrocolloids for Health*, 2. Available at: https://doi.org/10.1016/j.fhfh.2022.100086.

- Ramli, N. S., Brown, L., Ismail, P. and Rahmat, A. (2014) 'Effects of red pitaya juice supplementation on cardiovascular and hepatic changes in high-carbohydrate, high-fat diet-induced metabolic syndrome rats', *BMC Complementary and Alternative Medicine*, 14(1). Available at: https://doi.org/10.1186/1472-6882-14-189.
- Rebecca, O. P. S., Boyce, A. N. and Chandran, S. (2010). 'Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*)', *African Journal of Biotechnology*, 9(10), pp. 1450-1454.
- Reynolds, A. N., Akerman, A., Kumar, S., Diep Pham, H. T., Coffey, S. and Mann, J. (2022) 'Dietary fibre in hypertension and cardiovascular disease management: Systematic review and meta-analyses'. [Systematic review]. *BMC Medicine*, 20(1). Available at: https://doi.org/10.1186/s12916-022-02328-x.
- Roberfroid, M. B. (2002) 'Functional foods: Concepts and application to inulin and oligofructose', *British Journal of Nutrition*, 87(6), pp. 139-143. Available at: https://doi.org/10.1079/BJNBJN/2002529.
- Rocchetti, G., Giuberti, G., Lucchini, F. and Lucini, L. (2020) 'Polyphenols and sesquiterpene lactones from artichoke heads: Modulation of starch digestion, gut bioaccessibility, and bioavailability following *in vitro* digestion and large intestine fermentation', *Antioxidants*, 9(306). Available at: https://doi.org/10.3390/antiox 9040306.
- Rodrigues da Silva L. A., Santana, R. F., Achar, E., Ballester, G., Mota-Ortiz, S. R. and Fontanelle-Ribeiro-Junior, M. A. (2021) 'Standardization of experimental model regarding star fruit intoxication in Wistar rats suffering with nephropathy', *Acta Cirúrgica Brasileira*, 36(2). Available at: https://doi.org/10.1590/acb360204.
- Rodriguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L., Mena, P., Del Rio, D. and Crozier, A. (2014) "Bioavailability,

bioactivity and impact on health of dietary flavonoids and related compounds: An update', *Archives of Toxicology*, 88(10), pp. 1803-1853. Available at: https://doi.org/10.1007/s00204-014-1330-7.

- Ross, C. F. (2009) 'Sensory science at the human-machine interface', *Trends in Food Science & Technology*, 20(2), pp. 63-72. Available at: https://doi.org/10.1016/j.tifs.2008.11.004.
- Ruhan, A. M., Ullah, P., Uddin, M., Alam, J., Bari, S. and Chowdhury F. R. (2018) 'Acute kidney injury following star fruit (*Averrhoa carambola*) ingestion in a previously healthy young man', *Journal of Medicine*, 19(1), pp. 63-65.
- Sader, M., Pérez-Fernández, R., Kuuliala, L., Devlieghere, F. and De Baets, B. (2020) 'The constrained median: A way to incorporate side information in the assessment of food samples', *Mathematics*, 8(3). Available at: https://doi.org/10.3390/math8030406.
- Saghir, S. A. M., Sadikun, A., Khaw, K.-Y. and Murugaiyah, V. (2013) 'Star fruit (*Averrhoa carambola* L.): From traditional uses to pharmacological activities', *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 12(3), pp. 209-219.
- Saha, D., Guite, D. J. and Das, T. (2018) 'A complete review on the pharmacological evaluation of *Averrhoa carambola* plant', *World Journal of Pharmaceutical Research*, 7(11), pp. 199-210. Available at: https://doi.org/10.20959/wjpr201811-12201.
- Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V. and Salopek-Sondi, B. (2021) 'The role of polyphenols in abiotic stress response: The influence of molecular structure', *Plants*, *10*(1), 118. Available at: https://doi.org/10.3390/plants10010118.
- Santos-Sánchez, F. N., Salas-Coronado, R., Villanueva-Cañongo, C. and P-Carlos, B. (2019) 'Antioxidant compounds and their antioxidant mechanism', in Shalaby, E.

(ed.) *Antioxidants*. IntechOpen. Available at: http://dx.doi.org/10.5772/intechopen.85270.

- Sarkhosh-Khorasani, S. and Hosseinzadeh, M. (2021) 'The effect of grape products containing polyphenols on C-reactive protein levels: A systematic review and meta-analysis of randomised controlled trials'. [Systematic review]. *British Journal of Nutrition*, 125, pp. 1230-1245. Available at: http://dx.doi.org/10.1017/S0007114520003591.
- Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005) 'Dietary polyphenols and the prevention of diseases', *Critical Reviews in Food Science and Nutrition*, 45, pp. 287-306. Available at: https://doi.org./10.1080/1040869059096.
- Seck, S. M., Doupa, D., Dia D. G., Diop, E. H. A., Ardiet, D.-L., Nogueira, R. C., Graz, B. and Diouf, B. (2018) 'Clinical efficacy of African traditional medicines in hypertension: A randomized controlled trial with *Combretum micranthum* and *Hibiscus sabdariffa*', *Journal of Human Hypertension*, 32(1), pp. 75-81. Available at: https://doi.org/10.1038/s41371-017-0001-6.
- Sęczyk, L., Sugier, D., ´Swieca, M. and Gawlik-Dziki, U. (2021) 'The effect in the *in vitro* digestion, food matrix, and hydrothermal treatment on the potential bioaccessibility of selected phenolic compounds', *Food Chemistry*, 344:128581.
- Serna-Cock, L., Vargas-Muñoz, D. P. and Aponte, A. A. (2015) 'Structural, physical, functional and nutraceutical changes of freeze-dried fruit', *African Journal of Biotechnology*, 14(6), pp. 442-450. Available at: https://doi.org/10.5897/AJB14.14189.
- Serrano, A., Ros, G. and Nieto, G. (2018) 'Bioactive compounds and extracts from traditional herbs and their potential anti-inflammatory health effects', *Medicines*, 5(3). Available at: https://doi.org/10.3390/medicines5030076.

- Shahwan, M., Alhumaydhi, F., Ashraf, G. M., Hasan, P. M. Z. and Shamsi, A. (2022) 'Role of polyphenols in combating type 2 diabetes and insulin resistance', *International Journal of Biological Macromolecules*, 206, pp. 567-579. Available at: https://doi.org/10.1016/j.ijbiomac.2022.03.004.
- Shanmugam, S., Gomes, I. A., Denadai, M., Dos Santos-Lima B., de Souza-Araújo, A. A., Narain, N., Neta, M. T. S. L., Serafini, M. R., Quintans-Júnior, L. J. and Thangaraj, P. (2018) 'UHPLC-QqQ-MS/MS identification, quantification of polyphenols from *Passiflora subpeltata* fruit pulp and determination of nutritional, antioxidant, α-amylase and α-glucosidase key enzymes inhibition properties', *Food Research International*, 108, pp. 611-620. Available at: https://doi.org/10.1016/j.foodres.2018.04.006.
- Shiau, S.-Y. and Chang, G. W. (1983) 'Effects of dietary fiber on fecal mucinase and β-glucuronidase activity in rats', *The Journal of Nutrition*, 113, pp. 138-144.
- Shing, D., Corradi, M., Spinola, M., Papi, A., Usmani, O. S., Scuri, M., Petruzzelli, S. and Vestbo, J. (2017) 'Triple therapy in COPD: New evidence with the extrafine fixed combination of beclomethasone dipropionate, formoterol fumarate, and glycopyrronium bromide', *International Journal of COPD*, 12, pp. 2917-2928. Available at: https://doi.org/10.2147/COPD. S146822.
- Singh, A. K., Cabral, C., Kumar, R., Ganguly, R., Rana, H. K., Gupta, A., Lauro, M. R., Carbone, C., Reis, F. and Pandey, A. K. (2019) 'Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficiency', *Nutrients*, 11(9). Available at: https://doi.org/10.3390/nu11092216.
- Siow, L.-F. and Wong, Y.-M. (2017) 'Effect of juice concentration on storage stability, betacyanin degradation kinetics, and sensory acceptance of red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice', *International Journal of Food*

Properties, 20(3), pp. 623-632. Available at: https://doi.org/10.1080/10942912.2016.1172086.

- Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W. and De Freitas, V. (2013) 'Different phenolic compounds activate distinct human bitter taste receptors', *Journal of Agricultural and Food Chemistry*, 61(7), pp. 1525-1533. Available at: https://doi.org/10.1021/jf304198k.
- Song, H., Zheng, Z., Wu, J., Lai, J., Chu, Q. and Zheng, X. (2016) 'White pitaya (*Hylocereus undatus*) juice attenuates insulin resistance and hepatic steatosis in diet-induced obese mice', *PloS One*, 11(2). Available at: https://doi.org/10.1371/journal.pone.0149670.
- Stintzing, F. C., Schieber, A. and Carle, R. (2002) 'Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose', *Food Chemistry*, 77(1), pp. 101-106. Available at: https://doi.org/10.1016/50308-8146(01)00374-0.
- Streppel, M. T., Arends, L. R., van't Veer, P., Grobbee, D. E. and Geleijnse, J. M. (2005) 'Dietary fiber and blood pressure; A meta-analysis of randomized placebo-controlled trials'. [Systematic review]. *Archives of Internal Medicine*, 165, pp. 150-156.
- Stumpf, M. A., Schuinski, A. F., Baroni, G. and Ramthun, M. (2020) 'Acute kidney injury with neurological features: Beware of the star fruit and its caramboxin', *Indian Journal of Nephrology*, 30, pp. 42-46.
- Suárez, A. L., Thu, Z. M., Ramírez, J., León, D., Cartuche, L., Armijos, C. and Vidari, G. (2017) 'Main constituents and antidiabetic properties of *Otholobium mexicanum*', *Natural Product Communications*, 12(4), pp-533-535.
- Sudha, P., Zinjarde, S. S., Bhargava, S. Y. and Kumar, A. R. (2011) 'Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants', *BMC*

Complementary and Alternative Medicine, 11(1), pp. 1-10. Available at: https://doi.org/10.1186/1472-6882-11-5.

- Sulaiman, S. F., Sajak, A. A. B., Ooi, K. L., Supriatno, and Seow, E. M. (2011) 'Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables', *Journal of Food Composition and Analysis*, 24(4-5), pp. 506-515. Available at: https://doil.org/10.1016/j.jfca.2011.01.020.
- Tagliazucchi, D., Verzelloni, E., Bertolini, D. and Conte, A. (2010) '*In vitro* bio-accessibility and antioxidant activity of grape polyphenols', *Food Chemistry*, 120, pp. 599-606. Available at: https://doi.org/10.1016/j.foodchem.2009.10.030.
- Telli, A., Esnault, M.-A. and Ould-El-Hadj-Khelil, A. (2016) 'An ethnopharmacological survey of plants used in traditional diabetes treatment in south-eastern Algeria (Ouargla province)', *Journal of Arid Environments*, 127, pp. 82-92. Available at: https://doi.org/10.1016/j.jaridenv.2015.11.005.
- Temak, Y., Cholke, P., Mule, A., Shingade, A., Narote, S., Kagde, A., Lagad, R. and Sake, V. (2018) '*In vivo* and *in vitro* evaluation of antimicrobial activity of peel extracts of red dragon fruit (*Hylocereus polyrhizus*)', *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, 3(5), pp. 24-26.
- Tenore, G. C., Novellino, E. and Basile, A. (2012) 'Nutraceutical potential and antioxidant benefits of red pitaya (*Hylocereus polyrhizus*) extracts', *Journal of Functional Foods*, 4(1), pp. 129-136. Available at: https://doi.org/10.1016/ j.jff.2011.09.003.
- Tian, C., Hao, L., Yi, W., Ding, S. and Xu, F. (2020) 'Polyphenols, oxidative stress, and metabolic syndrome', *Oxidative Medicine and Cellular Longevity*, 2020:7398453. Available at: https://doi.org/10.1155/2020/7398453.
- Tian, J., Wu, X., Zhang, M., Zhou, Z. and Liu, Y. (2018) 'Comparative study on the effects of apple peel polyphenols and apple flesh polyphenols on cardiovascular risk

factors in mice', *Clinical and Experimental Hypertension*, 40(1), pp. 65-72. Available at: https://doi.org/10.1080/10641963.2017.1313851.

- Toomer, O. T., Vu, T., Pereira, M. and Williams, K. (2019) 'Dietary supplementation with peanut skin polyphenolic extracts (PSPE) reduces hepatic lipid and glycogen stores in mice fed an atherogenic diet', *Journal of Functional Foods*, 55, pp. 362-370. Available at: https://doi.org/10.1016/j.jff.2019.02.041.
- Tresserra-Rimbau, A., Guasch-Ferré, M., Salas-Salvado, J., Toledo, E., Corella, D., Castañer, O., Guo, X., Gómez-Gracia, E., Lapetra, J., Arós, F., Fiol, M., Ros, E., Serra-Majem, L., Pintó, X., Fitó, M., Babio, N., Martínez-González, M. A., Sorli, J. V., López-Sabater, M. C., Estruch, R. and Lamuela-Raventós, R. M. (2016) 'Intake of total polyphenols and some classes of polyphenols is inversely associated with diabetes in elderly people at high cardiovascular disease risk', *The Journal of Nutrition*, 146, pp. 767-777. Available at: https://doi.org/ 10.3945/jn.115.223610.
- Tsao, R. (2010) 'Chemistry and biochemistry of dietary polyphenols', *Nutrients*, 2, pp. 1231-1246. Available at: https://doi.org/10.3390/nu2121231.
- Van Trijp H. C. M. and Schifferstein H. N. J. (1995) 'Sensory analysis in marketing practice: Comparison and integration', *Journal of Sensory Studies*, 10(2), pp. 127-147. Available at: https://doi.org/10.1111/j.1745-459X.1995.tb00010.x.
- Venn, B. J. and Mann, J. I. (2004) 'Cereal grains, legumes and diabetes', *European Journal of Clinical Nutrition*, 58, pp. 1443-1461. Available at: https://doi.org/ 10.1038/sj.ejcn.1601995.
- Vidigal, M. C. T. R., Minim, V. P. R., Carvalho, N. B., Milagres, M. P. and Gonçalves, A. C. A. (2011) 'Effect of a health claim on consumer acceptance of exotic Brazilian fruit juices: açaí (*Euterpe oleracea* mart.), camu-camu (*Myrciaria dubia*), cajá (*Spondias lutea* L.) and umbu (*Spondias tuberosa arruda*)', *Food*

Research International, 44(7), pp. 1988-1996. Available at: https://doi.org/10.1016/j.foodres.2010.11.028.

- Vuong, Q. V., Hirun, S., Phillips, P. A., Chuen, T. L. K., Bowyer, M. C., Goldsmith, C. D. and Scarlett, C. J. (2014) 'Fruit-derived phenolic compounds and pancreatic cancer: Perspectives from Australian native fruits', *Journal of Ethnopharmacology*, 152(2), pp. 227-242. Available at: https://doi.org/ 10.1016/j.jep.2013.12.023.
- Wallace, T. C., Slavin, M. and Frankenfeld, C. L. (2016) 'Systematic review of anthocyanins and markers of cardiovascular disease'. [Systematic review]. *Nutrients*, 8(1). Available at: https://doi.org/10.3390/nu8010032.
- Walsh, J., Olavarria-Ramirez, L., Lach, G., Boehme, M., Dinan, T. G., Cryan, J. F., Griffin, B. T., Hyland, N. P. and Clarke G. (2020) 'Impact of host and environmental factors on β-glucuronidase enzymatic activity: Implications for gastrointestinal serotonin', *American Journal of Physiology – Gastrointestinal and Liver Physiology*, 318(4), pp. G816-G826. Available at https://doi.org/10.1152/ajpgi.00026.2020.
- Wang, Y.-C. L., Liu, B.-M., Supernaw, R. B., Lu, Y.-H. and Lee, P.-Y. (2006) 'Management of star fruit-induced neurotoxicity and seizures in a patient with chronic renal failure', *Pharmacotherapy*, 26(1), pp.143-146.
- What Works Clearinghouse. (2020) 'What Works Clearinghouse Standards Handbook', Version 4.1. Washington, DC:U.S. Department of Education, Institute of Education Sciences, National Center for Education Evaluation and Regional Assistance.
- Whelton, P. K. (2018) 'Sodium and potassium intake in US adults', *Circulation*, 137(3), pp. 247-249. Available at: https://doi.org/10.1161/CIRCULATIONAHA.117.031371.

