# NUTRITIONAL VALUE AND CONSUMER ACCEPTABILITY OF SMOOTHIES IN THE UK AND KSA

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# DECLARATION

I declare that this thesis is a result of my own work in the School of Biosciences, Faculty of Nutrition in The University of Nottingham, UK. This work has not been submitted to any other University, for a degree other than that for which I am now a candidate.

I started the PhD in 2009 under the supervision of Prof Greg Tucker and Dr Fiona McCullough; however, I did not fully complete the course due to some health circumstance. I resumed study again in 2016 to finish the thesis and submit by the end of September 2017.

**Basma Ismail Daffa**

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# ABSTRACT

Increased vegetable consumption could decrease the risk of cardiovascular disease (CVD), obesity and certain cancers. Smoothies are one of the ways to increase vegetable consumption. The aim of this study was to develop a vegetable-containing smoothie with improved nutritional value and consumer acceptability. A survey was conducted on university students (from UK and KSA) to assess their opinions, followed by tasting sessions with several potential smoothies. Following this, the most acceptable smoothie recipe was selected for further analysis. Some key nutritional parameters; ascorbate, antioxidants, phenolics, sugars and fibre of the novel smoothie were measured and compared with commercial smoothies available in the UK market. Also, the retention and stability, during 1, 3 and 5 days of storage at 4°C of these nutrients were analysed. The survey revealed that there was good awareness of the 5 A DAY requirement in the UK, but only fair awareness in the KSA student sample of increasing fruit and vegetable (F&V) intake. Price was a potential barrier for students. Tasting trials indicated that the UK student's acceptance was limited by firstly taste then texture and colour. The novel smoothie based on beetroot and red pepper appeared to be most acceptable. The nutritional value of this smoothie (ascorbate 30 mg/100mL, antioxidant capacity 1031μmoles Trolox Equivalents/100mL, phenolics 57mg Gallic Acid Equivalents/100mL and fibre 6g/100mL) appears to be higher than current commercial smoothies. It was also slightly lower in total sugars with 9g/100mL. This smoothie retained the nutritional benefits of the constituent F&Vs during processing. Also, the nutritional value of the designed smoothie was stable during storage at 4°C over 5 days, apart from ascorbate which declined dramatically. Potential commercial barriers included the high viscosity. Preliminary experiments were carried out to investigate the potential to reduce viscosity using enzyme cocktails. The smoothie containing 47% vegetables and 53% fruit could be one of the easiest ways of increasing F&V consumption, particularly amongst students, to maintain a healthy diet.

## ABBREVIATION LIST

AI: Adequate Intake

Antioxidants: Phytonutrients/Phytochemicals/Secondary Plant Products.

AGR: Arabian Gulf Region

BBC: British Broadcasting Corporation

BMI: Body Mass Index

CAA: Cellular Antioxidant Activity

CEE: Central and Eastren Europeans

COPD: Chronic Obstructive Pulmonary Disease

CVD: Cardiovascular Disease

DRIs: Dietary Reference Intakes

DRVs: Dietary Reference Values

EU: European Union

FAO: Food and Agriculture Organisation

FBDG: Food-Based Dietary Guidelines

FDA: The Food and Drug Administration

FM: Fresh Matter

FRAP: Ferric Reducing Antioxidant Power

FSU: Former Soviet Union

F&V: Fruit and Vegetables

FW: Fresh Weight

GCC: Gulf Cooperation Council

GDA: Guideline Daily Amounts

HHP: High Hydrostatic Pressure

HPP: High-Pressure Processing

IBD: Inflammatory Bowel Disease

KSA: Kingdom of Saudi Arabia

MENA: Middle East and North Africa

NCDs: Non-transmissible Chronic Diseases or Non-communicable Diseases

PEF: Pulsed Electric Field

PPO: Polyphenols Oxidase

PRI: Population Reference Intakes

RA: Rheumatoid Arthritis

RDAs: Recommended Dietary Allowances

RIs: Reference Intakes

RLVs: Reference Labelling Values

RNI: Reference Nutrition Intakes

SCF: Scientific Committee for Food

TAA: Total Ascorbic Acid

TAC: Total Antioxidant Capacity

T2DM: Type 2 Diabetes Mellitus

TE: Trolox Equivalents

TEAC: Trolox Equivalent Antioxidants Capacity

TNS Opinions: managing complex surveys, globally and including Eurobarometer. Based in Belgium

TPC: Total Phenolic Compounds

UAE: United Arab Emirates

UK: United Kingdom

WHO: World Health Organisation

WE: Western Europeans

# CHAPTER 1 INTRODUCTION & LITERATURE REVIEW

Several studies have recommended an increase in the daily consumption of fruit and vegetables (F&V), this is associated with reduction in the risk of a number of non-communicable chronic diseases related to nutrition worldwide (Warensjo et al., 2010). Two international organisations, the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) have recommended consumption of not less than 400g of F&V per day excluding potatoes and other starchy tubers (WHO, 2003).

Following the WHO recommendation, there have been numerous global initiatives to promote greater F&V intake. For instance, in the UK, the 5 A DAY campaign was introduced by the government via the National Health Service (NHS, 2015). No single vegetable or fruit can provide all the required nutrients, therefore consuming a variety of F&V everyday is important. A mixture of rainbow colours of F&V; dark leafy greens, yellow and orange vegetables, bright red, and cooked tomatoes are all nutrient sources and can act in a synergistic manner. Many studies concerning the consumption of F&V have highlighted the relatively higher importance of vegetables (Villegas et al., 2008). The WHO has recently reported vegetable consumption as being important for sustaining good health and preventing disease, specifically cardiovascular diseases (CVD) and some types of cancers (WHO, 2017). Furthermore, it has been suggested that during childhood, vegetable consumption encourages cognitive development (Contento et al., 1995), and along with physical exercise, it can help in preventing childhood obesity

(Aranceta-Batrina and Perez-Rodrigo, 2016). Also, studies have suggested that vegetable consumption at an early age can prevent the development of diseases such as hypertension, coronary disorders, and cancer in adulthood (Van Duyn and Pivonka, 2000, Dominguez, 2017). Health recommendations across the world encourage 3 to 5 vegetable portions per day, including the US Food Pyramid (Padget and Briley, 2005). Thus, in our present research work, the focus was on increasing vegetable intake by exploring both social and technical barriers to the introduction of a smoothie type drink containing vegetables. Also, a scoping survey was carried out to assess consumer acceptability of F&V based drinks, test out a number of potential recipes, and explore the nutritional composition and properties of these drinks.

## 1.1 ROLE OF FRUIT AND VEGETABLES CONSUMPTION IN REDUCING NON-COMMUNICABLE NUTRITION- RELATED DISEASES

F&V are rich sources of diverse nutrients, including vitamins, trace minerals, dietary fibre, and many other classes of biologically active compounds such as antioxidants and polyphenols (Fraga et al., 2019).

Epidemiological research has shown that high consumption of F&V is related with a lower risk of chronic diseases, in particular, Cardiovascular Disease (CVD), type 2 diabetes (T2DM) and some types of cancers including lung, mouth, stomach, pharynx, larynx, and oesophageal (EUFIC, 2012). The high prevalence of non-transmissible chronic diseases or non-communicable diseases (NCDs) has stimulated research into the potential relationship between diet and health. In particular, research has been conducted on the incidence of NCDs due to food consumption patterns. In general, a beneficial impact of high consumption of F&V has been observed, which along with the antioxidant content of these foods, was one of the main subjects of research in the early part of this century (Araya, 2006). Moreover, in 2019, a study confirmed that identifying the mechanism(s) by which bioactive compounds in vegetables decrease the risk of some diseases is challenging and some mechanisms remain unknown. Analysis of epidemiological relationships is further complicated by the fact that each vegetable family probably possesses a distinctive mixture of such bioactive compounds (Dias, 2019).

Despite these challenges, there have been several mechanisms proposed as to why the consumption of vegetables and fruit might slow or prevent the prevalence of some types of NCDs. The phytochemicals in F&V may exhibit several mechanisms of action, including but not limited to- modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, and antioxidant, antibacterial, and antiviral effects (Lampe, 1999). These possible effects have been investigated mainly in animal and cell-culture experimental models. However, dietary based research in humans has also shown the capacity of vegetables and fruit, and their components to modulate some of these mechanisms that are important for the reduction of NCDs incidence. The confirmation of any relationship between dietary studies in humans and laboratory-based research in animal models is essential for accurate assessment of the benefits of F&V in the human diet (Lampe, 1999).

Oxidative stress occurs due to the overproduction of reactive oxygen species and/or a lack of antioxidants. One suggested mechanism which affects aging and longevity is the prevention of oxidative stress, which would otherwise cause damage to cell membranes and DNA. Dietary antioxidants like vitamin C, A, and E, selenium, copper, and zinc contribute to the antioxidant defence by enhancing endogenous antioxidant enzyme systems, directly quenching free radicals, and inhibiting lipid peroxidation and DNA damage. Therefore, functional foods rich in antioxidants and anti-inflammatory agents have been identified, since benefits include exhibiting significant effects on the aging process and incidence of NCDs causing a positive impact on longevity (Wichansawakun et al., 2019). For example, Mediterranean and Okinawa-type diets can promote longevity and good health (Wichansawakun et al., 2019).

Other possible beneficial effects could arise due to the fact that dietary polyphenols have the ability to impact the composition of the gut microbiota. These gut bacteria metabolise polyphenols into bioactive compounds that are independently linked with health benefits (Fraga et al., 2019).

Assuming that F&V, with their phytochemicals, can influence not only the inflammatory processes, but also cellular redox, endothelial, and metabolic processes which are involved in the pathogenesis of various diseases. These mechanisms may be primarily responsible for the risk-reducing effect of F&V consumption in relation to single diseases (Wu et al., 2019). In total, based on the correlated relationships between F&V consumption and risk of several NCDs such as cardiovascular, obesity, and other diseases such as several types of cancer, cognitive, skin, eyes, lung, and bone diseases, it is reasonable to assume that these can be reduced by regular F&V intake (Yahia et al., 2019).

### 1.1.1 Cardiovascular Disease (CVD)

The impact of CVD yields enormous health and economic burdens globally (Mozaffarian et al., 2016). The Global Burden of Disease (GBD) research has shown that the CVD load decreased in the United Kingdom (UK) between 1990 and 2013. In particular, CVD death rates dropped in the UK by 52%, coronary heart disease (CHD) by 60%, and stroke by 46%. However, CVD was the second main cause of mortality in the UK in 2014 (Bhatnagar et al., 2016).

According to a survey carried out by the British Heart Foundation (BHF) in 2020, nearly 170,000 deaths each year which is an average of 460 people each day or one death every three minutes died of CVD. Also, there are around 7.4 million people living with heart and circulatory diseases in the UK.

Furthermore, CVD caused around 44,000 people under the age of 75 in the UK die from heart and circulatory diseases each year (BHF, 2020).

CVD is the first reason of death worldwide, approximately 17.9 million death per year (WHO, 2020). The recommendations to reduce cardiovascular risk include maintaining a healthy body weight, eating five or more portions of F&Vs each day, reducing intake of fat (particularly saturated fatty acids), reducing salt intake, and eating one portion of oily fish per week. Although some improvements have been made in recent years (e.g. a reduction in total fat intake), national studies suggest that more effective campaigns are required to increase awareness on the benefits of the dietary changes. Furthermore, it remains to be addressed by randomised controlled trials if phytochemicals can play a significant role in reducing vascular disease or not. The links between diet and CVD risk are still under investigation (Department of Health, 2000 in Kelly and Stanner, 2003).

The work of Hung et al., 2004 involved 71,910 females in the nurses' health study and 37,725 male health professions, none of whom had any major chronic diseases. In questionnaires administered in 1984 and 1986, they were asked about their daily food intake. The data were updated in 1994 and the entire group was examined for CVD, cancer or death in 1998. The study showed that the consumption of five portions of fruit per day can slightly reduce the risk of CVD but did not provide any statistically significant evidence to link it with other serious chronic diseases. On the other hand, the study confirmed a significant relationship between the intake of vegetables, especially those with green leaves and a decrease in the occurrence of CVD. There is strong evidence that vegetables might have a more powerful impact on health as compared with fruit and could have a slight positive impact on decreasing the risk of CVD (Kris-Etherton et al., 2002).

It should therefore be stressed that vegetables are of vital importance to human health and should be consumed daily, as strongly suggested by previous epidemiological studies. Vegetables have a more positive effect on health than fruit and can decrease the risk of major chronic diseases. This might be linked to a synergistic effect from phytochemicals like antioxidants which can be proactive and therapeutic.

In a systematic review by Esfahani et al., in 2011, 22 studies with commercially available mixed and concentrated F&V products were investigated. None of these studies reported any serious adverse effects suggesting that they may help certain individuals to improve their nutritional status. Clinical interventions were identified that examined the effect of commercially available concentrated mixed F&V supplements on CVD risk factors. The final conclusion of the review showed that daily consumption of F&V supplements significantly increased the serum concentrations of the major antioxidant pro-vitamins and vitamins found in plant foods (β-carotene, vitamins C and E) and folate. Functional changes, such as reduced serum homocysteine and markers of protein, lipid and DNA oxidation, were also reported. In addition, the health advantages in terms of markers of inflammation, immunity and endothelial function were promising (Esfahani et al., 2011).

A critical review by Boeing et al., in 2012 provided a comprehensive analysis of studies available and found convincing evidence to prove that increasing the consumption of F&V reduced the risk of chronic disease. These findings were for hypertension, coronary heart disease (CHD), and stroke, there is substantial evidence that increasing the consumption of F&V reduces the risk of these diseases. It is also likely that the risk of cancer in general is inversely associated with consumption of vegetables and fruit.

### 1.1.2 Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is a chronic condition, the percentage of people with T2DM is rising in most countries globally, affecting roughly 463 million adults age between (20-79) years living with diabetes; by 2045 this number will increase to nearly 700 million (IDF, 2019). The largest number of people with diabetes were between 40 and 59 years, furthermore, 1 in 2 (232 million) people with diabetes remain undiagnosed (IDF, 2019).

In Europe, T2DM is estimated at 19 million people or 4% of the total population. The number is predicted to increase to 26 million by 2030 (Daniells, 2008). Moreover, according to the NHS (2009), diabetes affects two million people in England and Wales. It has also been suggested by the NHS (2019) that T2DM is more common than type 1. In the UK, approximately 90% of all adults with diabetes have type 2.

A study by Villegas et al., in 2008 investigated 64,191 participants to study their consumption of F&V in relation to the risk of developing T2DM. The study discovered that while increasing fruit intake may not reduce the risk of T2D, a higher vegetable intake could reduce the risk of the disease by up to nearly 30% (Villegas et al., 2008). This may be due to the higher sugar levels in fruit compared to vegetables.

An increased intake of vegetables would result in a diet that is richer in fibre, antioxidants, magnesium, and a low glycaemic index. The diet will also contain higher levels of phytates, lignans, isoflavones, and natural components which can also have a positive influence on decreasing the risk of T2D. The indications, therefore, showed weak link between fruit consumption and reduced risk of T2D, the consumption of vegetables could offer a higher level of protection against the disease (Villegas et al., 2008). Moreover, a recent study found that native Australian (*Terminalia ferdinandiana* Exell., *Smilax australis* R. Br. and *Xanthorrhoea johnsonni* A.T. Lee) plants have functional properties which may be considered as the foundation of alternative control measures against T2DM (Njume et al., 2019).

However, Barclay in 2008, also sought to investigate links between diet and health. Barclay's study involved a group of 71,346 middle-aged female nurses who were free of CVD, cancer, and diabetes. After observing this group for 18 years, the study revealed three important findings. First, increasing fruit intake up to three whole portions a day might have an effect on decreasing the risk of T2D; second, an increase in the intake of green leafy vegetables to one portion per day was linked with a minor decline in the same risk and third, an intake of one portion of fruit juice a day may be related to a considerable increase in the risk of T2D. The reason behind this could be the amount of fibre that juices include mixed with flavonoids. Another study on apples, found evidence of synergistic relationship between the fibre and flavonoids found in the whole apple, which is likely to integrate with the microbiota in the gut (Bondonno et al., 2017).

The intake of beverages containing both flavonoids and dietary fibre has been found to result in restricted flavonoid digestion and absorption in the small intestine. This is due to flavonoids' interaction with dietary fibres and their subsequent entrapment by fibres would retard their hydrolysis by digestive enzymes in the small intestine. To elaborate, many flavonoids are found in the form of glycosides attached to a sugar moiety. These sugar residues are released from the flavonoids and can be absorbed in the small intestine, causing release of individual flavonoids (aglycones). The flavonoids (aglycones) are unstable, under these conditions, in the small intestine. The presence of dietary fibre could be a factor that influences the digestibility and bioavailability of flavonoids in the small intestine. The dietary fibres can bind to flavonoids through electrostatic, dipolar interactions, and hydrogen bonding, resulting in restricted flavonoid digestion and absorption in the small intestine. Therefore, undigested flavonoids would pass through to the large intestine where they are cleaved by colonic microflora and are either absorbed into the circulatory system or utilized to exert antioxidant activity in the intestinal environment (Thuengtung and Ogawa, 2019).

In another sub-study by the same lead author (Villegas et al., 2009), an association was found between dietary fibre intake and T2D risk. In addition, it is possible that an increased consumption of F&V will prevent body weight gain. As being overweight is the most important risk factor for T2DM, an increased consumption of F&V therefore might indirectly reduce the incidence of T2DM. Independent of overweight, there is probable evidence that increased consumption of F&V has no influence on the T2DM risk. However, sugar-sweetened beverages have an inverse association with obesity, and large epidemiological studies have been able to validate the relationship between consumption and weight gain, T2DM, and CVD risks (Malik et al., 2010). This may relate to the high amount of sugar in those beverages.

To endorse this, a recent pooled analyses and meta-analyses of cohort studies and randomised controlled trials associated with fruit consumption found that high consumption of canned fruit and sweetened fruit juice was definitely linked with the risk of T2DM and mortality (Fardet et al., 2019). It appears, therefore, that vegetables (particularly those with green leaves) are more likely to protect against the incidence of T2DM than fruit, and therefore, it is recommended that their intake should be increased.

### 1.1.3 Obesity

Obesity is the universal epidemic of the 21st century. One cause of obesity is exceeding the recommended daily calorie intake per day. In adulthood roughly 2000 kcal for women and 2500 kcal for men are recommended to be consumed daily. Maguire et al., in 2018 reported that, if the normal American adult can consume around 3770 kcal/day and the average individual in the UK can consume roughly 3400 kcal/day. With such daily increases in calories intake, there will be an increased load in glucose metabolism, and therefore obesity and related chronic diseases may increase in prevalence (Maguire et al., 2018).

The Body Mass Index (BMI) is commonly used as a descriptor of an individual being either overweight [BMI ($kg/m^2$) ≥25 and <30] or obese (BMI ≥30).Whereas the abdominal obesity, an independent predictor of health risk, can be defined by high waist circumference (Schlesinger et al., 2019). There is a strong relationship between obesity and some chronic NCDs. examples include diabetes and CVD in the Middle East and North Africa (MENA) countries (Nikoloski and Williams, 2016). Additionally, T2DM, CVD, non-alcoholic fatty liver disease (NAFLD), hypertension, and chronic kidney disease in general populations leading to increased mortality (Polyzos et al., 2019).

Incidence of obesity is high and appears to be increasing. In 2016, the WHO in a report on worldwide obesity showed this increase with over 600 million people in the obese range (BMI>30). Moreover, according to the statistical data from the WHO Regional Committee for the Eastern

Mediterranean, adults above 15 years of age in Kuwait, Bahrain, KSA, and UAE displayed the highest levels of obesity, varying from (74% to 86%) in females and (69% to 77%) in males (WHO, 2016). A review article showed a considerable variation in obesity in adults in the Arabian Gulf Region (AGR), (2% to 55%) in females and (1% to 30%) in adult males. Furthermore, there is a rising incidence of overweight and obesity levels among children and adolescents varying from (3% to 18%) in females and (5% to 14%) in males (Alnohair, 2014). Moreover, the occurrence of overweight and obesity in children and adolescents is growing in developed countries by around 25% and in the developing countries by around 13%. Around 1.5 billion adults are overweight, among them, nearly 200 million men and 300 million women are obese globally. In some countries, the prevalence of obesity has exceeded by 50%, especially in women of Kuwait (Polyzos and Mantzoros, 2019).

Results tend to confirm a higher obesity prevalence amongst females, whereas overweight is more noticeable among males in the AGR. Obesity occurrence in female is possibly due to increased age and being married, pregnancy, dietary behaviours, unemployment, and low in physical activity (Balhareth et al., 2019).

In general, there may be several significant reasons for obesity prevalence including nutrition patterns, education, age, gender, income, urbanisation and illiteracy (Nikoloski and Williams, 2016). Furthermore, thiamin and magnesium deficiency maybe be a factor in the progression of obesity and obesity associated chronic disease conditions (Maguire et al., 2018). In a recent systematic literature review, results found that sociodemographic factors such as low physical activity, sedentary behaviour, and unhealthy dietary habits were associated with overweight (Balhareth et al., 2019).

It is possible that an increased consumption of F&V will prevent body weight gain (Malik et al., 2010). A study in 2012, that used a nationally representative sample of 11,818 individuals from the Canadian Community Health Survey in 2004, concluded that increasing the intake of F&V maybe an effective dietary strategy to control weight and reduce the risk of obesity (Stukas, 2012). In a systematic review that investigated previous reviews on obesity prevention interventions until May 2017 globally found that school-based interventions that joined diet with physical activity components were most effective in avoidance of childhood prevalence overweight and obesity worldwide (Bleich, 2018).

In addition, a recent systematic review of natural experiments on childhood obesity prevention and control, it was found that assessing school-based policies concentrating on both factors i.e. food/beverage and physical activity environments constantly presented beneficial changes in the BMI (Bramante et al., 2019).

#### 1.1.4 Other Chronic Diseases

Possible evidence that increasing the consumption of F&V lowers the risk of other diseases was shown in a critical review by Boeing et al in 2012. He suggested a positive association between the high daily intake of vegetables and fruit and the reduction of major chronic diseases. The review contained considerable evidence, that increasing the consumption of F&V reduces the risk of disease such as hypertension, CHD, stroke, certain eye diseases, dementia, osteoporosis, and asthma. Nevertheless, there is a possible suggestion that the risk of cancer in general is inversely associated with the consumption of F&V. However, in a recent study on a Mexican American population, which investigated the outcome of F&V consumption on cancer risk and found unreliable evidence, partly due to limited data among this population. The F&V consumption were measured using questionnaire among 2,381 participants, 82 of them were cancer patients who self-reported it to the health care provider. The study showed that the participants who consumed five or more portions of F&V a day had a considerably 86% lower risk for reported cancer as compared with those who did not meet recommendations. The research concluded that there is an inverse linked with the cancer risk in Mexican Americans and suggested F&V consumption might be an effective area for future research as an approach for cancer reduction risk (Wu et al., 2019). There is also a high probability that increasing the F&V intake decreases the risk of certain eye diseases, dementia, osteoporosis, asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), glaucoma, and diabetic retinopathy (Wu et al., 2019). Moreover, high consumption of F&V is related to lower non-cardiovascular death (Saglimbene et al., 2019).

Fruit (especially berries), vegetables, tea and cocoa are rich in polyphenols. They are related to reduction in the risk of stroke, myocardial infarction, as well as improvements in lipids, endothelial-dependent blood flow and blood pressure, insulin resistance, and systemic inflammation (Fraga et al., 2019).

A systematic review for quantitative studies was accomplished to present a comprehensive evaluation of evidence on F&V consumption and health consequences. The study showed current evidence for fruit consumption on reduced risk of colon cancer, pancreatic, and depression diseases. In addition, evidence was also found for vegetable consumption; evidence to reduce colon and rectal cancer, hip fracture, stroke, depression, and pancreatic diseases (Angelino et al., 2019).

## 1.2 NUTRITIONAL COMPOSITION OF FRUIT AND VEGETABLES

The epidemiological studies discussed above show a potential link between F&V intake and health. This raises the question- what are the actual components of the F&V that are acting to confer this benefit? F&V provide the body with vital vitamins and minerals, they also provide fibre and energy the latter being mostly coming from sugar. In addition, some more specific phytonutrients, for example polyphenols, may have positive benefits for health. Each nutritional component will be discussed below.

### 1.2.1 Vitamins

The human body needs optimal nourishment daily. A combination of F&V can help to provide the body with minerals and both fat and water soluble vitamins, if consumed on a daily basis.

Water soluble vitamins including B-complex ($B_1$, $B_2$, $B_3$, $B_5$, $B_6$, $B_{12}$), biotin, choline, folic acid, and vitamin C are essential and need to be consumed everyday within the diet because the body cannot produce them and cannot store them in large amounts unlike fat soluble vitamins (Moreno and Salvado, 2000). Water soluble vitamins are continuously flushed from the body, therefore they need to be replaced daily (Light et al., 2017).

Fat soluble vitamins are (pro-vitamin A, carotenoids, D, E and K); vitamins C, E, and A are considered to be dietary antioxidants. Research has addressed the relationship of F&V intake with inflammatory and oxidative stress markers, the associations were found with F&V and vitamins C and E, but not specifically with provitamin A carotenoids (Yew et al., 2018).

#### 1.2.1.1 Vitamin C

It has been known for a long time that scurvy is a disease that results from an inadequate intake of vitamin C (Magiorkinis et al., 2011). It has since been found that vitamin C is an important nutrient that has several biochemical functions such as maintaining the integrity of tissues and synthesising collagen in human tissues (Enstrom, 1986). The failure of synthesising collagen results in scurvy. One conceivable reason for vitamin C loss in food has been linked to food cooking methods especially that used in care facilities. There have been recent cases of scurvy within health networks reported internationally (Armstrong et al., 2019).

During the 1990s there was an increased interest in studying the health benefits of vitamin C intake. One review looked at groups of elderly men who were smokers, diabetics, hypertensive, and at high risk of coronary heart disease and discovered that they had low plasma vitamin C levels. Also, some studies suggested that increasing the consumption of vitamin C may have a beneficial effect on total cholesterol, initiating studies into potential relationships between vitamin C and CVD. However, evidence relating vitamin C to a reduction in human CVD is basically circumstantial but taken as a whole is suggestive of an association (Simon, 1992).

Throughout the 2000s some reviews suggested that vitamin C (or ascorbic acid) was the most important water-soluble antioxidant in the human plasma and claimed that this nutrient had important intracellular functions (Duarte and Lunec, 2005). Most studies showed that vitamin C supplementation decreased oxidative DNA damage and could modify gene expression and cellular function, suggesting that there was thus a possible beneficial effect of vitamin C supplementation for the prevention of cancer and CVD (Duarte and Lunec, 2005). Studies have confirmed that vitamin C can play an important role in preventing a variety of diseases including CVD (Ye and Song, 2008). This may arise through it’s potential to act as an antioxidant, it’s use as a co-factor in some enzyme reactions or it’s effect on gene expression.

Levels of vitamin C in some selected fruit are shown in table 1.1. Citrus fruit, such as oranges and grapefruits are the richest sources of vitamin C. It has been found that grapefruit has slightly more vitamin C than orange (Proteggente et al., 2002). Other fruit, such as strawberries and raspberries are also considered to be excellent sources of vitamin C.

| **Fruit** | **Vitamin C Content (mg/100g FW)** |
|---|---|
| Guava | 491.6 ± 1.7mg |
| Blackcurrant | 179.50 ± 16.46 mg |
| Kiwi | 78.33 ± 17.15 mg |
| Strawberry | 64.81 ± 13.20 mg |
| Passion fruit | 54.3 ± 2.3 mg |
| Bitter orange | 44.5 ± 0.8 mg |
| Watermelon | 41.4 ± 0.3 mg/100g) |
| Orange | 41.2 ± 0.7 mg |
| Raw papaya | 39.3 ± 0.3 mg |
| Pineapple | 38.3 ± 1.0 mg |
| Melon | 27.2 ± 0.3 mg |
| Raspberry | 26.20 ± 2.67 mg |
| Lemon | 23.2 ± 0.9 mg/100g |
| Blueberries | 16.97 ± 10.09 mg |
| Tomato | 15.2 ± 0.2 mg |
| Cranberry | 11.60 ± 1.42 mg |

**Table 1.1: Vitamin C levels in selected fruit (adopted from: Ellong et al., 2015).**

| Vegetables | Vitamin C Content (mg/100g FW) |
| --- | --- |
| Red pepper | 191.2 ±2.3mg |
| Yellow pepper | 153.5±2.3mg |
| green pepper | 115.5 ±2.2mg |

**Table 1.2 Vitamin C levels in selected vegetables (adopted from: Zhang, & Hamauzu, 2003)**

Vitamin C can also be found in large amounts in green vegetables, such as broccoli and leafy vegetables (Proteggente et al., 2002). Additionally, peppers were reported to contain moderate to high levels of vitamin C. In particular, according to (Zhang, and Hamauzu, 2003) red pepper had higher content of vitamin C than yellow and green peppers (Table 1.2). These results were obtained using an HPLC analysis for ascorbic acid. According to the U.S. Department of Agriculture (USDA) in 2019, levels of ascorbic acid in raw beetroot were found to be 4.9mg/100g and 3.6mg/100g in cooked, boiled, and drained beetroot.

There are several analytical methods for measuring vitamin C (L-ascorbic and L-dehydroascorbic acids) (Hernández et al., 2006). Vitamin C level is significantly affected by processing techniques and it can be decreased by up to 100%, depending on the processing method used.

#### 1.2.1.2 Dietary reference values (DRVs) for vitamins and minerals

People need many different nutrients to maintain health and reduce the risk of diet-related diseases. The amount of each nutrient needed is called the nutritional requirement. Significant scientific progress has been made to identify the nutritional requirements needed to maintain good health. One of these requirement guidelines is the Dietary Reference Value (DRV) publication, which provides an estimate of the amount of energy and nutrients needed by different groups of healthy people in the UK population (British Nutrition Foundation, 2004). Each nutrient has a particular set of functions in the body and some are needed in larger quantities than others. For example, fibre is needed in gram quantities; while vitamin C is needed in milligrams and carotene is needed in micrograms. Individual requirements for each nutrient are related to a person's age, gender, level of physical activity, and state of health. In addition, some people absorb or utilise nutrients less efficiently than others and will have higher than average nutritional requirements (British Nutrition Foundation, 2004).

A study by Carr and Frei in 1999 looked at the recommended dietary allowance (RDA) for vitamin C at that time; for adult nonsmoking men and women it was 60mg/day,which is dependent on the average requirement of 46 mg/day to prevent the deficiency related disease scurvy. However, scientific evidence showed that an increased vitamin C intake was associated with a reduced risk of many chronic diseases such as cancer, CVD, and cataract. This is perhaps due to its antioxidant mechanisms. The study concluded that, probably the amount of vitamin C required to prevent scurvy is not sufficient to optimally protect against these chronic diseases. Consequently the RDA for vitamin C was not sufficient and was re-evaluated. A number of studies recommended an intake of 90–100mg vitamin C/day for optimal reduction of chronic disease risk in nonsmoking adult men and women adult. Therefore, they advised a new RDA of 120mg vitamin C/day.

Moreover, in 2000, there had been a widespread belief that consuming more than the Food and Drug Administration (FDA) recommended amount would be of advantage to many people. The Recommended Dietary Allowances (RDAs) for Vitamin C for adults were 90mg for male and 75mg for female, 85mg for pregnant women and 120mg/day in lactating women. Additionally, smokers require 35mg/day more vitamin C than nonsmokers (Institute of Medicine, 2000). However, in later years this has become a controversial issue since it has been suggested that consuming a large amount of vitamin C could be benificial to human health. *In vivo*, benefits of consuming vitamin C are many. It can prevent and may also cure the formation of a number of diseases as well as enhancing a number of functions and tissues in the body. It has been thought that it can be used to treat or/and prevent the formation of cancer (Proteggente et al., 2002).

Table 1.3 shows six sets of published nutrient requirements for adults (18 years and above), from different geographical regions. However, there are no specific guidelines for some other phytochemicals such as antioxidants and polyphenols.

| Nutrients for 18+y | US/Canada RDA. Acceptable Intakes (AI) 1997-2001 & Dietary Reference Intakes (DRIs) | | EU Population Reference Intakes (PRI) of nutrients 1993 | | UK Reference Nutrition Intakes 1991 | | Recommended Nutrient Intakes (RNI) for vitamins, FAO 2001 | | Nutrition Labelling Directive (90/496 /EEC) | Reference Labelling Values (RLVs)by Scientific Committee for Food (SCF) 2003 |
|---|---|---|---|---|---|---|---|---|---|---|
| | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | | |
| Vit A (µg) | 900 | 700 | 700 | 600 | 700 | 600 | 600 | | 800 | 800 |
| Vit B1 (mg) thiamin | 1.2 | 1.1 | 1.1 | 0.9 | 1 | 0.8 | 1.2 | 1.1 | 1.4 | 1.1 |
| Vit B2 (mg) riboflavin | 1.3 | 1.1 | 1.6 | 1.3 | 1.3 | 1.1 | 1.3 | 1.1 | 1.6 | 1.4 |
| Niacin (mg) | 16 | 14 | 18 | 14 | 17 | 13 | 16 | 14 | 18 | 16 |
| Vit B6 (mg) pyridoxine | 1.3 – 1.7 | 1.3 – 1.5 | 1.5 | 1.3 | 1.4 | 1.2 | 1.3 – 1.7 | 1.3 – 1.5 | 2 | 1.4 |
| Vit B12 (µg) cobalamin | 2.4 | | 1.4 | | 1.5 | | 2.4 | | 1 | 2.5 |
| Vit C (mg) | 90 | 75 | 45 | | 40 | | 45 | | 60 | 80 |
| Vit D (µg) | 5 – 15 (15 for 70+y) | | - | | 10 (50+y) | | 5 – 15 (15 for 65+y) | | 5 | 5 |
| Vit E (mg) | 15 | | - | | - | | - | | 10 | 12 |
| Vit K (µg) | 120 | 90 | - | | - | | 65 | 55 | - | 75 |
| Folic acid (µg) | 400 | | 200 | | 200 | | 400 | | 200 | 400 |
| Pantothenic acid (mg) Vit B5 | | | | | | | 5 | | 6 | 6 |
| Biotin (µg) | - | | - | | - | | 30 | | 150 | 50 |
| Calcium (mg) | 1000-1300 | | 700 | | 700 | | - | | 800 | 1000 |
| Phosphorus (mg) | 700 | | 550 | | 550 | | - | | 800 | 700 |
| Selenium (µg) | 55 | | 55 | | 75 | 60 | - | | - | 55 |
| Iron (mg) Fe | 8 | 8 - 18 | 9 | 16 | 8.7 | 14.8 | - | | 14 | |
| Zinc (mg) | 11 | 8 | 9.5 | 7 | 9.5 | 7 | - | | 15 | |
| Iodine (µg) | 150 | | 130 | | 140 | | - | | 150 | |
| Copper (mg) | 900 | | 1.1 | | 1.2 | | - | | - | |
| Magnesium (mg) | 400-420 | 310-320 | - | | 300 | 270 | - | | 300 | |
| Manganese (mg) | 2.3 | 1.8 | - | | - | | - | | - | |
| Chromium (mg) | 30 – 35 | 20 – 25 | - | | - | | - | | - | |
| Fluoride (mg) | 4 | 3 | - | | - | | - | | - | |
| Molybdenum (µg) | 45 | | - | | - | | - | | - | |
| Potassium (g) | 4.7 | | - | | - | | - | | - | |
| Chloride (g) | 2.3 | | - | | - | | - | | - | |
| Na (mg) Sodium | - | | - | | 1600 | | - | | - | |

**Table 1.3 Nutritional requirement intake for 18 years and over (adopted from: BENDER (2005) and European Union (2006) Directive 90/496/EEC). M= male, F= female.**

### 1.2.2 Dietary Fibre

Dietary fibre is obtained primarily from the polysaccharides (Slominski et al., 1990) of plant cell walls (pectin, hemicellulose and cellulose) that cannot be broken down by enzymes in the human digestive system (Anderson et al., 2009).

Vegetables are natural source of dietry fibre. A high fibre diet can reduce chances of developing coronary heart disease (CHD), blood cholesterol levels, incidence of heart disease, and body weight (Slavin, 2013).

There are two essential types of fibre: soluble and insoluble. Each of which has different characteristics and properties. Soluble fibres are, as the name suggests, water-soluble. Studies showed that soluble fibre reduces the total blood cholesterol levels, in particular low density lipoprotein (LDL) cholesterol, as they combine with the fatty acids to flush them out of the body. Also, they may improve blood sugar levels in diabetics (Anderson et al., 2009). Oats and vegetables such as legumes (dry beans, peas, and lentils) are the best sources of soluble fibre, followed by fruits (like apple, orange and grapefruit). Insoluble fibre is water-insoluble and kept unaffected throughout digestion. Benefits include the increased speed of movement of intestinal contents, moving waste out of the bowel to help prevent constipation (Slavin, 2013).

Insoluble fibre sources include F&V with edible peel or seeds, whole grain products like whole-wheat bread, rolled oats, brown rice and bran. Consumption of dietary fibre is vital to health. However, national dietary survey data has shown inadequate consumption of dietary fibre (Stephen et al., 2017).

There is no published dietary reference intake for soluble or insoluble fibre from the WHO. However, it has been suggested by experts in the American Heart Association that the intake should be between 25 to 30 grams of soluble fibre and from 6 to 8 grams of insoluble fibre per day (UCSF, 2016).

Resistant starch may be considered as a functional dietary fibre, as defined by the American Association of Cereal Chemists and the Food and Nutrition Board of the Institute of Medicine of the National Academics. Resistant starch has low water property capacity, small element size, and bland flavour. (Sharma et al., 2008). Resistant of starch has been described as that portion of starch and starch products that resist digestion in the small intestine. This type of starch may become resistant to digestion due to a number of reasons, it may be physically unreachable, retrograded, or chemically modified. Resistant starch seems to confer considerable health benefits such as reduction in risk of colon cancer and blood cholesterol level, modulation of blood glucose level, and may play a prebiotic role. These starches are being examined for both their potential health benefits as well as functional properties to produce high quality foods. However, the findings of different studies on resistant starch are not strong due to differences in experiment design, sources, types, and doses (Sharma et al., 2008).

### 1.2.3 Sugars

Sugar is a source of carbohydrate in the daily diet. Monosaccharides include fructose, galactose, and glucose are all simple sugars, with general formula $C_6H_{12}O_6$. They have five hydroxyl groups (−OH), a carbonyl group (C=O), and are cyclic when dissolved in water. Disaccharides such as sucrose, lactose, and maltose are all compound sugars, with the general formula $C_{12}H_{22}O_{11}$. They are formed by the mixture of two monosaccharide molecules with the segregation of a molecule of water (Nithiyanantham & Palaniappan, 2013). F&V are generally low in starch. Humans can utilise glucose, fructose, and sucrose from dietary sources. Fresh vegetables,specially leafy ones, are low in calories and as such their consumption will not contribute to excess energy, which can lead to obesity (CDC, 2015). Normally vegetables provide 50 calories or less per portion (cup); one cup of carrots provides 45 calories, one cup of broccolli or bell peppers provides 30 calories, and a cup of steamed green beans provides 44 calories. Whereas for fruit, one medium banana provides 105 calories, one medium apple provides 72 calories, and one cup of grapes provides 100 calories (CDC, 2015). Moreover, dried fruits such as raisins provide 400 calories per cup. Accordingly, vegetables are a naturally low-calorie group which means that they can be added to all meals during the day without worrying about weight gain.

### 1.2.4 Phytonutrients / Antioxidants

A number of phytochemicals have been discovered in vegetables, several of which have suggested nutritional benefits, and this is another crucial reason to position vegetables in the first and largest section of the food pyramid (Aune et al., 2017). These phytochemicals provide food with tasty flavours, vivid colours, unparalleled aromas, and many can be considered as phytonutrients in that they are linked with reduction of the risk of some chronic diseases including:

T2DM, CVD, stork, some types of inflammation and some type of cancer (Aune et al., 2017).

Two of the largest groups of phytonutrients are carotenoids and flavonoids both of which vegetables provide in large amounts. For example, spinach is one of the top sources of multiple phytonutrients (Pan et al., 2018).

The positive physiological impact of plant food consumption may be mostly due to the heterogeneous effects of plant components which exhibit anti-oxidative, anti-inflammatory, and anti-carcinogenic properties (Drossard, 2012). This impact has been partially attributed to various nutrients and bioactive compounds with antioxidant activity. The inclusion of extra phytonutrients present in vegetables may contribute synergistically to their beneficial effects, as higher vegetable intake could reduce the risk of major chronic diseases (Villegas et al., 2008).