- Wiardani, N. K., Moviana, Y. and Puryana, I. G. P. S. (2014) 'Red dragon fruit juice has potential to reduce glucose blood level in patient with diabetes mellitus type 2', *Journal Skala Husada*, 11(1), pp. 59-66.
- Winkler, J. and Ghosh, S. (2018) 'Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes', *Journal of Diabetes Research*, 2018:5391014, Available at: https://doi.org/10.1155/2018/5391014.
- Wojtunik-Kulesza, K., Oniszczuk, A., Oniszczuk, T., Combrzyński, M., Nowakowska, D. and Matwijczuk, A. (2020) 'Influence of *in vitro* digestion on composition, bioaccessibility and antioxidant activity of food polyphenols -A non-systematic review', *Nutrients,* 12(5):1401. Available at: https://doi.org/ 10.3390/nu12051401.
- Wootton-Beard, P. C., Moran, A. and Ryan, L. (2011) 'Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods', *Food Research International*, 44(1), pp. 217-224. Available at: https://doi.org/10.1016/j.foodres.2010.10.033.
- World Health Organization (WHO) (2023) *Diabetes*. Available at: https:// www.who.int/news-room/fact-sheets/detail/diabetes (Accessed: 27 September 2023).
- World Health Organization (WHO) (2023) *Hypertension*. Available at: https:// www.who.int/news-room/fact-sheets/detail/hypertension# (Accessed: 27 September 2023).
- World Health Organization (WHO) (2021) *Diabetes*. Available at: https://who.int/health-topics/diabetes#tab=tab_1 (Accessed: 15 May 2023).

- World Health Organization (WHO) (2019) *Global report on traditional and complementary medicine*. Available at https://www.who.int/publications/i/item/978924151536 (Downloaded: 27 September 2023).
- World Health Organization (WHO) (2016) *Global report on diabetes*. Available at: https://www.who.int/diabetes/global-report/en/ (Downloaded: 21 January 2019).
- World Health Organization (WHO) (2015) *Online Q&A on hypertension*. Available at: https://www.who.int/features/qa/82/en/ (Accessed: 22 January 2019).
- World Health Organization (WHO) (2000) *General guidelines for methodologies on research and evaluation of traditional medicin*e. Available at https://www.who.int/medicines/publications/traditional/en/ (Downloaded: 10 May 2019).
- Xiaohui, X., Tao, L., Qingwei, W., Xing, L., Jingzhi, T., Qiaoyun, Z., Liqun, T., Feifei, X. and Renbin, H. (2014) 'Protective effects of total extracts of *Averrhoa carambola* L. (*Oxalidaceae*) roots on streptozotocin-induced diabetic mice', *Cellular Physiology and Biochemistry*, 33(5), pp. 1272-1282. Available at: https://doi.org/10.1159/000358695.
- Xiong, L., Yang, J., Jiang, Y., Lu, B., Hu, Y., Zhou, F., Mao, S. and Shen, C. (2014) 'Phenolic compounds and antioxidant capacities of 10 common edible flowers from China', *Journal of Food Science*, 79(4), p. 517-525. Available at: https://doi.org/10.1111/1750-3841.12404.
- Xu, L., Zhang, Y. and Wang, L. (2016) 'Structure characteristics of a water-soluble polysaccharide purified from dragon fruit (*Hylocereus undatus*) pulp', *Carbohydrate Polymers*, 146, pp. 224-230. Available at: https://doi.org/10.1016/j.carbpol.2016.03.060.
- Yan, S. W., Ramasamy, R., Alitheen, N. B. M. and Rahmat, A. (2013) 'A comparative assessment of nutritional composition, total phenolic, total flavonoid, antioxidant capacity, and antioxidant vitamins of two types of Malaysian

underutilized fruits (*Averrhoa bilimbi* and *Averrhoa carambola*)', *International Journal of Food Properties*, 16, pp. 1231-1244. Available at: https://doi.org/10.1080/10942912.2011.582975.

- Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F. and Wang, Q. (2018) 'Response of plant secondary metabolites to environmental factors', *Molecules*, 23(4). Available at: https://doi.org/10.3390/molecules23040762.
- Yang, D., Xie, H., Jia, X. and Wei, X. (2015) 'Flavonoid C-glycosides from star fruit and their antioxidant activity', *Journal of Functional Foods*, 16(2015), pp. 204-210.
- Yasawardene, P., Jayarajah, U., De Zoysa, I. and Seneviratne, S. L. (2020) 'Mechanisms of star fruit (*Averrhoa carambola*) toxicity: A mini-review', *Toxicon*, 187(2020), pp. 198-202. Available at: https://doi.org/10.1016/j.toxicon.2020.09.010.
- Yen, T. T., Quan, T. H., Nhung, H. T. H., Tram, G. P. N., Karnjanapratum, S. and Benjakul, S. (2022) 'Development of antioxidative red dragon fruit bar using response surface methodology for formulation optimization', *Applied Food Research*, 2. Available at: https://doi.org/10.1016/j.afres.2022.100173.
- Yong, Y. Y., Dykes, G., Lee, S. M. and Choo, W. S. (2018) 'Effect of refrigerated storage on betacyanin composition, antibacterial activity of red pitahaya (*Hylocereus polyrhizus*) and cytotoxicity evaluation of betacyanin rich extract on normal human cell lines', *LWT-Food Science and Technology,* 91, pp. 491-497. Available at: https://doi.org/10.1016/j.lwt.2018.01.078.
- Yu, J. and Ahmedna, M. (2013) 'Functional components of grape pomace: Their composition, biological properties and potential applications', *International Journal of Food Science and Technology*, 48, pp. 221-237. Available at: https://doi.org/10.1111/ j.1365-2621.2012.03197.x.

- Yun, S., Habicht, J.-O., Miller, D. D. and Glahn, R. P. (2004) 'An *in vitro* digestion/Caco-2 cell culture system accurately predicts the effects of ascorbic acid and polyphenolic compounds on iron bioavailability in humans', *The Journal of Nutrition*, 134(10) pp. 2717-2721. Available at: https://doi.org/10.1093/jn/134.10.2717.
- Zainudin, M. A. M., Hamid, A. A., Anwar, F., Osman, A. and Saari, N. (2014) 'Variation of bioactive compounds and antioxidant activity of carambola (*Averrhoa carambola* L.) fruit at different ripening stages', *Scientia Horticulturae*, 172, pp. 325-331. Available at: https://doi.org/10.1016/j.scientia.2014.04.007.
- Zeng, Z., Li, Y., Yang, R., Liu, C., Hu, X., Luo, S., Gong, E. and Ye, J. (2017) 'The relationship between reducing sugars and phenolic retention of brown rice after enzymatic extrusion', *Journal of Cereal Science*, 74, pp. 244-249. Available at: https://doi.org/10.1016/j.jcs.2017.02.016.
- Zhan, J., Liu, Y.-J., Cai, L.-B., Xu, F.-R., Xie, T. and He Q.-Q. (2017) 'Fruit and vegetable consumption and risk of cardiovascular disease: A meta-analysis of prospective cohort studies', *Critical Reviews in Food Science and Nutrition*, 57(8), pp. 1650-1663. Available at: https://doi.org/10.1080/10408398.2015.1008980.
- Zhang, W., Lan, Y., Dang, B., Zhang, J., Zheng, W., Du, Y., Yang, X. and Li, Z. (2023) 'Polyphenol profile and *in vitro* antioxidant and enzyme inhibitory activities of different solvent extracts of highland barley bran', *Molecules*, 28. Available at: https://doi.org/10.3390/molecules28041665.
- Zhang, Y.-J., Wang, F., Zhou, Y., Li, Y., Zhou, T., Zheng, J., Zhang, J.-J., Li, S., Xu, D.-P. and Li, H.-B. (2016) 'Effects of 20 selected fruits on ethanol metabolism: Potential health benefits and harmful impacts', *International Journal of Environmental Research and Public Health*, 13(4). Available at: https://doi.org/10.3390/ijerph13040399.

- Zhu, K., Yao, S., Zhang, Y., Liu, O., Xu, F., Wu, G., Dong, W. and Tan, L. (2019) 'Effects of *in vitro* saliva, gastric and intestinal digestion on the chemical properties, antioxidant activity of polysaccharides from *Artocarpus heterophyllus* Lam. (Jackfruit) pulp' *Food Hydrocolloids*, 87, pp. 952-959. Available at: https://doi.org/10.1016/j.foodhyd.2018.09.014.
- Zi-Chao, W., Nisar, T., Lijun, S., Zhongxiang, F., Yuzhen, Y., Dan, L., Huichun, X., Huichun, W. and Yurong, G. (2019) 'Effect of *in vitro* gastrointestinal digestion on the composition and bioactivity of anthocyanins in the fruits of cultivated *Lycium ruthenicum* Murray', *CyTA-Journal of Food*, 17(1), pp. 552-562. Available at: https://doi.org/10.1080/19476337.2019.1613449.
- Złotek, U., Mikulska, S., Nagajek, M. and Świeca, M. (2016) 'The effect of different solvents and number of extractions steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts', S*audi Journal of Biological Sciences*, 23, pp. 628-633. Available at: https://doi.org/10.1016/j.sjbs. 2015.08.002.
- Zunft, K., Koebnick, C. and Haber, B. (2004) 'Dietary fibre: Bio-active carbohydrates for food and feed' in van der Kamp, J. W. (ed) *Dietary Fibre Conference Netherlands*, Wageningen:Wageningen Academic Publishers. Available at: https://doi.org/ 10.3920/978-90-8686-662-5.

Appendices

Appendix 1 'Investigating the sensory acceptance of tropical fruit based products'

Proceedings of the Nutrition Society (2022), **81 (OCE4)**, E101 doi:10.1017/S0029665122001306

Irish Section Conference 2022, 15–17 June 2022, Impact of nutrition science to human health: past perspectives and future directions

Investigating the sensory acceptance of tropical fruit based products

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Fruits have been used to design functional foods due to the presence of bioactive compounds and their antioxidant capacity. Hylocereus polyrhizus (dragon fruit) and Averrhoa carambola (star fruit) are tropical fruits that have been used to control diseases such as hypertension and type 2 diabetes in the traditional medicine of different countries [(1,2)]. Due to their high water content and their short shelf life, different preservation methods, such freezing and drying have been used to maintain their properties as much as possible and to allow their transport and storage to different regions [(3)]. However, several factors such as the effect of the food matrix, the release of nutrients, the design of products and their sensory perception need to be determined in products formulated with preserved fruit [(4)]. The aim of this study was to establish the sensory acceptance of beverages based on fresh, frozen and dried dragon fruit and star fruit. Six tropical fruit-based beverages were prepared. Three were formulated using dragon fruit in fresh, frozen and dried forms to contain 290 micrograms (μg) of Gallic acid equivalents (GAE) per milliliter (mL) of total phenolic content; three more based on fresh, frozen and dried star fruit were formulated with 490 μg GAE/mL total phenolic content. A hedonic test was conducted using Compusense Cloud software to determine the overall acceptance of the six beverages in order to evaluate five different attributes using a 9-point scale (9= 'like extremely', 1= 'dislike extremely'): appearance, colour, taste, viscosity and mouthfeel. Ethical approval was obtained from the University Research Ethics Committee (Reg. No. UREC 201379). IBM SPSS Statistics software, version 27, was used to carry out a Friedman non-parametric test and Wilcoxon post hoc test with a Bonferroni correction to compare the sensory acceptance of beverages.26 participants (8 males and 18 females) between 18 and 50 years were recruited. The overall acceptance was significantly different between tested products, $\chi 2$ (5) = 20.276, p = 0.001. Post hoc tests showed a significant difference (p = 0.003) between dried and fresh star fruit based products. Fresh and frozen dragon fruit-based beverages as well as the fresh star fruit product were the most preferred, ranking between 'like slightly' and 'like moderately' for overall acceptance. Attributes such as appearance, colour and taste influenced the like-dislike response. The results of this study provide valuable data to consider hedonic test as a tool to determine the attributes linked to acceptability, and to establish the influence of food matrix on sensory attributes. Fresh and frozen dragon fruit and fresh star fruit based products could be used in *in vivo* studies to evaluate the effect of polyphenols on health biomarkers.

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References

1. Ibrahim SRM *et al.* (2018) *J Food Biochem* **42**(2).
2. Dasgupta P *et al.* (2013) *Int J Pharm Res Rev* **2**(7), 54–63.
3. Barret DM & Lloyd B (2012) *J Sci Food Agric* **92**(1), 7–22.
4. Olivas-Aguirre FJ *et al.* (2017) *Int J Food Sci Technol* **52**(7), 1699–1709.

Appendix 2 'Effect of *in vitro* digestion on polyphenol release and antioxidant activity from three different species of dragon fruit (*Hylocereus* sp)'

Proceedings of the Nutrition Society (2021), **80 (OCE1)**, E49 doi:10.1017/S0029665121000501

Winter Conference Live 2020, 8–9 December 2020, Micronutrient malnutrition across the life course, sarcopenia and frailty

Effect of in vitro digestion on polyphenol release and antioxidant activity from three different species of dragon fruit (Hylocereus sp)

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The consumption of plants and derivatives rich in polyphenols is known to provide a positive effect on human health [1]. However, the phenolic compounds in a food matrix are not always completely accessible to digestive fluids. Therefore, *in vitro* digestion has been used as a mechanism to determine the bio-accessibility of polyphenols, and their possible health effects [2].

Dragon fruit is a tropical fruit that has been considered as an alternative treatment for diabetic people in different regions of the world and some studies have determined their content of phenolic compounds [3,4]. However, their bio- accessibility from different forms of the fruit has not been reported. The aim of this research was to quantify the polyphenol release and their antioxidant capacity during *in vitro* digestion of three different species of dragon fruit.

An *in vitro* digestion was carried out for red flesh dragon fruit (*Hylocereus polyrhizus*), white flesh dragon fruit (*Hylocereus undatus*), and yellow peel dragon fruit (*Hylocereus megalanthus*), under fresh, frozen, and dried conditions. Samples were taken at baseline, gastric phase, as well as 20, 60, and 120 minutes during intestinal phase. The amount of total phenolic compounds released was determined by Folin-Ciocalteu method and the antioxidant activity was evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging analysis. Depending on the normality of the results determined by Shapiro-Wilk test, a one-Way ANOVA with a Tukey's post hoc analysis or Kruskal-Wallis non-parametric test was carried out. Statistical difference was set at $p < 0.05$.

The results showed a significant difference in the amount of polyphenols released between digestion phases ($p < 0.001$) and an increase in their bio-accessibility as the *in vitro* digestion progressed. During intestinal phase, dried fruits presented lower release of total phenolic compounds and the white flesh dragon fruit exhibited the lowest value (538.54 ± 64.59 µg of gallic acid equivalent (GAE)/g fresh weight (FW)). On the other hand, fresh and frozen fruits had higher release of polyphenols with red flesh dragon fruit presenting the highest levels (2846.75 ± 333.10 µg GAE/g FW and 2737.18 ± 126.87 µg GAE/g FW, respectively).

Fresh and frozen dragon fruit samples showed a significant difference in antioxidant activity between the *in vitro* digestion phases recording the highest DPPH radical inhibition between 27.51% and 35.59% during the gastric phase. In contrast, dried dragon fruits showed DPPH inhibition lower than 10%.

This study demonstrated that the polyphenol content and the antioxidant activity of dragon fruits during an *in vitro* digestion is dependent on the species and the form evaluated. Dragon fruits showed potential to be used in human studies to evaluate their health effects.

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References

1. Coman MM *et al.* (2018) *Eur Food Res Tech* **244**, 735–745.
2. Lucas-González R *et al.* (2018) *Food Res Int* **107**, 423–436.
3. Ibrahim SRM *et al.* (2018) *J Food Biochem* **42**.
4. Choo W-S & Yong WK (2011) *Adv Appl Sci Res* **2**, 418–425.