Antioxidants are a wide group of substances which can be generically defined as working against the harmful effects of reactive oxygen species (ROS). ROS are highly reactive oxygen radicals formed during normal oxidative metabolism and in response to infection. They cause damage to fatty acids in the cell membranes, and can damage DNA (Bender, 2005). Consequently, ROS have been associated with the pathogenesis of various disorders such as cancer, stroke, diabetes, CVD, autoimmune diseases, neurodegenerative disorders, and may also be involved in aging (Ratnam et al., 2006). Antioxidants could thus play an important role in the prevention or treatment of several oxidative stress–related diseases. This could involve several mechanisms to either protect against or repair oxygen radical damage, for example, in scavenging ROS and preventing the damage they cause (Firuzi, 2011).

Antioxidants can be categorised into two main groups (Figure 1.1) enzymatic and non-enzymatic. Some antioxidants can be synthesised naturally in the human body which include enzymes, enzyme cofactors, and low molecular weight antioxidants. In the non-enzymatic category, a number of antioxidants can be acquired from the diet. Polyphenols are a major group of dietary antioxidants. Vitamins, carotenoids, organo-sulphural compounds, and minerals are the other classes of dietary antioxidants. Some non-enzymatic antioxidants like uric acid, vitamin E, glutathione, and CoQ10 are produced in the human body, but can also be obtained from dietary sources (Ratnam et al., 2006).

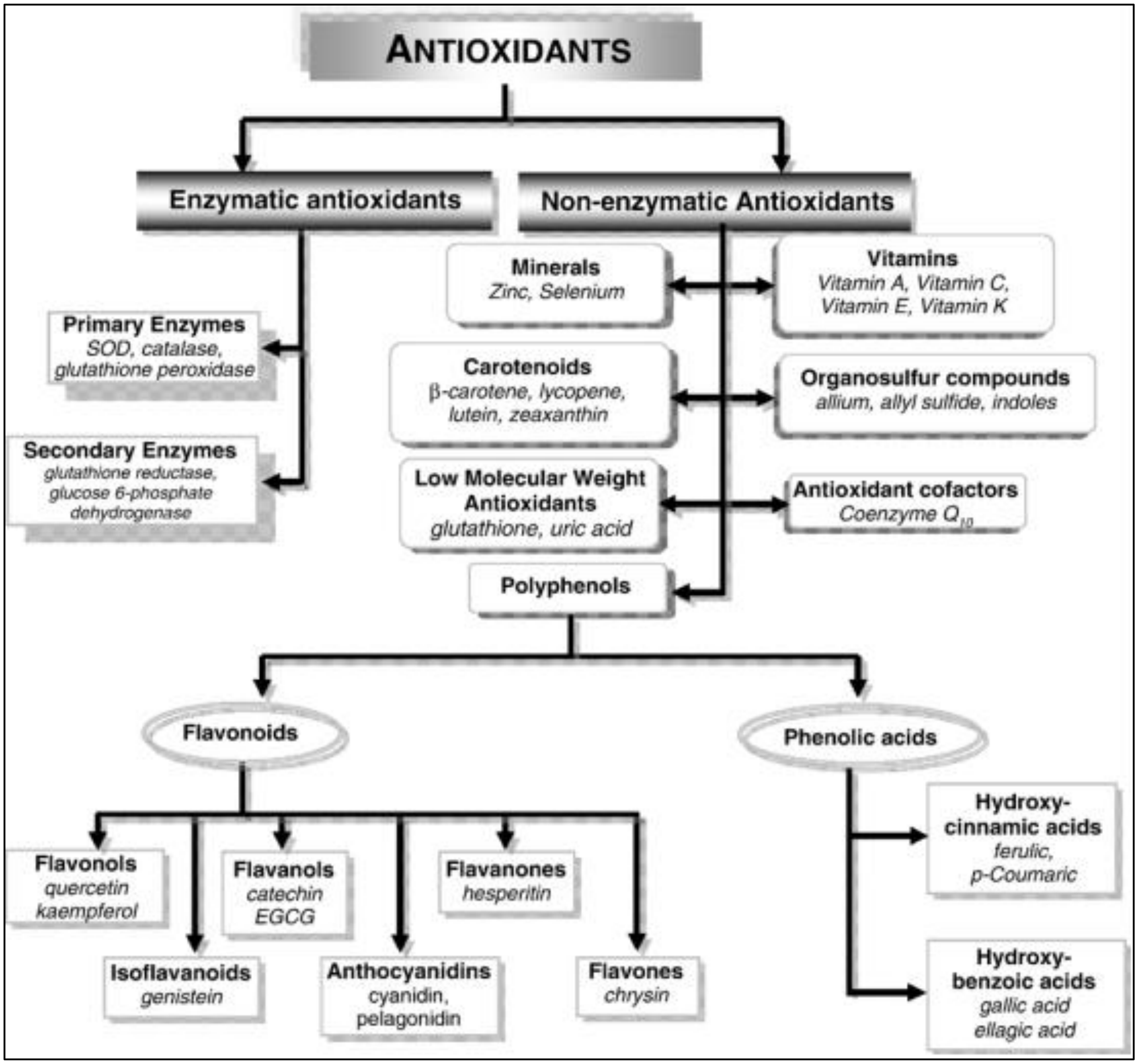



**Figure 1.1: Classification of Antioxidants.**
**Adopted from (Ratnam et al., 2006).**

Dietary antioxidant content can be assessed by a number of methods such as 1-ferric reducing antioxidant power (FRAP) assay (Holland, 2008), 2-Trolox equivalent antioxidant capacity (TEAC) assay (Carlsen et al., 2010), 3-the total radical-trapping antioxidant parameter (TRAP), and 4-oxygen radical absorbance capacity (ORAC) assay (Benzie and Strain, 1999). All of these assays have been used in the past to evaluate the antioxidant content of fruit, vegetables, or their products. They all work on a slightly different principle and can give differing results. There is, however, no general consensus among the scientific community as to which, if any, represents the best method of analysis.

Tables 1.4 and 1.5 shows the antioxidant content of various F&V as determined using the FRAP, TRAP, and TEAC methods. It appears that FRAP routinely gives the highest value for total antioxidant capacity (Pellegrini et al., 2003). Spinach and peppers (chilli and red bell) had the highest level of antioxidants (FRAP) among the measured vegetables listed in Table 1.4, whereas blackberry, redcurrant, and raspberry were the highest among the antioxidants (FRAP) measured fruit listed in Table 1.5 (Pellegrini et al., 2003). In particular, red and yellow peppers were higher in carotenoid content than green peppers (Zhang et al., 2003).

A study by Paganga, Miller, and Rice-Evans in 1999 assessed the antioxidant activities of 100g serving of fresh weight (FW) fruit, vegetables and compared it with previously reported findings for 150 mL beverage (500 ml portion in the case of beer), expressed in μmol Trolox equivalents. The results showed that the antioxidant activity of 1 glass (150 ml) red wine was equivalent to either 12 glasses white wine, 2 cups of tea, 4 apples, 5 portions of onion, 5.5 portions of eggplant, 3.5 glasses of blackcurrant juice, 3.5 (500 ml glasses) of beer, 7 glasses of orange juice, or 20 glasses of apple juice.

| Antioxidant Source | Antioxidant activity (estimated by three different methods) | | |
|---|---|---|---|
| | FRAP* *(mmol $Fe^{2+}$/ Kg FW*)* | TRAP* *(mmol Trolox/Kg FW*)* | TEAC* *(mmol Trolox/Kg FW*)* |
| Artichoke | 11.09 | 6.85 | 1.55 |
| Arugula | 14.30 | 4.22 | 3.55 |
| Asparagus | 10.60 | 9.71 | 3.92 |
| Avocado | 4.90 | 1.87 | 2.22 |
| Beet | 13.13 | 2.70 | 5.21 |
| Beetroot (cooked) | 15.31 | 7.67 | 2.94 |
| Broccoli | 11.67 | 3.07 | 3.04 |
| Cabbage(green) | 5.79 | 2.83 | 1.15 |
| Cabbage (Savoy) | 8.17 | 2.35 | 2.08 |
| Carrot | 1.06 | 0.70 | 0.44 |
| Cauliflower | 4.27 | 1.61 | 1.10 |
| Celery | 1.16 | 0.47 | 0.49 |
| Chicory | 6.72 | 1.88 | 1.86 |
| Cucumber | 0.71 | | 0.43 |
| Eggplant | 3.77 | 2.82 | 1.10 |
| Endive | 3.24 | 0.91 | 0.30 |
| Fennel | 2.33 | 0.78 | 0.43 |
| Green bean | 2.35 | 0.65 | 1.27 |
| Leek | 2.15 | 1.02 | 0.72 |
| Lettuce (green) | 4.94 | 2.31 | 1.33 |
| Mushroom | 16.39 | 6.26 | 4.93 |
| Onion (yellow) | 5.28 | 2.43 | 1.82 |
| **Pepper (chilli)** | **23.54** | 6.42 | 7.62 |
| **Pepper (red bell)** | **20.98** | 5.47 | 8.40 |
| Potato | 3.67 | 0.85 | 0.80 |
| Pumpkin | 4.00 | | 3.71 |
| Radicchio | 11.39 | 6.27 | 3.24 |
| Radish (red) | 3.77 | 3.62 | 2.22 |
| **Spinach** | **26.94** | 5.79 | 8.49 |
| Swiss chard (stalk) | 11.60 | 2.91 | 3.53 |
| Tomato (salad) | 5.12 | 1.31 | 1.65 |
| Tomato (sauce) | 6.15 | 1.69 | 1.47 |
| Turnip tops | 17.77 | 6.62 | 5.52 |
| Zucchini | 3.33 | | 2.86 |
| **FRAP = Ferric reducing antioxidant power; TRAP = total radical-trapping antioxidant parameter; TEAC = Trolox equivalent antioxidant capacity.*<br><br>**FW=Fresh weight* | | | |

**Table 1.4: Total antioxidant capacity of selected vegetables. Adopted from (Pellegrini et al., 2003).**

| Antioxidant source | Antioxidant activity (estimated by three different methods) | | |
|---|---|---|---|
| | FRAP *(mmol $Fe^{2+}$/Kg FW)* | TRAP *(mmol trolox/Kg FW)* | TEAC *(mmol trolox/ Kg FW)* |
| Apple (red Delicious) | 3.84 | 2.23 | 1.59 |
| Apple (yellow Golden) | 3.23 | 1.54 | 1.31 |
| Apricot | 4.02 | 2.29 | 1.44 |
| Banana | 2.28 | 1.05 | 0.64 |
| **Blackberry** | **51.53** | 21.01 | 20.24 |
| Blueberry | 18.61 | 9.30 | 7.43 |
| Cherry | 8.10 | 4.17 | 2.69 |
| Clementine | 8.88 | 2.74 | 3.10 |
| Fig | 5.82 | 2.06 | 2.47 |
| Grape (black) | 11.09 | 2.50 | 3.85 |
| Grape (white) | 3.25 | 1.59 | 2.48 |
| Grapefruit (yellow) | 10.20 | 4.04 | 3.05 |
| Kiwi fruit | 7.41 | 2.30 | 2.28 |
| Loquat | 2.70 | 1.73 | 0.75 |
| Melon | 5.73 | 0.95 | 1.20 |
| Melon (honeydew) | 2.27 | 1.12 | 0.65 |
| Olive (black) | 39.99 | 18.08 | 14.73 |
| Olive (green) | 24.59 | 14.64 | 10.43 |
| Orange | 20.50 | 5.65 | 8.74 |
| Peach (yellow) | 6.57 | 1.49 | 1.67 |
| Pear | 5.00 | 3.87 | 2.19 |
| Pineapple | 15.73 | 5.92 | 9.91 |
| Plum (red) | 12.79 | 8.09 | 5.11 |
| Prickly pear | 6.97 | 2.06 | 1.46 |
| **Raspberry** | **43.03** | 10.48 | 16.79 |
| **Redcurrant** | **44.86** | 12.14 | 14.05 |
| Strawberry(cultivated) | 22.74 | 8.56 | 10.94 |
| Strawberry (wild) | 28.00 | 10.34 | 11.34 |
| Tangerine | 9.60 | 2.76 | 4.16 |
| Watermelon | 1.13 | 0.46 | 0.69 |

**Table 1.5: Total antioxidant capacity of selected fruit. Adopted from (Pellegrini et al., 2003).**

All these antioxidant assays FRAP, TRAP, and TEAC methods measure the total antioxidant capacity of the food. However, it has more recently become apparent that it is unlikley that all antioxidants will exhibit beneficial bioactivity, hence measurements of more specific individual compounds are required. Moreover, there is no standard method for measuring TAC and the three methods reported in this table rely on slightly different mechanisms for their measurement. It is possible that each method thus favours a different class(es) of antioxidant. Thus, whilst the general ranking given by each method will be consistent the specific ranking of individual products within each method will vary. This can be exemplified by the readings for Asparagus which has the highest TRAP value, but whilst being near the top in the other two methods, it is not the highest. The precise reason(s) for this descrepency is not clear. However, these more general assays are still probably useful in providing an indication of the overall relative potential of individual foods or smoothie recipes, as in our present work.

### 1.2.5 Phenolic Components

Interest in dieatry phenolics/ total polyphenols content (TPC) has significantly increased, due to their potential antioxidant capacity (free radical scavenging and metal chelating activities) as well as their possible beneficial impacts on human health, such as in the cure and prevention of CVD, cancer, and other pathologies (Bravo, 1998). Perhaps the best studied phenol is resveratrol which is a polyphenol compound found in red grapes and red wine. Resveratrol has antioxidant properties and has been investigated for more specific possible anticarcinogenic effects (Jang et al., 1997). A large number of studies have shown that resveratrol can inhibit or reduce the progression of a wide selection of diseases, such as cancer , CVD, and ischaemic injuries as well as enhance stress resistance (Baur and Sinclair, 2006).

Polyphenols represent a large group of phytochemicals. They range from simple phenolic molecules to highly polymerized compounds with molecular weights greater than 30,000 Da (Bravo, 1998). Major phenolic antioxidant components include chlorogenic acid found in the flesh of eggplant and the flavonoid delphinidin conjugate in the skin. In the case of apple, the major phenolic antioxidants are chlorogenic acid, procyanidins/catechin compounds, rutin, and phloridzin. In addition, quercetin glycosides, another example of a flavenoid, are major phenolic components in onion (Paganga et al., 1999). A considerable number of studies have concentrated on the flavonoids, a major group of plant phenolics (Bravo, 1998). It has also been found that phenolics are major contributors to the antioxidant potential of the non-citrus juices (Gardner et al., 2000).

Although insufficient polyphenol intake may not cause an exact deficiency diseases, the sufficient consumption of polyphenols has been advised for the reduction of chronic diseases. Polyphenols might be related to enhance cerebral blood flow. Also, flavonoid quercetin and the stilbene resveratrol have also been related to cardio metabolic health (Fraga et al., 2019).

Looking specificly at the TPC of the smoothie composition of the major ingredients used in this thesis, beetroot can be eaten as a cooked vegetable or extracted for its juice (Wootton-Beard & Ryan, 2011 in Olumese, & Oboh, 2016).

Beetroot is a rich source of many nutrients that include folic acid, vitamin A, vitamin C, vitamin B6, niacin, and biotin. It is also rich in minerals such as iron, magnesium, selenium, potassium, calcium, zinc, phosphorus and sodium.

However, the bioactive compounds include phenolic compounds, saponins, and especially betalains, which are responsible for the characteristic colour of this tuber. Beetroot in either it's natural or processed form is a high source of antioxidants (Olumese, and Oboh, 2016).

Wootton-Beard and Ryan in 2011 studied the TPC of beetroot juice. TPC was measured via the Folin Ciocalteu (FC) method as gallic acid equivalents (GAE). They concluded that beetroot provided a high amount of bioaccessible TPC and perhaps represents a cost effective and convenient method of increasing antioxidants in food production, since beetroot juice had a TPC of 68.4 ± 0.3 mg GAE/70 mL. Olumese, and Oboh in 2016 assessed the TPC of Nigerian Beetroot (Beta vulgaris) in both the raw and processed forms. The Folin-Ciocalteau, potassium acetate and dinitophenyl hydrazine methods were used. Results were that raw beetroot and the beetroot juice contained a TPC of 98.08. ± 8.16mgGAE/g and 98.08. ± 5.77mgGAE/g, respectively.

Strawberry (*Fragaria* × *ananassa*) is an important source of bioactive phenolic compounds, most of which express significant antioxidant capacities *in vitro* and *in vivo*. Furthermore, strawberries are economically and commercially important and are widely consumed fresh or in processed forms, such as juices and jams. That is why they have been among the most studied berries from the agronomic, genomic, and nutritional points of view (Giampieri, et al., 2012). Phenolics in strawberry are well documented and include anthocyanins, flavonoids, and tannins (Cheng and Breen, 1991). In a recent study by Yang in 2016 eighteen individual phenolic compounds were shown to possess potent antioxidants scavenging activity. Glucosides and falandiosides were isolated from strawberry cv. Falandi which is widely cultivated in southern China.

Kelebek et al., in 2009 studied the phenolic composition of orange juice. The study reported that hesperidin, narirutin, and ferulic acid were the most abundant phenolic compounds within orange juice.  In addition, another 13 phenolic compounds in total were recognised, including hydroxybenzoic acids, hydroxycinnamic acids and flavanones.

In a study examined different coloured bell peppers (Capsicum annuum L.), it was found that, green pepper had the highest level of TPC then red and yellow peppers (Zhang et al.,  2003).

### 1.2.6 Factors Influencing Nutrient Levels of Fruit and Vegetables

There are many factors that can influence the nutrient composition of a plant. These include cultivation, growing conditions (soil type, water availability, nutrients, and climate), environmental factors, seasonal variation, stage of harvest, and conditions during time spent in transportation and marketing. Other factors include genotypic differences (species/genotype), pre-harvest climatic conditions, cultural practices, maturity, harvesting methods, postharvest handling procedures, and temperature management (Lee and Kader, 2000).

Post-harvest temperature is the most vital influence for the preservation of vitamin C i.e. losses are faster at higher temperatures and also with longer storage duration (Lee and Kader, 2000).

It is also important to consider factors during the preharvest period since they can affect the quality of F&V. These external factors can also influence the quality for consumers and supply chain stakeholders. Factors such as; optimisation of stage-specific production inputs, bio-fortification through targeted plant nutrition, application of accurate crop- and cultivar-specific harvest maturity indices, optimised application of controlled stress conditions that increase primary and secondary metabolites and improved organoleptic and functional aspects of quality, and re-direction of horticultural breeding towards improving flavour in horticultural products (Kyriacou, and Rouphael, 2018).

Furthermore, bruise damage can be a significant post-harvest problem and causes economic losses, decreases crop quality, and is of serious concern with regard to food safety. This damage is a major challenge in the agricultural industry, possibly the damage can take place at all stages of the harvest chain. (Hussein, Fawole and Opara, 2018).

In conclusion, a large number of factors can influence nutrients levels in F&V. Recognition is given to the fact that these might all contribute to 'seasonal' variation. For example, it seems inevitable that suppliers will be sourcing their products from different parts of the world at different times of the year and these maybe different cultivars as such genetics and growing conditions are likely to have major effects.

## 1.3 CURRENT FRUIT AND VEGETABLE CONSUMPTION

The epidemiological and nutritional composition results clearly indicate the need for a diet rich in fruit and vegetables. It is thus useful to consider the actual level of consumption of these food types across the world and particularly within the UK and KSA, as this is the focus of this thesis.

### 1.3.1 Factors Determining Fruit And Vegetable Consumption

There are a number of aspects that impact F&V consumption that are relevant to all geographical areas for instance age, gender, and socioeconomic status (EUFIC, 2012). These in turn can have an impact on other factors, such as: knowledge of food choice, affordability, and skills. On the other hand, there are also other individual factors such as, self-esteem, time management and, personal values. In addition, family issues, social networks and support, meal patterns, and atmosphere at meal time may determine food choices and dietary behaviour and as a result affect attitudes towards F&V consumption as well (EUFIC, 2012).

### 1.3.2 Measuring Food Intake

When measuring food intake, many different approaches are available such as food diaries and dietary recalls (i.e. questionnaires and interviews). Another approach is to calculate the average food supply of a household based on national statistics of spend. Every method has different characteristics and the accuracy varies among them. Fortunately, it is agreed that many national authorities continually implement surveys of F&V intake and thus, standardising survey methodology will enormously develop well-compared data between different countries in the EU (EUFIC, 2012).

The European Food Safety Authority (EFSA) presented a comprehensive food consumption database, namely the EU Menu, to provide comprehensive data about food consumption in a number of EU countries using a standard survey method (EFSA, 2015).

#### 1.3.2.1 Is it classed as a fruit or a vegetable?

A fundamental difficulty when assessing F&V consumption in Europe is the fact that F&V are defined differently in different Europeans countries. This represents a barrier when comparing data from diverse studies. The WHO consider potatoes a starchy vegetable and it is thus excluded from the broad vegetable definition. Some countries like Austria, Belgium, Denmark, Iceland, Netherlands, Portugal, Spain, and Sweden follow the WHO standard. In contrast Norway includes potatoes in their recommendations (EUFIC, 2012).

#### 1.3.2.2 Fruit and vegetables portion size

It is important to define the F&V size/portion to acquire precise comparable data on individual intake. In the EU, the recommendations are different among different countries, The WHO recommended to consume ≥400g F&V per day (without potatoes and starchy vegetables) and major EU countries follow this.

#### 1.3.2.3 Juice intake

Regarding juice intake, Iceland and Norway include juices in their F&V recommendations. On the other hand, Denmark, the Netherlands, and Sweden include it with restrictions i.e. counts as a maximum of one portion a day. Further, Belgium and Spain exclude juices from their recommendations and Austria and Portugal have no recommendations concerning juice (Yngve et al., 2005).

#### 1.3.2.4 Gender

Many studies have shown that, generally, females consume larger quantities of F&V than males (Bere, et al., 2008, Dibsdall et al., 2003, Rasmussen et al., 2006). Although the reasons for this seem to be unclear (Bere et al., 2008). Therefore, gender may have an impact even in the absence of nutrition education and knowledge (EUFIC, 2012). It has been shown that school girls consumed more F&V than boys. Girls in general having a higher awareness, of their daily F&V consumption and the nutritional benefits, than boys (Stukas, 2012).

The causes of this gender factor are complex but the traditional roles for women and men in society along with social structure could be an answer (Friel et al., 2005). Women as mothers care about preparing food, handle health, and nutrition at home and commonly do the groceris shopping for more than men. In addition, women are more likely to link a healthy diet with eating extra F&Vs (European Commission, 2007).

A study by Baker and Wardle in 2003, aimed to understand the differences in F&V intake between genders used a simple questionnaire with 1,024 adults in the UK. The study reported that men consumed fewer F&V than women and that nutritional knowledge about the F&V recommendations intake was higher in women than men. They, also reported that fewer men than women were aware of the relationship between F&V intake and disease prevention. Moreover, women rated their liking for F&V higher and men were less likely to be on a diet for weight loss.

#### 1.3.2.5 Age

Age can also affect F&V consumption. Consumption appears to be higher in preschool children (Cook et al., 2004), but it is likely to decline with age in children and adolescents (Rasmussen et al., 2006). Conversely in adulthood, consumption may increase with age. This is possible due to the fact that knowledge increases with age as well as income. Other aspects such as social eating habits and time dedicated to cooking may change with age and in particular effect vegetable consumption (Elfhag et al., 2008).

### 1.3.2.6 Education and knowledge

Higher education in general means higher income, also it could result in better awareness and knowledge about healthy eating behaviours. In addition, it seems that social influences, ideal, and principles related to income and education levels have a great deal of influence on eating habits as well as F&V consumption (Elfhag et al., 2008).

Potential links between nutritional knowledge and awareness has been extensively researched (Shaikh et al., 2008). It is the reason why particular groups may eat more healthily than others. It also influences what people eat to some extent. Nutritional knowledge is one of the most significant factors, among psychosocial influences, and is one of the powerful predictors of F&V intake (Shaikh et al., 2008).

On the other hand, absence of knowledge that provide skills to cook and prepare F&V can be a barrier to consumption and can also be an obstacle to their purchase (WHO, 2005). Women, in general, are more knowledgeable than men, as discussed earlier in the gender section.

### 1.3.2.7 Income and affordability

Numerous previous studies have showed that there is a relationship between income/salary level and F&V intake. One study reported that low income groups appeared to eat lower quantities of F&V than higher income groups (Dibsdall et al., 2003; Rasmussen et al., 2006).

Thus, affordability seems to have a strong effect as even in high income groups, high price and cost of F&V can be an obstacle to buying and consumption.

But generally, low income groups are identified as more price concerned groups (Kamphuis  et al., 2007).

#### 1.3.2.8 Availability and accessibility

In the home, availability and accessibility of F&V for both adults and children is essential for higher consumption (Bere, & Klepp, 2004; Kamphuis et al., 2007;  Rasmussen et al., 2006). School children may eat F&V at school every day. However, they consumed more F&V at home than at school (Stukas, 2012). The availability of attractive F&V displays in shops all year have  a positive impact on increased consumption especially in higher socioeconomic groups (Kamphuis et al., 2007). Conversely, it has been argued that poor quality and inadequate supply of F&V in shops and markets will cause difficulties and barriers in raising consumption (WHO, 2005).

#### 1.3.2.9 Family and social support

Family influences appear to have a powerful effect on male and female food habits, including F&V consumption, no matter whether they are children, adolescents, adults, or elderly (Shaikh et al., 2008). Social support may also improve F&V consumption (Shaikh et al., 2008). Generally marriage has a positive influence on the amount of F&V intake, particularly in men (Kamphuis et al., 2007; Friel et al., 2005).

Overall, it seems that family factors are stronger in men than in women. This may be related to the fact that it is the wife’s actions that have a positive impact on their husband/family consumption. Moreover, women usually pay more attention and care to family health matters in addition to their responsibility for shopping and food preparation (Kamphuis et al., 2007 ; Friel et al., 2005).

F&V intake in childhood may be linked to the quantity parents eat. When vegetables are introduced at an early age, their consumption is likely to be higher at preschool age (Cook et al., 2004). Moreover, children who consumed large quantity of F&V will continue with a higher consumption level at later ages (Sa and Lock, 2008; Kamphuis et al., 2007).

The pressure coming from parents, forcing the child to eat F&V, can have a negative influence on the child consumption. Hence, parents should instead be good role-models encouraging children to eat more F&V and increasing everyone's consumption (Pearson et al., 2009). In addition, family meal patterns and sharing family meals has been demonstrated to enhance F&V intake from childhood (Cook et al., 2004; Rasmussen et al., 2006). Availability of F&V at home, as discussed earlier, shared meals, and natural food preferences all affect the relationship between consumption levels (Cook et al., 2004).

#### 1.3.2.10 Food preference

Food preferences are initiated from an early age, from the time solid food is introduced to the baby. It is one of the important factors associated with F&V intake (Bere and Klepp, 2004; Rasmussen et al., 2006). Children might not like some food at the beginning when starting to consume solid food. Nonetheless, repetitive exposure can develop the preference. Because various vegetables have a savoury taste, the child can try them repeatedly before trying fruit to accept them. However, when parents use pressure and rewards for children trying to increase their F&V intakes' may affect the child preference negatively leading to strong aversions such as food "neophobia" - the opposition to try novel foods, in children as well as in adults. In children, repetitive exposure to vegetable could greatly decrease neophobia (Havermans et al., 2010).

It is recommended to use positive approaches in building the child's food habits; such as  parents acting as role models, they can consume plenty of F&V daily to encourage their child to eat in the same manner. Also, providing children frequently with a variety of foods in different taste, colors, and texture with patience in the repetitive foods they appear to dislike at the beginning (Benton,2004). Hence, food preference can be considerably developed during childhood and by time might be altered through adulthood (Havermans et al., 2010).

#### 1.3.2.11 Psychological factors-attitude and beliefs

Self-efficacy (e.g. the achievement of goals and belief in self to accomplish responsibilities) was found to be a strong predictor in adults of their attitude towards F&V intake (Shaikh et al., 2008).

Attitudes and beliefs also have an impact on consumption levels but this was less strong than self-efficacy (Shaikh et al., 2008).

Nutritional attitudes, such as, taste preferences and availability of F&V at home can both impact consumption rates. One study showed a dominant factor in increasing consumption rather than taste preference, availabilty , i.e. if low taste preference for F&V existed but they were available at home, the F&V intake would be increased (Neumark-Sztainer et al., 2003).

#### 1.3.2.12 Perceived barriers to higher F&V consumption

It has been proposed that one important barrier to increased F&V intake is that European's believe their diet is satisfactory (Dibsdall et al., 2003). Approximately 20% of Europeans reported that their eating habits were very healthy. Nevertheless, the wide majority of them thought what they consumed was good for their health, that it is easy to have a healthy diet, and that eating a healthy diet does not require the consumption of extra F&Vs (European Commission, 2006).

A study investigated the differences between high and low consumers of F&V and took into account food choice motivations and food choice behaviours in a cohort of women. Pollard et al., in 2002 reported that busy lifestyles and unbalanced working hours were barriers to eating F&V, particularly in case of vegetable intake. Time limitations to prepare and eat F&Vs characterise a commonly reported concern. There are indications that fruit is often considered a convenient food, whereas vegetables are not. Moreover, low consumers of F&Vs consider convenience factors, such as availability of shops, shopping time, simplicity of preparation, and available time for cooking of higher importance for their consumption as compared to high consumers of F&V. Besides, the study showed that the high F&V consumer group pointed to the health and natural content of the food as being the most important factor for their food choice (Pollard et al., 2002).

A lack of control over what is consumed has also been reported (European Commission, 2006).

### 1.3.3 Fruit And Vegetable Consumption in Europe

F&V consumption in almost all Western countries are well below the recommended 5 A DAY (Baker and Wardle, 2003). According to the WHO, F&V consumption in Europe is very low. It is estimated that in the majority of EU countries consumption is lower than 400g a day and the mean intake on average is lower than 300g a day (WHO, 2006). It is important to address the factors that impact the increase of F&V intake and develop initiatives to increase consumption.

### 1.3.4 EU Fruit and Vegetable Intake

A recent systematic review of cross-national studies by Stefler and Bobak (2015), compared the intake of F&V or levels of their indicators such as vitamin C and carotenoids between Eastern and Western European populations. This study utilized identical analysis and methods for data collection in both regions. The results indicated that CVD was a mortality risk factor possibly arising from insufficient intake of F&V.

The twenty-two studies revealed that fruit consumption in Central and Eastern Europeans (CEE) and the Former Soviet Union (FSU) was consistenly lower than the Western Europeans (WE). In terms of Northern and Southern Europe, it was noted that there were a limited number of published studies including surveys that included health behavioural results in Northern and Southern Europe in general. This information would help inform F&V consumption in these areas, and the data could be compared to those of Western Europe's.

Measurement of antioxidants in the twenty-two review studies identified a lower concetration of beta-carotene in CEE/FSU subjects and the results for vitamin C level were mismatched. There were no differences between males and females. Moreover, the review results were well-matched with the ecological dietary data of food availability based on food balance sheets (FBS) and household budgetary surveys (HBS).

In 2005, a standard Euro-barometer survey about health and food, commissioned by the Directorate for General Health and Consumer Protection, was carried out. The survey revealed that almost three out of five Europeans respondent considered that "eating a healthy diet" means a "balanced and varied diet". The majority (58%) also thought that the consumption of more F&Vs means a healthy diet and that their diet was healthy and that it is easy to be healthy (European Commission, 2006; EUFIC, 2012). Another interesting result was that the Europeans who consider themselves to be overweight are more conscious of the advantages of consuming a greater amount of vegetables and fewer high calories foods (European Commission, 2006).

The European Nutrition and Health Report in 2009, reported that in the EU, contributing countries and in every province of the EU the average intake of F&V increased from 1961 to 2003. However, a minor decrease of intake was identified in the Western EU countries (Elmadfa et al., 2009).

### 1.3.5 Fruit and Vegetable Consumption in UK

In 2000, 43% of consumers in the UK were aware of the 5 A DAY message and 26% claimed to be putting this into practice (Foods Standards Agency, 2001). Yet, consumption data from the 2000/01 National Diet and Nutrition Survey (NDNS) showed that less than 15% of adults met the 5 A DAY target. Thus, it appears that the gap between knowledge and action is quite wide (Ruxton, 2008). Moreover, on average, children eat only around 2 of the recommended 5 portions of F&Vs a day and children from the lowest socio-economic group tend to eat 50% less F&Vs than those from the highest group (NDNS, 2000).

From 2008 to 2017, unfortunately, the NDNS data showed that there was no significant change to the total F&V intake or the measurement meeting the 5 A DAY target. It remained low at around 30% for adults and around 10% in case of 11-18 age group. Current recommendation states that a 150ml of fruit juice can count as one serving of the 5 A DAY. However, this does not mean to avoid fruit juice completely to reduce free sugars, but it shows the need to be cautious in increasing the F&V intake even further to meet the 5 A DAY recommendation. The NDNS data presented a reduction in consumptions of fruit juices among the population and was only statistically significant in some age groups. It was estimated that over 9 years there was a decrease of consumption by 54g/day in in children aged 4-10 years (6g average yearly reduction), 48g/day in girls aged 11-18 years (5g average yearly reduction) and 32g/day in women aged 19-64 (4g average yearly reduction) (British Nutrition Foundation, 2019).

A qualitative study conducted in 2006 at St George's Medical School, University of London, distributing 300 questionnaires to assess the attitudes and behaviour of healthcare students regarding the consumption of five portions of F&Vs per day. The study showed that only 17% reported eating five or more portions of F&Vs on the previous day (Chahal, 2007). This data is similar to the rate identified in the 2005 Health Survey for England for 5 A DAY consumption on 16–24-years old, which was also 17% (NHS, 2005). Additionally, the study reported that over 80% of this group of UK healthcare students failed to eat five portions of F&Vs daily. It is not surprising that primary care physicians feel they must struggle to change the behaviour of many of their obese patients (Chahal, 2007). The evidence presented by these studies suggested that even the most informed person finds it difficult to consume the quantities of F&V recommended by health professionals (Crocker, 2003).

The Department of Health (2007) also confirmed that fewer than three portions of F&V a day were eaten by individuals in the UK; children and those with lower incomes consumed an even much lower than recommended quantity in 2007 (NDNS, 2007). In 2008–2009, 74% of consumers were aware of the 5 A DAY target, yet only 58% claimed to be putting this into practice (Food Standards Agency, 2008). Despite the slight improvement in attitude from 2007 to 2009, these levels do not seem to be satisfactory. These recommendations in the UK are much lower than those in some other countries; the US guidelines for example, suggest up to 13 portions of F&V should be consumed per day for an active male (Whitehead et al., 2012). Thus, the 5 A DAY goal should be attainable.

A study for the World Cancer Research Fund (WCRF) revealed that, on average, 17% of lower income households (social groups C2, D and E) have their 5 A DAY, and 27% of higher income households do so. The F&V consumption levels were higher in the south of England, where 26% reported they ate at least five portions. The lowest levels of consumption were reported in the north of England, where 18% had five or more portions a day. In London, it was found to be 21%, in Scotland 22% and in Wales 23%. The aid organisation WCRF invested in an awareness day called 'Fruity Friday' in May 2012 to increase fruit consumption awareness as part of Cancer Prevention Week (WCRF, 2012). Another finding published by the National Diet and Nutrition Survey (NDNS) in 2012 showed that, on average, adults ate only four portions a day. Only 30% of the population aged between 19 and 64 are eating the full five portions. The results were disappointing, as they were roughly similar to those of previous surveys in the NDNS series carried out between 1994 and 2001. The survey of 3,000 people was conducted for three years up to 2011. The NDNS involved an interview, a four-day dietary diary, and blood and urine samples. Results are used by the government to develop a policy, monitor progress on diet and nutrition, and to assess whether the UK population is meeting expert recommendations on diet and nutrient intakes (DH, 2012).

Globally, annual reporting has shown that population growth rates have decreased (FAO, 2017). It's peak was in the late of 1960s, with global growth rates extending to 2% yearly then rates declined to 1.2%. At present, the absolute annual percentage rise in population is to some extent under 80 million people (FAO, 2017).

Moreover, in a recent research it was proposed that the effects of the advertising campaigns on F&V was able to put into practice only around half a serving per adult per day to be purchased after 10 years of beginning the programme (Castiglione and Mazzocchi, 2019). From what has been stated to date, adherence to the 5 A DAY consumption in the UK remains low and has never reached 50% in any part of the UK. Therefore, scientists, nutritionists, and health professionals are searching for ways to raise the consumption of F&Vs in an easy, convenient, and affordable manner. Hence, in this thesis work, smoothie consumption has been suggested as a potential contribution to solve this problem.

### 1.3.6 Fruit and Vegetable Consumption in The Arabian Gulf Region

In the Middle East, the Arab nation consists of 22 countries spreading from the north west of Africa to the Arabian Gulf region (AGR), namely: Morocco, Algeria, Mauritania, Tunisia, Libya, Egypt, Sudan, Djibouti, Somalia, the Comoros Islands, Palestine, Lebanon, Jordan, Syria, Iraq, Kingdom of Saudi Arabia (KSA), Kuwait, Bahrain, Qatar, United Arab Emirates (UAE), Oman, and Yemen (ADC, 2009).

From an economical point of view, they can be distributed into 3 classifications. The first is high per capita income category which is the AGR and includes 6 countries: Bahrain, KSA, Kuwait, Oman, Qatar, and the UAE, which formed the Gulf Cooperation Council (GCC). The second is middle per capita income category which includes Iraq, Jordan, Syria, Tunisia and Lebanon. And lastly, the low per capita income category which includes Djibouti, Egypt, Mauritania, Somalia, Sudan and Yemen (Musaiger, 1993).

From the health point of view, in general the nutritional status in the Arab provinces has experienced radical changes in the food intake patterns over the past five decades. Even though each of the Arab countries has different population, socio-economic status, geographical, weather, health status, trade, and industry factors they all suffer from the same two issues relating to poor nutrition. The first issue is malnutrition, in particular iron deficiency anaemia in those who are in the under-developed countries. The other is over-nutrition related mainly to wealth and linked to changes in lifestyle. Both are related to common chronic diseases for instance CVD, diabetes, hypertension, and obesity as well as some types of cancer (Yahia et al., 2019).

The health services are mostly responsible for combating under-nutrition. Some common factors for under-nutrition are lack of safe water, unreliable dietary habits, poor hygiene, poverty, illiteracy and lack of nutrition knowledge. Generally, inactivity as well as lack of nutritional awareness in dietary habits is linked to incidences of the diet-related non-communicable diseases (Musaiger, Hassan, and Obeid, 2011). In the Middle East, obesity in particular and its incidence is a major health concern and it is increasing in most of the population (Ahmad et al., 2015).

The AGR has changed rapidly from rural to urban. i.e. they live modernise lifestyle. As mentioned earlier, the AGR, which has high per capita income, consist of 6 countries that make up the Gulf Cooperation Council (GCC). They are oil-producing countries that are categorised by higher health standards and low infant deaths (Musaiger, 1993).

The revenue from oil and petroleum products in the 1960s has allowed farming and fishing to shift to modernist methods (Todd, 2016). In addition, it has resulted in continually expanding populations, especially in Qatar and the UAE which have had rapid increase in population, per capita income and wealth (NG et al., 2011). Population growth has an impact not only on food demand in the region but also on food exports. At present, a considerable amount of food is imported to meet the expectations of the population growth, mainly foreign workers on temporary visas who represent more than half of the citizens in many of these Gulf countries (Todd, 2016).

There are several factors affecting the food consumption patterns in AGR, these include but not limited to, population growth, political conditions, deterioration in the price of oil, the Gulf crisis, and repatriation of an enormous number of the work force from the oil exporting countries in their home countries (Musaiger, 1993; Alnohair, 2014). Other factors include income, education, prices, women's employment, the media, geographical differences, immigration, religious differences, food preferences, food beliefs, family dietary habits, cultural, socioeconomic, urbanisation, lifestyles, nutritional, and social aspects. All these complex factors influence food choices and can contribute to the tendency of increasing occurrences of nutritionally related non-communicable diseases (Musaiger, 1993; Alnohair, 2014).

In the last two decades the incidence of obesity in the AGR particularly in the high income, oil-producing countries has increased (Musaiger et al., 2012). This was probably due to changes in the pattern of food consumption which has shifted from traditional and local foods such as dates, wheat, and vegetables to a greater consumption of fast foods, processed foods, and sugar-dense beverages like soda drinks. Together with these changes in lifestyle; high technological developments, such as remote activation, cars, elevators, and escalators, have led to a more sedentary lifestyle and a reduction in the level of physical exercise.