Appendix 3 Ethics Full Approval for Sensory Evaluation

OXFORD BROOKES UNIVERSITY

Dr Sangeetha Thondre
Director of Studies
Faculty of Health and Life Sciences
Oxford Brookes University

15th April 2020

Dear Dr Thondre,

UREC Registration No: 201379 Verastegui
Study Title: Sensory evaluation of beverages based on Dragon Fruit and Star Fruit

Thank you for your email of 9th April 2020 outlining the response to the points raised in my previous conditional approval letter regarding the PhD study of your research student, Mildred Verastegui and attaching the revised documents. I am pleased to inform you that, on this basis, UREC is happy to grant full approval for this study.

The UREC approval period for the data collection phase of the study is two years from the date of this letter, so until 15th April 2022. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Dr Alaaddine El-Chab, Supervisory Team
Mildred Verastegui, Research Student
Dr Adam Bibbey, Research Ethics Officer
Ms Jill Organ, Research Degrees Team

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Appendix 4 Recruitment Poster for Sensory Evaluation

OXFORD BROOKES UNIVERSITY

SENSORY EVALUATION OF BEVERAGES BASED ON DRAGON FRUIT AND STAR FRUIT

Research approved by the Oxford Brookes University Research Ethics Committee (UREC 201379)

Looking for Volunteers

From October 2020 – April 2022

Are you aged between 18-50 years?
Not allergic to fruits?
Non-smoker?
No renal disease?
No medication that may affect your sensory taste?
Non-pregnant or breastfeeding?

If you do not have a cold or Covid symptoms, come and help us in our research!

Visit our Sensory Lab at Oxford Brookes Centre for Nutrition and Health

Spend an hour, test our beverages and tell us how much you like or dislike their attributes!

Mouthfeel

Taste

Colour

Viscosity

If you would like to participate and earn **£8 Amazon voucher** or just need more information, please contact

PhD Research Student: Mildred Flores Verastegui at
18031126@brookes.ac.uk

Appendix 5 Participant Information Sheet for Sensory Evaluation

OXFORD BROOKES UNIVERSITY

Participant Information Sheet

Contact details:
Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Study title
Sensory Evaluation of Beverages Based on Dragon Fruit and Star Fruit.

Invitation paragraph
You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?
The purpose of this study is to determine the sensory acceptance of non-alcoholic drinks of dragon fruit (*Hylocereus polyrhizus*) and rehydrating beverages based on star fruit (*Averrhoa carambola*).

Dragon fruit and star fruit have been related with positive effects on health because of their content of polyphenols. In different countries both fruits have been used as a natural medicine for diabetes and hypertension.

In this project, the formulation of different edible products based on dragon fruit and star fruit will be done to evaluate the role of polyphenols on their sensory attributes such colour, taste, viscosity, and mouthfeel, as well as appearance and overall acceptance, by a hedonic test with a 9 points scale.

The aim of this study is to test, in one visit, beverages based on dragon fruit and star fruit. During one hour session held at Oxford Brookes Centre for Nutrition and Health (OxBCNH), two sets of three samples will be given to each participant, in balanced order with a random code. The participants will be asked by written instructions to taste each sample and evaluate them.

In the event of restrictions to attend OXBCNH for testing, the samples will be given to participants to take away and test at home during an online meeting.

Why have I been invited to participate?
You and other 49 participants have been invited to this study, as you fulfil the following inclusion criteria:

- You are an adult aged 18-50 years
- You do not have allergy to fruits
- You are a non-smoker
- You do not have renal disease
- You are not on medication that may affect the sensory taste
- You are non-pregnant or breastfeeding
- You do not have a cold on test day
- You have not had Covid-19 related symptoms for the last 7 days

  Age, smoking, pregnancy, lactation, medication and cold are known to influence sensory perception of foods.

Do I have to take part?
It is up to you to decide whether or not to take part in this research study. If you do decide to take part you will be given this information sheet along with a privacy notice that will explain how your data will be collected and used, and be asked to give your consent. If you decide to take part you are still free to withdraw at any time and without giving a reason.

For students of Oxford Brookes University: choosing to either take part or not take part in the study will have no impact on your marks, assessments or future studies. If you are a staff member working at Oxford Brookes University it will have no impact on your current/future employment.

What will happen to me if I take part?
You will be asked to visit the Oxford Brookes Centre for Nutrition and Health, located at Sinclair Building, 3rd floor in Headington Campus for 1 hour session in the sensory lab. You will be required to wear a face covering indoors and have to avoid any food or drink other than water for 2 hours before the start of the test session.

You will receive, in an individual tasting booth, two sets of three beverage samples, of 10 ml each one, in balanced order and randomly coded. You will be asked to evaluate the acceptability of each sample through a 9 point scale, given to you, for different attributes such appearance, colour, taste, viscosity, mouthfeel, and overall acceptance. You must fill out the online evaluation form and clean your palate between samples with a sip of water and a bite of cracker to avoid interferences. You will be asked to wash hands before and after handling test samples.

At the end of the session you will receive an electronic £8 Amazon voucher.

If there is a mandatory lockdown, the samples will be sent to your doorstep or take away the samples to conduct the test at home during an online meeting with the researcher.

What are the possible disadvantages and risks of taking part? (where appropriate)
Possible disadvantages of taking part in this study could be the hour that you need to spend at Oxford Brookes Centre for Nutrition and Health and/or having to test products you do not usually consume.

Also, there is a low but possible risk of developing an allergy, in case of sensitivity to similar tropical fruit products.

What are the possible benefits of taking part?
As a participant you will receive £8 Amazon voucher upon participation in the sensory evaluation. You will contribute to this study to obtain valuable data to continue understanding the role of polyphenols in sensory attributes and human health. In addition, you could receive a summary of results if requested.

Will what I say in this study be kept confidential?
All information collected will be kept strictly confidential (subject to legal limitations).

Research data will be kept securely at all times, electronic devices are password protected.

- Data may be stored in Google Drive, for which the University has a security agreement.
- Data generated by the study will be retained in accordance with the University's policy on Academic Integrity.
- Data generated in the course of the research will be kept securely in paper or electronic form for a period of ten years after the completion of a research project.

If you withdraw from the study your data will be destroyed.

What should I do if I want to take part?
If you want to take part in this research study, you should contact:

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

What will happen to the results of the research study?
The results obtained in this study will be used in a PhD thesis and they will be published in peer-reviewed journals and or as a conference paper / presentation.

Who is organising and funding the research?
I am conducting the research as a PhD research student at Oxford Brookes University, within the Oxford Brookes Centre for nutrition and Health (OxBCNH) in the Department of Sport, Health Sciences and Social Work at Faculty of Health and Life Sciences.

PRODEP-Mexico is funding this research through the PhD scholarship 511-6/18-7113.

Who has reviewed the study?
The research has been approved by the University Research Ethics Committee, Oxford Brookes University.

Contact for Further Information

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Dr. P. Sangeetha Thondre – Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: pthondre@brookes.ac.uk

If you have any concerns about the way in which the study has been conducted, they should contact the Chair of the University Research Ethics Committee on ethics@brookes.ac.uk.

Thank you for taking time to read this Information Sheet

Appendix 6 Consent Form for Sensory Evaluation

OXFORD BROOKES UNIVERSITY

CONSENT FORM

Full title of Project:
Sensory Evaluation of Beverages Based on Dragon Fruit and Star Fruit.

Name, position and contact details of Researchers:

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Dr. P. Sangeetha Thondre – Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: pthondre@brookes.ac.uk

	Please initial box
1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason.	☐
3. I agree to take part in the above study.	☐
4. I agree to follow all the health and safety measures as instructed by the researcher.	☐
5. In the event of another lockdown, I agree to do the home testing of the samples during an online meeting with the researcher.	☐

Name of Participant	Date	Signature
Name of Researcher	Date	Signature

Appendix 7 Health Questionnaire for Sensory Evaluation

OXFORD BROOKES UNIVERSITY

Subject Number: _____

Sensory Evaluation of Beverages based on Dragon Fruit and Star Fruit

Health Questionnaire

Age: ____ Sex:	☐ Female	☐ Male	☐ Other category

Please tick as appropriate:

	YES	NO
1. Have you had high temperature, a new continuos cough, a loss or change to your sense of smell or taste for the last 7 days? If yes, please give the start date: _____	☐	☐
2. Do you have allergies to any food types? If yes, please identify them: _____	☐	☐
3. Are you a smoker? If yes, how many cigarettes per day do you smoke? _____	☐	☐
4. Do you have any kidney disease? If yes, specify: _____	☐	☐
5. Have you had cold for the last 5 days? If yes, please give the start date: _____	☐	☐
6. Do you take any medication that change your taste perception? If yes, please give details: _____	☐	☐
7. Are there any foods you dislike? If yes, which one(s)? _____	☐	☐
8. Are you pregnant?	☐	☐
9. Are you breastfeeding?	☐	☐

Signature: _Dipika Kothari_____ Date: 04 /08 /20221_

Thank you for taking time to answer this questionnaire

Appendix 8 Ethics Full Approval for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Dr Sangeetha Thondre
Director of Studies
Faculty of Health and Life Sciences
Oxford Brookes University

8th November 2021

Dear Dr Thondre,

UREC Registration No: 211527
Study Title: Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes

Thank you for the email of 3rd November 2021 outlining the response to the points raised in my previous conditional approval letter regarding the PhD study of your research student, Mildred Verastegui and attaching the revised documents. I am pleased to inform you that, on this basis, UREC is happy to grant full approval for this study.

The UREC approval period for the data collection phase of the study is two years from the date of this letter, so until 8th November 2023. If you need the approval to be extended please do contact me nearer the time of expiry.

As Director of Studies, your responsibilities include:

- Ensuring that (where applicable) all the necessary legal and regulatory requirements in order to conduct the research are met, and the necessary licenses and approvals have been obtained
- Reporting any ethics-related issues that occur during the course of the research or arising from the research (e.g. unforeseen ethical issues, complaints about the conduct of the research, adverse reactions such as extreme distress) to the University Research Ethics Officer
- Submitting details of proposed substantive amendments to the study to the Research Ethics Officer for approval.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely,

Prof. David Evans
Chair of the University Research Ethics Committee

cc. Dr Alaaddine El-Chab, Supervisory Team
Dr Shelly Coe, Supervisory Team
Miss Mildred Verastegui, Research Student
Dr Catherine Graham, Research Ethics Officer
Dr Robyn Curtis, Research Ethics & Integrity Officer
Mrs Jill Organ, Research Degrees Team

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Appendix 9 Recruitment Poster for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

We are looking for volunteers

To participate in our research to find the effect of dragon fruit consumption on blood glucose levels and blood pressure.

Research approved by the Oxford Brookes University Research Ethics Committee (UREC **211527**)

If you are 18 - 65 years, do not have Covid-symptoms, and fulfil the following:

- Have a parent or sibling with type 2 diabetes
- Have low/moderate physical activity*
- Are overweight or obese*

*We can measure this for you

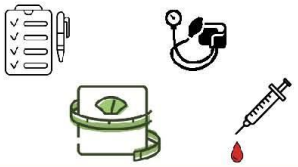
OR

- Have a body mass index (BMI) between 18.5 and 24.9
- Do not have medical conditions
- Are not pregnant or breastfeeding
- Do not have allergy to dragon fruit

Come to the Oxford Brookes Centre for Nutrition and Health (OxBCNH) to take part in our 4 weeks study:

- Consume daily a dragon fruit product or follow a general health guidelines
- Visit the OxBCNH 3 times (3 hours per session).
  - ✓ Health questionnaire and food recall.
  - ✓ BMI and blood pressure measurements.
  - ✓ Venous and finger prick blood samples will be collected. (*)

  (*) You may opt to give finger prick blood samples only.



Your time and participation will be compensated with Amazon vouchers as below:

Venous and capillary blood taking	Capillary blood sampling
£15 at the end of the first session	£15 at the end of the first session
£25 at the end of the second session	£40 at the end of the third session
£40 at the end of the third session	

For more details, please contact

Robyn Bridle
19232226@brookes.ac.uk

Haythim Hamed
19049195@brookes.ac.uk

Mildred Flores-Verastegui: 18031126@brookes.ac.uk

Appendix 10 Participant Information Sheet (At Risk) for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Participant Information Sheet
-Individuals at Risk of Type 2 Diabetes-

Contact details:
Robyn Bridle – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19232226@brookes.ac.uk

Haythim Hamed – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19049195@brookes.ac.uk

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Study title
Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals.

Invitation paragraph
You are being invited to take part in a PhD research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?
The purpose of this study is to determine the effect of dragon fruit (*Hylocereus polyrhizus*) on blood glucose levels and blood pressure in people at risk of type 2 diabetes and in healthy individuals.

Dragon fruit has been related with positive effects on health because of its content of polyphenols (compounds in plants) and has been used in different countries as a natural medicine for diabetes and hypertension.

In this project, a beverage based on frozen dragon fruit will be tested to evaluate the role of polyphenols on blood glucose levels and blood pressure and compare its effect with following general health guidelines, over the a period of four weeks.

The aim of this study is to test a dragon fruit based product to determine its effect on blood glucose levels and blood pressure in healthy people and those who may be at risk of type 2 diabetes. One group will receive general health guidelines and the other will be given a frozen dragon fruit based beverage to consume daily for four weeks. Three testing sessions, lasting three hours each, for both groups will be held at Oxford Brookes Centre for Nutrition and Health (OxBCNH) to determine blood pressure and blood glucose levels. You will be asked to drink a glucose beverage and blood samples will be taken from both your vein and from finger pricks. You have the option to give only finger prick blood samples.

Why have I been invited to participate?
You and other 31 participants have been invited to this study, as you fulfil the following inclusion criteria:

- You are an adult aged 18-65 years
- You have a parent or sibling with type 2 diabetes, or you are overweight or obese, or you have a sedentary lifestyle (low/moderate physical activity)
- You have not had Covid-19 related symptoms for the last 7 days
- You do not have allergy to dragon fruit
- You do not have diabetes, hypertension or kidney disease
- You are not on medication that requires prescription
- You are not-pregnant or breastfeeding

Do I have to take part?
It is up to you to decide whether or not to take part in this research study. If you do decide to take part you will be given this information sheet along with a privacy notice that will explain how your data will be collected and used, and be asked to give your consent.

If you decide to take part, you are still free to withdraw at any time and without giving a reason. If you withdraw from the study after the first visit your data will be destroyed after your first visit. If you withdraw from the study after the second visit, it will not be possible to destroy your data from the study.

For students of Oxford Brookes University, choosing to either take part or not take part in the study will have no impact on your marks, assessments or future studies. If you are a staff member working at Oxford Brookes University it will have no impact on your current/future employment.

What will happen to me if I take part?
You will be asked to visit the Oxford Brookes Centre for Nutrition and Health, located at Sinclair Building, 3rd floor in Headington Campus for 3 testing sessions (3 hours each) in the consulting room. You will be fasted for 12 hours before the start of each session, you are allowed to drink a moderate amount of water. Please wear comfortable clothing.

On the first session you will be asked to sign a consent form and fill a health and Covid-19 screening questionnaires and a physical activity questionnaire. Your height and weight will be measured to determine your body mass index (BMI). If your BMI is lower than 25 and you do not have a low/moderate physical activity in accordance with the physical activity questionnaire you will not take part in the study.

If you fulfil the inclusion criteria, in each session, you will record a 24 hour dietary recall. Blood pressure will be measured on arrival, after 10 minutes of rest three times at 0, 5 and 10 minutes. A blood sample from your vein in the arm will be taken to determine blood glucose levels before and after 2 hours of drinking a standard glucose solution(*). Between the 2 hours finger-prick blood samples will be taken at 0, 15, 30, 45, 60, 90 and 120 minutes. The treatment will be given to you to start the intervention during the first session; an advice on health guidelines if you are randomly assigned to group 1 or a dragon fruit beverage fresh made by the researcher if you are assigned to group 2.

(*) You will be given the option to give venous and finger-prick blood samples or just finger-prick blood samples.

If you are part of the group following the general health guidelines, you will be given advice and supporting material to take home. If you are part of the group testing the beverage, the frozen fruit will be given to you to take away and information on how to make and consume the beverage will be given to you in order to do this at home on a daily basis. You will be asked to visit the OxBCNH two more times to collect the fruit. If this is not possible, the researcher will send the samples to your doorstep by post. A paper log will be given to you to record your treatment follow up and comments.

At the end of the sessions you will receive an Amazon voucher. If you gave venous and finger prick samples: £15 after the first session, £25 after the second testing session and £40 at the end of third session. If you select the option to give just finger-prick blood samples you will receive £15 after the first session, and £40 at the end of third session.