Regarding F&V intake in the AGR, the Arab Centre of Nutrition advises the consumption of not less than three portions of vegetables and at least two portions of fruit every day. However, it can be concluded that, similar to the case in the EU, the majority of the AGR population fail to consume adequate quantities of F&V. In particular, children and teenagers consume limited portions of F&V and this prevents them from obtaining vital nutrients (Musaiger et al., 2012).

A recent systematic review by Aljefree and Ahmed in 2015, using available articles between 1990 and 2014, aimed to investigate the evidence for the association of Western dietary pattern with coronary heart disease (CHD) and its associated risk factors among adults in the MENA region including Gulf countries. The research analysed 15 studies mainly among adults. The significant findings showed that there were relationships between the Western dietary pattern (high fat dairy products, hydrogenated fats, refined grains, rich in red meat, sweets, soft drinks, eggs, and pizza, but low in F&Vs, whole grains, fibre and sea food) and the increased risk of high BMI, diabetes, dyslipidaemia, hypertension, and metabolic syndrome (MetS). In contrast, very few studies found a relation between the Mediterranean diet and MetS. The Mediterranean dietary pattern contains a high intake of F&V, wholegrain, legumes, nuts, olive oil, and seafood. It also includes a reasonable intake of dairy products, eggs, meat and wine. This Mediterranean diet offers numerous benefits for CVD prevention, as it is full of antioxidants, fibre, polyphenols, monounsaturated fatty acids, and magnesium. A recent review found a strong evidence of greater adherence to the Mediterranean diet and a risk of overall mortality reduction, CVD, CHD, overall cancer, neurodegenerative diseases and diabetes. However, there was no evidence for bladder, endometrial, or ovarian cancers, in addition for LDL cholesterol levels (Dinu et al., 2017).

In a review paper which investigated the prevalence of obesity in the Gulf contries, the researchers found that obesity was common among women; whereas, men have a prevalence of being overweight (NG et al., 2011). Both overweight and obesity rates are high in Kuwait, Qatar, and KSA among adults between 30 to 60 years, but prevalence is lower in the younger and elderly population (NG et al., 2011). However, there is a noticeable increase in obesity in pre-schooled age children in both Kuwait and KSA, whereas, overweight and obesity was the highest among adolescents. Kuwait has the highest occurence (NG et al., 2011). It is also important to note that hypertension and diabetes levels are also exteremly high in the Gulf Region, and they tend to rise with age. The worst levels of hypertension and diabetes were reported in the UAE (NG et al., 2011). Moreover, the Global School Health Survey of 2010 in the UAE stated that 40% of students between 12-15 years old were either obese or overweight, which is unfortunately double the 2005 data. This urged the UAE government to impose obligatory school health education sessions and drafted strict guidelines on the provision of food and drinks in schools.

A recent cross-sectional study of 1,022 students, with 539 boys and 483 girls aged between 12-16 years, was carried out in Dubai, UAE. This study aimed to acquire information about adolescents' dietary and activity habits, and their link with the increasing prevalence of obesity. Using short self-completed questionnaire, the study showed that residents of Dubai seem to eat healthier in comparison to other Emirati citizens, especially in case of girls. Generally, 15% of students reported that they never ate fruit, 31% drank sugar sweetened beverages daily, 16% never ate breakfast, 18% never drank milk, 37% played sport or exercised daily, and 60% watched more than 2 hours of TV daily. The study concluded that although there had been a recent change in school policies, there is a need for further interventions encouraging changes in the daily lifestyle among adolescents, and improved provision of healthy food habits in schools to be more suitable for students. This was because the students were still failing to eat a healthy diet and participate in physical activities (Haroun et al., 2016).

### 1.3.7 Fruit and Vegetable Consumption in KSA

One cross-sectional study in 2015 from University of Dammam in KSA, included a total of 367 student participants with 223 (60.76%) male and 144 (39.23%) female. The participants were randomly chosen to assess F&V consumption using a questionnaire covering predictive parameters on the frequency and types of food intake. The study found that the consumption of F&V was not equivalent to the WHO recommendation. Moreover, the findings suggested there was a significent relationship ($p < 0.001$) between moderate/high intake of F&V and family food habits, awareness about the WHO F&V consumption guidelines, fitness consciousness and regular exercise. However, there was no significant association between gender and BMI in the F&V consumption (Alsunni and Badar, 2015).

## 1.4 RECOMMENDATIONS FOR FRUIT AND VEGETABLE CONSUMPTION

It is generally believed that many populations, including the nations mentioned above, consume an insufficient amount of F&V to maintain a healthy lifestyle. Thus, health organisations and governmental entities have collaborated to draft the consumption recommendations. However, different organisations and countries came up with slightly different recommendations. For instance, in 2000 the US Department of Agriculture encouraged consumers to consume at least two portions of fruits and at least three portions of vegetables a day regularly, which can be fresh, frozen, dried, or canned types with variation in colours and varieties such as dark-green leafy vegetables, orange fruits, and peas. On the other hand, in some other countries, consumers are encouraged to eat at least 10 portions of F&V a day. There is an evidence that consumption of whole foods is better than isolated food components such as dietary supplements and nutraceuticals (Oguntibeju, Truter and Esterhuyse, 2013).

The following section will elaborate on such recommendations:

### 1.4.1 Recommendations For Fruit and Vegetables Consumption Worldwide

The following is a list of some of the recommendations worldwide: "5 al dia" in Argentina, "Go for 2 and 5" in Australia (Western Australia), 5 to 10 a day in Canada, "Vegefru-7" in Japan, "5 x dia" Mexico, "5 A DAY" in New Zealand, "5 por dia" in Uruguay, and "5 A DAY" and "5 to 9 a day for better health" in United States of America (WHO, 2005).

### 1.4.2 Recommendations for Fruit and Vegetables Consumption in Europe

The initiatives of the European countries are clear in conducting national nutrition policies towards increasing the F&V consumption. In fact, most European countries indicated inadequate consumptions of F&V and one of their aims is to raise it (WHO, 2006). However, The WHO Regional Committee for Europe permitted decision EUR/RC57/R4 in September 2007 which authorises the Action Plan for Member States to implement and enhance their national food and nutrition policies and health system governance. Also, for international organisations to reflect at the regional and worldwide levels, aiming to accomplish the population nutrition and health goals. Therefore, goals should be implemented according to Food and Agriculture Organisation (FAO) and WHO recommendations (WHO, 2008).

In March 2005, the EU Platform for Action on Diet, Physical Activity, and Health recognised the growth in the prevalence of obesity in EU schools' children and its associated illness. One reason was the low F&V intake. Consequently, in June 2007 the EU commission presented a proposal for a school fruit scheme highlighted with the modification of the Common Market Organisation (CMO) for F&Vs. The White Paper on Diet, Physical Activity, and Health aiming to increase the F&V consumption in children across the EU schools and in return decreasing obesity and chronic diseases associated with poor diet (European Commission, 2007).

In 2010, the "School Fruit Scheme" was introduced widely in EU schools as a long-term programme that gave free F&V. It also was aimed at raising awareness by conducting educational events to show the significance of good eating habits and to encourage children to make F&V part of their daily consumption. Not only children, but it also included parents and teachers. Moreover, the school fruit scheme was supported financially by the European Commission with some EU countries participations. The scheme includes companions from public health, education divisions, and agriculture divisions to ensure efficiency and monitoring to develop and improve the schemes during the programme (European Commission, 2010). Furthermore, an annual report was published to demonstrate a summary of each meeting that included the accomplishments of the Platform and the observed progress of the Platform's members. The Platform consists of 32 active members including selected elite nutritionists (EU Platform, 2009).

There are some national EU initiatives targeting increase in the F&V consumption within the local population and particularly children. For instance, 10 portions recommended daily in France, called "Un fruit pour la récré" / "10 par jour in France"; which have greater F&V recommendations. Also, in Denmark, ≥600g per day namely; "6 om dagen" was recommended (Yngve et al., 2005; WHO, 2008). In addition, "3x3" which is 3 a day in Hungary, "5 am Tag" in Germany, "SchoolGruiten 2+2" in the Netherlands, "5 om dagen" (schools fruit programme) in Norway, "5 A DAY" in Poland, "Fruitness" in Italy, "5 al día" in Spain, "5 om dan" in Sweden, "5 am tag/par jour/ al giorno" in Switzerland, "Food dudes" in Ireland, and "5 A DAY" in the UK (WHO, 2005; European Commission, 2010; EUFIC, 2012) "6 a day" campaign in Denmark, where Danish population is consuming very low F&V, were recommended (European Commission, 2008).

This research will be focusing on the UK's 5 A DAY campaign which is the policy applied for general population to increase their F&Vs intake.

### 1.4.3 The 5 A DAY Recommendation in UK

The UK government introduced the 5 A DAY programme after the NHS Plan 2000. The aim of this plan was to encourage the public to increase their F&V consumption (The Department of Health, 2004).

The programme identifies one portion as being equal to 80g and states that the daily total consumption of F&V should include a variety of types and colours, the darker the better. A portion thus equals two or more small fruits, i.e. satsumas, one medium-sized fruit like an apple or banana, half a grapefruit, or one large slice of melon. A portion of vegetable would be two broccoli spears or three heaped tablespoons of cooked vegetables such as carrots, peas, or sweet corn. Whereas, potatoes, yams, cassava, and plantain do not count because they are considered to be starchy foods rather than vegetables. In addition, F&Vs used can be fresh, frozen, tinned, or dried. Pulses and beans count as one portion, no matter how much is eaten. Also, the NHS in 2015 announced that a glass of smoothies 250ml is equal to one portion of the 5 A DAY total of drinks from fruit and vegetable juices. Smoothies was also mentioned but with no more than 150ml a day. However, innocent smoothies claims that 250ml is equal to two whole portions of the 5 A DAY; this might be considered acceptable because they were able to provide evidence that their smoothie products contained all the edible parts of the pulped F&V, in a sufficient amount and did not include dairy product (Innocent, 2009 in ASA, 2014)

There was already a great demand for F&Vs in the UK market by 2009 and continued to increase over all the three main sectors (fresh, frozen and canned).

Much of this increase in demand has resulted from the trend towards healthier eating and the encouragement from the UK government, through campaigns such as 5 A DAY, to consumers to consume greater quantities of F&Vs (Mintel, 2009). Over the years, the 5 A DAY recommendation has continued to be a feasible target, despite research suggesting more portions would be optimal for health (NHS, 2017). In 2011, the Department of Health (DH) devoted around £10 million to encourage people to eat their 5 A DAY through the "Change4Life" campaign. This scheme promoted 5 A DAY by providing "supermeal" recipe ideas and reducing prices of F&V in retail stores. A pilot study of the scheme showed that F&V sales increased by roughly 40% (DH, 2011).

Moreover, in 2019 the UK organic market acknowledged a stable and healthy development. Sales grew by 5.3%, and thus by 2020 the UK organic market will possibly be worth £2.5 billion.  Home delivery of organic, by online and box schemes, is increasing rapidly and is at 14.2%. Additionally, by 2023, home delivery will probably achieve a quarter of all sales of organic foods in the UK. Also, the amount of organic certified farming was increased by 1.9%, the first rise since 2008 (Soil Association, 2019).

A recent study investigated the children's sugar consumption in the UK using "Change4Life Sugar" Smart application. The authors of the study have high awareness of the necessity of sugar reduction in children. However, there was a complication in behavioural change as public health actions were not usually observed as effective plans for health upgrade. There was a little disbelief in the government, the public health officials, and the food industry. The researchers recommended simple methods owning to sugar reduction and more reliable sources of information can, consequently, be accepted by the UK public (Swift et al., 2018).

Furthermore, current systematic review assessed around 14 quantitative articles examining healthy eating campaigns interventions. It claimed to have found the most effective method to improve health which is the "stop or go behaviours" through the "Change4Life" campaign. An example of this would be is in substituting fast food eating with increased F&V consumption. Nevertheless, the outcomes were on the contrary, the method might have not been the greatest way for healthy eating encouragement. One example could be in recognising one's waist size that might lead to further successful results. Further, they also found evidence of successful campaigns if done for six months or longer (Abril and Dempsey, 2019).

### 1.4.4 Recommendations for Fruit and Vegetable Consumption in The Arabian Gulf Region (AGR)

Unlike the EU, there are no dietary programs on food policies in the Arab nations. This is primarily due to the great efforts on therapeutic care and the cost of preventative health care services, lack of studies and researches tackling the dietary habits factors in details, lack of epidemiological studies, lack of nutritional supervision, and lack of assessment on the cost-effectiveness of nutrition intervention programs (Musaiger, Hassan, and Obeid, 2011).

In general, the AGR Nutritionists and dieticians have been dependent on the food-based dietary guidelines (FBDG) established for other countries, for instance, the USA, Canada, and the UK, for delivering nutritional data to the Arab public. Consequently, adopting such policies is required to stimulate healthy lifestyle, better nutrition behaviours, and most importantly to reduce non-communicable diseases related to nutrition. This has been confirmed in a number of Arab Gulf meetings and since 1992, the FAO and WHO have encouraged the perception of FBDG. Accordingly, in 1997, a primary workshop on Diet, Nutrition, and a Healthy Lifestyle was set up in Manama, Bahrain to develop FBDG for the AGR. Although, the Gulf countries have comparable nutrition and socioeconomic conditions, they made an important recommendation that each Arabian country should evolve their dietary guidelines based on their specific need of public health concerns, religious background, lifestyle, culture, and age differences. Hence, a basic Arabic booklet of FBDG for the AGR was published and distributed to various health authorities and the Arabic community and was made available online (Musaiger et al., 2012).

Numerous comments were received from nutritionists, professionals, and the public on rephrasing and illustrating the first version. As a result, a second version was issued. The present FBDG is a simple guide for the AGR, however, such guidelines need to be reviewed occasionally (every 3 to 5 years) with the advancement of researches and scientific evidence in the context of the risk factors of non-communicable diseases associated with nutrition (Musaiger et al., 2012).

## 1.5 BACKGROUND TO THE SMOOTHIE

Smoothies are fruit and/or vegetable-based beverages, which might be considered as sugar free and additive-free products with high nutritional interest and convenience to consumers. In a market where consumers seek high quality, suitable products, simple composition, minimal processing, high organoleptic, and nutritional quality, smoothies have a prominent place. In fact, a smoothie is considered a typical example of a 'superfood', defined as natural foods that may have special benefits for improving health. Smoothies have become widespread among buyers and may considerably contribute to daily antioxidant consumptions (Keenan et al., 2010).

Smoothies are typical examples of beverages in the food industry that responded to the awareness growth of consumers to have healthier foods with humble ingredients and clean labels (Nunes et al., 2016).

The smoothie market has grown at a rapid rate in the 21$^{st}$ century. This was due to the increased awareness on the importance of nutrition, particularly F&V intake. The smoothies share in the chilled juice market have been increasing annually. According to a new report by the global industry analysts Incorporated (a worldwide business strategy and market intelligence source), the global market for smoothies is predicted to reach US$15.8 billion by 2022. The focus being kept on growing popularity of juice cleanses for detoxification and evergreen consumer preference to ensure popularity of detox and green smoothies (Castillejo et al., 2016).

A smoothie can be a meal replacement or a snack as it has fulfilling thick texture. And, with increasing health consciousness and busy lifestyles, smoothies have transformed from indulgent treats to convenient meal replacements for on-the-go consumers.

Nowadays convenience plays a vital role in increasing the daily consumption of F&V, particularly in vegetables. Convenience in this context means something is available with less effort and in less time. The availability of smoothies is one convenient and easy approach to raise the consumption of F&V. Hence, it is important to review some of the history behind the smoothies, especially in the UK and KSA.

From the principal investigator's knowledge, it had been observed from 2008 to 2013 that sales of fruit smoothies were increasingly common. After 2013 there was some existence of F&V smoothies in the UK beverage market e.g. the Innocent brand "Gorgeous Greens" smoothie and Naked brand "Beetroot" smoothie. According to the BBC, the £100 million UK smoothie market is doubling yearly. Alongside the smoothie stalls were getting more popular presumably as a mean to meet the increasing eagerness of the population to fulfil the 5 A DAY recommendation. Vegan requirements and raw food concepts, such as London's Crush, may have been significant driving factors as well. The London's Crush recently opened its 15th branch in London making it one of the UK's biggest smoothie chains. Another major smoothie outlet would be Boost Juice, opening stalls widely across the globe from Australia to Kuwait and have more than 200 outlets and bars in Manchester, Oxford, and Nottingham.

Increased awareness, obesity advices, and the importance of consuming five fruit a day have driven a high sugar content in a healthy fruit smoothie. Despite the widespread criticism against sugar, the increased use of vegetables as ingredients in smoothies would be nutritionally advantageous. Furthermore, vegetable became more popular in smoothies than/with fruit varieties (Shieh et al., 2017). However, it has been of interest to investigate this phenomena throughout a primary scoping survey among health students who are knowledgeable and educated to justify the kind of vegetable-based smoothies that might help increase vegetable consumption. Students are normally health conscious, have a busy lifestyle, have limited time to prepare healthy meals, prefer food to-go, and rely mainly on fast foods may thus find a smoothie a convenient meal replacement or a snack option for on-the-go breakfast/meal. Students are thus the perfect group for the researcher to use as a sample population.

In contrast, in the KSA a very limited number of brands of smoothie have been observed in retail outlets. In terms of F&V beverages, only the V8 vegetable-based juice is available in hyper-supermarkets. Additionally, nowadays several smoothie and juice bars have been set up alongside coffee shops. The options available for smoothies incorporating vegetables are mainly limited to the inclusion of carrot, beetroot, spinach and cucumber. To the best of the researcher's knowledge, the popularity of vegetable dominated smoothie products is negligible. There are products sold in both the UK and KSA beverage markets that contain vegetables and despite the smoothie claim, the ingredients list reports either the use of concentrated juices or that they contain minimal amounts of vegetables.

Therefore, the vegetable-based smoothie product that has been the core of this research would be a new-comer as a commercial product in the line of F&V beverages.

### 1.5.1 The UK Smoothie Market

The 'quick hit' from a smoothie helps satisfy customers that they have consumed a healthy product with natural ingredients (Johnson, 2008). According to the Mintel Group, an independent market analysis company, smoothies were primarily designed to appeal the age group of 15 to 40 years old. In particular, the market targeted teenagers, offering real fruit thick feel and taste. Smoothies were developed to take advantage of the growth in the fresh juice market. Some of the more fashionable juice bars and outlets, such as Pret a Manger and Starbucks, have been capitalising on the popularity of the smoothie within an ever-evolving consumer lifestyle.

For many, the pace of modern living forces the 'time-poor' working people to eat breakfast on their way to or at work, plus sometimes having lunch in the office as well. Smoothies providing a nutritious meal replacement option could be a solution to those working people. Sainsbury's has built smoothie bars into some of its city-centre outlets – Centrals – offering speed and convenience to those wanting sustenance on the go (Mintel, 2002).

Pete and Johnny Smoothies (PJ's) was launched in the UK in October 1994 after Harry Cragoe and Patrick Folkes noted the product's success in California.

Originally, the company adopted the homespun cottage-industry positioning of Ben and Jerry’s ice cream (Johnson, 2008). PJ’s claims that each 250 ml bottle provides two of the recommended five portions of fruit because they blend whole crushed fruit and fruit juice together (PJ's, 2007).

Innocent was set up in 1998, and represents the UK's leading smoothie brand, with a 63% market share. Innocent smoothies are now sold in 12 European countries (Alarcon, 2008). The company states that its smoothies always contain 100% natural ingredients (Innocent, 2009).

PJ’s smoothies are cheaper than Innocent smoothies by about 60p per 250ml. This may reflect the fact that Innocent uses only fresh juices, whereas PJ’s uses partly concentrated juices in some of their smoothies. Thus, in terms of nutritional value, Innocent smoothies might be better. Innocent had 72% of the UK market share in 2007 (Johnson, 2008). However, this rose to 74% in 2008 as the company became a smoothie market leader (Ruxton, 2008). Between 2008 and 2010, however, Innocent faced cost inflation on fruit and international investment, and the financial crisis had a detrimental effect on the economy. Hence, Coca-Cola reclaimed an 18% share from Innocent at the end of 2009.

Although Innocent faced fierce competition, the company was worth £32 million and increased it’s stake to 58% in 2010, when it was valued at around £180 million. The year 2010 was a successful year, as Innocent sold more than 2 million smoothies per week. Their share of the UK market rose to 77% with £150 million. The company also launched orange juice in 2010 (Ball, 2011).

In 2008, the sales value of smoothies had increased by 513% to reach £282 million. Growth had been driven primarily by increased household penetration, up from 31% in 2006 to 57% in 2008 (Mintel, 2008). Also, 'You Gov' survey in 2008 suggested that 37% of consumers occasionally or regularly drank smoothies (Ruxton, 2008). The year 2008 was the year of change for the smoothie market. Economic constraints had slowed the phenomenal rates of growth experienced earlier and the arrival of newcomer Tropicana threatened to erode the overwhelming dominance of market leader Innocent (Mintel, 2008). Moreover, in 2012, the British Soft Drinks Association (BSDA) reported that the industry prosperity of the UK smoothie market had grown in retail value by 4.2% to reach £1.8 billion, with a volume of 1160 million litres providing 124 servings of fruit juice per person (BSDA, 2012). Moreover, according to the Telegraph, British consumers spent £1.5bn on juice drinks and smoothies in 2015.

From 2012 to 2017, total sales of fruit drinks were estimated to be £5.1 billion and smoothie sales grew over by 6.6%, whereas volumes dropped by 9.6% in the same period. The increase in average prices, as well as the decline in fruit juice intake is the reason behind this (Mintel, 2017).

In 2018, juices with added sugar were taxed based on total sugar content but 100% juice including F&V smoothies will be exempt from tax (Murrin et al., 2018).

By 2018-2019 healthy eating habits and in particular the pursuit of 5 A DAY plan became the driving force behind the persistent rates of exceptional growth.

### 1.5.2 The KSA Smoothie Market

A massive change in direction within the beverage products consumed in Saudi Arabia between juices and smoothies has been reported (Wilson et al., 2016). Smoothie has showed up to be available to consumers and most educated people had known the differences between smoothie and juice. It was observed that the Shaker, Smoothie Factory, Bubble Nation, Cup and Couch, My Shake, Warm and Frosty Café, Shakeliciouse, and RudeShake, are all places that sell varieties of smoothies in Jeddah, Saudi Arabia. These are mainly franchised along with Starbucks that has a wide selection of smoothie products depending on the fruit season and they have recently launched the matcha green tea smoothie. In addition, in KSA, due to lack of market research analysis in the literature, the researcher's observation is required. Smoothie popularity in KSA has raised from a 5 stars complimentary drink to a smoothie with detoxifying properties for all. This made a lot of consumers use smoothie as a detox drink for around 3-6 days. Some of the sport experts, nutrition health coaches, and people with business background started local smoothie business selling homemade smoothies products using social media (Instagram) as free marketing channels. For example, "Quench Me" uses fresh smoothies for their detox plans and "Cleanse and Glow" uses cold-pressed juices and fresh salads for their dietary plans. They both claim their products increase immunity, improve digestion, enhance skin and nails health, and are high in antioxidants. Their popular marketing moto is "Change your food, change your life''. This can be observed and recognised as an increase in awareness when compared to the past 5 years. This increase was likely influenced by the positive change in lifestyle, the improved interest in F&V, and the widespread knowledge on health benefits when linked to F&V consumption. This change in awareness can also be linked to deferent health and social trends or events; outdoor activities (e.g. hiking, motorbike riding, camping), sports classes and competitions.

However, a significant gap in knowledge remains, as there are no studies on the level of smoothie consumption in KSA. Similarly, there is a complete lack of published analytical marketing research around the smoothie sales performance and over shelves availability. Also, very limited scientific research papers and studies on the actual up-to-date statistical values on daily F&V intake and physical activity among the population of KSA.

## 1.6 SMOOTHIE NUTRITIONAL COMPOSITION

The introduction of smoothies is potentially a decent way to enhance F&V consumption. A 250 ml serving of F&V smoothies covers a large portion of the recognised recommended daily nutrient intakes for dietary fibre, some minerals, and vitamins for different population groups (Castillejo et al., 2016).

It is therfore of interest to invistegate the nutritional composition of some popular smoothies in the UK market and identify possible deficiencies and potential improvments.

One definition of what a smoothie is (NHS, 2007) defined a smoothie as 'a delicious and healthy drink made up of fruit that's been blended with fruit juice, milk, or yoghurt'. Furthermore, Ruxton in 2008 described commercial smoothies as 'combinations of homogenised fruit and juice'. Single servings of commercially-available smoothies contain at least 1 portion of whole crushed fruit (80g) plus 1 portion of juice (150ml) or contain more than 80g of whole crushed fruit with the remainder as juice'.

The researcher's smoothie definition is: a mixture drink that is made from F&V usually with added liquids such as juices, milk, or yoghurt and sometimes with additional flavourings such as honey, spices, or protein powder blended until liquified.

One theory about where smoothies came from, is that they originated on the beaches of California, where warm weather and cool healthy drinks have long been part of the culture. The first drink served on the beach was a blend of orange juice, bananas, and strawberries. Such 'coolers' later became recognised as smoothies. During the late 1960s, Ann Seranne and Eileen Gaden produced a cookbook which included a range of smoothie recipes such as 'Orange-Pineapple Smoothie' and 'Pineapple Banana Smoothie', made with fruit juices, fresh bananas, and ice cream (Crocker, 2003).

Others argue that smoothies were originally a US phenomenon, their roots dating back to 1940s Hollywood when the Hansen family started distributing crushed fruit drinks to the superstars on film sets. Smoothies' association with a contemporary lifestyle was promoted at the 1984 Los Angeles Olympic Games, where the nutritious and fruit-based drink was created for athletes. It's smooth consistency is said to have given the drink its name (Mintel, 2002).

Smoothies were originally classified as juices, and their benefits were underestimated, they were only considered as one portion of the 5 A DAY. It was claimed that they did not contain the same amount of fibre as found in whole F&V (Ruxton, 2008). However, in April 2009, the Department of Health amended the smoothie guidelines such that 250 ml of smoothie was recognised as providing a maximum of two portions of the targeted 5 A DAY if it contained 100% fruit, including all of the edible pulped fruit and/or vegetable (Innocent, 2012).

For a particular smoothie to meet the requirements of providing two portions of the targeted recommendation, it needs to:

- contain a minimum of 80 g of crushed whole fruit and/or vegetable; or
- contain at least 80 g of one variety of whole fruit and/or vegetable and at least 150 ml of 100% fruit and/or vegetable juice of a different variety (NHS, 2011).

Health scientists used to classify smoothies within the juice category, but juices are mainly high in calories and sugar compared with whole fruit. Previous studies argued that consumption of sugar-sweetened juices and 100% fruit juice is linked with weight gain (Malik et al., 2006; Libuda et al., 2008). As a result, they discouraged smoothie consumption to avoid high sugar intake (Ruxton, 2008). However, major differences between juices and smoothies have been discovered since the latter became acceptable as a portion of the 5 A DAY. The composition of a typical smoothie is discussed below:

### 1.6.1 Sugar

The energy and sugar contents in smoothies are usually similar to those found in whole fruit. A study by Ruxton in 2008 evaluated energy, carbohydrate, sugar, starch, and fibre content of an average smoothie with three different fruit combinations (banana and strawberry, banana and cherries, and banana and mango). The findings demonstrated no significant difference between the nutrient compositions across all three drinks. This suggests that the total sugar intake of consumers choosing a smoothie as part of their 5 A DAY would not be significantly higher than that of consumers selecting a fresh fruit combination instead. Around 29% of the Guide Daily Allowance (GDA) for total sugars would be provided by a single portion of smoothie, although no sugar is added (Ruxton, 2008).

Two studies by Nicklas, O'Neil and Kleinman in 2008 and 2010 respectively, showed no evidence of increased weight gain associated with the consumption of 100% fruit juice in children and adolescents. Additionally, another study showed that 100% fruit juice can improve diet quality in children and adults (O'Neil et al., 2011).

The sugar and energy contents of some commercial smoothies in the UK are shown in Table 1.6. These smoothies have no added sugar, but contain natural sugar from fruit and the total sugar is thus very high. Consequently, the smoothies are also moderately high in calories/total energy.

| Brand | Smoothie | Ingredients (as shown on the labels) | Total sugar 100mL/g | Energy 100mL/kcal | Fibre 100mL/g | vitamin C 100mL/mg |
|---|---|---|---|---|---|---|
| Innocent | Strawberries & banana | Not available in the market currently | 12.2 | 53 | 0.6 | 10 |
| Innocent | Seriously Strawberry | strawberries (32%), ½ a pressed apple, 13 pressed white grapes, ½ of a mashed banana (20%), a slice of a squeezed orange & 6 crushed blackcurrants | 11 | 52.1 | 0.8 | 6.5 (8% NRV) |
| Innocent | Gorgeous Green | some squeezed orange, some crushed spinach (2%), some crushed kale (2%), splash of safflower and spirulina extract (0.4%), a splash of baobab extract (0.02%) & a squeeze of lemon juice | 10 | 49.5 | 0.9 | 12 (15% NRV) |
| Innocent | Magnificent Mango | 1 pressed apple, ⅓ of a crushed mango (16%), ½ a mashed banana,⅓ of a squeezed orange, a dash of passion fruit (3%),a piece of crushed peach & a squeeze of lime | 11 | 54 | 1.4 | 23 |
| Innocent | Pomegranate Magic | 27 pressed red & white grapes, ¾ of a mashed banana, ¼ of a pressed pomegranate (14%), ½ a squeezed orange, 24 crushed blueberries (3%), a squeeze of lemon juice & 6 peeled acai berries (0.7%) | 13 | 62 | 1.5 | N/A |
| Innocent | Totally Tropical | ¼ of pressed pineapple (39%), 1 mashed banana (37%), ⅓ of a pressed apple, a squeeze of orange, some creamy coconut milk (3%) & a squeeze of lemon juice | 12 | 67 | 1.1 | 28 (35% NRV) |
| Innocent | vanilla & raspberry ripple | 21 crushed raspberries, a dash of pressed | 11 | 50 | 0 | 19 (24% NRV) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | beetroot, some bourbon-vanilla extract, 4 pressed apples, 2½ crushed bananas & ¼ of a pressed rhubarb stalk | | | | |
| Naked | Green Machine | 2 3/4Apples, 1/3Mango 1/2Pineapple 1/2Banana 1/3 Kiwi Boosted with: 1335mg Spirulina 409m Alfalfa 102mg Broccoli 102mg Spinach 76mg Barley Grass 76mg Wheatgrass 51mg Garlic 51mg Ginger 51mg Kale 51mg Parsley | 12 | 60 | N/A | 9.3 |
| Naked | Bright Beets | 1¾ Apple, ½ Beets, 1/3 Carrot, ¼ Coconut's Water, 1/3 Lemon & a hint of ginger | 8 | 38 | N/A | 0 |

**Table 1.6 Ingredients, total sugar, energy, fibre and vitamin C content of some well-known smoothies' brand in the UK (from the label).**
NRV = Nutrient Reference Value

### 1.6.2 Fibre

The higher fibre content in smoothies makes them a preferable health drink when compared to juice. Ruxton in 2008 compared eight samples of Innocent smoothie with orange juice, as Innocent is a market leader with a 74% market share in 2008. The results showed that smoothies contained 1.7g of fibre per 100g, due to the inclusion of whole homogenised fruit, compared with 0.1g per 100g obtained from orange juice. Hence, assuming that 250 ml was consumed, the potential contribution of smoothies to the GDA for fibre is 18% which was estimated to be superior to fibre in the equivalent portion of freshly squeezed orange juice 1% of the GDA. (Ruxton, 2008).

### 1.6.3 Micronutrients

F&V smoothies are usually a rich source of vitamins, folates, polyphenols, antioxidants, and some minerals, among others, which can be added to the daily diet if ingredients were properly selected.

In a systematic review by Ruxton et al., in 2006 comparing pure F&V juices with whole fruit, it was suggested that the similar health benefits observed in both cases may be due to comparable antioxidant and/or polyphenol contents.

In addition, it was revealed in a study by Ruxton in 2008 which compared eight samples of Innocent smoothie with orange juice that smoothie samples were richer in vitamin C (41 mg/100 g) than orange juice (30 mg/100 g). Hence, smoothies could provide a potentially important contribution to the GDA of vitamin C >100% reference nutrient intake (RNI), assuming that 250 ml of smoothie to be consumed. In the same study, antioxidants were compared using the oxygen radical absorbance capacity (ORAC) method (vitamin E equivalent) between the eight smoothies and orange juice. The results showed that smoothie samples had significant amounts of antioxidants, at 1566 (μmol) ORAC Trolox equivalents (TE) compared to only 900 (μmol) ORAC TE in orange juices (Ruxton, 2008).

Another study by Hagl et al. in 2011 was performed to determine the amounts of polyphenols and D-(-)-quinic acid reaching the ileostomy bags of probands (and thus the colon in healthy humans) after ingestion of apple smoothie, a beverage containing 60% cloudy apple juice and 40% apple puree. Ten healthy ileostomy subjects were recruited, each ingested 0.7 L of apple smoothie (one bottle). Their ileostomy bags were collected directly before and 1, 2, 4, 6 and 8 h after smoothie consumption. The polyphenols and D- (-)-quinic acid contents of the ileostomy fluids were examined using HPLC-DAD and HPLC-MS/MS. The total polyphenols and D-(-)- quinic acid content of the apple smoothie was determined to be 1955.6+/-124.6 mg/0.7 L, which is very high compared to cloudy apple juices. Therefore, the conclusion of the authors of the study was that apple smoothies could be more useful in the prevention of chronic colon diseases than cloudy apple juice and apple cider (Hagl et al., 2011).

In a study, thermal treatment of fresh blended green vegetable smoothie was used to preserve quality and reduce microbial load. Total vitamin C of the smoothie deteriorated during storage time at 5ºC and significantly decreased when stored at 15ºC. Original total phenolics content were (151.1 mg 29 $kg^{-1}$ fw) but improved by 44% and 36% after thermal treatments (Castillejo, 2016).

## 1.7 FACTORS AFFECTING SMOOTHIE NUTRITIONAL VALUE AND STORAGE

### 1.7.1 pH

The pH unit measures the degree of acidity or alkalinity in a solution. In specific, pH is the measurement of the hydrogen ion concentration [H+]. Every aqueous solution can be measured to determine its pH value. The pH scale is logarithmic and approximates the negative of the base 10 logarithm of the molar concentration (measured in units of moles per litre) of hydrogen ions in a solution. It is measured on a scale of 0 to 14.

The consumption of fresh fruit or vegetables can be a source of acidity that may have a dental impact. Two clinical studies comparing the effect of whole and processed fruit on teeth found no differences in plaque pH following the consumption of either fresh or processed fruit (Hussein et al., 1996). Additionally, Issa et al., in 2011 reported a similar enamel demineralisation potential when either whole fruit or fruit juices were consumed. Moreover, a study by Beighton et al., in 2004 showed that the amount of fruit acid and sugar released into the saliva after consumption of whole or crushed fruit was equivalent.

In summary, smoothies offer similar nutritional composition as F&V, while juices from concentrate usually have less. Smoothie consumption is not specifically related to obesity, as the sugar content is equivalent to that of whole fruits. Taking this into account, smoothies are superior sources of fibre and have higher antioxidant levels, including vitamin C, compared to juice from concentrate. Finally, smoothies have the same pH levels as whole F&Vs; hence, they present no increased risks for dental health.

### 1.7.2 Effects of Processing Operations

There are several thermal and non-thermal approaches used in the food industry to preserve beverages. Technologies include a microwave and infrared heating along with the non-thermal High-pressure processing (HPP), pulsed electric field (PEF), and ultrasound. All of these could be beneficial ways to accomplish healthier and safer products with possible longer shelf life (Ozkan et al., 2019).

The development of thermal treatment is one of the safest ways to guarantee that no additives are added. Although pasteurisation preserves bioactive compounds like flavonoids and phenolics in beverage products, it can turn its natural colour to brownish which is an unappealing attribute to consumers. In one study pasteurisation of a juçara, banana, and strawberry smoothie at 90 °C for 35s had a negative effect on the smoothie's colour (de Oliveira Ribeiro et al., 2018).

Mild thermal treatment and low temperature storage can significantly increase the shelf life of smoothies but often degrade vitamin C content. According to a study of two fresh blended vegetable smoothies consisting of tomato, carrots, pepper, and broccoli, their shelf lives were 28 days at 5 °C. Whereas thermally treated ones (3 min at 80 °C) could be stored for up to 40 days at 20 °C and 58 days at 5 °C. These mild heat-treated smoothies showed a total vitamin C reduction of 2-fold during storage at 20°C as compared with samples stored at 5 °C. While the total carotenoids, lycopene, and total chlorophyll contents were not significantly affected (Castillejo et al., 2016).

A further investigation on the same smoothie as used in the previous study, showed that thermal treatment resulted in the deactivation of quality-degrading enzymes whilst some health-promoting compounds, such as total phenolics content (TPC) were not significantly affected, surprisingly, the total antioxidant capacity (TAC) of the smoothies has increased by 62% after the thermal treatment. In general, TPC and TAC were well preserved during storage for 40 days at 20°C and for 58 days at 5°C after this mild thermal treatment (Rodríguez-Verástegui et al., 2016).

High hydrostatic pressure (HHP) (450 MPa/20 °C/5 min) (HHP-450) or (600 MPa/20 °C/10 min) (HHP-600) was used in a study on fruit smoothie samples over a storage period of 10 h at 4°C. Treatment was found to reduce levels of TAC, TPC, and ascorbic acid. Also, the colour of the smoothie samples deteriorated as compared to fresh samples. The process also led to the total inactivation of polyphenols oxidase (PPO). HHP-450 samples had higher ($p < 0.001$) levels of TAC, TPC and anthocyanin than HHP-600 samples. Ascorbic acid levels were reduced in all the treated smoothies. Thus, the researchers suggested that HHP processing at moderate temperatures could be an appropriate substitute for traditional thermal processing (Keenan et al., 2012).

Additionally, in a more recent study the researchers used the HPP treatment (630 MPa, 6min, 20 °C) and did the storage at 25 °C to ensure the microbiological and antioxidant stability of a range of F&V smoothie products. They found that total soluble solids and pH remained the same during treatment and storage. TAC actually increased by 5–75% following treatment but some products showed a distinct orange-brownish colour change during the storage period perhaps owing to the considerable decrease of betacyanin. Thus, for this kind of treatment, it is essential to make modifications in pigmentation stability (Fernandez et al., 2019).

Thermal processes can result in the reduction of some bioactive compounds in F&V smoothies, particularly vitamin C, whereas non-thermal processes might not have such major negative effects on phytochemicals and indeed in some circumstances could even enhance their bio-availability and bio-activity (Al-Juhaimi et al., 2018).

Enzyme treatment would be considered a non-thermal technique and its use depends on the needs of the process. The use of enzymes, in general, has a number of benefits both commercially and environmentally. For example, pectinolytic enzymes are commonly used in fruit juice production, they can break down complex polysaccharides of fruit tissues into simple molecules. Consequently, degradation of pectin by enzyme treatment increases the efficiency of juice extraction from raw material (enzyme-assisted extraction), reduces energy costs and waste, and generates visually more attractive products with better-quality (Danalache et al., 2018).

Enzymes can be used to improve the rheological properties of products in the beverage industry. Commercial enzymes typically used to reduce the viscosity of fruit juices and purées are pectinases, amylases, cellulases, and xylanases. Pectinases holds a 25% share in global food enzyme sales and industrial demand is still rising (Uzuner and Cekmecelioglu, 2019).

In one study, a commercial enzyme preparation consisting of pectinases and cellulases was used to reduce the viscosity of mango purée. It was found that there was a considerable reduction in the number of insoluble solids within minutes using a moderate amount of enzyme. Therefore, the fruit purées viscosity can be controlled based on enzyme concentration and incubation time (Brito and Fabrice, 2012).

Despite there being very little published information relating to any potential impact on the nutritional composition of enzymatically treated smoothies, it is obvious that such enzyme treatment will have an impact on the fibre content of the smoothie. There may also be an impact on the sugar content since digestion of cellulose, for example, could release monomeric glucose. This will be covered in more detail in the discussion (section 3.24).

## 1.8 THE RESEARCH GAP

There are several products in the market that promote an increase of fruit consumption. Prior to 2013, fruit smoothies such as Innocent (strawberry and banana) were familiar products in the UK market. In 2007, "Solero" by Walls (Unilever) UK launched two ice-cream flavours: strawberry and banana, and blackberry and banana as high-quality ice-cream. In 2011, the company decided to revitalise its varieties of ice creams and focus on selling two favourite flavours: Exotic and Berry which contained 50% fruit and fruit juice and less than 4% fat at only 90 calories per stick (Unilever, 2012). However, to the best of the researcher’s knowledge, there were very few products on the market that promoted increased vegetable intake.

After 2013, beverage companies in the UK started to produce F&V or vegetable containing smoothies such as Innocent “Gorgeous Greens” and Naked “Green Machine” and “bright beets”. More recently Naked company has produced some vegetable-based drinks, their contents is mentioned in Table 2.2. Additionally, the V8 company also makes smoothies which only contain carrot and orange juice. Moreover, there are only few examples of equivalent vegetable or mixed F&V drinks such as V8 juice, which is a blend of water and concentrates of seven vegetables - beetroot, celery, carrots, lettuce, parsley, watercress, and spinach - and one fruit, tomato. These were available in both the UK and KSA. However, smoothies should not contain juice concentrates but should be made of 100% pure fruit and/or vegetables, unlike normal juices which contain very low fibre content.

Similarly, there are few reports in the literature concerning acceptability and nutritional composition of vegetable smoothie products. The study of Rodríguez-Verástegui et al., in 2016 examined two red fresh F&V smoothies containing tomato, red pepper, broccoli, and carrot. Also, the work of Castillejo et al., in 2016 studied the effect of thermal treatment on a green vegetable smoothie and determined the impact on some nutrients during storage but the fresh smoothie was not investigated.

More research is required to assess consumer awareness of the benefits of F&V smoothies and their acceptability. In particular how this may vary between different regions and cultures. As well as the acceptability of such products in KSA is an area in which there is a lack of suitable studies.

Nutrient retention and stability may be influenced by different F&V combinations within the smoothie, yet little is known about these aspects which are important for both home and commercially processed products. Texture is likely to be a major concern for the acceptability of smoothies with higher vegetable content. The use of cell wall degrading enzymes, used commercially in fruit juice production, has not been research enough on their potential for smoothie manufacture. Similarly, the potential impact of enzyme treatment on nutrient content is poorly understood.

## 1.9 AIM AND OBJECTIVES

The aim of this project is to generate a smoothie that contains a higher proportion of vegetables, compared to commercial alternatives, and which is acceptable to the consumer and has improved nutritional value. This will be achieved through the following approach:

1) A survey (by questionnaire) in the UK and KSA to assess and compare students' opinions and attitudes regarding vegetable and fruit smoothies.
2) The survey information obtained, together with potential nutritional values of the ingredients will be used, to design potential smoothie recipes that will be evaluated by a small tasting panel in the UK. Followed by the selection of the

"optimal" smoothie based on consumer acceptance and nutritional enhancement.

3) The evaluation of this "optimal smoothie" in terms of content, retention during processing, seasonal variability, and stability over 5 days of storage of selected nutritional factors.

**The hypotheses to be tested during this thesis are as follows:**

H1. Acceptance of vegetable containing smoothies will differ between populations in the UK and KSA.

H2. A smoothie can be designed with increased vegetables content that will be acceptable to consumers in the UK. This will be nutritionally superior to current commercial alternatives.

H3. The nutrient levels in the fresh ingredients for the smoothie will vary due to seasonality but will be largely retained during processing. However, some nutritional factors will be lost during storage at 4° for 5 days.

H4. The texture is going to be a major barrier to consumer acceptance of vegetable containing smoothies. However, treatment with plant cell wall degrading enzymes will overcome adverse texture.

# CHAPTER 2 NUTRITIONAL & AESTHETIC QUALITIES OF SMOOTHIES – INVESTIGATION USING QUESTIONNAIRE

The questionnaire objectives, in the UK were to investigate students' thoughts, favourite fruit, vegetables or F&V based smoothies, and to inform them about the F&V smoothie recipes. In the KSA the results could serve as a foundational base to an insight into the Saudi student's perceptions and consumer potential of smoothies in general. Hence, this study will partially aim to assess whether a novel F&V smoothie product would be a lucrative business idea in the Saudi Arabian market. In addition, the potential acceptancy of vegetable-based smoothie products in both countries.

The two-primary hypotheses in this part of the thesis research were

H1. Acceptance of vegetable containing smoothies will differ between populations in the UK and KSA.

H2. A smoothie can be designed with increased vegetable content that will be acceptable to consumers in the UK.

These hypotheses were acceptable as the criteria used to define 'what was acceptable for the consumer?' this involved questionnaires followed by a tasting session in this research work. The criteria that defined consumer acceptability was validated through subjects' tasting of the smoothie samples. The questionnaire gathered key data from the health students' samples and their willingness to consume a vegetable-based smoothie to define what was acceptable to the consumer in both the UK and the KSA. As the researcher expected the sample's participants had the knowledge related to F&V consumption, therefore, the question was to investigate whether students are applying this knowledge to dietary behaviour or not. Also, the result of the questionnaire provided a comparison between the UK and the KSA students on F&V awareness and intake. The objective of the study was to compare awareness between two completely groups from different countries and culture, but similar in educational level and comparable in age.

A higher level of education should indicate higher knowledge and awareness, and consequently higher consumption of F&V. Elfhag and his colleagues in 2008 indicated that higher education could relate to better awareness and better knowledge on healthy eating behaviours. According to Shaikh and his fellow worker in 2008, nutritional knowledge is one of the powerful predictors of F&V intake. In addition, according to the researchers Shive and Morris in 2006, university students have consistently shown that their diets were low in F&V and high in fat, sodium, and added sugars. Furthermore, in European population, student lifestyle and lack of time were key difficulties that made it hard to achieve healthy eating (European Commission, 2006). Likewise, in the KSA, a recent study carried by Washi and Ageib in 2010 in the city of Jeddah found that Saudi adolescents between the ages of 13 to 18 years were under insufficient dietary habits and consequently higher body weight status. Moreover, this thesis represents one of the limited number of studies that have examined consumer acceptability of F&V smoothies. Hence, the aim of this chapter was to investigate the preferences and acceptability of consuming vegetable-containing smoothies in student populations from Nottingham city, UK and Jeddah city, KSA by conducting a primary questionnaire.

F&V smoothie especially the green smoothies had attracted attention, especially from vegans, students, raw eaters, detoxing and weight management consumers. Smoothies are currently found in gyms, hospitals, café bars, sandwich bars, malls, and shopping centres, as well as it has been observed on the shelves of major supermarket chains in the UK and in few places in KSA.

## 2.1 THE QUESTIONNAIRE DESIGN AND METHODOLOGY

The whole study, including the survey and the tasting session, was approved by the Ethics Committee reference number (A/4/2009) the University of Nottingham UK. Analyses were carried out using SPSS v.16.0© (SPSS Inc., Chicago, IL, USA). The study aimed to recruit 100 Health and Science students as subjects from each country. The design and the criteria of the study are described below.

### 2.1.1 Inclusion criteria

The samples population were healthy adult students from the University of Nottingham, UK and the King Abdulaziz University (KAU), KSA. Participants were between 18 to 35 years old with the ability to consent in health and sciences departments.

### 2.1.2 Exclusion criteria

Participants not within the age range mentioned in the Inclusion criteria were excluded.

### 2.1.3 Study Design

In the UK, two pilot studies were completed in order to refine the English questionnaire for coherence. Each pilot study consisted of 10 native speaker participants. As a result, the designed questionnaire (Appendix 1) was first distributed in hard copy format to the subject sample who were health students from the University of Nottingham. The participants were asked to answer 25 questions, no confidential information was needed other than gender and age. In total, 300 copies were filled by the subject sample.

Secondly, in the KSA, the questionnaire was directed online to university students of health and science faculties located in Jeddah, KSA. The KSA sample responded to the same UK questionnaire. However, due to uncertainty on the student's English language ability, the researcher translated the questionnaire from English to Arabic to suit the Arabian participants. This made it comfortable to respond to the questionnaire with their first language (Arabic), to achieve accurate responses. Before publishing the questionnaire, a pilot study was conducted with 10 questionnaires in Arabic, which were given to native Saudis' for comment about language clarity and coherence. The questionnaire was published online via a Survey Monkey account.

### 2.1.4 Compliance

One hundred subjects fully completed the questionnaire from each country. The researcher has eliminated incomplete surveys (questions with missing/no answers). Some of the complete questionnaires were rejected because they did not fit in the inclusion criteria of the research e.g. students, not from the health and science field.

### 2.1.5 Statistical Analysis

The analysis was carried out using IBM SPSS Statistics software version 16. The data was non-parametric, hence presented as chi-square analysis to determine the differences between the two countries, the UK and KSA. Values at $p<0.05$ were considered statistically significant.

### 2.1.6 Sample Size

The sample size was determined by an equation calculated on Raosoft, which is a database web survey software (Raosoft, 2011). It provided required number of individuals that are needed to obtain a representative sample and maintain a realistic approach in accordance with the statistical probabilities of the study. The sample size was calculated using an acceptable margin of error of 1%, the confidence level of 95%, and the response distribution of 50%. The recommended minimum sample size given was 198. Hence, 200 participants, 100 from each country were selected as an appropriate number. Therefore, the margin of error would be 9.79%.

In terms of the numbers selected above, the sample size (n) and margin of error E are given by:

x = Z(c/100)2r (100-r)

n = N x/ ((N-1) E2 + x)

E = Sqrt [(N - n) x/n (N-1)]

Where, N is the population size, r is the fraction of responses, and Z(c/100) is the critical value for the confidence level c.

### 2.1.7 Demographic Data of the Study

The vast majority of the volunteers were female from both the UK (71%) and KSA (81%) respectively. The age of both the UK and KSA participants were between ≥18 and ≥35 years. All the subjects were in good health and were student from different scientific and health colleges.

## 2.2 THE QUESTIONNAIRE RESULTS

| | | | group | | Total | Chi-square | p-value | Significance |
|---|---|---|---|---|---|---|---|---|
| | | | UK | KSA | | | | |
| Gender | FEMALE | n | 71 | 82 | 153 | 3.365a | 0.067 | NS |
| | | % | 71.0% | 82.0% | 76.5% | | | |
| | MALE | n | 29 | 18 | 47 | | | |
| | | % | 29.0% | 18.0% | 23.5% | | | |
| Total | | n | 100 | 100 | 200 | | | |
| | | % | 100.0 % | 100.0 % | 100.0 % | | | |

**Table 2.1 Gender of the questionnaire results in KSA and UK samples.**

The results in Table 2.1 showed that in the UK and the KSA females are more likely to participate in F&V smoothies and health awareness questionnaire than male. Statistically, there is no significant difference between the KSA, and the UK participates. Moreover, the results showed that 75% of the KSA sample were between 18 to 22 years old; while, in the UK 53% of the sample were from the same age group as the KSA. Additionally, the same number of participants, 19% in both UK and KSA were aged between 23-25 years old. Moreover, 28% of the UK participants and 6% of the KSA sample were between 26 and 35 years old. Overall, the highest numbers of participants' in both samples were from 18-

22 year age group. Statistically, there was no significant difference between ages in the UK and KSA, with P value of 0.000 and Chi-square value of 27.74.

The question about universities was an open question inquiring which University the student came from. The majority (80%) of the KSA participants were from KAU and the remaining 20% were from other universities located in Jeddah, Saudi Arabia. This was not intentional but was due to the online survey method. Unlike the KSA sample, in the UK sample all students were from Nottingham University. In regard to the open questions on the faculty name and the student's major subject, the results revealed that all students from both countries were from a plethora of different scientific and health-related colleges or schools. These included animal science, nutrition and food sciences schools in the UK. While, the KSA participants were from Medical, Applied Sciences, Clinical Nutrition, and Pharmacology colleges.

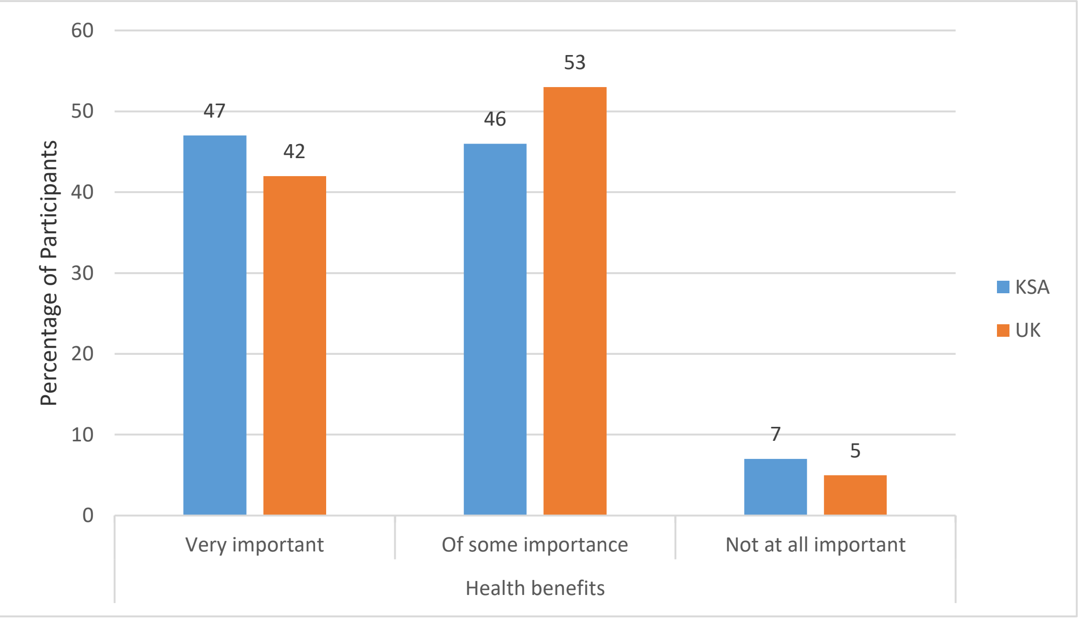



**Figure 2.1 Health benefits as a reason for choosing drinks in KSA and UK samples and UK samples.**

Figure (2.1) shows that health benefits were one of the factors affecting the choice to purchase a drink in the KSA and UK. The majority of the KSA group (47%) consider health benefits a "very important" reason that can affect drink choice. In contrast, in the UK group, the majority (53%) consider health benefits "of some importance". Statistically, there was no significant difference between the UK and the KSA samples with P value of 0.574 and Chi-square value of 1.109.

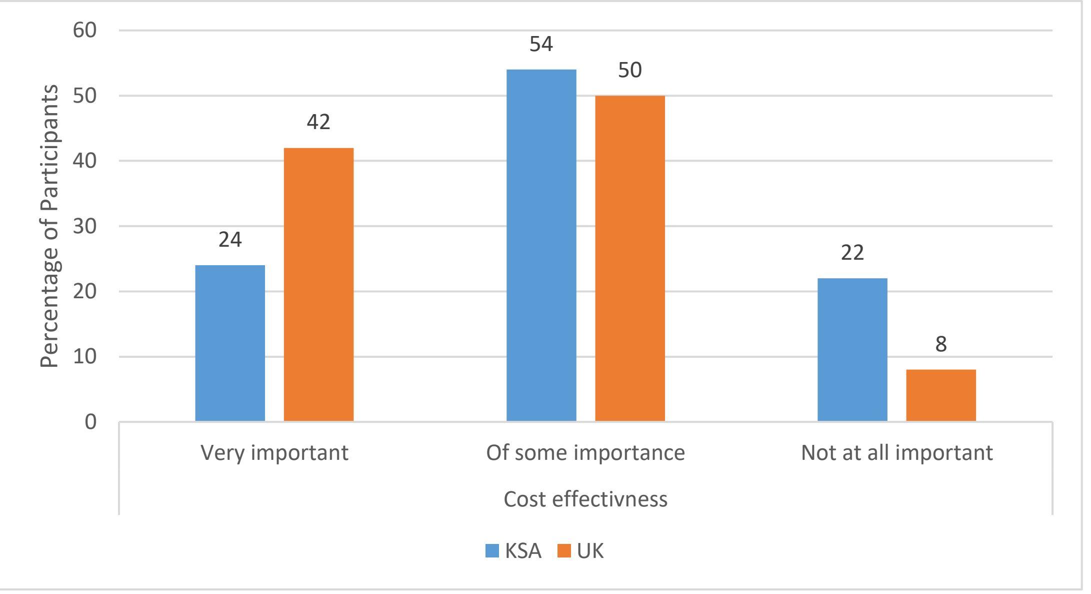



**Figure 2.2 Cost effectiveness as a reason for choosing drinks in KSA and UK samples.**

Figure 2.2 shows that cost has an effect on purchasing a drink in both the UK and the KSA. Nearly 50% of the volunteers in the UK and the KSA indicted that cost was “of some importance” when they purchased a drink. Statistically, there was a significant difference between the two samples on the tendency to think that cost is very important. The UK sample had 42% in comparison to only 24% from the KSA believe in cost-effectiveness. The P value was 0.003 and the Chi-square was 11.596.

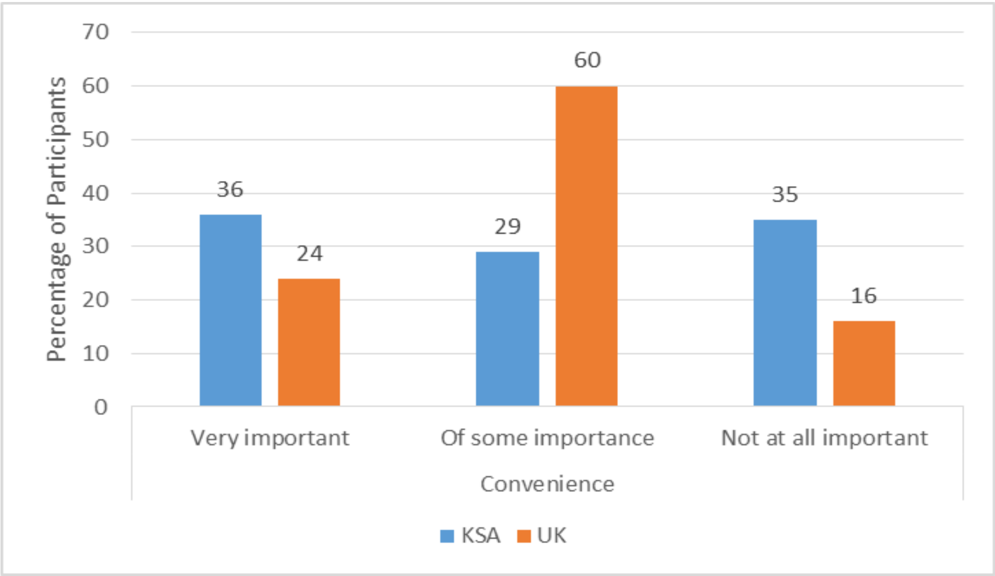



**Figure 2.3 Convenience as a reason for choosing drinks in KSA and UK samples.**

Figure 2.3 shows that convenience could be a reason to decide whether to purchase a drink or not in the UK and the KSA. The results showed that there were differences in the opinion of the KSA participants about the convenience factor. Almost similar percentages of 36% and 35% of participants indicated that this factor was either "very important" or "not at all important" when choosing a drink. Whereas, more than half of the UK sample at 60% revealed convenience as "of some importance". Statistically, there is a highly significant difference between the UK and the KSA samples with P value as 0.000 and Chi-square as 20.276. The majority of the UK participants think convenience is a strong motive but the KSA participants think the opposite.

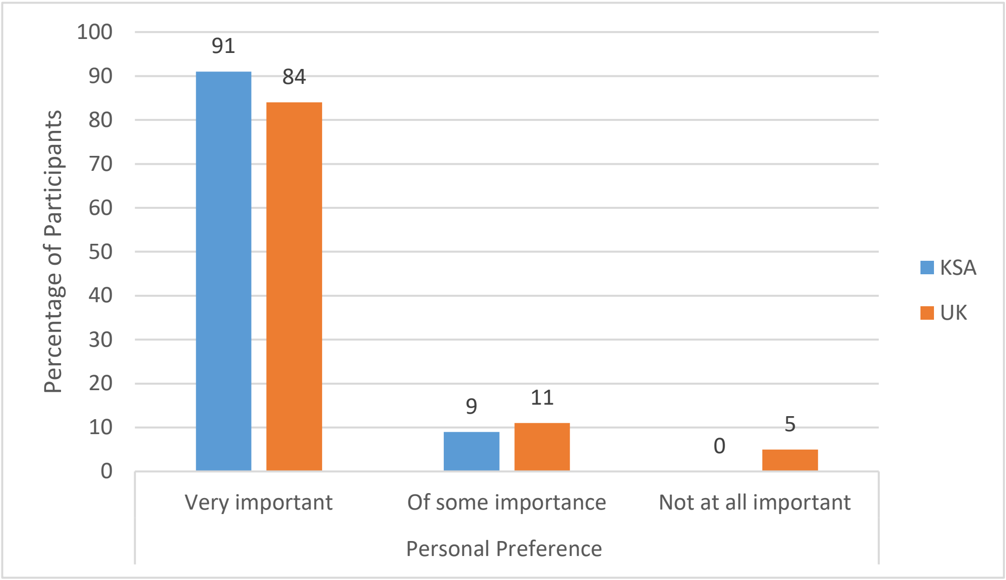



**Figure 2.4 Personal preference as a reason for choosing drinks in KSA and UK samples.**

Figure 2.4 shows that personal preference was one of the main factors that affect purchasing drinks in the KSA and the UK. The majority of both the country's sample indicated that personal preference is a very important factor related to purchasing beverages. It is very important to mention that "non-alcoholic beverage" was written on the UK questionnaire only, due to religious reasons. Overall, the personal preference seemed to play the most important role among all factors. These results were seen in both the KSA and the UK. Statistically, there was no significant difference between the UK and the KSA sample with P value of 0.065 and Chi-square of 5.48.

The results of the question "What are your 3 most favoured fruits to eat?", revealed that apple, banana followed by strawberry were the most favourite fruit to eat in the UK sample. Similarly, the KSA participants also chose the same fruit but in a different order; strawberry, banana followed by apple as the most favourite fruit to eat.

Regarding the most preferred fruits to be mixed in a smoothie:

| Fruit | Prefer a lot % | | Prefer a little % | | Do not prefer % | |
|---|---|---|---|---|---|---|
| Sample | KSA | UK | KSA | UK | KSA | UK |
| Apple | 25 | 33 | 40 | 54 | 35 | 13 |
| Banana | 55 | 68 | 25 | 20 | 20 | 12 |
| Kiwi | 30 | 29 | 36 | 39 | 34 | 32 |
| Lemon | 42 | 18 | 37 | 49 | 21 | 33 |
| Melon | 37 | 29 | 33 | 44 | 30 | 27 |
| Orange | 38 | 50 | 34 | 38 | 28 | 12 |
| Pear | 23 | 26 | 33 | 53 | 43 | 21 |
| Pineapple | 50 | 56 | 26 | 31 | 24 | 13 |
| Strawberry | 70 | 71 | 17 | 24 | 14 | 5 |
| Tomato | 8 | 70 | 10 | 22 | 82 | 8 |

**Table 2.2 Participants opinions about the preferable fruits to be in a smoothie in the KSA and UK samples.**

Table 2.2 shows the participant's preferences of fruits to be in a smoothie among both samples. Fruits that scored highly in both samples were strawberry, banana and pineapple. However, lemon was more appealing to the KSA participants and orange to the UK group. Interestingly, the UK sample showed preference of 92% towards the inclusion of tomato in a smoothie, whereas it scored very low in the KSA sample with only 8%. Additionally, a minority of the fruits listed in the table were disliked by the UK sample. In contrast, the majority of the KSA sample disliked the thought of including pears or tomatoes in a smoothie.

Regarding the response to the question on the top three most favoured vegetables to eat; among the UK participants in the order of preference were carrots, broccoli and then cucumber. Unlike the UK sample, cucumber, lettuce and then carrot were the three most favourable vegetables to eat in the KSA group. To sum up, carrot was the most favoured vegetable to eat among both samples.

| Vegetable | Prefer a lot % | | Prefer a little % | | Do not prefer % | |
|---|---|---|---|---|---|---|
| Sample: | KSA | UK | KSA | UK | KSA | UK |
| Beetroot | 28 | 24 | 27 | 22 | 45 | 54 |
| Broccoli | 19 | 18 | 24 | 30 | 57 | 52 |
| Cabbage | 10 | 12 | 20 | 19 | 70 | 69 |
| Carrot | 57 | 60 | 31 | 28 | 12 | 12 |
| Cauliflower | 14 | 14 | 18 | 25 | 68 | 61 |
| Celery | 14 | 16 | 26 | 29 | 60 | 55 |
| Green pepper | 20 | 16 | 23 | 27 | 57 | 57 |
| Lettuce | 41 | 17 | 24 | 25 | 35 | 58 |
| Parsnip | 9 | 13 | 24 | 16 | 67 | 71 |
| Radish | 8 | 9 | 16 | 20 | 76 | 71 |
| Red pepper | 12 | 21 | 24 | 33 | 64 | 46 |
| Spinach | 39 | 25 | 22 | 22 | 39 | 53 |
| **Arugula/ Spring green** | 33 | 15 | 34 | 24 | 33 | 61 |
| Sweet potato | 35 | 35 | 18 | 25 | 47 | 40 |
| Turnip | 6 | 7 | 22 | 13 | 72 | 80 |

**Table 2.3 Participant's opinions about the preferable vegetables to be in a smoothie in the KSA and UK samples.**

Table 2.3 shows the participant's preferences of vegetables in the smoothie among both the KSA and the UK samples. The vegetable that scored the majority of positive opinions in the "prefer a lot" category in both samples was carrot. Also, lettuce scored a 41% as a preferred a lot vegetable and red pepper was disliked at 64% in the KSA sample only. However, the most disliked vegetables to be included in a smoothie among both the UK and the KSA groups were broccoli, cabbage, cauliflower, celery, green pepper, parsnip, radish turnip, and sweet potato as they all scored high percentages in the "do not prefer" category. In addition, beetroot, lettuce, and spring green are the most disliked vegetables to be in smoothie among the UK sample only. Moreover, spinach and arugula (rocket plant) gave mixed opinions among the KSA sample, both having equal responses- in "appealing a lot" and "disliked" of 33%.

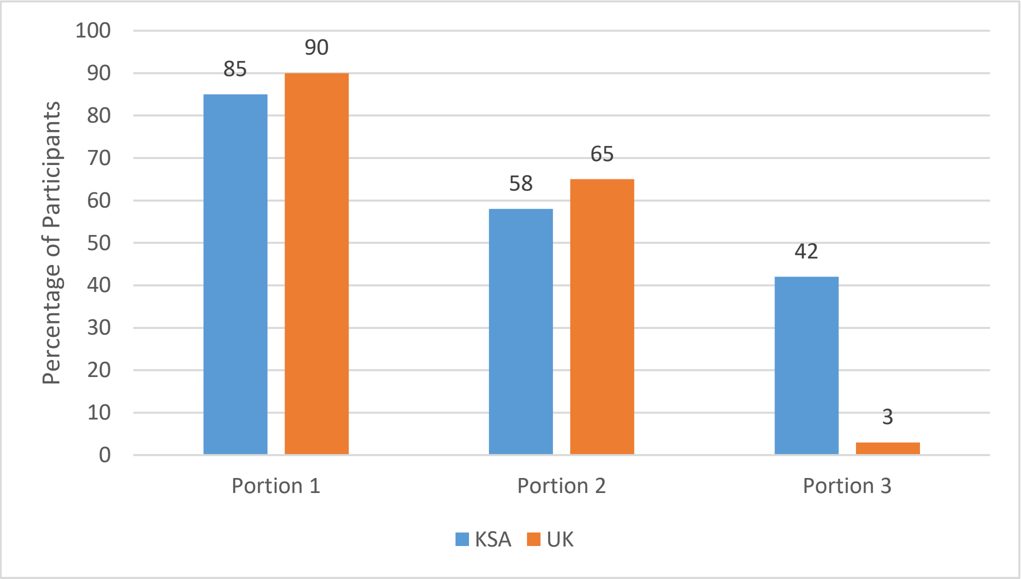



**Figure 2.5 Percentage of participants eating at least 1, 2 or 3 portions of fruits daily by both KSA and UK samples.**

Figure 2.5 illustrates the percentage of participants eating 1,2, or 3 portions of fruit daily in both the UK and the KSA samples. In the KSA, the majority, 85% of the sample consumed at least one portion of fruit, nearly half of the sample a 58% consumed two portions of fruit, and a 42% of the sample consumed a total of 3 portions of fruit per day. In contrast, the majority, 90% of the UK sample consumed one portion of fruit per day. Moreover, 65% of the UK sample consumed two portions of fruit per day and only 3% consumed 3 portions of fruits a day.

In summary, as per the answers to this question, the KSA participants were consuming more fruit than the UK participants and date consumption was seen in the KSA group only. In addition, both groups were aware of the portion size (i.e. one cup of fresh fruit juice is equivalent to one whole fruit or 1/4 cup of dried canned fruits).

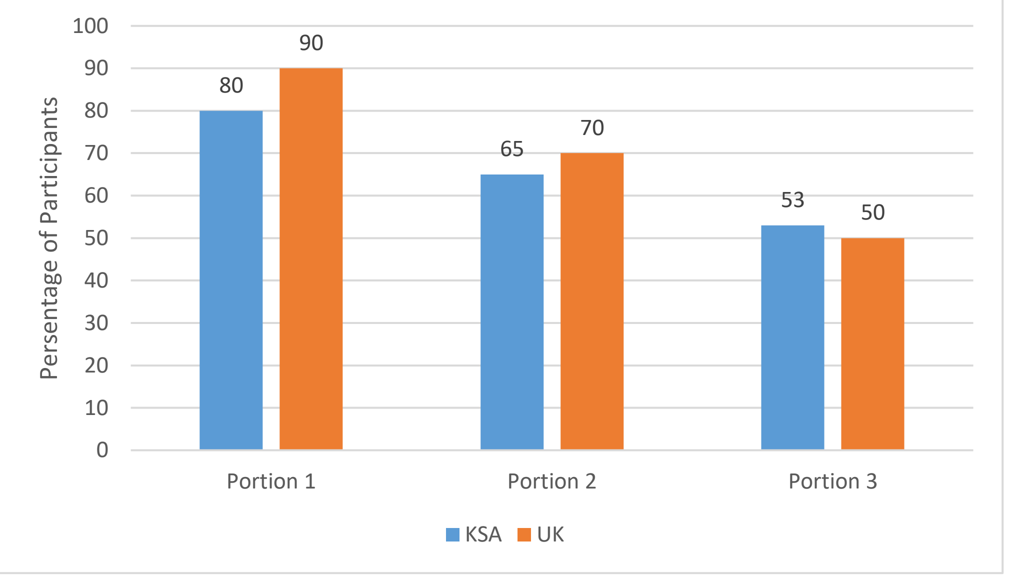



**Figure 2.6 Percentage of participants eating at least 1, 2 or 3 portions of vegetables daily by both KSA and UK samples.**

In reference to vegetable's intake, figure 2.6 shows the amount of vegetables eaten per day by participants in both the KSA and the UK samples. The majority at 80% of the KSA sample participants stated that they consumed one portion of vegetables, whereas 65% of the sample consumed two portions of vegetables, and 53% of the sample consumed three portions of vegetables per day. In comparison, the figure also illustrates that the majority of the UK sample at 90% consumed one portion of vegetables per day. Likewise, the KSA sample, 70% of the UK sample consumed two portions of vegetables daily. While, 49% of the UK sample eat three portions daily.

The question measured the vegetable consumption, recalling from the previous day intake. UK participants consumed more vegetables than the KSA participants. Also, both groups were aware that mixed salad, vegetable soup and frozen vegetable are included within the vegetable intake.

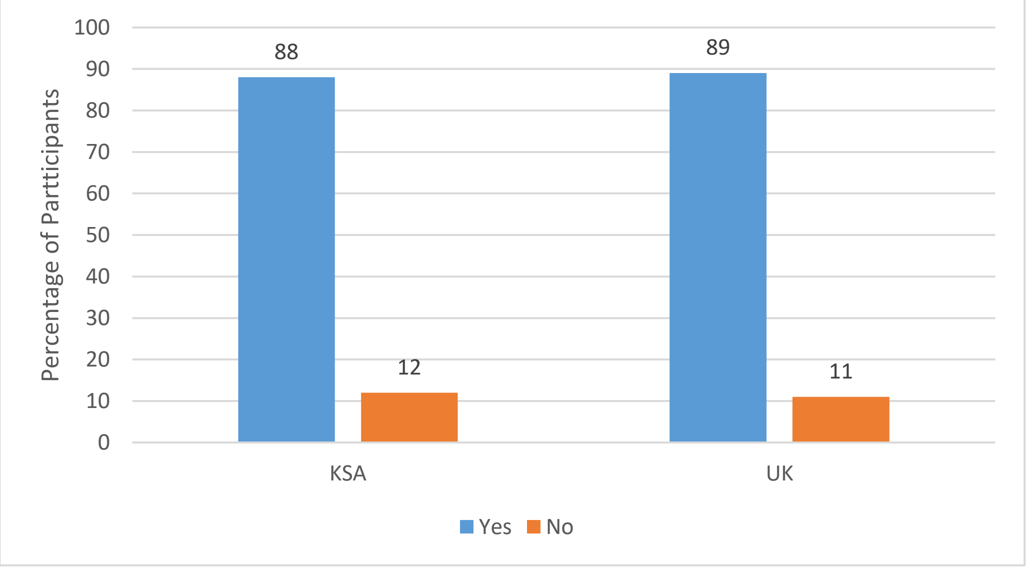



**Figure 2.7 Percentage of participants who have previously tried a smoothie in KSA and UK samples.**

Figure 2.7 shows the percentage of participants who had tried a smoothie product in both the KSA and the UK samples. The majority of the KSA and the UK samples have tried a smoothie product before. Whereas, nearly ⅛ of the sample had never tried a smoothie before. Statistically, there was no significant difference between both samples with P value of 0.825 and Chi-square of 0.049.

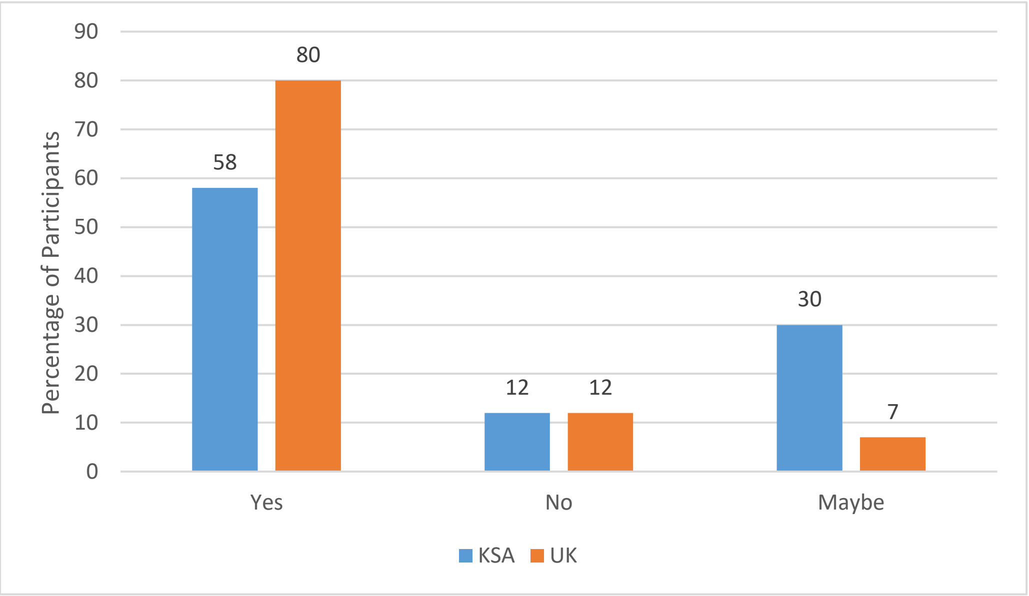



**Figure 2.8 Percentage of participants who like the smoothie in KSA and UK samples.**

Figure 2.8 shows the percentage of participants who enjoy smoothies in the KSA and the UK samples. The UK sample liked smoothies by 22% more than the KSA sample. In addition, equally 12% of the participants in both the KSA and the UK disliked smoothies. Moreover, about a third of the KSA sample were unsure of their preference towards smoothies compared to a low 7% in the UK sample (7%). Statistically, there was a high significant difference between the UK and the KSA samples, with P value of 0.000 and Chi-square of 19.87.

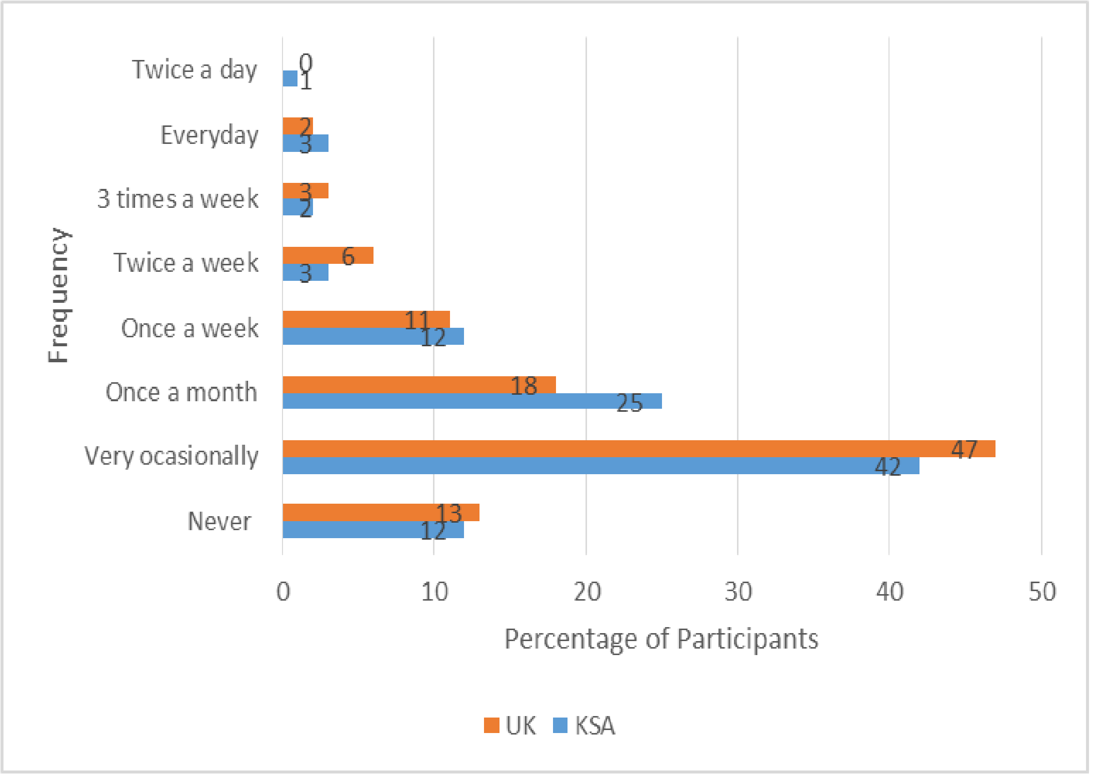



**Figure 2.9 Frequency of smoothie intake among participants in KSA and UK samples.**

Figure 2.9 illustrates the frequency of smoothie consumption by participants in both the KSA and the UK samples. The majority of both the KSA sample at 42% and the UK sample at 47% drank smoothies very occasionally. Around 13% of both groups "never" consider smoothie as part of their drinking habits. In addition, a quarter of the KSA sample drink smoothie once a month and only one person from the KSA group consumed smoothies often "twice a day".

In summary, half of the subjects consumed smoothies very occasionally, in both KSA and UK.