During the last session you will be asked to return the follow-up record and to complete an exit questionnaire.

What are the possible disadvantages and risks of taking part? (where appropriate)
Possible disadvantages of taking part in this study could be the time that you need to spend at Oxford Brookes Centre for Nutrition and Health and/or having to test products you do not usually consume.

Also, there is a low but possible risk of having an allergy, in case of sensitivity to similar tropical fruit products. This can be checked during the first session.

There may be a concern about knowing you are a risk of developing type 2 diabetes, based on the measurements taken.

What are the possible benefits of taking part?
As a participant you will receive Amazon vouchers upon participation: A total of £80 if you gave venous and finger-prick samples (£15 after the first session, £25 after the second session and £40 at the end of the intervention), or a total of £55 if you gave only finger-prick samples (£15 after the first session, and £40 at the end of the intervention). You will contribute to this study to obtain valuable data to continue understanding the role of polyphenols in dragon fruit on human health. In addition, you could receive a summary of the study results if requested.

Will what I say in this study be kept confidential?
All information collected will be kept strictly confidential (subject to legal limitations).

Research data will be kept securely at all times, electronic devices are password protected.

- Data may be stored in Google Drive, for which the University has a security agreement.
- Data generated by the study will be retained in accordance with the University's policy on Academic Integrity.
- Data generated in the course of the research will be kept securely in paper or electronic form for a period of ten years after the completion of a research project. Electronic data will be stored in Arkivum as the University provides access to it for long term storage of data.

If you withdraw from the study after the first visit your data will be destroyed after your first visit. If you withdraw from the study after the second visit, it will not be possible to destroy your data from the study.

What should I do if I want to take part?
If you want to take part in this research study, you should contact:

Robyn Bridle – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19232226@brookes.ac.uk

Haythim Hamed – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19049195@brookes.ac.uk

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

What will happen to the results of the research study?
The results obtained in this study will be used in a PhD thesis and they will be published in peer-reviewed journals and or as a conference paper / presentation. Some of the results will be used to write the MSc student's project report.

Who is organising and funding the research?
We are conducting the research as a PhD research student and MSc Applied Human Nutrition students at Oxford Brookes University, within the Oxford Brookes Centre for Nutrition and Health (OxBCNH) in the Department of Sport, Health Sciences and Social Work, Faculty of Health and Life Sciences.

PRODEP-Mexico is funding this research through the PhD scholarship 511-6/18-7113.

Who has reviewed the study?
The research has been approved by the University Research Ethics Committee, Oxford Brookes University.

Contact for Further Information

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Dr. P. Sangeetha Thondre – Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: pthondre@brookes.ac.uk

If you have any concerns about the way in which the study has been conducted, you should contact the Chair of the University Research Ethics Committee on ethics@brookes.ac.uk.

Thank you for taking time to read this Information Sheet

Appendix 11 Participant Information Sheet (Healthy) for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Participant Information Sheet
-Healthy Individuals-

Contact details:
Robyn Bridle – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19232226@brookes.ac.uk

Haythim Hamed – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19049195@brookes.ac.uk

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Study title
Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals.

Invitation paragraph
You are being invited to take part in a PhD research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?
The purpose of this study is to determine the effect of dragon fruit (*Hylocereus polyrhizus*) on blood glucose levels and blood pressure in people at risk of type 2 diabetes and in healthy individuals.

Dragon fruit has been related with positive effects on health because of its content of polyphenols (compounds in plants) and has been used in different countries as a natural medicine for diabetes and hypertension.

In this project, a beverage based on frozen dragon fruit will be tested to evaluate the role of polyphenols on blood glucose levels and blood pressure and compare its effect with following general health guidelines, over the a period of four weeks.

The aim of this study is to test a dragon fruit based product to determine its effect on blood glucose levels and blood pressure in healthy people and those who may be at risk of type 2 diabetes. One group will receive general health guidelines and the other will be given a frozen dragon fruit based beverage to consume daily for four weeks. Three testing sessions, lasting three hours each, for both groups will be held at Oxford Brookes Centre for Nutrition and Health (OxBCNH) to determine blood pressure and blood glucose levels. You will be asked to drink a glucose beverage and blood samples will be taken from both your vein and from finger pricks. You have the option to give only finger prick blood samples.

Why have I been invited to participate?
You and other 31 participants have been invited to this study, as you fulfil the following inclusion criteria:

- You are an adult aged 18-65 years
- You have a body mass index between 18.5 and 24.9.
- You have not had Covid-19 related symptoms for the last 7 days
- You do not have allergy to dragon fruit
- You do not have diabetes, hypertension or kidney disease
- You are not on medication that requires prescription
- You are not-pregnant or breastfeeding

Do I have to take part?
It is up to you to decide whether or not to take part in this research study. If you do decide to take part you will be given this information sheet along with a privacy notice that will explain how your data will be collected and used, and be asked to give your consent.

If you decide to take part, you are still free to withdraw at any time and without giving a reason. If you withdraw from the study after the first visit your data will be destroyed after your first visit. If you withdraw from the study after the second visit, it will not be possible to destroy your data from the study.

For students of Oxford Brookes University, choosing to either take part or not take part in the study will have no impact on your marks, assessments or future studies. If you are a staff member working at Oxford Brookes University it will have no impact on your current/future employment.

What will happen to me if I take part?
You will be asked to visit the Oxford Brookes Centre for Nutrition and Health, located at Sinclair Building, 3rd floor in Headington Campus for 3 testing sessions (3 hours each) in the consulting room. You will be fasted for 12 hours before the start of each session, you are allowed to drink a moderate amount of water. Please wear comfortable clothing.

On the first session you will be asked to sign a consent form and fill a health and Covid-19 screening questionnaires and a physical activity questionnaire. Your height and weight will be measured to determine your body mass index (BMI).

If you fulfil the inclusion criteria, in each session, you will record a 24 hour dietary recall. Blood pressure will be measured on arrival, after 10 minutes of rest three times at 0, 5 and 10 minutes. A blood sample from your vein in the arm will be taken to determine blood glucose levels before and after 2 hours of drinking a standard glucose solution(*). Between the 2 hours finger-prick blood samples will be taken at 0, 15, 30, 45, 60, 90 and 120 minutes. The treatment will be given to you to start the intervention during the first session; an advice on health guidelines if you are randomly assigned to group 1 or a dragon fruit beverage fresh made by the researcher if you are assigned to group 2.

(*) You will be given the option to give venous and finger-prick blood samples or just finger-prick blood samples.

If you are part of the group following the general health guidelines, you will be given advice and supporting material to take home. If you are part of the group testing the beverage, the frozen fruit will be given to you to take away and information on how to make and consume the beverage will be given to you in order to do this at home on a daily basis. You will be asked to visit the OxBCNH two more times to collect the fruit. If this is not possible, the researcher will send the samples to your doorstep by post. A paper log will be given to you to record your treatment follow up and comments.

At the end of the sessions you will receive an Amazon voucher. If you gave venous and finger prick samples: £15 after the first session, £25 after the second testing session and £40 at the end of third session. If you select the option to give just finger-prick blood samples you will receive £15 after the first session, and £40 at the end of third session.

During the last session you will be asked to return the follow-up record and to complete an exit questionnaire.

What are the possible disadvantages and risks of taking part? (where appropriate)

Possible disadvantages of taking part in this study could be the time that you need to spend at Oxford Brookes Centre for Nutrition and Health and/or having to test products you do not usually consume.

Also, there is a low but possible risk of having an allergy, in case of sensitivity to similar tropical fruit products. This can be checked during the first session.

There may be a concern about knowing you are a risk of developing type 2 diabetes, based on the measurements taken.

What are the possible benefits of taking part?
As a participant you will receive Amazon vouchers upon participation: A total of £80 if you gave venous and finger-prick samples (£15 after the first session, £25 after the second session and £40 at the end of the intervention), or a total of £55 if you gave only finger-prick samples (£15 after the first session, and £40 at the end of the intervention). You will contribute to this study to obtain valuable data to continue understanding the role of polyphenols in dragon fruit on human health. In addition, you could receive a summary of the study results if requested.

Will what I say in this study be kept confidential?
All information collected will be kept strictly confidential (subject to legal limitations).

Research data will be kept securely at all times, electronic devices are password protected.