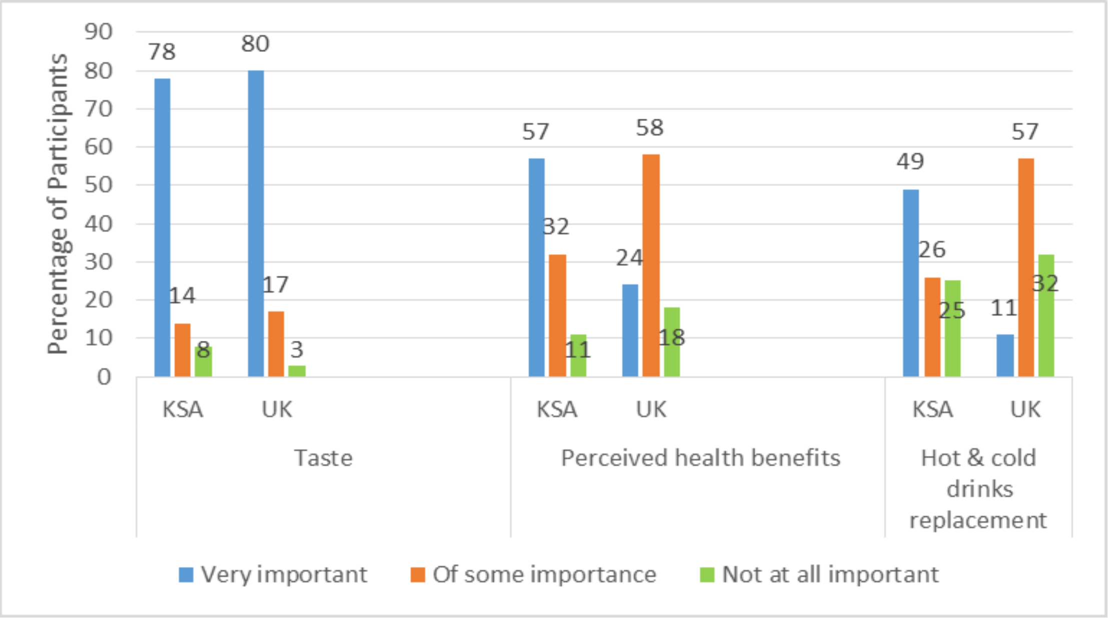



**Figure 2.10 Reasons affecting smoothie consumption among participants in KSA and UK samples.**

Figure 2.10 shows three of the factors affecting the decision to drink a smoothie in both the KSA and the UK samples. The vast majority at 80% of the samples consider taste to be the most important factor when choosing to drink smoothies. Statistically, there was no significant difference between the UK and the KSA samples with P value of 0.288 and Chi-square of 2.49.

In the KSA group, it was very important for 57% of the study sample to choose a smoothie based on "health benefits". Whereas, in the UK group it was of some important for 58% of study sample. Statistically, there was a high significant difference between the samples with P value of 0.000 and Chi-square value of 16.95.

It was also very important for 49% of the study sample in the KSA group to drink the smoothie instead of hot and cold drinks like tea, coffee, or fizzy drinks. On the other hand, the majority at 57% of the UK sample expressed that it is "of some importance". Statistically, there was a high significant difference between the UK and the KSA samples with P value of 0.000 and Chi-square of 29.32.

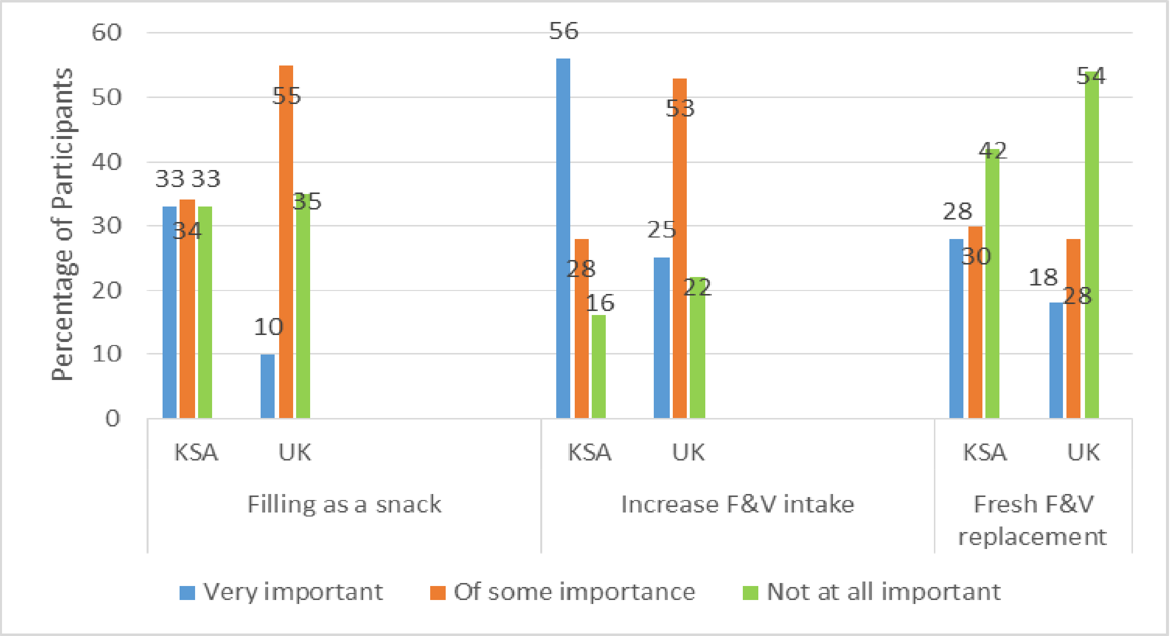



**Figure 2.11 Reasons affecting smoothie consumption among participants in KSA and UK samples.**

Figure 2.11 shows some other factors that could affect the consumption of smoothies in both the KSA and the UK samples. Only 10% of the UK participants indicated that their reason for consuming smoothies was that it would be as good as a healthy snack. Whereas, in the KSA sample, there were varied opinions in response to this question. Each of the responses covered third the KSA sample, whether very important, of some importance, or not at all important. Statistically, there was a significant difference between both the UK and the KSA samples with P value of 0.002 and Chi-square value of 12.88.

“Increasing F&V intake” was the most important reason for consuming smoothies among 56% of the KSA sample as compared to the UK sample. The majority at 53% of the later sample state that increasing F&V consumption is “of some importance”. Moreover, in the UK the “Increase F&V intake” was written in relation to “helping to achieve 5 A DAY” as it might be more appropriate while maintaining the essence of the question.

Statistically, there was a high significant difference between the UK and the KSA samples with P value of 0.000 and Chi-square of 15.36.

Additionally, the majority of both the UK and the KSA samples considered that smoothies are not important as a replacement for fresh F&V. Statistically, there was a significant difference between the UK and the KSA samples with P value of 0.004 and Chi-square value of 11.02.

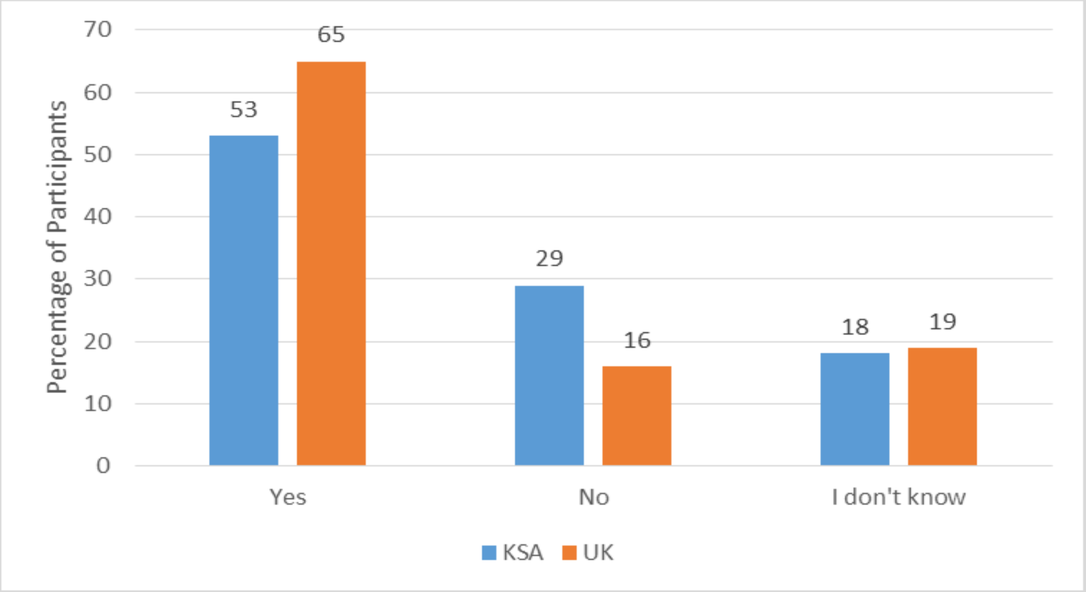



**Figure 2.12 Participant's opinions about smoothies as a part of F&V in UK (5 A DAY) and KSA samples.**

Figure 2.12 shows the percentage of participant's opinions on considering smoothie as a part of F&V intake in both UK and the KSA samples. More than half of the KSA sample at 53% and the UK sample at 65% reported that they consider smoothies to be part of their F&V consumption (in the UK part of the 5 A DAY). Additionally, just less than a quarter of each sample did not know whether smoothies were considered as part of the F&V intake or not. Interestingly, 29% and 16% of the KSA and the UK samples, respectively, did not consider smoothie as part of their F&V consumption. Statistically, there was no significant difference between the UK and the KSA samples with P value of 0.129 and Chi-square value of 4.09.

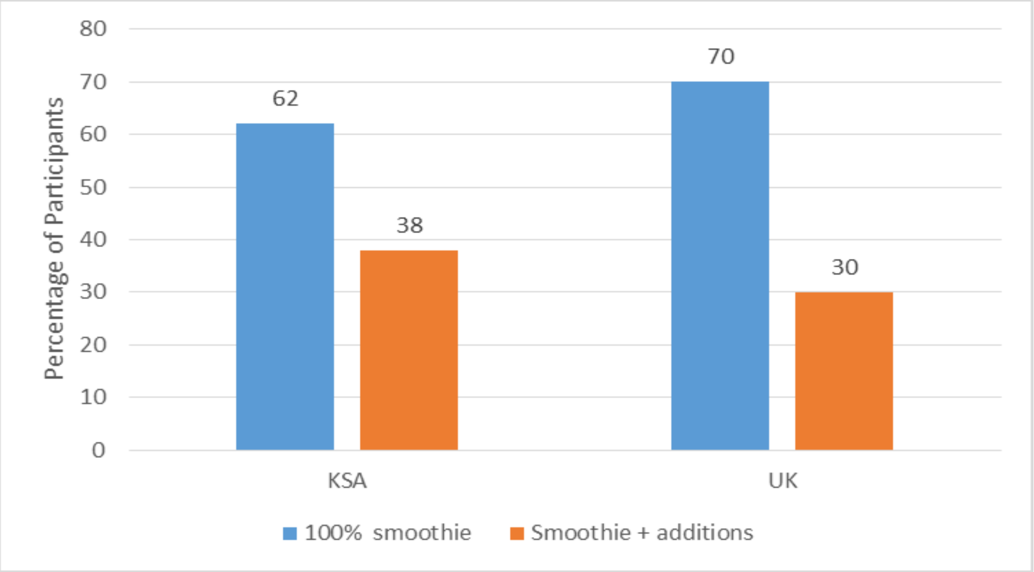



**Figure 2.13 Participant's preference about type of smoothies in UK and KSA samples.**

Figure 2.13 shows the percentage of participant's preference on the type of smoothies preferred in both the UK and the KSA samples. The most significant outcome was that a high percentage of participants in both the KSA and the UK samples prefer pure smoothie. However, nearly a third of both the KSA and the UK samples preferred smoothies with additions like yoghurt, oat, and honey. Statistically, there was a significant difference with P value of 0.007 and Chi-square value of 9.863 between the 2 samples in both of the acceptability areas that were 100% smoothies and smoothies with additions.

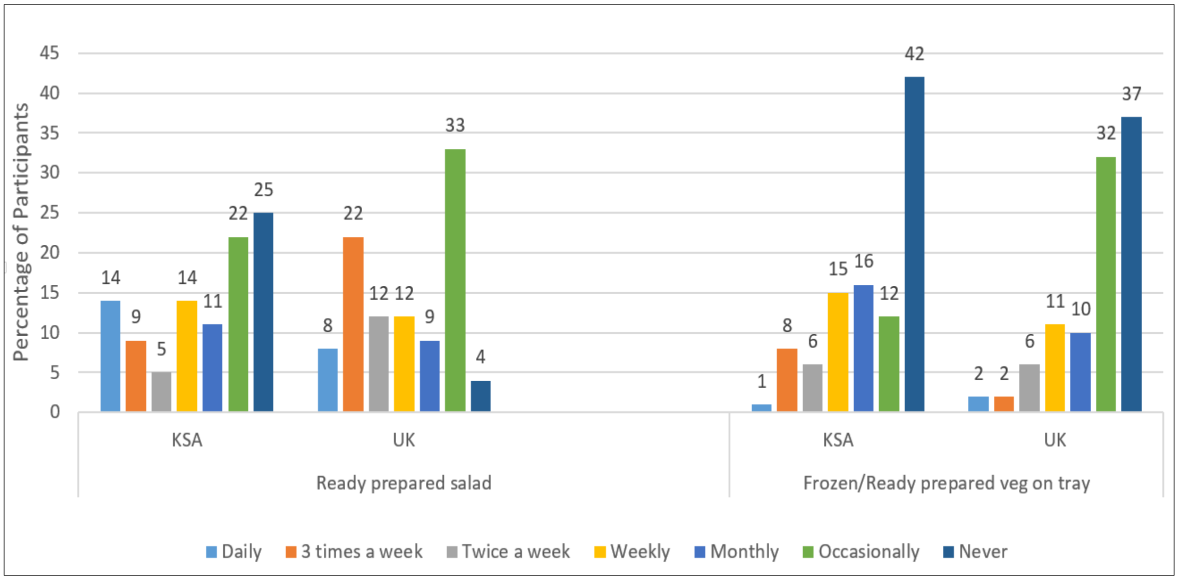



**Figure 2.14 Percentage of vegetable products intake among KSA and UK samples.**

Figure 2.14 shows the percentage of vegetable products intake among the KSA and the UK samples. A high 25% of the KSA sample never ate ready prepared salads. While a third of the UK sample consumed ready prepared salad occasionally. The majority at 42% of the KSA and a third of the UK participants "never" consumed frozen vegetables/ready prepared vegetables. "Frozen vegetables" was written in the KSA questionnaire due to unavailability of the "ready prepared vegetables" in the Saudi market.

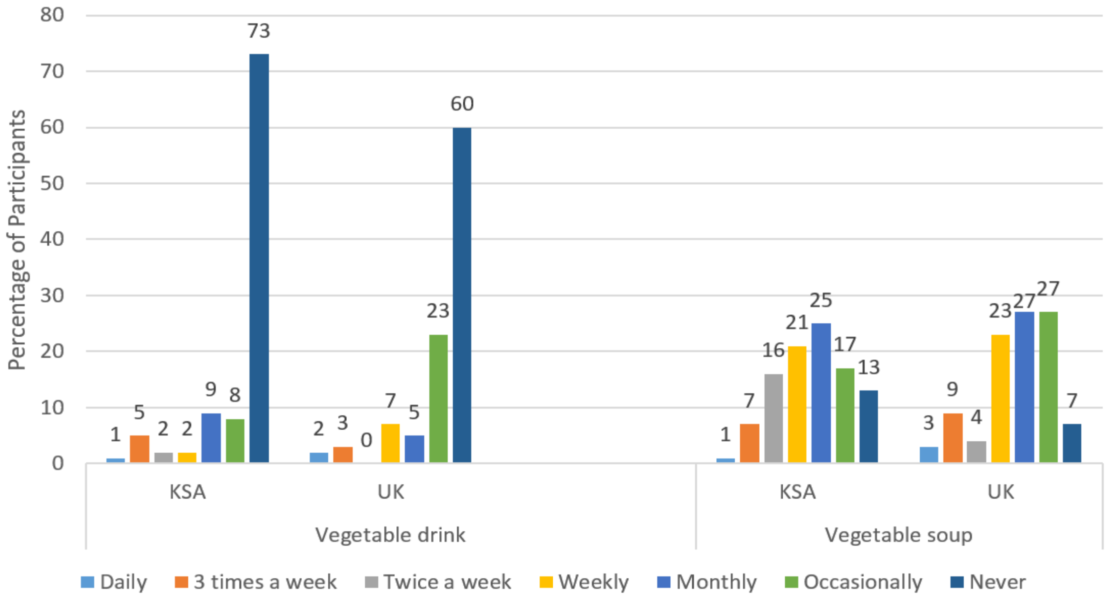



**Figure 2.15 Percentage of vegetable products intake in the KSA and UK samples.**

Figure 2.15 shows the percentage intake of two other vegetable products in the KSA and the UK samples. More than half of both samples have "never" drunk a vegetable drink, while in the UK, 23% of participants "occasionally" drank it.

Additionally, the majority of participants in the KSA group consumed vegetable soup, 21% on weekly basses and 25% on monthly basses. Whereas, there were mixed results about vegetable soup among the UK group with exactly the same number of participants of 27% falling into "monthly" and "occasionally".

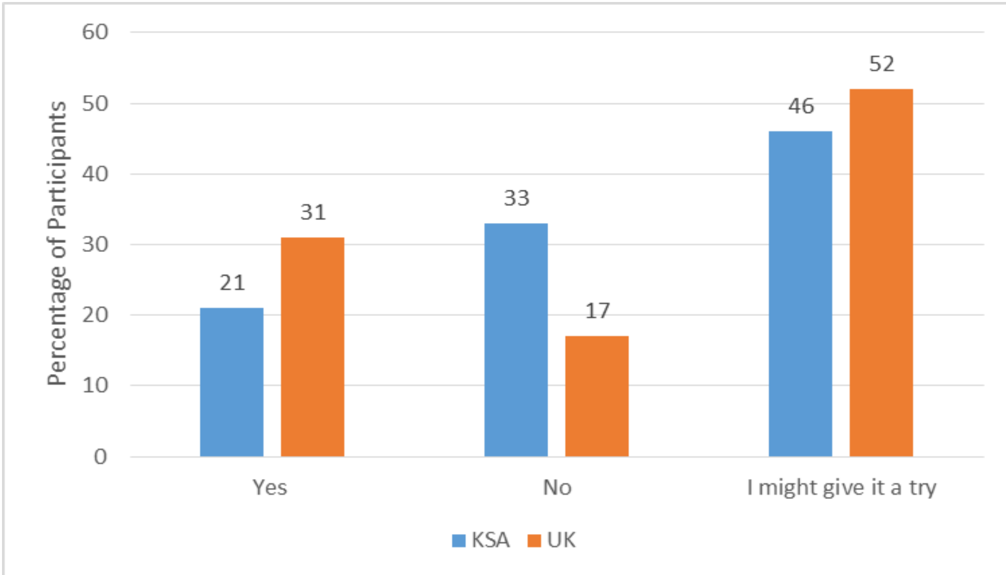



**Figure 2.16 Percentage of participant's acceptance of combined vegetable and fruit smoothie in KSA and UK samples.**

Figure 2.16 shows the acceptance of combined F&V smoothie in both the KSA and the UK samples. An almost similar high percentage of participants in the KSA at 46% and the UK at 52% groups would like to try F&V smoothies. Also, almost a third of the UK sample and 21% of participants in the KSA sample were accepting the concept of a combined vegetable and fruit smoothie.

However, in contrary, a third of the KSA sample did not accept mixing F&V in one smoothie and only 17% of the UK sample did not accept the F&V smoothie combination. Statistically, there was a significant difference with P value of 0.027 and Chi-square value of 7.19 between the samples in terms of acceptability of F&V smoothies.

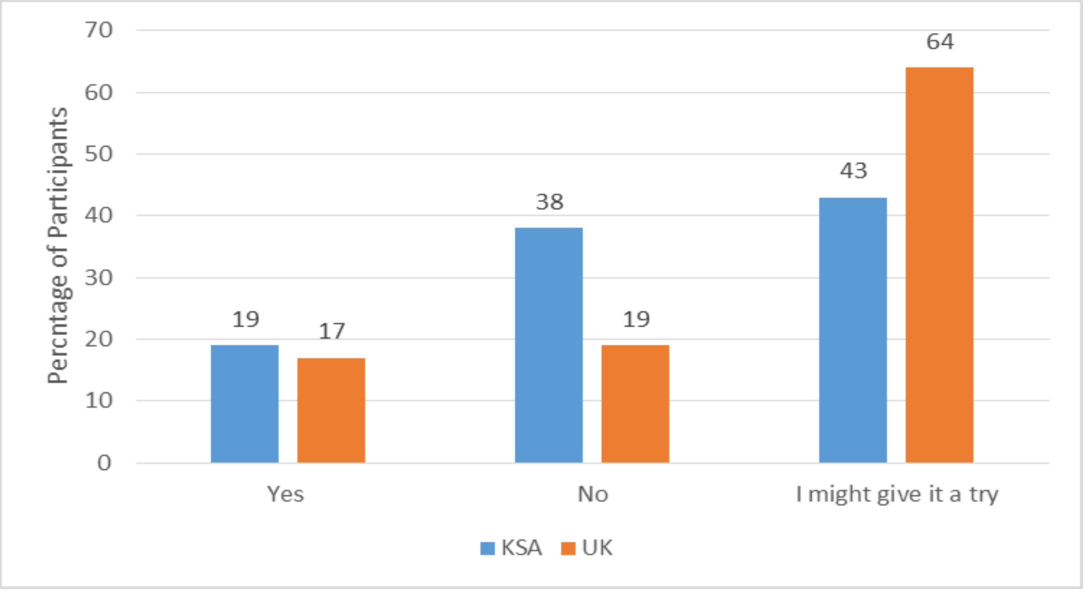



**Figure 2.17 Percentage of participant's acceptance of vegetable-based smoothie in KSA and UK samples.**

Figure 2.17 represents the acceptance of vegetable smoothies in both samples. More than half at 64% of the UK group would like to try a vegetable smoothie, but only 43% of the KSA sample would like to do so. More than a third at 38% of the KSA sample does not accept the idea of drinking vegetable-based smoothies. Statistically, there was a significant difference with P value of 0.007 and Chi-square value of 9.863 between the samples in regard to acceptability of 100% vegetable smoothies.

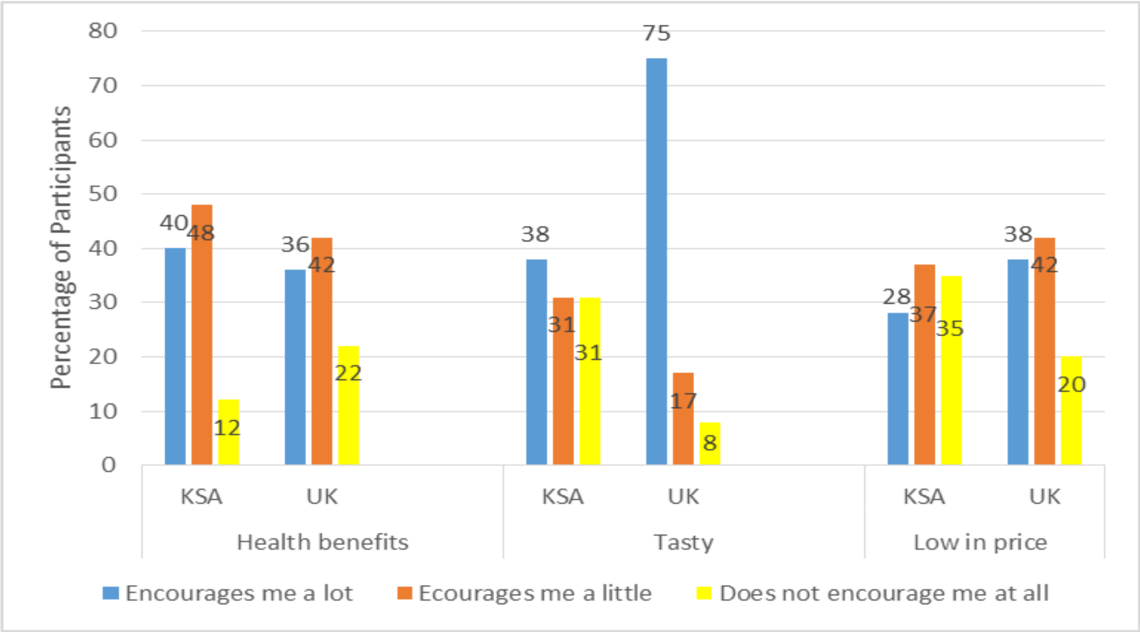



**Figure 2.18 Percentage of participant's motives to buy and consume vegetable smoothie.**

Figure 2.18 illustrates three of the motives that could encourage people to buy and drink smoothies containing vegetables; taste, benefits, and price. The motive "perceived health benefits" would encourage the majority of both samples a little. However, it would be more encouraging to the KSA group at 40% than to the UK group at 36%. Statistically there was no significant difference between the samples with P value of 0.257 and Chi-square value of 4.04.

Taste was the most important motivation that would drive 75% of the UK sample to purchase smoothie containing vegetables and 38% of the KSA participants were motivated by it. Statistically there was a high significant difference between the samples with P value of 0.000 and Chi-square value of 28.12.

Moreover, “Low price” as a motive would encourage 38% of the UK participants and 28% of the KSA participants. However, the majority in both samples find this motive encouraging them a little. Statistically, there was no significant difference between both samples with P value as 0.058 and Chi-square value of 5.7.

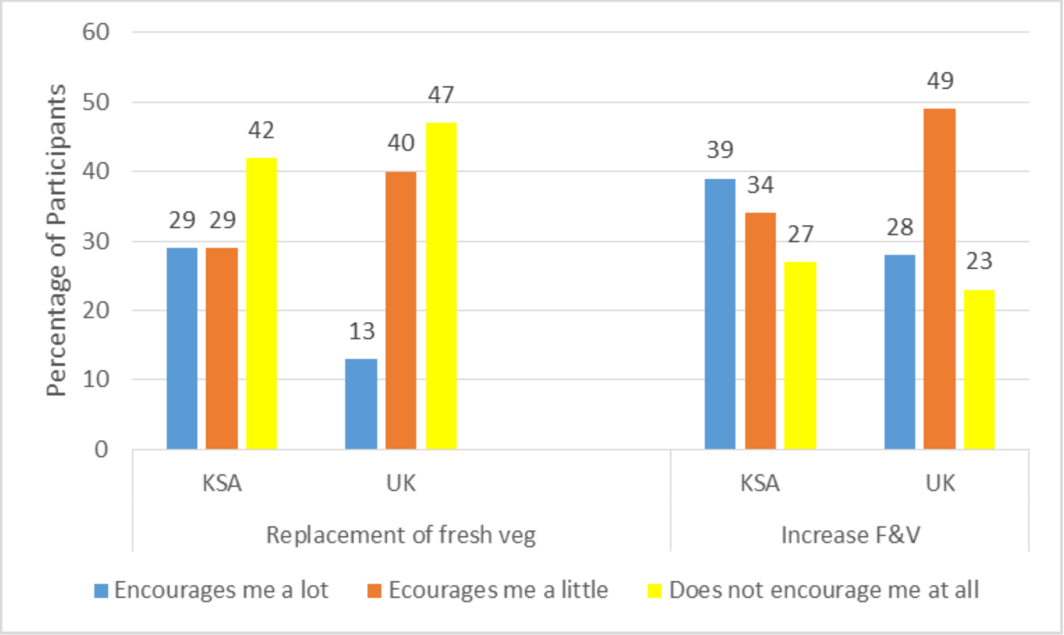



**Figure 2.19 participant’s motives to buy and consume vegetable smoothie.**

Figure 2.19 demonstrates two motives that can encourage people to buy and consume smoothie containing vegetables. The motive “increasing F&V intake” (helps meet the 5 A DAY for UK participants) encouraged 39% of the KSA participants a lot and almost 50% of UK participants said that it encouraged them a little. Statistically there was no significant difference between the samples with P value of 0.141 and Chi-square value of 3.917.

In contrast, "smoothie replacing fresh F&V" does not encourage the majority of volunteers in either the UK or the KSA with 42 % and 47% respectively. Statistically there was a significant difference between the samples with P value of 0.032 and Chi-square value of 6.87.

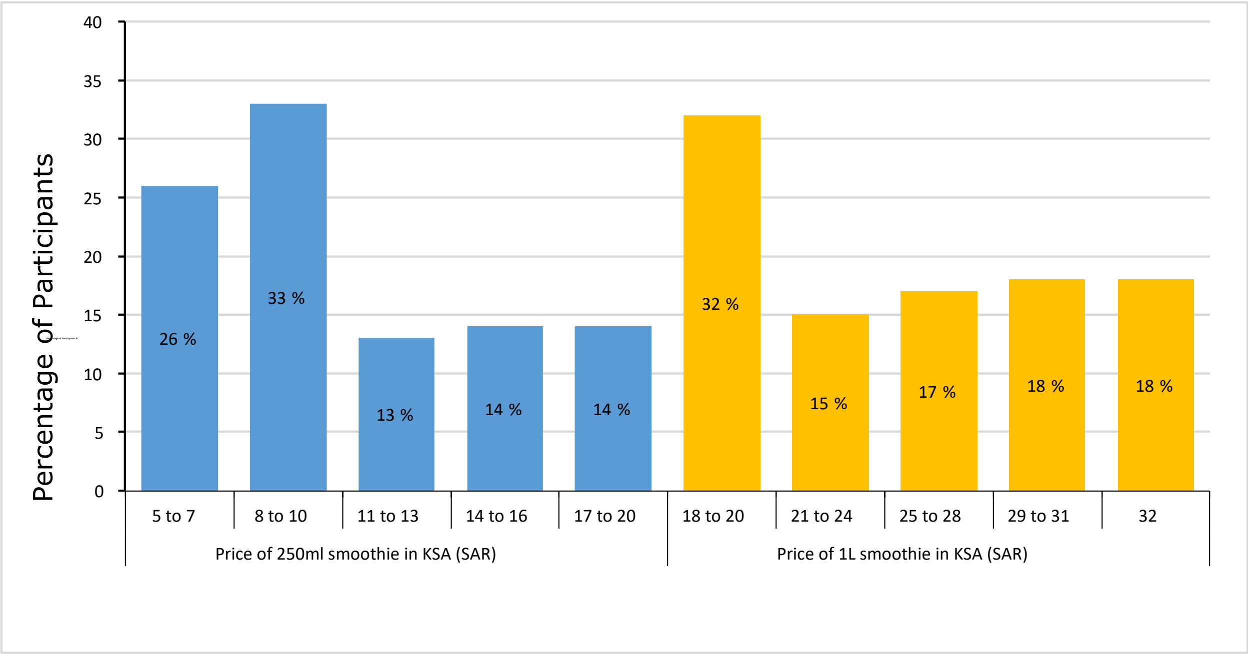



**Figure 2.20 Percentage of KSA participant's prepared to pay maximum price (SAR) for 250ml and 1L of smoothies.**

Figure 2.20 shows the maximum price that KSA participants were ready to pay for 250ml and 1L smoothies. One third of the KSA sample was willing to pay a maximum of 10 Saudi riyals (SAR) for a 250ml smoothie bottle. And almost another third of them were ready to pay a maximum of 20 SAR for a 1L smoothie bottle/carton.

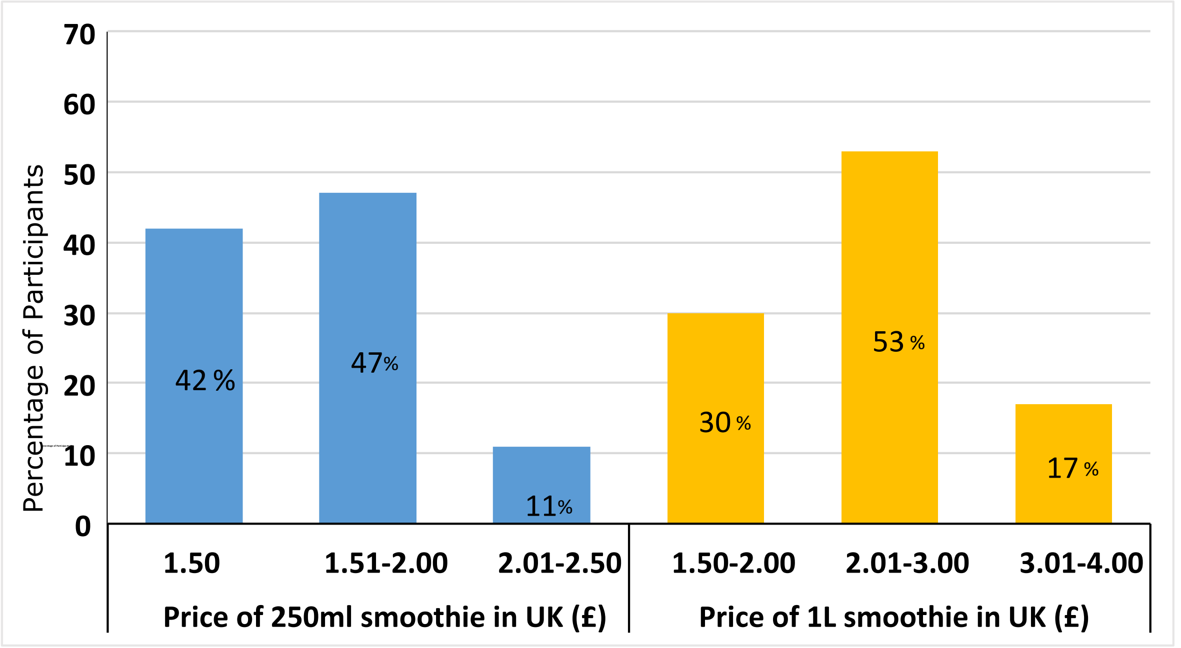



**Figure 2.21 Percentage of UK participant's prepared to pay maximum price (£) for 250ml and 1L of smoothies.**

Figure 2.21 shows the maximum price that UK participants were ready to pay for 250ml and 1L smoothies. 47% of the participants were willing to pay a maximum of £2 for a 250ml smoothie bottle and 53% of the samples were willing to pay a maximum of £3 for a 1L smoothie bottle/cartoon.

## 2.3 STUDENT TASTING PANEL IN THE UK

Using the information from the questionnaire's results, several potential smoothie recipes were designed. These recipes were then assessed for potential nutritional value by a theoretical approach using the reference data values for nutrient composition. Lastly, they were tested by a taste panel to assess consumer acceptability. This was first carried out using a small and focus group.

### 2.3.1 Preliminary Tasting Session

The aim of the focus group was to try different smoothie mixtures, designed from the questionnaire data, before the recipes were introduced to real panels in the tasting session.

The tasting session was aimed to identify consumer acceptability of overall liking and preference. This included colour, taste, and consistency of the six selected vegetable–based smoothies. The tasting session was to confirm whether members found the smoothies to be acceptable and enjoyable without requiring an extra effort to drink in relationship to fruit-based smoothies. Ten smoothie combinations were suggested by more than one respondent in the questionnaire. These combinations were produced in the dietary laboratory for a focus group consisting of four persons, including the researcher.

Some of the mixes were excluded because of thickness or undesirable taste or colour. The excluded mixes were:

- Orange, carrot and sweet potatoes
- Orange, carrot and sweet corn
- Apple, carrot and green pepper; and
- Apple, carrot and broccoli.
-

| no. | Smoothie/250ml | Vita C mg | Vita A µg | Vita E mg | Folic acid µg | Magnesium mg | Anti-Oxidant µmol | Potassium mg | Sodium mg |
|---|---|---|---|---|---|---|---|---|---|
| 348 | % 47 Vegetable+%53 Fruit. Orange, Carrot & Beetroot | 75.8 | 880 | 0.64 | 127.5 | 28 | 34.5 | 46 | 85 |
| 267 | % 50 Vegetable+% 50 Fruit. Apple, Orange, Carrot & Beetroot | 37.5 | 822 | 0.93 | 99 | 23.8 | 21.5 | 630 | 98 |
| 551 | % 47 Vegetable+%53 Fruit Strawberry, Carrot & Celery | 108 | 865 | 0.73 | 53 | 21 | 31.29 | 535.8 | 65 |
| 134 | %76 Vegetable+%24 Fruit. Orange, Beetroot, Carrot & Red pepper | 113 | 979 | 0.82 | 142 | 27 | 36.79 | 749 | 124 |
| 729 | % 100 Vegetables. Beetroot, Carrot & Cucumber | 8.5 | 693 | 0.34 | 139.9 | 23.5 | 18.52 | 821 | 147 |
| 901 | %47 Vegetable+%53 Fruit. Strawberry, Beetroot , Red pepper & Orange | 139 | 207 | 0.53 | 133 | 27 | 48.5 | 692 | 102 |

**Table 2.4 the six smoothies chosen for the tasting session with nutritional composition (McCance and Widdowson, 2008).**

The focus group chose 6 smoothie combinations as being potentially acceptable following the preliminary tasting session. These are listed in Table 2.4 along with their potential nutritional composition calculated based on the data reference values in McCance and Widdowson in 2008.

### 2.3.2 Student Tasting Panel Materials and Methods

The tasting sessions were publicised through advertising in University of Nottingham campuses. They were held in the dietetic laboratory of the Sutton Bonington campus for those who wanted to participate and with no obligation to contribute.

F&V were purchased the previous day to the tasting session from Tesco. Appropriate attention to food safety and hygiene was carried out. The six smoothie mixes, as identified in the preliminary session, were made fresh the day of the tasting session using a Kenwood food processor. Glass bottles were filled with smoothies and stored at 4°C. The homogeneity of representative samples was uniform, since the smoothie ingredients would not separate due to water not being one of the ingredients. Trials were designed to mimic consumer consumption of the smoothie. Samples were kept in the fridge until required. It was not feasible to either monitor or control the temperature during the tasting sessions, however, samples were not left out of the fridge for an extended length of time.

The samples were served in small, transparent, and disposable plastic cups placed on trays with a three-digit random code label representing the smoothie number as listed in Table 2.4. Participants were not told the ingredients of the smoothies. Each participant was given an evaluation sheet to fill in, found in Appendix 2, for the six different mixtures that they have tried. Between tasting the different samples, participants rinsed their mouths with water. The procedure was standardised in terms of sample preparation and verbal instructions given to the participants by the researcher and was carried out over two days.

The evaluation sheet consisted of Likert-type scales ranging from 1 (Like very much) to 5 (Dislike very much) for each sample to determine acceptability. Three questions were asked about preferences for the following features:

- Colour: 1 (attractive) to 3 (unattractive);
- Flavour: 1 (acceptable) to 3 (unacceptable); and
- Mouth feel/texture: 1 (smooth) to 3 (very thick).

Furthermore, two open-ended questions asked respondents to comment on the sample and their willingness to pay £1.50 for a 250 mL smoothie.

#### 2.3.2.1 Smoothie Preparation

Since the project was assessing “home-made” smoothies it was felt appropriate to use a commercially available blender. The smoothie was thus made using a Kenwood food mixer set at maximum speed. The ingredients are listed in Table 2.5. All ingredients were blended together for around 2 min until it has homogenised, this being assessed visually. The same preparation method was used for all the smoothie types listed in Table 2.4.

Other purchased commercial smoothies are described in Table 2.6. The smoothie ingredients and the commercial smoothies were purchased from Tesco supermarket.

| 901 Sample | Description |
|---|---|
| **Beetroot 83g** | Tesco Cooked Beetroot (No vinegar)<br>250g vacuumed pack<br>Grown in the UK |
| **Red pepper 30g** | Tesco Fresh Red Pepper<br>Grown in Holland<br>Class I |
| **Strawberry 70g** | Tesco Strawberries<br>227g<br>Vitamin C = 77mg / 100g (128% RDA) |
| **Orange Juice 60mL** | Tesco Orange Juice with the bits<br>100% pure squeezed juice (not from concentrate)<br>1L |

**Table 2.5: 901 Smoothie Ingredients.**

| Sample | Description |
| --- | --- |
| Innocent Seriously Strawberry | Pure fruit smoothie<br>Ingredients: Strawberries, banana, apple, white grapes, blackcurrants & squeezed orange.<br>Available in 750mL or in 250mL. |
| Innocent Gorgeous Greens | Finest fruits and green stuff<br>Ingredients: Apple, pear, spinach, kale, safflower& spirulina extract, baobab extract, peach, squeeze of lemon juice, squeezed orange juice, courgette.<br>Available in 750mL or in 250mL. |
| Naked Green Machine | 100% juice smoothie<br>Ingredients: Apple, mango, pineapple, banana, kiwi, spirulina, alfalfa, broccoli, spinach, barley grass, wheatgrass, garlic, ginger, kale & parsley.<br>Available in 450mL. |
| Naked Bright Beets | 100% juice smoothie<br>Ingredients: Beets, carrot, apple, coconuts water, lemon, ginger,<br>Available in 450mL. |

**Table 2.6 Composition of Popular Smoothies in the UK (data from labels).**

## 2.4 STUDENT TASTING PANEL RESULTS IN THE UK

A total of 41 students participated. The participants were 7 male and 34 female in the age group ≤18 to ≥50 year. Most subjects were British. Table 2.7 shows the combined results of the two tasting sessions. The number of participants responding to each attribute is given in the table as well.