- Data may be stored in Google Drive, for which the University has a security agreement.
- Data generated by the study will be retained in accordance with the University's policy on Academic Integrity.
- Data generated in the course of the research will be kept securely in paper or electronic form for a period of ten years after the completion of a research project. Electronic data will be stored in Arkivum as the University provides access to it for long term storage of data.

If you withdraw from the study after the first visit your data will be destroyed after your first visit. If you withdraw from the study after the second visit, it will not be possible to destroy your data from the study.

What should I do if I want to take part?
If you want to take part in this research study, you should contact:

Robyn Bridle – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19232226@brookes.ac.uk

Haythim Hamed – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19049195@brookes.ac.uk

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

What will happen to the results of the research study?
The results obtained in this study will be used in a PhD thesis and they will be published in peer-reviewed journals and or as a conference paper / presentation. Some of the results will be used to write the MSc student's project report.

Who is organising and funding the research?
We are conducting the research as a PhD research student and MSc Applied Human Nutrition students at Oxford Brookes University, within the Oxford Brookes Centre for Nutrition and Health (OxBCNH) in the Department of Sport, Health Sciences and Social Work, Faculty of Health and Life Sciences.

PRODEP-Mexico is funding this research through the PhD scholarship 511-6/18-7113.

Who has reviewed the study?
The research has been approved by the University Research Ethics Committee, Oxford Brookes University.

Contact for Further Information

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Dr. P. Sangeetha Thondre – Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: pthondre@brookes.ac.uk

If you have any concerns about the way in which the study has been conducted, you should contact the Chair of the University Research Ethics Committee on ethics@brookes.ac.uk.

Thank you for taking time to read this Information Sheet

Appendix 12 Consent Form for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

CONSENT FORM

Full title of Project:
Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals.

Name, position and contact details of Researchers:

Mildred Inna Marcela Flores Verastegui – PhD Research Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 18031126@brookes.ac.uk

Dr. P. Sangeetha Thondre – Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: pthondre@brookes.ac.uk

Robyn Bridle – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19232226@brookes.ac.uk

Haythim Hamed – MSc Applied Human Nutrition Student
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences.
Oxford Brookes University
Email: 19049195@brookes.ac.uk

		Please initial box	
1.	I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.		
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason.		
		YES	NO
3.	I agree to give finger prick and venous blood samples, body measurements and food records.		
4.	I opt to give finger prick blood samples only, body measurements and food records.		
5.	I agree to follow all the health and safety measures as instructed by the researcher.		
6.	I agree to take part in the above study.		

Name of Participant	Date	Signature
Name of Researcher	Date	Signature

Appendix 13 Screening Questionnaire for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Subject Number: _____

Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals

Screening Questionnaire

Age: _____ Sex: ☐ Female ☐ Male ☐ Other category
Ethnicity background: ☐ Asian ☐ Black ☐ Mixed ☐ White ☐ None of these

Please tick as appropriate:

	YES	NO
1. Have you had high temperature, a new continuos cough, a loss or change to your sense of smell or taste for the last 7 days? If yes, please give the start date: _____	☐	☐
2. Do you have allergies to any food types? If yes, please identify them: _____	☐	☐
3. Do you have a parent, brother or sister with type 2 diabetes? If yes, please specify: _____	☐	☐
4. Do you have any health conditions? If yes, specify: _____	☐	☐
5. Do you take any medication that requires prescription? If yes, please give details: _____	☐	☐
6. Do you take any vitamins, minerals or supplements? If yes, please give details: _____	☐	☐
7. Do you exercise or participate in any sport? If yes, please give details: _____	☐	☐
8. Are you a smoker? If yes, how many cigarettes per day do you smoke? _____	☐	☐
9. Are there any foods you dislike? If yes, which one(s)? _____	☐	☐
10. Are you pregnant or breastfeeding?	☐	☐

Signature: _____ Date: ___/___/_____

Thank you for taking time to answer this questionnaire

Appendix 14 Covid-19 Screening Questionnaire for Dragon Fruit Study

Subject Number: _____

Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals

Covid-19 Screening Questionnaire

Please tick as appropriate:

	YES	NO
1. Have you had high temperature, a new continuos cough, a loss or change to your sense of smell or taste for the last 7 days? If yes, please give the start date: _____	☐	☐
2. Have you had head or body aches? If yes, please specify: _____	☐	☐
3. Have you been abroad within 10 days? If yes, please give details: _____	☐	☐
4. Have you been in contact with anyone who show the above mentioned symptomes?	☐	☐
5. Have you been tested for Covid-19? If yes, please give details (when and what was the result): _____	☐	☐

Signature: _____ Date: ___ /___ /____

Thank you for taking time to answer this questionnaire

Appendix 15 International Physical Activity Questionnaire (IPAQ) for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Subject Number: _____

Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals

International Physical Activity Questionnaire
IPAQ: Short last 7 days self-administrated format

The following questions are from the final Short Last Day Self-Administered version of IPAQ from the 2000/01 Reliability and Validity Study.

Please answer each question even if you do not consider yourself to be an active person.

In answering the following questions,

- **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.
- **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

1a. During the last 7 days, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

Think about *only* those physical activities that you did for at least 10 minutes at time.

_____ **days per week** ⇨

Or

_____ **none**

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities?

_____ **hours** _____**minutes**

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did yo do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week** ⇨

Or

_____ **none**

2b. How much time in total did you usually spend on one of those days doing moderate physical activities?

_____ **hours** _____**minutes**

3a. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ **days per week** ⇨

Or

_____ **none**

3b. How much time in total did you usually spend walking on one of those days?

_____ **hours** _____**minutes**

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at desk, visiting Friends, Reading, traveling on a bus or sitting or lying down to watch television.

4. During the last 7 days, how much time in total did you usually spend sitting on a week day?

_____ **hours** _____**minutes**

This is the end of the questionnaire, thank you for your participation

Signature: _____ Date: ___/___/_____

Appendix 16 General Health Guidelines and Treatment Follow-up for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Subject Number: _____

Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals

Appendix 1: General Health Guidelines and Treatment Follow-up

Examples of the material used as base for the general health guidelines that will be given as part of the four weeks study.

Dietary Advice

From NICE guidance: Type 2 diabetes: prevention in people at high risk. Public health guideline [PH38]

Encourage people to:

Increase the consumption of foods that are high in fibre, such as wholegrain bread and cereals, beans and lentils, vegetables and fruit.

Choose foods that are lower in fat and saturated fat, for example, by replacing products high in saturated fat (such as butter, ghee, some margarines or coconut oil) with versions made with vegetable oils that are high in unsaturated fat, or using low-fat spreads.

Choose skimmed or semi-skimmed milk and low-fat yoghurts, instead of cream and full-fat milk and dairy products.

Choose fish and lean meats instead of fatty meat and processed meat products (such as sausages and burgers).

Grill, bake, poach or steam food instead of frying or roasting (for example, choose a baked potato instead of chips).

Avoid food high in fat such as mayonnaise, chips, crisps, pastries, poppadums (papads) and samosas.

Choose fruit, unsalted nuts or low-fat yoghurt as snacks instead of cakes, biscuits, bombay mix or crisps. [2012]

This form will be used to record the follow-up of your treatment.

Please record the date, at the end of each day, and tick if you are following or not the guidelines given to you. If you did not follow them, please let us know the reason in the comments section.

At the end of the intervention please return this form to the researcher.

Day	Date	Following Y	Following N	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				

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Day	Date	Following Y	Following N	Comments
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

Signature: _____ Date: ___ /___ /_____

Thank you for your time!

Appendix 17 Treatment Intake Log for Dragon Fruit Study

OXFORD BROOKES UNIVERSITY

Subject Number: _____

Effect of Dragon Fruit Consumption on Glycaemic Response and Blood Pressure in Individuals at Risk of Type 2 Diabetes and Healthy Individuals

Appendix 2: Treatment Intake Log

This form will be used to record the daily dragon fruit based beverage intake.

Please write the date, time and tick if you take or not your treatment during the four weeks study. If you did not take it, please let us know the reason in the comments section. If you feel any symptom or discomfort, please report it.

At the end of the intervention please return this form to the researcher.

Day	Date	Time	Intake		Comments
			Y	N	
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					

Signature: _____ Date: ___/___/_____

Thank you for your time!