In terms of colour, smoothie 901 was attractive to most participants, 39 out of 41, followed by 134 and 348. Smoothies 267, 551 and 729 were the least attractive. In terms of flavour, smoothie 901 was acceptable to most participants ,32 out of 41, followed by 348 and 267 which were acceptable to half of the sample. It was followed by smoothies 551 and 134. Smoothie 729 was found to be the least acceptable. Finally, in terms of texture or mouth feel; smoothie 901 ranked best by 35 participants out of 41 followed by 348 and 551. Smoothies 134, 729, and 267 had the worst texture as listed in Table 2.7.

| **Smoothies** | 348 | 267 | 551 | 134 | 729 | 901 |
|---|---|---|---|---|---|---|
| **Attributes** | **Total of 41 panels** | | | | | |
| **Colour** (Attractive) | 33 | 27 | 25 | 34 | 25 | 39 |
| Neither | 6 | 8 | 8 | 5 | 9 | 2 |
| Unattractive | 2 | 6 | 8 | 2 | 7 | 0 |
| **Flavour** (Acceptable) | 20 | 20 | 16 | 12 | 7 | 32 |
| Neither | 10 | 6 | 8 | 14 | 7 | 6 |
| Unacceptable | 11 | 15 | 17 | 15 | 26 | 3 |
| **Mouth feel** (Smooth) | 19 | 11 | 19 | 16 | 12 | 35 |
| Neither | 6 | 5 | 6 | 5 | 7 | 3 |
| Very thick | 16 | 24 | 16 | 19 | 22 | 3 |
| **Over all acceptability** Like very much | 7 | 6 | 5 | 3 | 3 | 10 |
| Like slightly | 11 | 12 | 8 | 9 | 5 | 19 |
| Neither like nor dislike | 8 | 4 | 8 | 4 | 6 | 6 |
| Dislike slightly | 13 | 10 | 10 | 15 | 4 | 6 |
| Dislike very much | 2 | 9 | 10 | 10 | 23 | **0** |
| **Would you buy it?** (Yes) | 13 | 14 | 12 | 10 | 6 | 26 |
| No | 28 | 26 | 29 | 30 | 34 | 15 |

**Table 2.7 Evaluation sheet analysis**

In terms of acceptability, smoothie 901 was rated best overall. A total of 10 participants out of 41 liked it very much, and there were no participants who disliked it very much. This was followed by 348, 267, 551, and 134, respectively. Smoothie 729 was the most undesirable. Twenty-six participants out of 41 said they would buy smoothie 901. However, 34 out of 41 would not buy smoothie 729.

Question 7 asked participants which sample they preferred overall. Figure 2.22 shows the order of overall smoothies' preference.

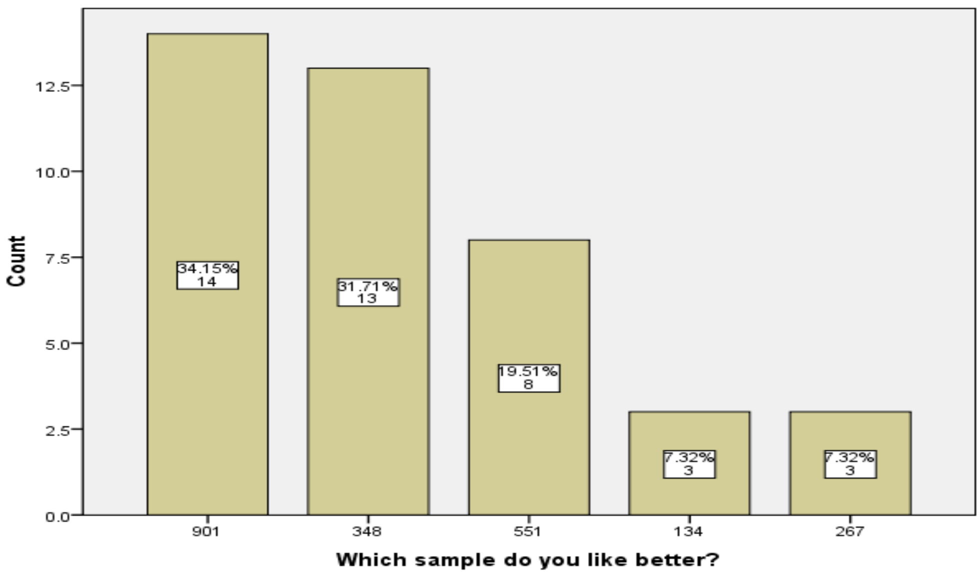



**Figure 2.22 Responses to question 7 on the evaluation sheet.**

To sum up, out of all 6 smoothies that were used in the taste session, 901 was selected as the one with the best consumer acceptance factor(s)in terms of colour, flavour, texture, and over all acceptability.

## 2.5 QUESTIONNAIRE AND TASTING SESSION DISCUSSION

Like most published surveys, the questionnaire began with gender exploration as it is important to study both. Manifestly, 82% were female participants in the KSA sample and 71% were female participants in the UK sample but there was no significance difference between the KSA and the UK participants with a P value of >0.05 (p= 0.067). The survey in the UK was randomly distributed in hard copy format to health faculties' students in Nottingham University and digitally answered online by the KSA sample. The survey was focused on health faculties' students with no targeted gender in mind. From the researcher observation female tend to respond to surveys more than males do, especially these kinds of questionnaires that concern health, wellbeing, and fitness awareness. According to some studies, women correspond to surveys more likely than male (Smith, 2008; Curtin et al., 2000; Moor and Tarnai, 2002; Singer, and et al., 2000). In the literature, many studies showed that female prefer to consume F&V more than male (Bere et al., 2008; Dibsdall et al., 2003; Rasmussen et al., 2006). Girls in general are mindful about the daily F&V consumption and the nutritional benefits more than boys do (Stukas, 2012). Overall, the high females respond to this survey is an explanation of all the above motives.

The questionnaire was mostly answered by students in the age group of ≥18 to ≤35 years old, with no intention to target a specific age group. This is perhaps due to the fact that most universities students are within this age group. The age of this sample group showed the compatibility of comparison between samples age group of both countries.  Youth like to participate in the surveys more than older age groups (Smith, 2008; Moore and Tarnai, 2002; Goyder, 1986). In regard to reasoning, when making the choice to purchase a drink, the researcher noted that it would be best to state clearly "non-alcoholic drink" for the UK participants while conducting survey. However, in the KSA survey there was no need to state this due to the fact the alcoholic drinks are prohibited in the KSA by law for religious purposes. The majority of the UK sample rated health benefits as somewhat important as opposed to the KSA sample who rated it as very important and this may trace back to the fact that they are science students. In a study by Pollard et al., in 2002, it was found that the most significant encouraging factors for food choice were health and natural content of the food. This was in the women cohort who consumed higher quantities of F&V. In addition, cost effectiveness had a medium importance in both groups. However, statistically, there was a significant difference between both samples. This may relate to an average socioeconomics level among all university students. As in this study, income levels were not assessed as mainly both sample groups rely on satisfactory income. Furthermore, the UK students mainly depend on their parents, scholarships, or part time jobs for paying their university fees. On the other hand, in the KSA group, education in public universities such as KAU is free and full-time students are granted monthly financial reward during their studies. Whereas private university like Ibn Sina, students depend mostly on their parents or scholarships for university fees.

Convenience means shop and product availability and simplicity/ease of preparation and cooking. This attribute has medium importance among the UK group whereas in the KSA group there were mixed opinion, with no one opinion being significantly higher than the other (very important is partially equivalent to not at all important). This might be because of the high availability of drink products in almost all stores with existing problems such as transportation. Despite the availability of some drinks may be low in some stores in the UK, leading UK customers tend to choose an alternative drink. This also indicates the ease of preparing a homemade smoothie as being of some importance. The availability and accessibility of F&V vary between the UK and the KSA markets. However, there are similarity of some F&V existing in both countries such as tomato, strawberries, oranges, mango, cucumber, lettuce, and broccoli which are locally harvested or imported. In the KSA, importing of F&V in the AGR has increased over the years due to an increase in population and the hot climate, particularly in the summer, hence many vegetables can't grow, as previously discussed in Chapter1. In addition, the display of F&V in supermarkets in both countries are similar and well organised with high quality local and imported exotic varieties. Statistically there is a high significant difference between the UK and the KSA samples with P value of 0.000. Furthermore, in a study by Pollard et al, it was found that a group who had low intake of F&V has indicated that the convenience factor is of higher importance for their intake when compared with than high intake consumers (Pollard et al., 2002).

However, looking at the main finding of the questionnaire, both groups relied mostly on personal preference when choosing to purchase a drink i.e. taste, colour, and aroma as personal food preferences were important factors linked with F&V intake as the studies by of Bere and Klepp in 2004 and Rasmussen et al., in 2006 have demonstrated.

Concerning the daily fruit portions consumed by participants, this question was an example of a rough estimation of the daily fruit intake in both the UK and the KSA samples recalled from the previous day's intake. The number of students in both the UK and the KSA that have not consumed any fruit is very small, which is good. This indicated that fruit consumption is high in both samples. The KSA participants consumed more fruits (as 3 portions a day) than the UK participants. As well as a great deal of dates consumption was perceived in the KSA group only. However, there is a staggering difference between the number of those who ate 3 fruits in the KSA and the UK, with 42% of the KSA sample eating 3 fruits the previous day and a mere 3% of the UK sample eating a third fruit. This indicates that the KSA sample may prefer sweet taste more than the UK sample. Besides, as expected from health students at this level of education, both groups are aware of the portion size such as one cup of fresh fruit juice is equivalent to one whole fruit or 1/4 cup of dried canned fruits. Observing that, both groups considered fruit juice as one portion of their fruit intake.

When comparing the vegetable intake eaten the day before in both groups, it was found to be very close. However, regarding the intake of 1 or 2 portions of vegetables a day, the UK's intake was higher. While, the KSA participants are more likely to go for a third portion when compared with the UK participants. The question measured an estimation of vegetables portion consumption recalled from the previous day's intake. Hence, as expected, the UK participants consumed slightly more vegetables than the KSA participants. Also as expected, both groups were aware of mixed salad, vegetable soup, and frozen vegetable and included them within their vegetable intake.

All in all, most of the students did not achieve the minimum recommended daily five servings. The majority only had an average of two portions a day. In addition, a reliance on fast, calorie-dense, and nutrient-poor foods combined with insufficient funds could put this population at risk for chronic related diseases due to poor nutrition later in life. As mentioned in the UK background studies, the Health Survey in England reported only 17% citizens consumed the 5 A DAY and were aged between 16 and 24 years of age (NHS, 2005). Also, in a study by Chahal in 2007, the results reported from the questionnaire study on the F&V consumption per day stated that only 17% consumed five or more portions of F&V on the previous day. Similarly, it is not surprising, that the study by Chahal found that over 80% of this group of the UK healthcare students failed to eat five portions of F&V daily. The evidence presented by these studies suggested that even the most disciplined person finds it difficult to consume the quantities of F&V recommended by nutrition experts. This was also confirmed in 2003 by a study done by Crocker, in the west including the UK and a study by Musaiger et al in 2012 in the Arab Gulf countries including the KSA.

Moreover, if only vegetable consumption data is highlighted; the number of systematic review assured that its consumption is inadequate and clearly vegetables are less eaten in Europe. Correspondingly, the lower level of antioxidants such as polyphenols, vitamin C, and carotenoids indicated the same results in both the UK and the KSA. Similarly, the KSA public schools have a lack of fruit supply and seldom vegetables in meals provided to their students unlike the UK public schools. In addition, to what have been discussed in Chapter 1, the AGR population fail to consume adequate quantities of F&V, particularly, in children and teenagers in the study by Musaiger et al in 2012.

In Germany and the Netherlands raw and cooked vegetables intake were higher, although none of them provided school lunches for free and children reported that high F&V consumption was encouraged by their family's environmental factor. Therefore, the study findings showed a stronger relation between vegetables intake and providing no free school lunches. Thus, it was proposed that parental intervention is essential when schools offer no vegetables. Moreover, in a study by Domínguez in 2017, pregnant women who were introduced to non-bitter vegetables consequently lead to greater vegetable intake by their children at early stages of life.

This study found that around 88-89% of the UK and the KSA respondents had previously tried a smoothie product and that 80% of the UK students liked smoothies. However, only 58% in the KSA participants like smoothie drink. The UK samples preferred smoothies more than the KSA sample. Also, there were hesitant participant in the KSA sample more than in the UK sample in preference to smoothie. It can be, thus, interpreted that smoothie is a popular drink in both samples but more preferred within the UK students. Statistically speaking, there is a high significant difference between the UK and the KSA samples.

This is a clear indication, as expected, that the smoothie in the both country's markets is a popular drink, but it is more acceptable within the UK. A study by Holland in 2007 identified smoothies as a fairly new product, as they had only been available since 2001, to the majority of the UK population. The introduction of smoothies increased consumer choices considerably and the persuasive marketing has suggested to consumers that these drinks are more of a convenient way to get their 5 A DAY. The findings also corresponded with a study by Drossard et al in 2012 concerning the acceptance of anthocyanin-rich juices amongst children and adolescents. In this study, smoothies received higher positive ratings than juices among 45% to 68% of the student participants. The results also indicated that consuming smoothie is not a regular habit in both samples, as it is been drunk very occasionally.

Optimistic results showed more than half of both samples considered smoothie a part of F&V consumption, which mean that there is an adequate knowledge in both students' samples. However, there is still a need for more awareness as nearly a quarter of each country's sample were not sure whether smoothie can be considered as a F&V intake or not. This was expected, as the work of Ruxton in 2008 revealed that the gap between knowledge of the 5 A DAY and action is widening. This suggesting that greater awareness that smoothies are part of the 5 A DAY recommendation and that 250mL equals one portion should be promoted. According to NHS in 2015 and Department of Health in England, in the Eat-well Guide in 2016, it was confirmed that 100% fruit juice, vegetable juice, and unsweetened smoothies can count as a maximum of one portion of the 5 A DAY only and if F&V is combined in a smoothie it should not be more than 150mL a day. However, the company Innocent claimed that a 250mL smoothie containing F&V is equal to 2 portions of the 5 A DAY.

A close focus to the KSA sample was needed as 29% of them are confident that smoothies are not part of their F&V intake. In Saudi, more awareness is needed on smoothies being part of F&V intake.

The survey also resulted in that overall the UK participants consumed more vegetable products than the KSA sample. In addition, the majority of the two samples never drank vegetable drinks, while in the UK 23% participants occasionally drank it. A study by Memish et al in 2014 found a strong relation between obesity in and low vegetables intake in KSA.

The participants' responses suggested that the consumption of vegetables is notably low among the student's population (see Figures 2.11 and 2.12). A study by Blacker and Creanor in 2011 compared two fruit-based smoothies and demonstrated that they both had low pH levels and relatively high titratable acidity. This led the researchers to suggest that consumption of fruit smoothies, which are made from pure fruit blended with 100% fruit juice, should be limited to meal times. Furthermore, it should be consumed through a straw, similar to other acidic soft drinks. A vegetable-based smoothie may help to resolve these problems by increasing vegetable consumption and perhaps reducing the pH levels of the smoothie.

The responses received in the questionnaires demonstrated that consumers would accept a novel drink which includes vegetables. More than half the UK questionnaire population at 62% accepted the idea of consuming 100% vegetable-based drink and 50% of the sample is ready to try a vegetable combined with fruit-based smoothie.

According to the questionnaire results, the majority of participants in both the KSA and the UK samples prefer pure smoothie. Also, the acceptability among participants of vegetables to be in a smoothie is more in the UK sample than in the KSA sample (Figure 3.13). Therefore, the tasting session and the business introductory investigation of such commercial smoothies were done in the UK. As expected, 60% of the UK sample had never consumed vegetable drinks such as V8. Likewise, 73% in the KSA sample never consumed vegetable drinks.

The results indicated that it is probably desirable to make a combined F&V–based smoothie, as respondents indicated that their preference was toward a fruity drink which had a sweet taste but was neither savoury nor salty. Furthermore, the respondents preferred pure smoothies without additions such as yoghurt. Based on these results, a pure smoothie was made without any additions.

Taste was confirmed to be the most important motivating factor, which would encourage both countries' samples to drink vegetable-based smoothie (Figure 3.18). Food preferences can be developed considerably during childhood and by time might be altered through adulthood (Havermans et al., 2010). In addition, in the KSA sample, "perceived health benefit" and "increase fruit and vegetable" were encouraging motives more than in the UK sample.

Only 10% of the UK participants chose to have a smoothie drink as a snack. Whereas in the KSA sample there were mixed opinions, as the same number of participants consider smoothie as a snack to be “very important” and “not at all important”. In general, the reasons “health benefits” and “increasing fruit and vegetable” consumption are more important among the KSA sample than the UK sample. Additionally, smoothies are not important to replace fruit and vegetable in both samples. In the KSA group, it is “very important” to drink smoothie instead of hot and cold drinks i.e. tea, coffee, and fizzy drinks but in the UK sample it is “of some importance”.

After finding out the most favoured fruit of the two groups, it was clear to the researcher that they are almost mandatory ingredients in order to achieve the majority’s preference. Strawberry was the most appealing fruit to be in a smoothie in both samples; hence, the researcher used strawberry, orange, and apple in different smoothies preparing for the tasting session whereas banana was excluded in order to avoid high viscosity.

This study recommended that lemons, melon, and guavas could be possible ingredients in smoothie for the KSA in future studies. While, pineapple and tomato could be used for further smoothie study in the UK.

Looking at the results outcome for the most favoured vegetable to eat and the most appealing to be in a smoothie in the both the UK and the KSA samples, the response was carrot. Hence, it was used in the design of the smoothie in order to achieve the majority preference. Cucumber, on the other hand, was excluded because it was evaluated as undesirable ingredient during the pilot tasting session.

To further determine the eventual composition of an acceptable smoothie, the tasting sessions were organised. Six out of 71 smoothies suggested from the questionnaire were presented to participants to evaluate. The tasting session was designed to determine which of the six potential smoothie recipes would be acceptable to the consumers. The final smoothie chosen (901) was made of 47% vegetables consisting of 83g beetroot and 30g red pepper and 53% fruit consisting of 70g strawberry and 60mL orange juice.

Studies such as those by Villegas et al., in 2008 and Barclay in 2008 have revealed a positive association between an increased consumption of green leafy vegetables and a reduction in chronic disease risk. However, the smoothie with green leafy vegetables was not popular in the focus group due to the gritty texture and the green bits in the drink. Instead, a beetroot and red pepper smoothie was chosen. There have been limited reports in the literature concerning the creation of new vegetable smoothies or drinks, some of which will be discussed later.

The estimated prices that participants from the KSA sample could invest on a smoothie were, 10SR for a 250mL bottle and 20SR for 1L. Similarly, the UK sample estimated prices were, £2 for a 250ml bottle and £3 for 1L of a smoothie drink.

On the commercial point of view, student's limited budget must be taken into account when preparing smoothies. Low price as a motive encouraged the UK participants more than the KSA ones. While, the motive "smoothie replacing fruit and vegetable" was not encouraging to both samples when choosing to drink vegetable smoothies.

### 2.5.1 Commercial Potential Discussion

The hypothesis for the commercial approach is that the designed smoothie will be a commercial product in the UK. The smoothie 901 could potentially be used in a commercial brand in the UK market after resolving the barriers from the producer's questionnaire disseminated to two large UK companies – one for beverages company and the other one for grocery company. The barriers included high pH, technical difficulties, and consumer acceptability. This study can partially help companies with data about consumer acceptability, measurement of smoothie pH level, and stability of smoothie nutrients and consequently shelf life.

In terms of the price, £2.99 for a 250 ml could be suitable for specialised brands such as Innocent and M&S, which offer impulse but not grocery prices. However, under-utilised and low-grade F&V could be used as an economic means to produce vegetable-based smoothies at low prices, as noted in the recent review by Bhardwaj and Pandey in 2011.

Emphasis on the marketing and sales of smoothie makers and smoothie recipe books is a positive movement, since home-made vegetable and fruit smoothies have been shown to be nutritionally beneficial and cost effective in this thesis. An initiative in Newcastle was launched in 2006 by Jamie Sadler as part of the East End Community Health Project (EECHP) to educate children in healthy eating by setting up smoothie bars in local schools. The drinks were made up of crushed fruit and fresh fruit juices, and each represented three portions of fruit per serving to each child (Holland, 2008).

A recent review by Bhardwaj et al in 2011, considered convenient and economic approaches to consuming underutilised and spoiled fruits and vegetables. They suggested blending two or more underutilised F&V juices with spices extract for the preparation of natural F&V based nutritive beverages. Most F&V juices turn bitter after extraction due to the conversion of chemical compounds. In spite of being underutilised, the consumption of highly nutritive F&V mixtures is very limited due to high acidity, astringency, bitterness, and some other factors. While improving the flavour, palatability and nutritive and medicinal value of various fruit juices such as mango, papaya, pineapple, citrus, pear, apple, and watermelon and vegetables including carrot, beetroot, and bitter gourd and medicinal plants like Aloe Vera and spices can also be used for juice blending. All these natural products are valued very highly for their refreshing juice, nutritional value, pleasant flavour, and medicinal properties (Bhardwaj and Pandey, 2011).

Taking into account that breaking into the commercial market is complex and requires a strong product to stand any chance of being a competitor or player in a new market. Hence, the work of this present project will indicate some of the smoothie market barriers.

## 2.6 QUESTIONNAIRE AND TASTING SESSION CONCLUSION

The project commenced with scoping surveys in the UK and the KSA to ascertain consumer awareness and acceptability of fruit & vegetable-based smoothies. This was followed by a taste panel to test a range of potential recipes. From this panel a compromise between consumer acceptance and potential nutritional improvement was made and a recipe was selected for more detailed study.

From the questionnaire, it has been found that vegetable-based drinks would be acceptable to the UK consumers (university students).

To conclude, the tasting sessions results confirmed that the (901) smoothie containing 47% vegetable (beetroot and red pepper) and 53% fruit (fresh strawberry and orange juice) was acceptable to the consumer sample i.e. students in the UK.

# CHAPTER 3 NUTRITIONAL & AESTHETIC QUALITIES OF SMOOTHIES - LABORATORY ANALYTICAL STUDIES

The previous chapter identified some “acceptable” recipes for vegetable containing smoothies, with the 901 smoothie being the most acceptable. Laboratory studies were then carried out to address the final aim of the thesis, as described in the aim and objectives earlier - to evaluate this smoothie in terms of content, variability with season and retention and stability of some selected nutrients. These tests were broken down into phases, each designed to address the various hypotheses from section 1.9.

H2 was that the 901 smoothie would be nutritionally superior to those commercially available. The first phase was thus concerned with measuring the levels of Total Ascorbic Acid (TAA), Total Antioxidant Capacity (TAC), Total Phenolics Content (TPC), sugars and fibre in the 901 smoothie and comparing these values to those in selected commercially available products.

H3 was that the nutritional content would vary with seasons. Thus, the second phase of tests was concerned with monitoring the nutritional content of the 901 smoothie at different times of the year. The ingredients were obtained from the same commercial source at four sampling points throughout the four seasons of the year. Since any observed variation may be due to seasonal variations in the ingredients used for the smoothie, a preliminary investigation was also carried out to ascertain the potential variations in the ingredients throughout the year.

H3 also stated that the various nutrients would be retained during processing but since some are potentially labile their levels may not be stable during storage. Therefore, in phase three and four the retention and stability over 5 days of storage at 4°C of TAA, TAC, TPC, total sugars, and pH in the 901 smoothie were determined.

H4 was that the texture would be a major barrier to smoothie acceptability. The tasting sessions, discussed in Chapter 2, confirmed this assertion. H4 also stated that treatment with plant cell wall degrading enzymes may be a way to overcome this barrier. To test this hypothesis the last phase examined the effect on the rheological properties of the smoothie following treatment with enzymes. The 134 smoothie was included in this last phase, along with the 901. The 134 smoothie contained a higher amount of vegetable (76%) and was thus more viscous than the 901 smoothie and as such a potentially better candidate for this preliminary investigation. It was considered that their viscosity levels needed to be reduced in order to reach a suitable consistency that would be closer to a commercial smoothie. Thus, a commercial smoothie was included as the comparator.

# 3.1 MATERIALS AND METHODS TO MEASURE THE NUTRITIONAL CONTENT OF SMOOTHIE 901

The ingredients for smoothie 901 were purchased from Tesco and prepared using a Kenwood food mixer. The ingredients consisted of 83g beetroot, 30g red pepper, 70g strawberry, and 60ml orange juice as listed in Table 2.5. All the ingredients were blended together with no water added for 2 minutes on the highest speed, until all the ingredients were homogenised as assessed visually (section 2.3.2.1). Then, five gram samples were added to 25mL of 1.5% meta-phosphoric acid for the vitamin C and FRAP buffer for the total antioxidant assay and methanol for total phenol assay. The extracts were then centrifuged for 5 min at 2000g. For sugar analysis, 1g of smoothie was homogenised in 99mL of distilled water.

Total Ascorbic Acid (TAA) was measured immediately (dilution 1:10). This was followed by determination of Total Antioxidant Capacity (TAC) through FRAP assay (dilutions 1:10 and 1:20). The Total Phenolic Compounds (TPC) (dilutions 1:5 and 1:10) and sugar content (dilution 1:100) were measured the following day. The pH was measured on the day of preparation and on the next 5 days of storage at 4°C. Extracts were stored at 2–4°C to measure fibre. The smoothies were subjected to extensive homogenisation prior to analysis. Replicate samples showed minimal standard deviations between readings in most cases suggesting that further replication was not required.

## 3.2 MATERIALS AND METHODS OF MEASURING NUTRIENTS IN INDIVIDUAL INGREDIENTS OF SMOOTHIE 901

The individual ingredients of 901 smoothie (beetroot, red pepper, strawberry and orange juice) were analysed independently for their nutritional composition in terms of ascorbic acid, phenols, antioxidant activity, sugar and fibre. Nutrient extraction and assays in each case used tissue from three different packs, (or beetroot, red pepper or strawberry) that were combined and chopped. Five grams samples of each tissue were then added to 25mL of 1.5% meta-phosphoric acid for the vitamin C, FRAP buffer for the total antioxidant assay or methanol for total phenol assay and homogenised separately using a Kenwood smoothie maker until the sample was fully broken down. The extracts were then centrifuged for 5 min at 2000g. Five millilitres of Innocent orange juice were tested without dilution for vitamin C, FRAP and phenol analysis. For sugar analysis, 1g of tissue was homogenised in 99mL of distilled water. All nutrients were then analysed as described above for the 901 smoothie.

The smoothie content was analysed on 5 separate occasions, Oct. 2011, Nov. 2011, Oct. 2012, Jan. 2017 and Feb 2017. The homogenisation was carried out and a number of replicates were used to provide an indication of deviation. The deviations in most cases were considered to demonstrate adequate homogenisation. Similarly, realistic values for nutrient recovery were also indicative of a suitable sampling regime.

In terms of the retention of nutrients during the preparation of the smoothie 901. The nutrient composition of the basic ingredients was determined at the same time as the smoothie 901 was prepared for the nutrient stability assays (i.e. on day 1) and analysed by the same methods as described above. This experiment was repeated over three consecutive weeks and each week was analysed independently to establish repeatability.

For stability, the smoothie 901 was prepared and nutrients analysed directly at day 1. The smoothie was then stored at 4°C for 5 days and nutrients were measured on day 3 and then day 5. The preparation of the 901 smoothie and nutrient analyses were carried out as described above. The n refers to the number of replicates taken for each ingredient at each time.

### 3.2.1 Total L-Ascorbic Acid (TAA)

Ascorbic acid was measured using the BOEHRINGER MANNHEIM/R-BIOPHARM Enzymatic BioAnalysis/Food Analysis – Colorimetric Method.

The kit comes with the following ready prepared material:

- Solution 1 (undiluted), consisting of sodium phosphate/citrate buffer, with pH approx. 3.5, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT);
- Ascorbate oxidase (AAO) spatulas; and
- Solution 3 (undiluted), containing approx. 4 ml 5-methylphenazinium methosulfate (PMS) solution.

#### 3.2.1.1 Assay procedure

Ascorbate was measured following the manufacturer's instructions as summarised in Table 3.1.

| Pipette into cuvettes | Blank(B) | Sample (S) |
|---|---|---|
| Distilled water (warmed to ≈37ºC) | 1.50mL | 1.52mL |
| Solution 1 (buffer) | 0.50mL | 0.50mL |
| Sample | 0.10mL | 0.10mL |
| Suspension 4 (Ascorbic acid oxidase) | 0.02mL | - |
| Mix & incubate for 3 min at 37ºC in a water bath. While incubating mix the content of the blank (B) and sample (S) assays every 1 min for about 5s, then add: | | |
| Solution 2 (MTT buffer) | 0.20mL | 0.20mL |
| Mix & incubate for 2-3 min at 37ºC. While incubating, mix the contents every 1 min for about 5s. then read the absorbance of (B) and (S) assays ($A_1$). The reaction mixtures, at this stage are sensitive to light, avoid standing these cuvettes in the light. Start the reactions by addition of: | | |
| Solution 3 (PMS) | 0.20mL | 0.20mL |
| Mix & read the absorbances of the solutions ($A_2$) immediately one after other at the end of the reaction (approx. 8min at 37ºC). | | |

**Table 3.1: Assay procedure for measuring L-ascorbic acid**

The absorbance was read at 578nm. The L-ascorbic acid content was calculated by determining the absorbance differences ($A_2$-$A_1$) for both sample and blank. The absorbance difference of B was subtracted from the absorbance difference of S to obtain

$$\Delta A_{\text{L-ascorbic acid}}: \Delta A = (A_2 - A_1).$$

The measured absorbance difference should, as a rule, be at least 0.100 absorbance units to achieve sufficiently reliable results.

The concentration of L-ascorbic acid was then calculated as follows:

$$C = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{\text{L-ascorbic acid}}\ [g/L]$$

V = final volume [mL]

MW = molecular weight of L-ascorbic acid [g/mol]

$\varepsilon$= extinction coefficient for MTT-formazan at 578nm

= $16900[l \times mol^{-1} \times cm^{-1}]$

d = light path [cm]

v = sample volume [mL]

It follows:

$$C = \frac{2.52 \times 176.13}{16900 \times 1.0 \times 0.1} \times \Delta A_{\text{L-ascorbic acid}}\ [g/L]$$

$$= 0.2626 \times \Delta A_{\text{L-ascorbic acid}}\ [g/L]$$

The concentration of L-ascorbic acid obtained from this formula was then converted to (mg/100 mL) for smoothies and (g/100g) for solid samples.

$$= \frac{c_{\text{L-ascorbic acid}}\ [g/L \text{ sample solution}] \times 100\ [g/100g]}{\text{Weight}_{\text{sample}}\ [g/L \text{ sample solution}]}$$

This varies according to the dilution utilised.

### 3.2.2 Total Antioxidant Capacity (TAC)

TAC was measured using the Ferric Reducing Antioxidant Power (FRAP) assay and was carried out as described by Benzie and Strain (1977). This method was chosen because it was available in the university lab and it is widely used in the literature.

#### 3.2.2.1 Solutions

1) Acetate buffer (300 mM): 1.55g sodium acetate trihydrate + 8 ml glacial acetic acid, made up to 500 ml with distilled water and adjusted to pH 3 using NaOH

2) Triphenyltetrazolium chloride (TPTZ) solution (10 mM): 0.0156g TPTZ + 5 mL 40 mM Hydrogen chloride (HCL)

3) Iron lll chloride hexahydrate (20 mM): 0.0271 g iron lll chloride + 5 mL distilled water.

The FRAP reagent was made up fresh each day by mixing 25 mL acetate buffer with 2.5 mL TPTZ solution and 2.5 mL iron lll Cl.

4) Trolox standard stock solution (1mM): 0.0050g Trolox + 20mL ethanol.

#### 3.2.2.2 Assay procedure

A series of Trolox standards were prepared from the Trolox stock, as shown in (Table 3.2).

| Trolox Standards | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
|---|---|---|---|---|---|---|
| Trolox (mL) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| Ethanol(mL) | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0 |
| Concentration (μmoles/mL) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1 |

**Table 3.2 Trolox standard curve for FRAP assay.**

A 96 well micro assay plate was prepared by loading each of the following in duplicate: 10 μl of ethanol (blank), 10 μl of each Trolox standard, and 10 μl samples. Then, 100 μl of FRAP regent was dispensed into all wells. The plate was left to stand for 10 min and then read at 550nm in a microplate reader. Values obtained were expressed as μmoles Trolox equiv (TE) /100mL. A typical standard curve is shown in Figure 3.1.

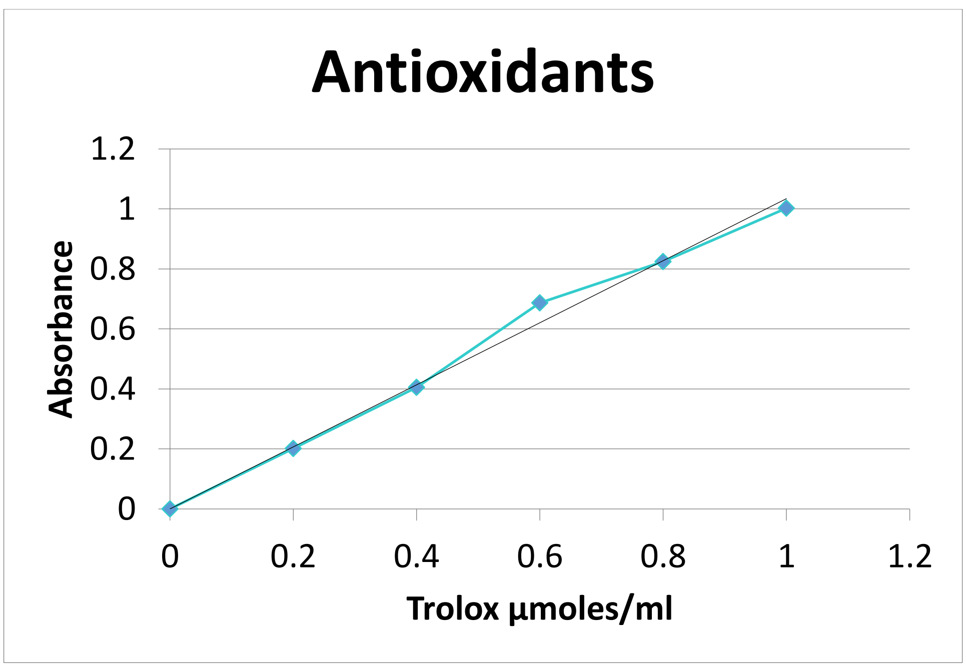



**Figure 3.1 Typical standard curve for measuring FRAP assay**

### 3.2.3 Total Phenolic Compounds (TPC)

The Folin-Ciocalteu method was used to measure total phenolic concentration (Ignat et al., 2011).

#### 3.2.3.1 Solutions

1) Standard solution of Gallic acid (GA): dissolved 10mg GA + 2mL ethanol + distilled water make up to 10mL, to give final concentration of 1000µg/mL.

2) Undiluted Folin-Ciocalteu reagent

#### 3.2.3.2 Assay procedure

Standards were prepared from the standard (GA) mg/mL, as shown in Table 3.3.

| GA (mL) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
|---|---|---|---|---|---|---|
| Distilled Water (mL) | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0 |

**Table 3.3 Gallic acid standard curve for measuring total phenolics**

0.1mL of the standards and the samples were placed into large glass tubes in duplicate. For the blank, 0.1mL of distilled water was added. It was followed by adding 6mL distilled water to each tube. Then 0.5mL of undiluted Folin reagent was added to all tubes. Assays were left to incubate at 30°-40°C for 2 hrs. Transfer the assays into semi-micro cuvettes in duplicate to read absorbance at 765nm, using the blank as a reference.

A typical standard curve is shown in Figure 3.2. The values obtained were expressed as mg GA equivalence (GAE)/100 mL.

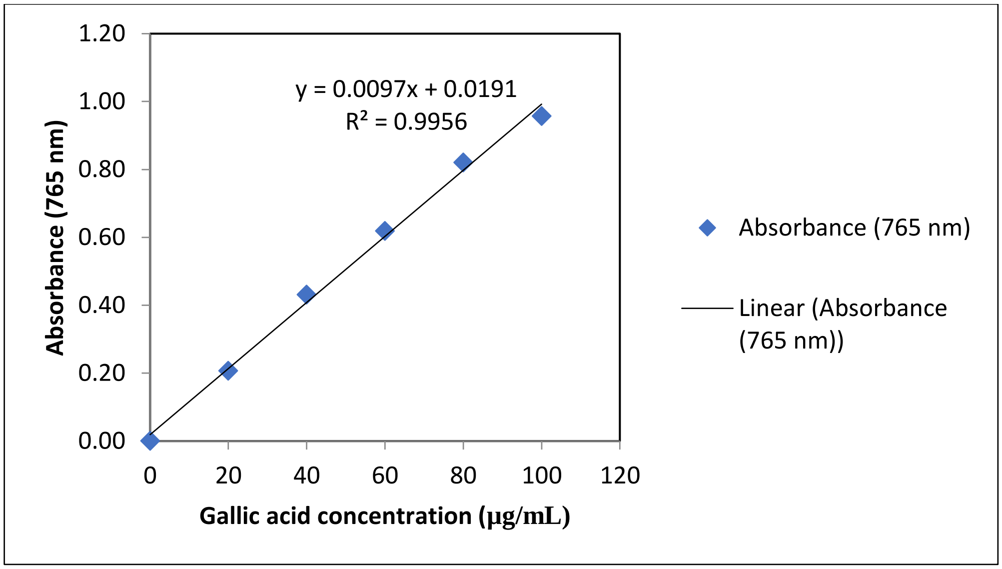



**Figure 3.2: Typical standard curve for total phenol assay**

### 3.2.4 Sugar Content

The amount of sugar present was monitored using a sucrose/D-glucose/D-fructose kit supplied by Boehringer Mannheim.

#### 3.2.4.1 Assay procedure

The samples were prepared and diluted, using 1 ml of sample to 99 ml of re-distilled water. The assay was then carried out as shown in Table 3.4.

| | **Blank sucrose sample** | **Sucrose sample** | **Blank D-glucose/D-fructose sample** | **D-glucose D-fructose sample** |
|---|---|---|---|---|
| **Solution 1 ***<br>**Sample solution** | 0.200mL<br>- | 0.200mL<br>1.6mL | -<br>- | -<br>1.6mL |
| Mix by gentle swirling, incubate for 5 min at 37°C (before pipetting warm up solution 1 to 37°C). Addition of: | | | | |
| **Solution 2** | 1.000mL | 1.000mL | 1.000mL | 1.000mL |
| Mix with a plastic spatula, read absorbances of the solutions after approx. 3min at 340nm ($A_1$). Start reaction by addition of: | | | | |
| **Suspension 3** | 0.020mL | 0.020mL | 0.020mL | 0.020mL |
| Mix with a plastic spatula, wait for completion of the reaction (approx. 15 min) and read absorbances of the solutions at 340nm ($A_2$).<br>If the reaction has not stopped after 15 min, continue to read the absorbances at 2 min intervals until the absorbance increases constantly over 2 min. Addition of: | | | | |
| **Suspension 4** | - | - | 0.020mL | 0.020mL |
| Mix with a plastic spatula, read absorbances of the solutions after 15 min ($A_3$) | | | | |

**Table 3.4: Methodology for sugar estimation**

*Pipette solution 1 and each sample solution onto the bottom of cuvette and mix by gentle swirling. The calculation was performed as follows. The absorbance differences (A2–A1) for both blanks and samples was determined. The absorbance difference of the blank was subtracted from the absorbance difference of the corresponding sample.

$$\Delta A = (A_2\text{-}A_1)_{\text{sample}} - (A_2\text{-}A_1)_{\text{blank}}$$

The difference between $\Delta A_{\text{total D-glucose}}$ (from the sucrose sample) and $\Delta A_{\text{D-glucose}}$ (from the D-glucose sample) yielded $\Delta A_{\text{sucrose}}$.

D-fructose was then determined as follows. The absorbance differences ($A_3$–$A_2$) for the blank and sample (D-glucose/D-fructose) were determined. The absorbance difference of the blank was subtracted from the absorbance difference of the sample. This resulted in $\Delta A_{\text{D-fructose}}$. The measured absorbance differences should, as a rule, measure at least 0.100 absorbance units to achieve sufficiently precise results.

According to the general equation for calculating the concentrations:

$$c = \frac{V \times MW}{\epsilon \times d \times v \times 1000,} \times$$

V = final volume (mL).

MW = molecular weight of the substance to be assayed (g/mol),

d = light path (cm),

Є = extinction coefficient of NADPH at 340 nm = 6.3 [L × $mmol^{-1}$ × $cm^{-1}$],

v = sample volume (mL).

*For sucrose it follows:*

$$c = \frac{3.020 \times 342.3}{\epsilon \times 1.00 \times v \times 1000} \times \Delta A_{sucrose} = \frac{Z}{\epsilon} \times \Delta A_{sucrose}$$ [g sucrose/L sample solution].

*For D-glucose it follows:*

$$c = \frac{3.020 \times 180.16}{\epsilon \times 1.00 \times v \times 1000} \times \Delta A_{glucose} = \frac{B}{\epsilon} \times \Delta A_{glucose}$$ [g glucose/L sample solution].

*For D-fructose it follows:*

$$c = \frac{3.020 \times 180.16}{\epsilon \times 1.00 \times v \times 1000} \times \Delta A_{frucrose} = \frac{C}{\epsilon} \times \Delta A_{fructose}$$ [g fructose/L sample solution].

The concentration of sugars obtained from the formula was converted to g/100 mL.

### 3.2.5 Determination of Insoluble Matter as an Estimate of Fibre in Food

Using a home based method, a 50ml lidded tube was weighed, and then 30ml sample added and reweighed. It was centrifuged at 3000rpm (~650g) for 10 minutes and the supernatant carefully removed and discarded it. Then 30ml water was added (pure/Elix) and the pellet re-suspend. It was centrifuged at 3000 rpm for 10 minutes and then the supernatant was discarded. This step was repeated twice using 2 x 30ml water. Next, the pellet was re-suspended using 30ml ethanol and then centrifuged. This step was repeated twice using 2 x 30ml 80% ethanol. The supernatant was discarded, and then the tubes were inverted in the rack (over tissue paper) for 30 minutes and placed in an oven at 100°C for 48h or to a constant weight by grams. The values are /100g. The calculation is Y/100g=Reading x100/30.

## 3.3 MATERIALS AND METHODS OF RHEOLOGICAL ANALYSIS OF SMOOTHIES AND EFFECT OF ENZYMES

The viscosity in this study was measured using a computer-controlled 'Anton Paar' rheometer, which monitors viscosity at an increasing shear stress and generates a flow curve from which the intrinsic viscosity value can be calculated. The shear viscosity of the smoothies was evaluated at 20°C using a rotational rheometer (MCR301, Anton Paar, Graz, Austria) fitted with a concentric cylinder geometry (bob diameter: 27 mm, bob length: 40 mm, cup diameter: 29 mm). The shear rate was decreased from 500 to 1 $s^{-1}$ recording steady state viscosity data at 14 logarithmically spaced shear rate values. The data were fitted with the power law model utilising the instrument's software and used to generate K and n values.

A commercially obtained Innocent smoothie was used as a reference for the viscosity studies. All analyses were carried out at 20°C as this was technically more appropriate. It should be noted that the methodology did not mimic the chilled temperature at which the smoothie would usually be consumed. However, it was felt that since the aim was to achieve a reduction in viscosity and make a comparison with commercial smoothies', temperature was not the major concern. The two enzyme cocktails were selected based on their predicted target cell wall substrates. "Pectinase" was a commercial cocktail from Sigma based around pectinases and "Celltec2", a commercial cocktail from Novazyme based on cellulases. These are the two major cell wall polymers. The precise composition of each cocktail was not available.

## 3.4 RESULTS OF NUTRITIONAL COMPARISON CONTENT OF SMOOTHIE 901 AND COMMERCIAL SMOOTHIES

The average nutritional value of the smoothie 901 is shown in table 3.5 and this was compared with some of the most popular commercial smoothie products in the UK market, namely Innocent banana and "serious strawberry". Also, smoothie 901 was compared with some recent comparable F&V

smoothies which are popular products in the UK market such as Innocent gorgeous greens smoothie, Naked green machine and Naked bright beets. All smoothies were purchased from Tesco or from the student café at Nottingham University, UK. The same methods for the 901 smoothie described above, were repeated using the commercially obtained Innocent and Naked smoothies and these were compared with the average values of the 901 smoothie (Table 3.5).

| Nutrient /100mL | Smoothie 901 | Innocent seriously strawberry | Innocent gorgeous green | Naked green machine | Naked bright beets |
|---|---|---|---|---|---|
| TAA (mg) | 30 ±5.6 | 12 | 12.4 | 10 | 0 |
| TAC (µmoles) | 1031 ± 163 | 553 | 253 | 396 | 935 |
| TPC (mg) | 57 ±27 | 16 | 48 | 58 | 93 |
| Sugar (g) | 9 ±0.68 | 11 | 8 | 20 | 8 |
| Fibre (g) | 5.9 ±0.4 | 2.4 | 1.7 | 4.2 | 2.3 |

**Table 3.5 Nutritional comparison between the smoothie 901 (n=5) and a "representative" sample from commercial smoothies.**

The range of values for the smoothie 901 were used for comparison with commercially obtained Innocent seriously strawberry, Innocent gorgeous green, Naked green machine, Naked bright beets smoothies (Table 3.5). Total ascorbic acid was two and a half times higher in 901 than in the innocent smoothies and three fold higher than the Naked green machine. Interestingly the Naked bright beets contained no detectable ascorbate. Total antioxidant capacity was much greater in 901 than in the innocent smoothies but comparable to the level found in Naked bright beets. The other three smoothies had an intermediate level. Total phenols were three times higher in 901 than innocent serious strawberry and lower than Naked bright beets, but comparable with the other two smoothies. Total sugar in 901 was lower than Naked green machine but comparable with the other smoothies. Dietary insoluble fibre was much higher in 901 smoothie than the four Innocent and Naked smoothies. The manufacturers of the smoothies placed the nutrient information on their products. The nutritional information for the four commercial smoothies used in this investigation is shown in Table 3.6.

| Vitamin C/mg | On the label /100mL | Lab value /100mL | Lab value /250mL |
|---|---|---|---|
| Smoothie 901 | - | 30 | 75 |
| Innocent seriously strawberry | 10 | 12 | 30 |
| Innocent gorgeous green | 23 | 12 | 30 |
| Naked green machine | 9 | 1 | 35 |
| Naked bright beets | 0 | 0 | 1.5 |

**Table 3.6 Comparison of actual lab values of vitamin C in smoothie 901 and commercial popular smoothies in the UK market from package labels (Innocent seriously strawberry & Innocent gorgeous green n=4) (Naked green machine & Naked bright beets n=6)**

Comparison of the nutrient content written on the commercial smoothie labels with the actual laboratory-derived values showed similar values. The comparison of amount of vitamin C in mg/250mL is shown in Table 3.6. Only one commercial smoothie i.e. Innocent gorgeous green gave significantly different results between the label and the measured values.

### 3.4.1 Comparison of Theoretical Values from McCance and Widdowson and Actual Smoothie Content

It was important to establish how accurately the nutritional content of the 901 smoothie, as measured in the laboratory, could be predicted through the use of the theoretical nutrient composition values from McCance and Widdowson (2008). Similarly, since the Innocent (strawberry and banana) smoothies had a nutritional label, it was decided that it would be interesting to compare the label value for the nutritional composition of this smoothie with the actual lab values, and the theoretical values calculated from McCance and Widdowson (2008). The values for each nutrient in smoothie 901 were gathered from McCance and Widdowson tables, which were added together and then compared to the laboratory values (Table 3.7).

| Nutrients | Actual Lab value/100mL | Estimated from M&W/100ml |
|---|---|---|
| **Total Sugar** | 8.75g | 7.6 g |
| **Fibre** | 1.26g | 1.48 g |
| **Vitamin C** | 31mg | 55.6 mg |

**Table 3.7 Comparison of 901 nutrient composition as determined by laboratory analysis and estimated from McCance & Widdowson's reference values**

The estimated values for sugar and fibre were very similar to those determined experimentally. It can be thus accepted that the nutrient databases are an accurate predictor of sugar and fibre levels for smoothies. However, the vitamin C value as estimated using McCance and Widdowson gave a higher value than the laboratory result. This probably reflects the greater instability, and variability of vitamin C in the various ingredients.

The equivalent comparisons for the Innocent smoothie are shown in table 3.8. The Innocent label only listed carbohydrate, total sugar, fibre and vitamin C content. In this case the values on the label and those measured in the laboratory were very similar. The values as estimated from the nutritional database are also very similar, apart from fibre, which is much higher, possibly as a result of blending process.

| Nutrient | From the label /100mL | Estimated from M&W/100mL | Actual Lab value /100mL |
|---|---|---|---|
| **Carbohydrate** | 12g | 14.8 g | 12g |
| **Of which Sugar found naturally in the fruit** | 10.5g | 14.35 g | 11g |
| **Fibre** | 0.8g | 1.3 g | 0.86 g |
| **Vitamin C** | 10mg | 21 mg | 12mg |

**Table 3.8 Comparison of laboratory measured, theoretically calculated and label values for Innocent (strawberry & banana) nutrient composition**

The results indicate that the quality control at Innocent probably does not rely on the use of nutritional databases such as McCance and Widdowson. More importantly, it shows that the laboratory techniques used in this project provided values very similar to those reported on the commercial label for most nutrients. In both the cases, the predicted nutritional composition from McCance and Widdowson, in terms of vitamin C, was very different from those actually measured in the laboratory or reported on the label. It was also noted that the nutritional composition of the 901 smoothie was variable throughout the year (Table 3.9). One possible reason for this was that the nutritional value of the various ingredients is variable. Thus, the next set of experiments was designed to look at nutrient content in the raw material over time.

## 3.5 RESULTS MEASURING NUTRIENTS IN INDIVIDUAL INGREDIENTS OF SMOOTHIE 901

The smoothie content was analysed on 5 separate occasions, Oct. 2011, Nov. 2011, Oct. 2012, Jan. 2017 and Feb 2017. The nutritional contents for the individual smoothie "901" on the 5 separate occasions are shown in Table 3.9 and their averages in Table 3.10.

| **Nutrient /100ml** | **Oct 2011** | **Nov 2011** | **Oct 2012** | **Jan 2017** | **Feb 2017** |
|---|---|---|---|---|---|
| TAA (mg) | 36 ±4 | 32 ±2 | 25 ±8 | 27 ±12 | 31 ±2 |
| TAC (µmoles) | 1047 ±0.04 | 1050 ±0.07 | 930 ±0.05 | 1309 ±0.06 | 820 ±3.8 |
| TPC (mg) | 49 ±0.35 | 28 ±0.8 | 32 ±1.02 | 90 ±0.4 | 87 ±0.2 |
| Sugar (g) | 8.6 ±0.55 | 9.3 ±0.3 | 8.7 ±0.19 | 9.7 ±0.7 | 9.6 ±1.7 |
| Fibre (g) | 6.6 ±0.47 | 5.9 ±0.1 | 5.8 ±0.77 | 6.2 ±0.3 | 6.3 ±0.4 |

**Table 3.9 Nutritional analysis of smoothie 901. (The 901 smoothie was prepared on five separate occasions and the nutrient content analysed).**

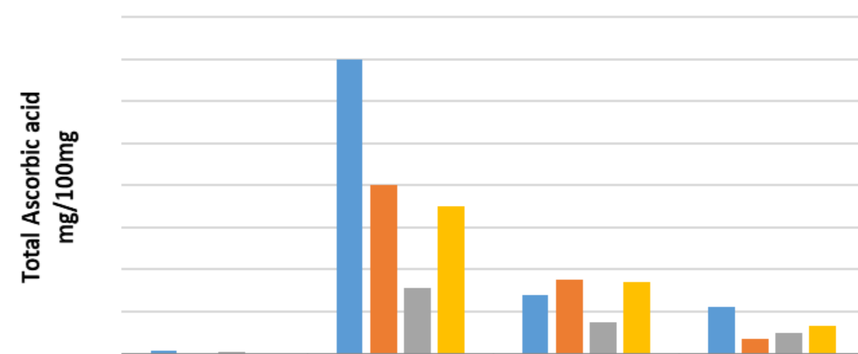
| Nutrients | 901 Smoothie Average/100mL |
|---|---|
| **TAA** mg | 30.2 |
| **TAC** µmoles | 1031 |
| **TPC** mg | 57 |
| **Total Sugar** g | 9.18 |
| **Fibre** g | 6.16 |
| **pH** | 4 |

**Table 3.10 Average nutritional composition of smoothie 901 (n=6)**

The following sections present the results for the individual ingredients assayed at four different seasons.

### 3.5.1 RESULTS OF VARIATION IN TOTAL ASCORBIC ACID (TAA)

The results for total ascorbic acid content are shown in Figure 3.3.



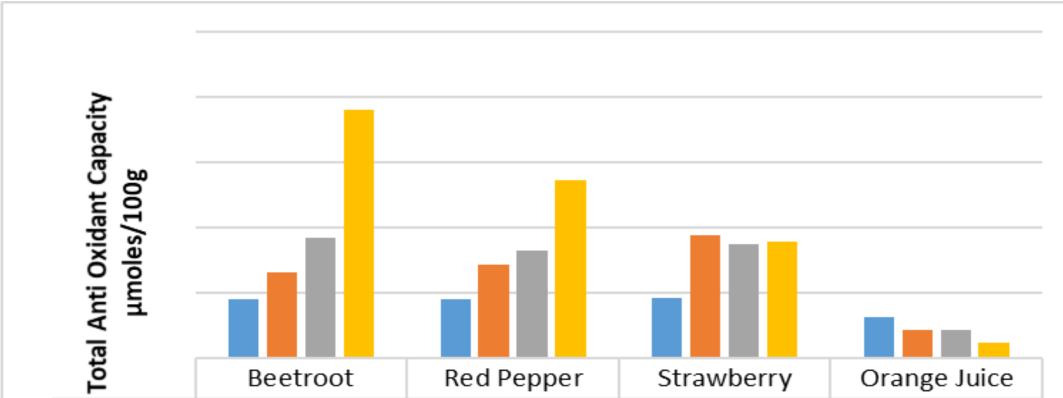

| | Beetroot | Red Pepper | Strawberry | Orange Juice |
|---|---|---|---|---|
| Winter | 1.6 | 140 | 28 | 22 |
| Spring | 0 | 80 | 35 | 7 |
| Summer | 1.1 | 31 | 15 | 10 |
| Autumn | 0.2 | 70 | 34 | 13 |

**Figure 3.3 Variation in TAA of the ingredients used for preparing 901 smoothie (n=1).**

The above results showed that there was variation in ascorbate content of all four ingredients. There were very low levels of ascorbic acid in the beetroot. Red pepper generally contained the highest ascorbic acid content especially in the winter and had the greatest variation in levels showing the lowest TAA in the summer. The season for red peppers is from August to October (late summer to mid-autumn). Total ascorbic acid in strawberry was highest during spring and autumn, as the season for strawberries is late spring and early summer. The season for orange juice is winter and early spring (March). The laboratory results showed that total ascorbic acid levels increased in the winter. However, generally, orange juice had a lower content of ascorbic acid as compared to the red pepper and strawberry. There was no obvious general trend in ascorbate levels, but the lowest levels in both strawberry and pepper were found in the summer. The reason for this is unclear but may be related to the ambient temperature during transportation being higher.

### 3.5.2 RESULTS OF VARIATION IN TOTAL ANTI OXIDANT CAPACITY (TAC)

The results for antioxidant activity are shown in Figure 3.4.



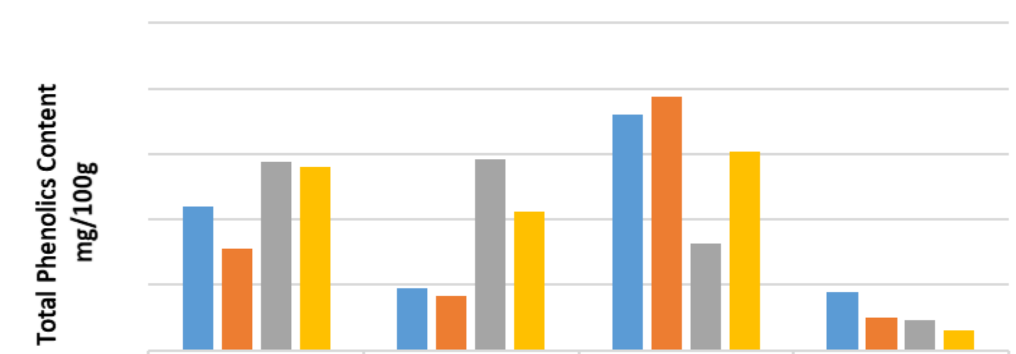

| | Beetroot | Red Pepper | Strawberry | Orange Juice |
|---|---|---|---|---|
| Winter | 450 | 450 | 465 | 310 |
| Spring | 656 | 718 | 937 | 217 |
| Summer | 921 | 822 | 870 | 216 |
| Autumn | 1907 | 1359 | 893 | 117 |

**Figure 3.4 Variation in TAC of ingredients used for preparing 901 smoothie (n=1)**

The antioxidant activity in beetroot and pepper showed very similar profiles. The lowest levels were found in winter and this increased through the seasons to give the highest values in autumn. There were no considerable differences between antioxidant activity in red pepper and beetroot during the four seasons. The antioxidant activity in strawberry was also lowest in winter, but was relatively stable throughout spring, summer and autumn. Orange juice had the lowest antioxidant activity compared to the other ingredients but reached it's peak in the winter.

### 3.5.3 RESULTS OF VARIATION IN TOTAL PHENOLICS CONTENT (TPC)

The results for total phenols are shown in Figure 3.5.




| | Beetroot | Red Pepper | Strawberry | Orange Juice |
|---|---|---|---|---|
| Winter | 110 | 47 | 180 | 45 |
| Spring | 78 | 42 | 194 | 25 |
| Summer | 144 | 146 | 82 | 23 |
| Autumn | 140 | 106 | 152 | 15 |

**Figure 3.5 Variation in TPC content of ingredients used for the 901 smoothie (n=1)**

There were no general trends in TPC among the four ingredients. Total phenolic content in the strawberry was highest in spring which started to decrease in summer and then again increase in autumn and winter. Red pepper had the highest level of TPC in the summer. Orange juice in general had the lowest phenol content among all the ingredients but reached the highest level in winter. Beetroot had fairly stable amounts of TPC through the year except perhaps for a slight decline in spring.

## 3.6 RESULTS FOR RETENTION OF TOTAL ASCORBIC ACID (TAA) DURING PRODUCTION OF SMOOTHIE 901

The content of ascorbic acid in the individual ingredients and it's retention over the three weeks is shown in Table 3.11.

| Week 1<br>Ingredient | TAA mg/100g or 100mL* | Amount added to smoothie (mg) |
|---|---|---|
| Beetroot | 0 | 0 |
| Strawberry | 17.96 | 12.6 |
| Pepper | 80.35 | 24.1 |
| Orange juice | 25.89* | 15.5 |
| Expected total in smoothie | | 52.2 |
| Actual amount in smoothie | 34* | 72.1 |
| % recovery | 73 | |

| Week 2<br>Ingredient | TAA mg/100g or 100mL* | Amount added to smoothie (mg) |
|---|---|---|
| Beetroot | 2.5 | 2 |
| Strawberry | 17 | 11.9 |
| Pepper | 126 | 37.8 |
| Orange juice | 30* | 18 |
| Expected total in smoothie | | 69.7 |
| Actual amount in smoothie | 31* | 65.7 |
| % recovery | 94 | |

| Week 3<br>Ingredient | TAA mg/100g or 100mL* | Amount added to smoothie (mg) |
|---|---|---|
| Beetroot | 0 | 0 |
| Strawberry | 16 | 11.2 |
| Pepper | 95.8 | 27.7 |
| Orange juice | 26.9* | 16.1 |
| Expected total in smoothie | | 55 |
| Actual amount in smoothie | 23.4* | 49.6 |
| % recovery | 90 | |

**Table 3.11 Total ascorbic acid content of individual ingredients and recovery in smoothie 901.**

### 3.6.1 Example of Calculating Retention of TAA:Week-1

- 0 mg of TAA /100g of beetroot

Only 83g for making the smoothie 901

= 0mg of ascorbic acid /83mL of beetroot

- 17.96mg of TAA /100g of strawberry

Only 70g for making the smoothie 901

= 12.6mg of ascorbic acid /70mL of strawberry

- 80.35mg of TAA /100g of red pepper

Only 30g for making the smoothie 901

= 24.1mg of ascorbic acid /30mL of red pepper

- 25.89mg of TAA /100mL of orange juice

Only 60mL for making the smoothie 901

= 15.5mg of ascorbic acid /60mL of orange juice

Hence a total of 52.2 mg of ascorbic acid was present in the "901 smoothie" as ingredient. However, the actual total ascorbic acid in 901 smoothie was:
an average of 3 replicate samples in day 1= 18 mg/100mL

Total volume of the smoothie= 212mL*18/100= 38mg TAA. So 38/52.2*100= represents a 73% TAA recovery.

Beetroot had very little ascorbate, ranging from undetectable to 2.5mg/100g. Pepper had the highest ascorbate content and showed the highest week to week fluctuation which ranged from 80 to 126mg/100g. The ascorbate content of both the strawberry and orange juice were fairly consistent over the three weeks which was around 16mg/100g and 30mg/100mL, respectively.

These values were used to calculate an expected amount of ascorbate in the final smoothie and this was then compared with the actual ascorbate content. The recovery fluctuated from 73% in week 1 to 90% and 94% in the week 2 and week 3 samples. This demonstrates that in general there was very good retention of the ascorbate in the home made smoothie “901”.

## 3.7 RESULTS FOR RETENTION OF TOTAL ANTIOXIDANT CAPACITY (TAC) DURING PRODUCTION OF SMOOTHIE 901

The total antioxidant capacity of the individual ingredients and it's retention over the three weeks is shown in Table 3.12.

| Week 1<br>Ingredient | TAC µmoles /100g or 100mL* | Amount added to smoothie (µmoles ) |
|---|---|---|
| Beetroot | 1760 | 1461 |
| Strawberry | 1815 | 1271 |
| Pepper | 1606 | 482 |
| Orange juice | 1485* | 891 |
| Expected total in smoothie | | 4105 |
| Actual amount in smoothie | 1672* | 3545 |
| % recovery | 86 | |

| Week 2<br>Ingredient | TAC µmoles /100g or 100mL* | Amount added to smoothie (µmoles ) |
|---|---|---|
| Beetroot | 1045 | 867 |
| Strawberry | 825 | 578 |
| Pepper | 1100 | 330 |
| Orange juice | 550* | 330 |
| Expected total in smoothie | | 2105 |
| Actual amount in smoothie | 847* | 1796 |
| % recovery | 85 | |

| Week 3<br>Ingredient | TAC µmoles /100g or 100mL* | TAC amount added to smoothie ((µmoles ) |
|---|---|---|
| Beetroot | 2189 | 1817 |
| Strawberry | 759 | 531 |
| Pepper | 1210 | 363 |
| Orange juice | 726* | 436 |
| Expected total in smoothie | | 3147 |
| Actual amount in smoothie | 840* | 1780 |
| % recovery | 66 | |

**Table 3.12: Total antioxidant capacity of individual ingredients and recovery in smoothie 901.**

### 3.7.1 Example of Calculating Retention of TAC:Week-1

- 1760 µmoles of TAC /100g of beetroot

Only 83g for making the smoothie 901

=1460.8 µmoles of TAC /83g of beetroot

- 1815 µmoles of TAC /100g of strawberry

Only 70g for making the smoothie 901

=1270.5 µmoles of TAC /70g of strawberry

- 1606 µmoles µg of TAC /100g of red pepper

Only 30g for making the smoothie 901

=481.8 µmoles of TAC /30g of red pepper

- 1485 µmoles of TAC /100mL of orange juice

Only 60g for making the smoothie 901

= 891 µmoles of TAC /60mL of orange juice

So total of 4100 µmoles of anti oxidants was present "901 smoothie" as ingredient. However, actual average of TAC of smoothie 901 was in this week with 1672 µmoles TAC/100mL.
Total volume of the smoothie= 212mL*1672/100= 3544.6 µmoles/212mL of smoothie 901.

Thus recovery is actually 4100-3544.6= 555µmoles TAC loss. So 3544.6/4100*100= represents 86% TAC recovery.

In the case of antioxidant capacity, the ingredients in general showed a much greater fluctuation than for the ascorbate. Pepper showed the least fluctuation over the three weeks ranging from 1100 to 1606 µmoles/100g.

Beetroot ranged from 1045 to 2189 μmoles/100g; strawberry from 759 to 1815 μmoles/100g and orange juice from 550 to 1485 μmoles/100mL. Apart from the beetroot, the other three ingredients showed the highest antioxidant capacity in week 1. This fluctuation in TAC of the ingredients was reflected in the amount of TAC recorded in the smoothies and recoveries on the three weeks were 86%, 85% and 66% for weeks 1, 2 and 3, respectively. Again this demonstrates a satisfactory retention of antioxidant capacity in the home made “smoothie 901”.

## 3.8 RESULTS FOR RETENTION OF TOTAL PHENOLICS CONTENT (TPC) DURING PRODUCTION OF SMOOTHIE 901

The content of phenolic acids in the individual ingredients and it's retention over the three weeks is shown in Table 3.13.

| Week 1<br>Ingredient | mg GAE /100g or 100mL* | mg GAE /amount added to smoothie (mg) |
|---|---|---|
| Beetroot | 109 | 90 |
| Strawberry | 78 | 55 |
| Pepper | 87 | 26 |
| Orange juice | 50 * | 30 |
| Expected total in smoothie | | 201 |
| Actual amount in smoothie | 88* | 187 |
| % recovery | 93 | |

| Week 2<br>Ingredient | mg GAE /100g or 100mL* | mg GAE /amount added to smoothie (mg) |
|---|---|---|
| Beetroot | 128 | 106 |
| Strawberry | 88 | 61 |
| Pepper | 101 | 30 |
| Orange juice | 51* | 31 |
| Expected total in smoothie | | 228 |
| Actual amount in smoothie | 83* | 176 |
| % recovery | 77 | |

| Week 3<br>Ingredient | mg GAE /100g or 100mL* | mg GAE /amount added to smoothie (mg) |
|---|---|---|
| Beetroot | 138 | 114 |
| Strawberry | 86 | 60 |
| Pepper | 87 | 26 |
| Orange juice | 54* | 33 |
| Expected total in smoothie | | 233 |
| Actual amount in smoothie | 90* | 191 |
| % recovery | 82 | |

**Table 3.13 Total phenolic acid content of individual ingredients and recovery in smoothie 901.**

### 3.8.1 Example of Calculating Retention of TPC:Week-1

**Beetroot:**

From the standard curve, a concentration of 1000 µg/mL gave the absorbance of 0.853. Thus, the equation:

Absorbance of 0.869 = 1000 µg/mL

Thus, absorbance of 1 = 1000/0.853µg/mL

Average absorbance of beetroot =0.317

Thus, absorbance = 0.317x1000/0.853 µg/mL

Thus, the concentration =

But I used 0.2mL not 0.1mL of sample so 365/2= 182 µg/mL.

Sample dilution: x600 = 109436 µg GAE/100mL$^{-1}$

109436/1000= as 109 mg GAE/100g$^{-1}$ beetroot, but only 83g for making the "smoothie 901" =

So, 109/100*83 = 90mg GAE/83mL$^{-1}$ beetroot.

**Strawberry:**

From the standard curve a concentration of 1000 µg/mL gave the absorbance of 0.869. Thus, the equation:

Absorbance of 0.869 = 1000 µg/mL

Thus, absorbance of 1 = 1000/0.869 µg/mL

Average absorbance of strawberry =0.227

Thus, absorbance = 0.227x1000/0.869 µg/mL

Thus concentration = 261

0.2mL was used not 0.1mL of sample, so /2= 131μg/mL

Sample dilution: x600 = 78365.934 μg GAE/100mL$^{-1}$

/1000= as 78 mg GAE/100g$^{-1}$ strawberry, but only 70g for making the "smoothie 901" =

So, 78/100*70= 55mg GAE/70mL$^{-1}$ strawberry.

**Red pepper:**

From the standard curve a concentration of 1000 μg/mL, showed absorbance of 0.869. Thus, the equation:

Absorbance of 0.869 = 1000 μg/mL

Thus, absorbance of 1 = 1000/0.869 μg/mL

Average absorbance of red pepper= 0.252

Thus, absorbance = x1000/0.869 μg/mL

Thus, concentration = 290

0.2mL used not 0.1mL of sample, so /2= 145 μg/mL

Sample dilution: x600 = 86996.544 μg GAE/100mL$^{-1}$

/1000= as 87 mg GAE/100g$^{-1}$ red pepper, but only 30g for making the "smoothie 901" =

So, 87/100*30= 26mg GAE/30mL$^{-1}$ red pepper.

**Orange juice:**

From the standard curve a concentration of 1000 μg/mL gave the absorbance of 0.869. Thus, the equation:

Absorbance of 0.869 = 1000 μg/mL

Thus, absorbance of 1 = 1000/0.869 μg/mL

Average absorbance of orange juice= 0.145

Thus, absorbance = x1000/0.869 μg/mL

Thus, concentration = 167

0.2mL used not 0.1mL of sample so /2= 83 μg/mL

Sample dilution: x600 = 50057.5 μg GAE/100mL$^{-1}$

/1000= as 50 mg GAE/100mL$^{-1}$ orange juice, but only 60mL for making the "smoothie 901" =

So, 50/100*60= 30mg GAE/60mL$^{-1}$ orange juice.

**Smoothie 901:**

From the standard curve a concentration of 1000 μg/mL gave the absorbance of 0.869. Thus, the equation:

Absorbance of 0.869 = 1000 μg/mL

Thus, absorbance of 1 = 1000/0.869 μg/mL

Average absorbance of smoothie 901= 0.256

Thus, absorbance = x1000/0.869 μg/mL

Thus, concentration = 295

0.2mL used not 0.1mL of sample so /2= 147 μg/mL

Sample dilution: x600 = 88377 μg GAE/100mL$^{-1}$

/1000= as 88 mg GAE/100mL$^{-1}$ smoothie 901.

So, 201mg of total phenolic content in "901" ingredients.

However, actual average of TPC of smoothie 901 was 88 mg/100mL.

Total volume of the smoothie= 212*88/100= 187mg GAE /212mL$^{-1}$ of smoothie 901.

Thus, recovery is actually 201-187mg = 14mg TPC loss.

So, 187/201*100= represents a 93% TPC recovery.

In the case of phenolic content, all four ingredients showed relatively little fluctuation over the three weeks. Beetroot phenolic content ranged from 109 to 138mg/100g, strawberry from 78 to 88mg/100g, pepper from 87 to 101mg/100g and orange juice from 50 to 54mg/100mL. This was reflected in the amount of TPC observed in the smoothies which was relatively consistent with an average of 87+3mg/100mL. The recovery of TPC was 93%, 77% and 82% for weeks 1, 2 and 3, respectively. This again demonstrates reasonable recovery of TPC in the smoothies.

A summary of the recoveries of TAA, TAC and TPC is shown in Table 3.14.

| | TAA% | TAC% | TPC% |
|---|---|---|---|
| Week 1 | 73 | 86 | 93 |
| Week 2 | 94 | 85 | 77 |
| Week 3 | 90 | 66 | 82 |

**Table 3.14 Percentage recoveries of ascorbate (TAA), total antioxidant capacity (TAC) and total phenolics (TPC) in smoothie 901.**

## 3.9 RESULTS FOR RETENTION OF SUGARS DURING PRODUCTION OF SMOOTHIE 901

The glucose, fructose and sucrose contents in the individual ingredients and their retention over the three weeks are shown in Tables 3.15 - 3.17.

| Week 1 Ingredient | Glucose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 0.45 | 0.37 |
| Strawberry | 3.25 | 2.28 |
| Pepper | 2.95 | 0.89 |
| Orange juice | 3.35* | 2.01 |
| Expected total in smoothie | | 5.55 |
| Actual amount in smoothie | 4.4* | 9.33 |
| % recovery | 168 | |

| Week 2 Ingredient | Glucose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 3.35 | 2.78 |
| Strawberry | 3.15 | 2.20 |
| Pepper | 2.7 | 0.81 |
| Orange juice | 2.0* | 1.2 |
| Expected total in smoothie | | 6.99 |
| Actual amount in smoothie | 3* | 6.36 |
| % recovery | 91 | |

| Week 3 Ingredient | Glucose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 0.45 | 0.37 |
| Strawberry | 2.65 | 1.86 |
| Pepper | 2.75 | 0.82 |
| Orange juice | 2.65* | 1.59 |
| Expected total in smoothie | | 4.64 |
| Actual amount in smoothie | 4.3* | 9.1 |
| % recovery | 196 | |

**Table 3.15: Glucose content of individual ingredients and recovery in smoothie 901.**

| Week 1 Ingredient | Fructose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 0.45 | 0.4 |
| Strawberry | 3.7 | 2.6 |
| Pepper | 4.75 | 1.4 |
| Orange juice | 3.45* | 2.1 |
| Expected total in smoothie | | 6.5 |
| Actual amount in smoothie | 4.8* | 10.2 |
| % recovery | 157 | |

| Week 2 Ingredient | Fructose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 3.35 | 2.78 |
| Strawberry | 5.65 | 3.96 |
| Pepper | 3.95 | 1.19 |
| Orange juice | 2.05* | 1.23 |
| Expected total in smoothie | | 9.11 |
| Actual amount in smoothie | 3.4* | 7.2 |
| % recovery | 79 | |

| Week 3 Ingredient | Fructose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 0.45 | 0.37 |
| Strawberry | 3.05 | 2.14 |
| Pepper | 4.15 | 1.25 |
| Orange juice | 2.8* | 1.68 |
| Expected total in smoothie | | 5.44 |
| Actual amount in smoothie | 4.7* | 10 |
| % recovery | 183 | |

**Table 3.16 Fructose content of individual ingredients and recovery in smoothie 901.**

| Week 1<br>Ingredient | Sucrose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 9.8 | 8.13 |
| Strawberry | 0.25 | 0.18 |
| Pepper | 0.35 | 0.11 |
| Orange juice | 4.25* | 2.55 |
| Expected total in smoothie | | 10.97 |
| Actual amount in smoothie | 1.65* | 3.5 |
| % recovery | 32 | |

| Week 2<br>Ingredient | Sucrose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 6.0 | 6.60 |
| Strawberry | 0.2 | 0.14 |
| Pepper | 0.15 | 0.05 |
| Orange juice | 2.4* | 1.44 |
| Expected total in smoothie | | 8.23 |
| Actual amount in smoothie | 1.5* | 3.18 |
| % recovery | 38 | |

| Week 3<br>Ingredient | Sucrose g/100g or 100mL* | Amount added to smoothie (g) |
|---|---|---|
| Beetroot | 7.1 | 5.89 |
| Strawberry | 0.35 | 0.25 |
| Pepper | 0.15 | 0.05 |
| Orange juice | 3.3* | 1.98 |
| Expected total in smoothie | | 8.17 |
| Actual amount in smoothie | 1.8* | 3.82 |
| % recovery | 46 | |

**Table 3.17: Sucrose content of individual ingredients and recovery in smoothie 901.**

The sugar content of the four ingredients showed some fluctuation over the three weeks, with a couple of data points that were extremely variable. Beetroot, as expected contained the highest amount of sucrose and had very little glucose and fructose. However, this was evident only for weeks 1 and 3. The values for these three sugars in beetroot on week 2 may represent an experimental error. Strawberry contained mainly glucose and fructose with equal amount of each sugar. Again, the results for week 2 where there is a much greater amount of fructose may represent an experimental error. The level of sucrose in strawberry on all the three weeks was very low. Pepper had a similar sugar profile to that of strawberry. It primarily had glucose and fructose, with the latter being present at slightly higher amounts in each week with very little sucrose. Orange juice showed the most consistent sugar profile, with approximately equal amounts of all three sugars each week.

Again, these values obtained above were used to estimate the expected sugar content of the smoothie and then compared with the actual recorded values in the respective smoothie on each week. A summary of the sugar recoveries in the smoothie 901 is shown in Table 3.18. These values were expressed as a percentage recovery as shown in Table 3.19.

| | | Glucose | Fructose | Sucrose | Total |
|---|---|---|---|---|---|
| Week 1 | Expected | 5.55 | 6.5 | 10.97 | 23.02 |
| | Observed | 9.33 | 10.2 | 3.5 | 23.03 |
| Week 2 | Expected | 6.99 | 9.11 | 8.23 | 18.23 |
| | Observed | 6.36 | 7.2 | 3.18 | 16.75 |
| Week 3 | Expected | 4.64 | 5.44 | 8.17 | 18.25 |
| | Observed | 9.1 | 10 | 3.82 | 22.92 |

**Table 3.18: Summary of sugar recovery in smoothie 901(g).**

| | Glucose % | Fructose % | Sucrose % |
|---|---|---|---|
| Week 1 | 168 | 157 | 32 |
| Week 2 | 91 | 79 | 38 |
| Week 3 | 196 | 183 | 46 |

**Table 3.19: Percentage recoveries of sugars in smoothie 901**

For the levels of total sugars, the recovery was 100%, 92% and 126% for weeks 1, 2 and 3, respectively. This data might suggest that the values for the individual ingredients were indeed correct. However, looking at the recovery of the individual sugars, the recoveries of glucose and fructose were both higher than expected over three weeks and the recovery of sucrose was lower. This suggests that sucrose is being converted to glucose and fructose, by enzymatic action during the preparation of the smoothie.

## 3.10 RESULTS FOR STABILITY OF TOTAL ASCORBIC ACID (TAA) IN SMOOTHIE 901 DURING STORAGE

The results for TAA are shown in Figure 3.6.

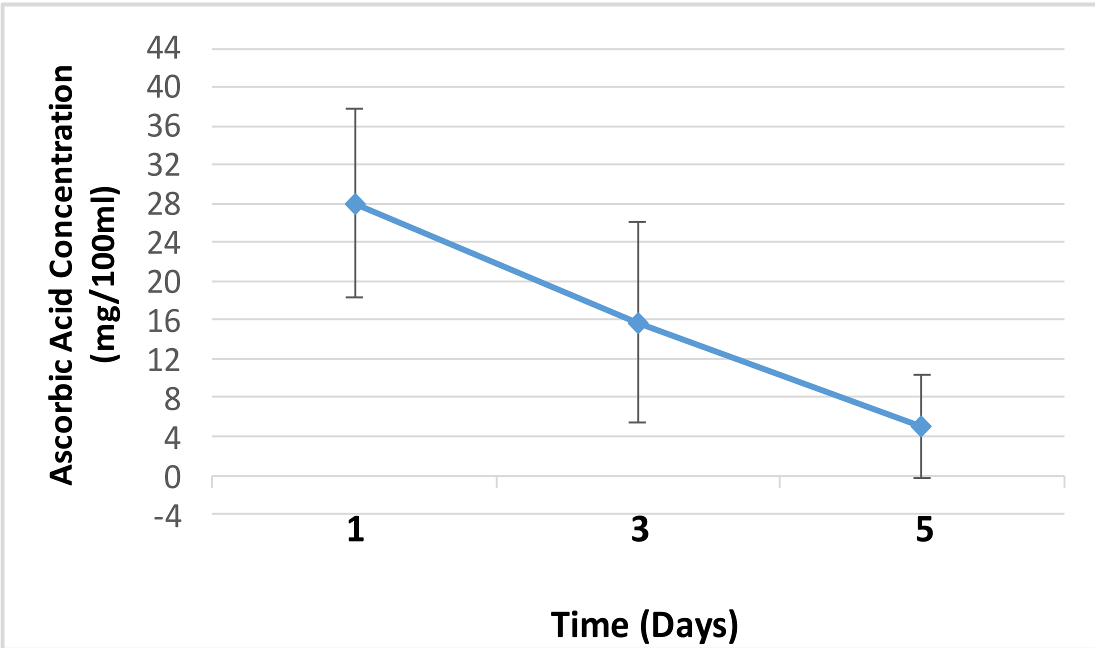



**Figure 3.6 TAA and stability in 901 smoothie during 5 days of storage at 4°C. Smoothie was made and total ascorbic acid measured immediately on the day of preparation (day 1). The smoothie was then stored at 4°C and measured again on days 3 and 5. Results are expressed as the mean ±SD (n=4).**

The total ascorbic acid concentration at zero time (the day of making the 901 smoothie) was an average of 28±9.8 mg/100ml. After 3 days of storage the ascorbic acid concentration declined sharply by 56.2%. Moreover, the percentage loss of ascorbic acid after 5 days of storage at 4°C was 82% and the TAA was only around 5±5.3 mg/100ml. The data was analysed using independent t-tests which showed significant differences between 0 and 3 days and also between 0 and 5 days ($p=0.012$, $p=0.00$), respectively.

## 3.11 RESULTS FOR STABILITY OF TOTAL ANTIOXIDANT CAPACITY (TAC) IN SMOOTHIE 901 DURING STORAGE

The results for TAC are shown in Figure 3.7.

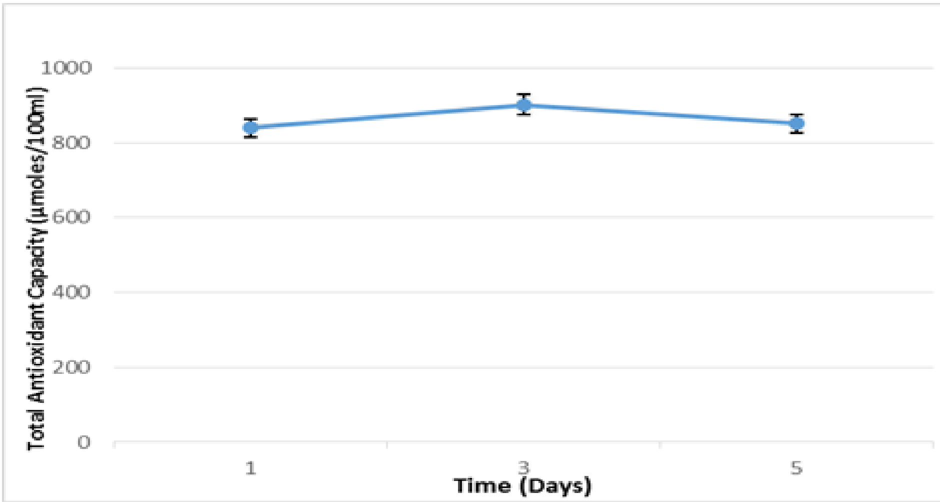



**Figure 3.7 Levels of TAC and stability in 901 smoothie during 5 days of storage at 4°C. Smoothie was made and antioxidant capacity measured in the day of preparation (day 1). The smoothie was then stored at 4$^{o}$C and antioxidant activity measured on day 3 then on day 5. Results are expressed as the mean ±SD (n=4).**

The TAC in the smoothies 901 on day 1 was an average of 1064 ±450 µmoles/100mL. However, TAC was slightly raised on day 3 of storage to an average of 1092 ±382 µmoles/100ml. The TAC then declined on day 5 to reach an average of 1039 ±382 µmoles/100mL. Analysis of the results showed that these differences were not statistically significant (p=0.995,p=0.996) which means that there were no significant diffrences between 1, and 3 or 5 days and TAC levels in the smoothie appear to be stable over the 5 days of storage at 4$^{o}$C.

## 3.12 RESULTS FOR STABILITY OF TOTAL PHENOLIC COMPOUNDS (TPC) IN SMOOTHIE 901 DURING STORAGE

The results for the TPC are shown in Figure 3.8.

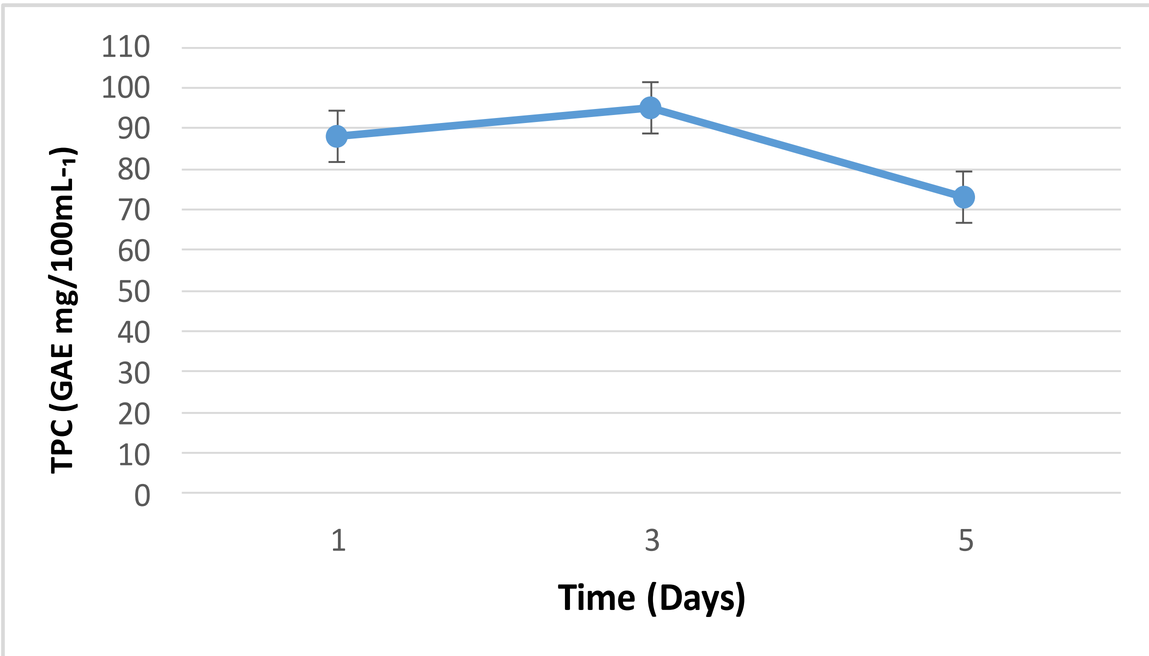


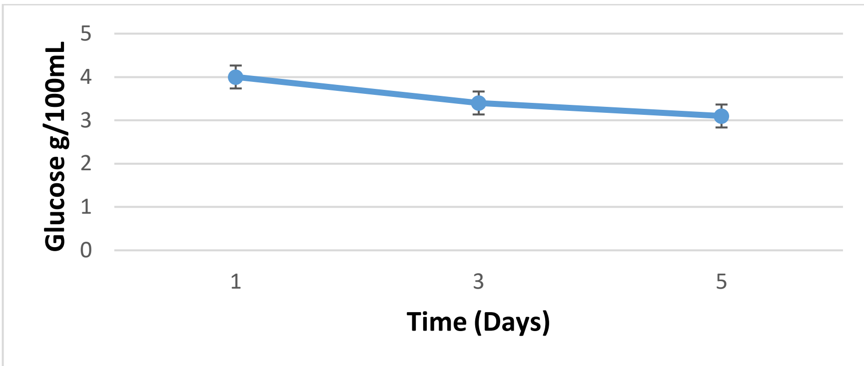

**Figure 3.8 Stability of TPC in smoothie 901 stored at 4°C for 5 days. Smoothie was prepared and TPC measured immediately at day 1. The smoothie was then stored at 4°C and TPC re-measured on day 3 and 5. Results are expressed as the mean ± SD (n=4).**

The TPC of the smoothies on day 1 was an average of 88±3.86 mgGAE/100mL. The trend during storage, however, varied slightly between replicates. On day 3 of storage the average TPC increased slightly to 95±43.3 mgGAE/100ml but this was due to an increase in only 2 of the 4 replicates with the other two samples showing either a slight decline or stable levels of TPC. The TPC then declined on day 5 to reach an average of 75±25.6 mg GAE/100ml. However, this was only true in 3 out of 4 replicates. The analysis of the combined results showed that these differences were not statistically significant ($p=0.947, p=0.740$) which means that there were no significant differences between day 1, and day 3 or 5 days. Thus, the TPC levels in the smoothie appears to remain constant throughout the 5 days of storage at $4^{o}C$.

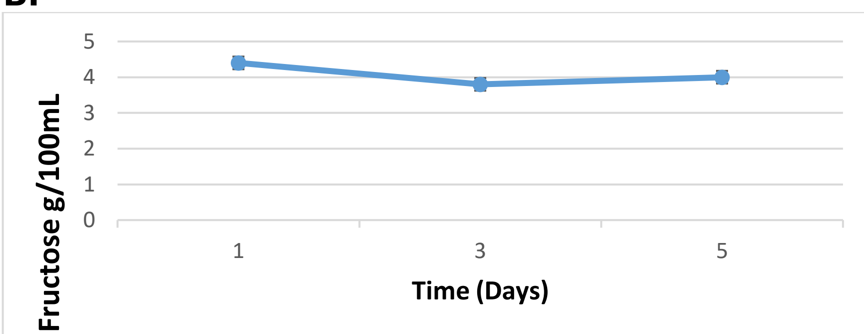
## 3.13 RESULTS FOR STABILITY OF SUGARS IN SMOOTHIE 901 DURING STORAGE

The results for the 3 types of sugars are shown in Figure 3.9 a,b,c.

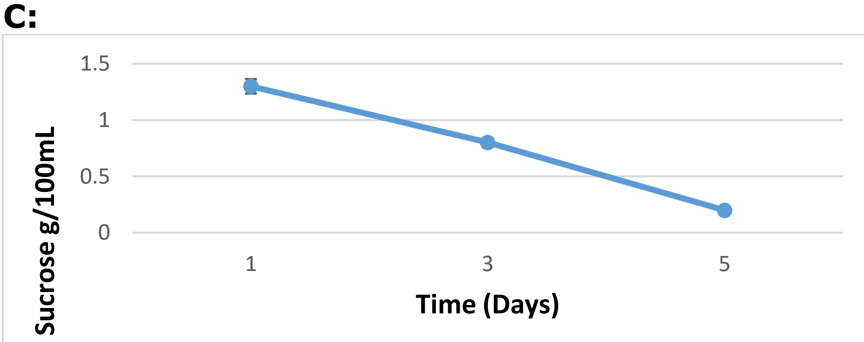
**A:**




**B:**




**C:**




**Figures 3.9 Levels and stability of sugars in 901 smoothie during 5 days of storing at 4°C. Smoothie was prepared and content of (A) glucose, (B) fructose and (C) sucrose measured immediately (day 1). The smoothie was then stored at 4ºC and sugars re-measured on day 3 and then on day 5. Results are expressed as the mean and standers error (± SD) (n=4).**

The glucose level consistently showed a value of approximately 4±0.4 g/100mL at day 1. This was similar to fructose, with the level was estimated at 4.4±0.3 g/100mL. However, the level of sucrose was lower at 1±0.5 g/100mL. The change during storage, showed a decline in all the three sugars on day 3. On day 5, there appeared to be a further decline in glucose and sucrose only, with fructose showing a slight increase.

However, statistical analysis of the results of glucose and fructose showed that there are no significant differences ($p=0.348, p=0.093$) ($p=0.264$, $p=0.767$ respectively) which implies that there are no significant diffrences in glucose and fructose levels in the "901 smoothie" between 1 and 3 or 5 days. However, statistical analysis showed that there are significant differences between the levels of sucrose ($p=0.331, p=0.012$) which implies that there was a statistically significant change in the sucrose level in the "901 smoothie" between 1 and 5 days of storage.

## 3.14: RESULTS OF MEASURING pH IN SMOOTHIE 901

The pH of the smoothie is an important characteristic for both taste and stability and safety of the product. Thus the pH of the 901 smoothie (Table 3.20) and it's variation over storage (Figure 3.10) were measured.

| pH | Smoothie 901 |
|---|---|
| **January 2017 n=6** | 4 $\pm$0.01 |
| **February 2017, n=4** | 4 $\pm$0.02 |

**Table 3.20 pH measurements of smoothie 901.**

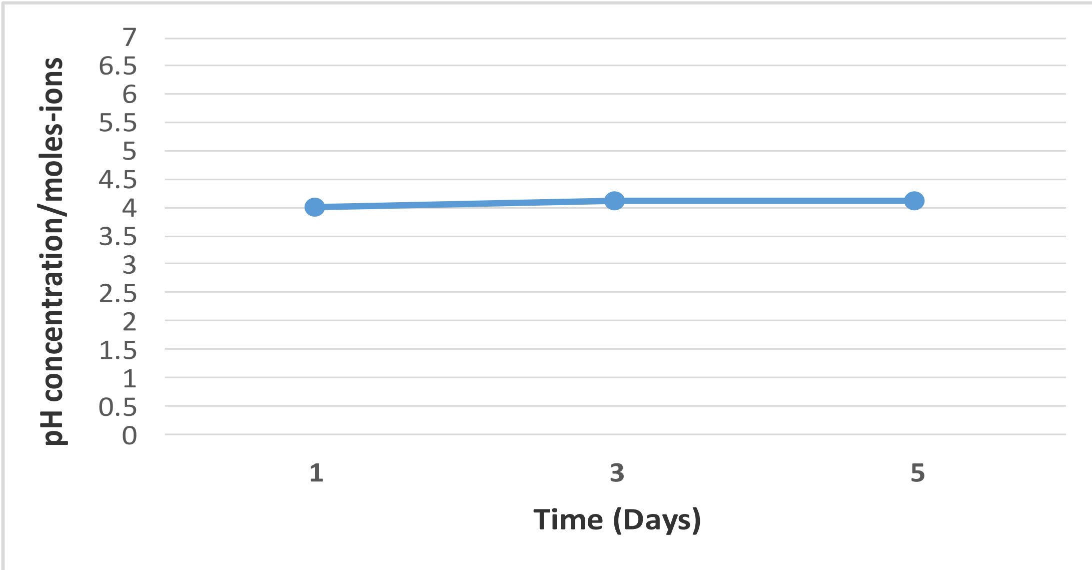



**Figure 3.10 Stability of pH concentration in 901 smoothie during 5 days of storing at 4°C. Smoothie was prepared and pH measured immediately at day 1. The smoothie was then stored at 4ºC and pH re-measured on day 3 and then on day 5. Results are expressed as the mean (n=4).**

The pH of the smoothie 901 was acidic (pH=4) and there was no statistically significant changes ($p=0.708, p=0.052, p \geq 0.05$) during the storage.

## 3.15 RESULTS OF PRELIMINARY EXPERIMENT TO DETERMINE ENZYME TREATMENT CONDITIONS

The aim of this preliminary experiment was to determine a suitable enzyme cocktail and conditions, prior to the full rheological analysis. The "Smoothie 901" was prepared as described previously in Section 2.4.2.1) and stored at 2–4°C until required. Samples (30 mL) of the smoothie were allocated to four glass beakers; 50µl of distilled water was added to the first beaker as a control, while 50µl aliquots of commercial enzyme cocktails – pectinase (Sigma), cellulase ('CelTec2' Novazyme) or a mix of both enzymes were added to the remaining three beakers. For the enzymes these were both commercial enzyme cocktails from either Sigma or Novozyme. Their trade names are "pectinase" and "celtec 2". A crude estimate of viscosity was determined by measuring the time taken for the smoothie to flow between the 10 and 15mL marks on a 25mL pipette. The viscosity was measured 10 and 30 min after the addition of enzymes in all four samples. The results are presented in Table 3.21.

| Smoothie | After 10 min. | After 30 min. |
|---|---|---|
| **Control** | **>1 min.** | **>1 min.** |
| **Pectinase** | **49 sec.** | **12 sec.** |
| **Cellulase** | **45 sec.** | **13 sec.** |
| **Mix** | **17 sec.** | **3 sec.** |

**Table 3.21 Effect of cell wall hydrolytic enzymes on the viscosity of a 901 smoothie.** Enzyme cocktails were added at time 0 and viscosity was estimated as time of travel between marked points on a 25ml pipette.

Samples of 901 smoothie were incubated at room temperature with enzyme cocktails. The viscosity changes were monitored by flow time through a 25mL pipette (n=1). From Table 5.14 it can be observed that the control smoothie with no added enzyme, retained a high viscosity over 30 min, as shown by the flow-out rate of greater than 1 min. The addition of either enzyme cocktail reduced the viscosity, as the flow-out rate was significantly reduced. There was no apparent difference between the flow rates with either the cellulase or pectinase enzyme. Interestingly, a greater reduction in viscosity was observed when these were added together. The pectinase enzyme mix was selected for further investigation because this may be more readily adaptable for commercial practice. While the use of combined enzymes may result in huge reduction in viscosity, this might not be acceptable in terms of the consistency of the smoothie, as the viscosity appeared to be approaching that of water and therefore the drink would have a consistency more similar to a juice than a smoothie.

### 3.15.1 MEASUREMENT OF SMOOTHIE VISCOSITY USING THE ANTON PAAR RHEOMETER

Smoothies 901 and 134 were prepared as described previously. Both were stored at 2–4°C for 2 days. Samples (50 mL) of each smoothie were allocated in two glass beakers. First was untreated and second were samples treated with pectinase enzyme and were analysed separately in the Anton Paar rheometer to measure their viscosity within 2-4 hrs. Measurements of shear and viscosity derived from the raw data were transferred to Microsoft Excel. Shear and viscosity where then converted to log shear and log viscosity. Plotting log viscosity versus the log shear rate allows the calculation of K and

n. These can then be used to calculate the intrinsic viscosity using the following equation:

$$\eta = K * \dot{\gamma}^{n-1}.$$

In this equation, n provides an indication of Newtonian behaviour. A value of 1 indicates a perfect Newtonian liquid, where viscosity is independent of shear, while n < 1 indicates shear thinning and n > 1 shear thickening.

A typical result for the 901 smoothie is shown in Figure 3.11. A summary table of K and n values are given in Tables 3.22 and 3.23), respectively.

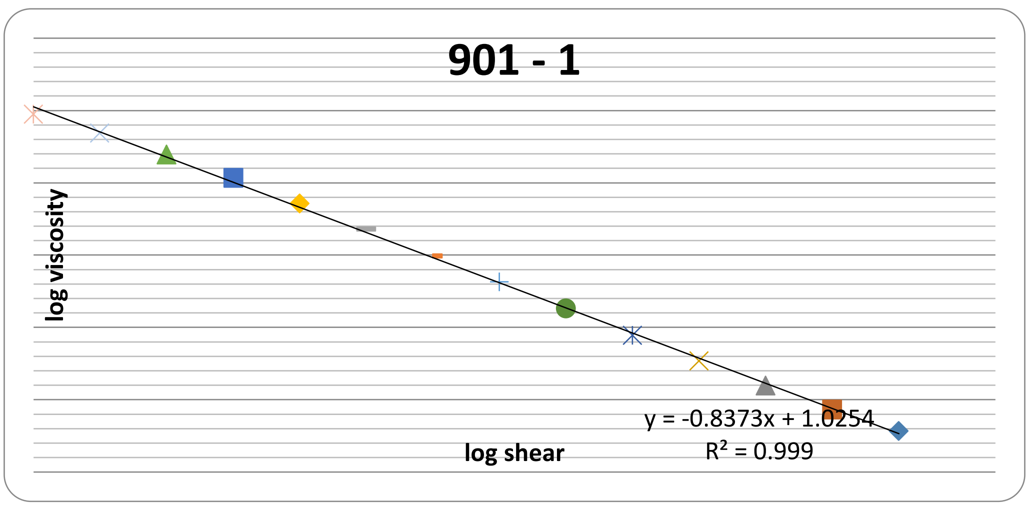



**Figure 3.11 Smoothie 901 viscosity.**

Viscosity was measured at 14 different shear rates. The different coloured symbols indicate each individual reading.

| Smoothies | **K** | **mean** |
|---|---|---|
| 901 untreated | 10.60 - 9.39 | 9.99 |
| 134 untreated | 8.46 - 7.9 | 8.2 |
| 901 treated | 1.9 - 2.22 | 2 |
| 134 treated | 3.90 - 3.41 | 3.7 |
| Innocent | 1.40 -1.45 -1.34 | 1.4 |

**Table 3.22: Log K values for 901, 134 and Innocent smoothies (n=2)**

| Smoothies | **n** | **Mean** |
|---|---|---|
| 901 untreated | 0.16 - 0.19 | 0.18 |
| 134 untreated | 0.27 - 0.27 | 0.27 |
| 901 treated | 0.29 - 0.26 | 0.28 |
| 134 treated | 0.24 - 0.26 | 0.25 |
| Innocent | 0.37-0.36-0.37 | 0.36 |

**Table 3.23: The n values for 901, 134 and Innocent smoothies (n=2).**

The K values in Table 3.22 showed that the Innocent smoothie had a lower viscosity than both the untreated 109 and 134 smoothies. The enzyme treatment has successfully reduced the viscosity of both 109 and 134 smoothies. The n values show that all smoothies are showing shear thinning but again with a clear difference between the commercial smoothie and in particular the 901 smoothie. Treatment with enzymes shifts the value of n for the 901 smoothies towards values of reference “Innocent”. The pectinase enzyme cocktail had thus reduced the viscosity of both experimental smoothies. Treated smoothie 901 showed viscosity behaviour that was more similar to that of the reference Innocent smoothie (Table 3.23).

### 3.15.2 MEASUREMENT OF SMOOTHIE VISCOSITY OVER TIME

A second set of experiments was designed to directly demonstrate the viscosity change over 30 min following the addition of the enzyme. The pectinase enzyme cocktail (50μL) was added directly to 50mL of untreated smoothie in the rheometer. The rheometer was set at a constant shear rate of 100d (gamma), and the temperature of the rheometer chamber was maintained at constant 20°C. The viscosity was recorded over 30 min. The results for smoothie 901 are shown in Figure 3.12, while those for smoothie 134 are given in Figure 3.13. The results showed that viscosity decreased with time after the addition of the enzyme. But it was observed in each case that viscosity reached a state of little or no change after about 1000s. A further experiment was thus carried out where the smoothie was incubated with the pectinase cocktail for 3h and then the viscosity was determined as described in Section 5.7.3. The values of K and n from this experiment are shown in Table 3.24.

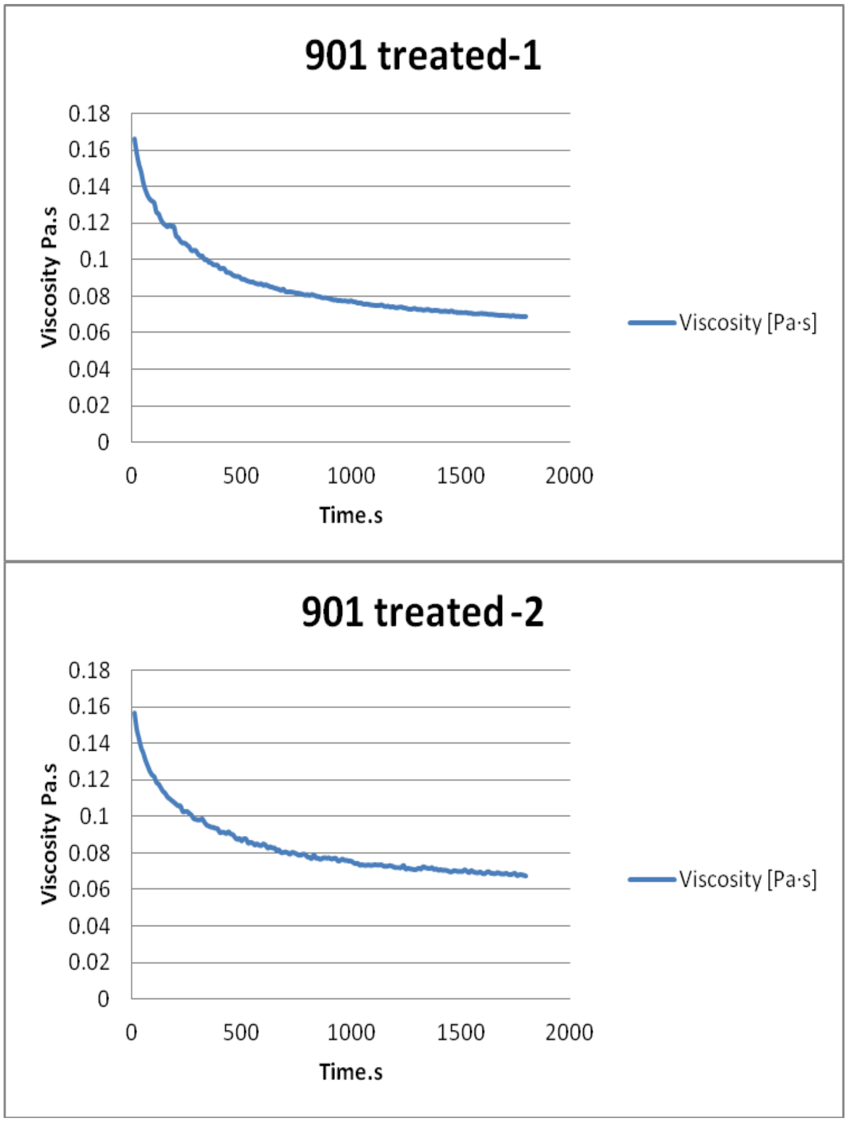



**Figure 3.12 Smoothie 901 viscosity versus time (2 replications).** Smmothie was placed in the rheometer and enzyme cocktail added at time 0. Viscosity was then recorded continuously at fixed shear rate.

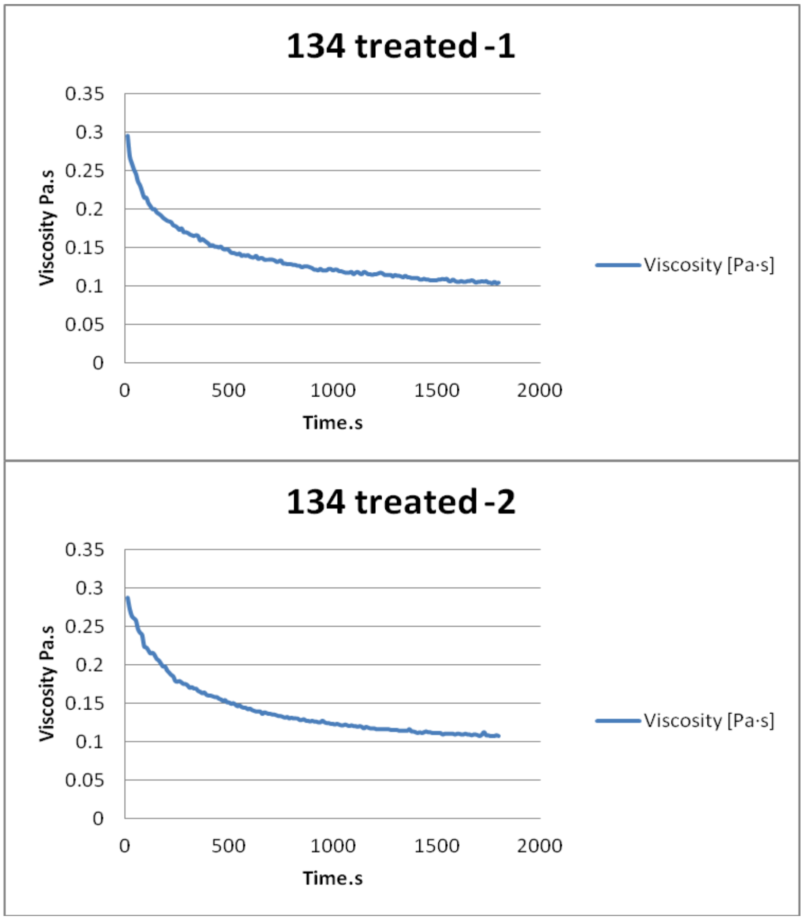



**Figure 3.13 Smoothie 134 viscosity versus time (2 replications)**
Smmothie was placed in the rheometer and enzyme cocktail added at time 0. Viscosity was then recorded continuously at fixed shear rate.

Surprisingly, after 3 hrs, the treated “901 smoothie” still had similar viscosity to Innocent, and was no less viscous than at 30 min.

| Smoothies | **K** | **n** |
|---|---|---|
| 901 treated after 3hrs | 2.28 | 0.32 |
| Innocent | 1.4 | 0.36 |

**Table 3.24: Log K and n values for 30 mL of smoothie 901 treated in the rheometer (3 replications)**

## 3.16 DISCUSSION OF NUTRITIONAL COMPARISON CONTENT OF SMOOTHIE 901 AND COMMERCIAL SMOOTHIES

The main hypothesis in the present research was to design a smoothie that would be nutritionally superior to current commercially available fruit/F&V smoothies in the UK market. The study explored the smoothie 901's nutrient levels and then compared the findings with other smoothie products in the UK market. Comparison was with available, leading brand smoothies in the UK, as evidenced by the availability of those smoothies in the Nottingham University cafes and also in the local shops and supermarkets such as, Tesco and Asda. Innocent is currently one of the leading smoothie brands in the UK along with Naked juice smoothies, therefore, both brands were chosen for the nutritional comparison. Based on the laboratory experiments, it was found that ascorbic acid and antioxidant capacity were all higher in smoothie 901 (Table 3.5) than in the Innocent smoothie products (seriously strawberry, gorgeous green) and Naked smoothie products (green machine, bright beets). However, levels of phenolics in the 901 smoothie were intermediate between the Innocent and Naked commercial smoothies.

Vitamin C is a water-soluble vitamin found in food and in dietary supplements. The human body cannot produce this vitamin and it is mostly obtained from plant foods; it can be found in approximately 90% of F&V. It is a water-soluble vitamin which means there is no risk of toxicity from overdose which may pose a problem with fat-soluble vitamins such as vitamin A. Even large amounts of vitamin C such as 700mg of vitamin C are unlikely to cause harm (Sparman and Thompson, 2017). Excessive doses of vitamin C in the body can be excreted in the urine. Ascorbate is possibly the most ubiquitous antioxidant found in F&V, however, it can be oxidised quickly when exposed to light or heat. Vitamin C is also used in food factories as it can be added to processed foods because of its antioxidant and preservative properties. The recommended dietary reference intake for vitamin C for an adult is from a minimum of 65 to 90mg per day, to a maximum of 2,000mg per day. Additionally, in a recent systematic review by Cuhls et al., in 2017 it was observed that doses of 20g of vitamin C supplementation in terminal cancer patients was linked to improvement of quality of life including physical and cognitive function, reduced fatigue and appetite loss, as well as nausea. Hence, no serious adverse effects have been reported with dietary supplementation.

Daily consumption of vitamin C is essential, since it is not stored within the body. The "smoothie 901" contained approximately 30mg of vitamin C per 100mL; this is around 75mg/250mL, which is equivalent to 188% of the RNI per 250mL and 125% of the Nutrition Labelling Directive per 250mL. Hence, from a nutritional point of view, smoothie 901 contains an adequate amount of vitamin C, which is more than 100% of either recommendation.

The study of Thomas and Thomas in 2008 described a method of producing three different vegetable-based beverages from chick peas, butternut squash and split chickpea or chana dal (Chana dal is a type of lentil that comes from India, looks just like yellow split peas but is quite different) with purified water, flax seed, almond extract, vanilla extract, aromatic bitters, soy milk, condensed milk and added vitamin C. The final products contained approximately 50% pulverised vegetable base. The resulting mixture was then frozen immediately and stored to give texture-enhancing frozen smoothie consistency and for the purpose of preservation. This invention was patented in 2008. This beverage is distinct from ours in that it contains added vitamin C, whereas smoothie 901 contains natural vitamin C. Moreover, a study by Gardner et al., in 2000 showed that the health benefit of fruit juices, in particular phenolic antioxidants, found that vitamin C accounts for 65–100% of the antioxidant potential of drinks derived from citrus fruit but less than 5% of those from apple and pineapple juice.

The majority of the nutrient contents as estimated from the reference nutrient values, were also similar but had a tendency to get overestimated by about 20%. The exception was in vitamin C, where the estimated value was actually around twice of what was indicated on the label or measured in the laboratory. This could be due to pasteurisation, which resulted in a loss of vitamin C. These results show that the quality control at Innocent and Naked is very good, and these companies may not rely simply on the use of nutritional databases such as McCance and Widdowson. It also shows that the laboratory techniques used in this project were good and provided values very similar to those reported on the commercial label.

The smoothie 901 was found to have 1031μmoles Trolox Equivalent (TE) /100mL which is equivalent to 2578μmoles TE/250mL = 1 cup, which was much higher than the values for the commercial (Innocent and Naked) smoothies used in this research. Besides, a recent study by Rodríguez-Verástegui et al., in 2016 investigated two red fresh vegetable smoothies containing tomato, red pepper, broccoli and carrot and found that TAC was around 301/373 mg Trolox Equivalent $kg^{-1}$ fw whilst smoothie 901 roughly provides 10mg TAC/1L. Thus, the 901 smoothie appears to provide more antioxidant capacity than the commercial smoothies, but this may be further enhanced by the inclusion of a greater amount or range of vegetable ingredients.

The nutritional benefit of enhancing antioxidant intake is not entirely clear. Dietary antioxidants include vitamins C and E, carotenoids, organo-sulphural compounds, phenolics and minerals. To date, there is no recommendation for daily intake or any calculated composition in food table analyses for antioxidants. Nonetheless, many studies have shown that foods rich in antioxidants and phytochemicals provide protection against the harmful effects of stress, boost immune function, as well as reducing the risk of chronic health conditions such as CVD and cancer. Robinson and Ogawa (1999), found a correlation between managing stress and consumption of high levels of antioxidants from food and smoothie intake.  Furthermore, it has been recommended in a number of old and recent studies, and also in the recent International Symposium on Immuno-nutrition held in Madrid in 2017; to increase the intake of food rich in antioxidants as this may be associated with benefits to human health since oxidative stress is related to a number of not only diet-related non-communicable diseases such as cardiovascular but also cancer, neurodegenerative disease and aging. However, a recent study by Vauzour et al., (2017) argued that results in terms of protection from cognitive deterioration are unreliable and fragmented and more research is necessary to define the fundamental brain mechanisms.

Measuring antioxidant capacity in natural products has been investigated extensively (Aune et al., 2017). Also, different chemical methods *in vitro* have been established including ferric reducing ability of plasma (FRAP) assay to measure the antioxidant power in biological samples such as smoothies. It is a simple chemical-based method that has low cost, is highly reproducible and produces an index value expressed as equivalents of Trolox that allows different products to be compared (Benzie and Strain, 1996). Also, ORAC is a common method that assesses the probability of a sample as an inhibitor of a target molecule oxidation (López-Alarcón and Denicola, 2013). Again, there is no real consensus on which, if any, of these assays can be considered as the most indicative of nutritional benefit.

One of the main bioactive substances in fruit and vegetables are polyphenols. These are present in different parts of the plant and have been shown to have anti-inflammatory and antimicrobial abilities. There is an increasing interest in natural antioxidants, particularly phenols, present in medicinal and dietary plants, that might help prevent oxidative damage.

In the present research, the average TPC content of smoothie 901 was 57mg GAE equivalents /100mL. The TPC was higher in smoothie 901 than in both Innocent smoothie products (seriously strawberry and gorgeous green). However, this was not the case with both Naked smoothie products as total phenolics content was found to be marginally higher in the Naked green machine smoothie and significantly higher in the Naked bright beets smoothie. A study by Chun et al., (2005) determined that the daily consumption of phenols from the most commonly consumed fresh fruit (14 types) and vegetables (20 types) in the American diet was 450 mg GAE/day. The study reported orange to provide the highest amount of total phenolics (117 mg GA equivalent person$^{-1}$day$^{-1)}$. Thus, the 901 smoothie could make a significant contribution to the normal intake of phenolics. However, there are no recommended intake values for total phenolics.

It is important to point out that the TPC assay is not totally specific for phenolics, but is actually an antioxidant assay. In fact, TAC and TPC values do not directly demonstrate the nutritional benefit but could be used as an indicator of the relative prospects for benefit in the different smoothies.

There have been a number of studies investigating the nutritional composition of F&V or their juices and the impact of processing methods. Proteggente et al., (2002) analysed the regularly consumed F&V available in the UK market for the composition of their major phenolic components. The total phenolic content (applying the Folin assay) and vitamin C levels were determined. The results were calculated in terms of 100g fresh weight (FW) uncooked portion sizes. The fruit and vegetables rich in anthocyanins (e.g. strawberry, raspberry and red plum) demonstrated the highest antioxidant activities, followed by those rich in flavanones (e.g. orange and grapefruit) and flavonols (e.g. onion, leek, spinach and green cabbage), while hydroxycinnamate-rich fruit (e.g. apple, tomato, pear and peach) consistently elicited lower antioxidant activities. The TEAC, FRAP and ORAC values for each extract were relatively similar and correlated well with the total phenolic and vitamin C contents.

A study by Pyo et al., in 2014 on four Korean homemade fruit juices containing apple, pear, persimmon, and mandarin examined polyphenol compounds. The authors found that the juices containing mandarin orange showed the maximum TPC (862.3 mg GAE /serving) and that this was about 6.8 fold higher than that of pear juice. The research also suggested that it is crucial to know that TPC is affected by different processing methods. Thus, the main findings were that blending might be a superior approach that provided significantly higher levels of TPC. Also, it was specified that the juice extraction method significantly influences the phytochemical levels and antioxidant capability of fruit juices. A study by Bahorun et al., (2004) analysed ten vegetables including chilli pepper, for vitamin C content and showed that it varied between 25 to 748 µg $g^{-1}$ FW. The TAC of the vegetables ranged from 0.43 to 3.68 µmol $g^{-1}$ FW Trolox equivalent antioxidant capacity (TEAC) and from 0.60 to 8.47 µmol $g^{-1}$ FW FRAP. The TPC in the vegetables varied between 132 and 1189 µg $g^{-1}$ FW and total flavonoids between 45 and 944 µg

$g^{-1}$ FW, whereas pro-anthocyanidins were discovered in just a few vegetables in very low levels. The maximum TAC values were detected in Chinese cabbage, onion, mugwort and broccoli. According to Uckoo et al., (2012) blended grapefruit juice, which had a higher flesh content than juiced grapefruit juice, also had higher TPC.

The results of the present study and others suggests that processing techniques considerably influence the levels of phytochemicals and blending is the better technique for obtaining higher levels of phytochemicals for health benefits from drinks. Also, according to Ma et al., (2012) fruit flesh generally contain large amounts of organic acids, sugars, minerals, and vitamins but the peels contain higher amounts of phenolic compounds. A study of McCarthy et al., (2013) showed that antioxidant activity, as measured by the FRAP assay, increased significantly after addition of extracts of cranberry juice to fruit beverages. However, in *vitro* TPC and FRAP activity was considerably reduced during digestion of the fruit beverages. According to Gardner et al., (2000) phenolics appear to be the chief contributors to the antioxidant potential of non-citrus juices. Further, according to Pyo, et al., in 2014 blending persimmon, mandarin orange and apple juice resulted in higher levels of ascorbic acid (233.9 mg/serving), total polyphenols (862.3 mg Gallic acid equivalents/serving), and flavonoids (295.1 mg quercetin equivalents/serving). A recent study by Formica-Oliveira et al., in 2017 found that incubation of shredded carrot, under hyperoxia conditions, resulted in a smoothie with higher phenolic content and good levels of microbiological and physio-chemical quality.

Insoluble fibre content was higher in 901 smoothie (approximately 15g/250mL) than in all the commercial smoothies. The Naked green machine smoothie had the highest level among the commercial varieties with 10g/250mL of fibre. Moreover, the levels in our study was found to be similar to studies by Holland (2008) and Ruxton (2008) i.e., the amounts of fibre for our study were equal to the amount of fibre in other fruit smoothies. While there is no dietary reference intake for insoluble or soluble fibre, the American Heart Association's Diet and Lifestyle, suggested to increase the F&V consumption to reach a total dietary fibre intake of between 25g to 30g per day with around one-fourth or 6g to 8g soluble fibre (UCSF,2016). As a result, only 1 glass of smoothie 901 (250mL) would provide approximately half of the recommended daily allowance of dietary fibre needed per day for an adult. Whereas, an apple or 1 cup of blueberries would provide 3 to 4 grams of fibre and a half cup of peas or 1 cup of carrots would provide 3 to 4 grams of fibre.

The relationship between high dietary fibre intake and the prevention of some chronic diseases such as CHD, moderate blood cholesterol levels and lower body weights has been reported for some time (Slavin, 2005 and 2013). As fibre sources are limited to foods of plant origin, the intake of fruit, vegetables, and complex carbohydrates is an accepted indicator for fibre intake. The GDA for fibre intake is 24 g/day. One serving (250 ml) of smoothie 901 provides 18% of daily recommended fibre intake. Dietary fibre intakes are not provided by any of the national dietary survey data and also only a small number of countries in Europe deliver recommendations on the types of fibre that are preferable to achieve recommended intakes (Stephen et al, 2017). In addition, the intake of dietary fibre is low in the Arab Gulf countries and Middle East countries due to low consumption of vegetables and fruits (Musaiger, 2002; Musaiger, 2012).

It is important to note, that the results for fibre in the present thesis are an estimate of insoluble fibre. The full fibre analysis through the Englyst method, is very time consuming and was considered not to be appropriate within the current study. The major limitations being that some soluble fibre is likely to be lost during the washing and insoluble fibre may be overestimated. However, since the estimation of fibre in both the "smoothie 901" and the commercial smoothies were carried out using the same method, a comparison between these two is applicable.

The total sugar in smoothie 901 was around 9g/100mL whereas in the commercial smoothie's sugar ranged between 8–20 g/100 mL. The sugar content of the 901 smoothie was slightly lower than that of Innocent seriously strawberry smoothie and much lower than that of Naked green machine smoothie ((20g/100mL)). It is recommended that an individual's average daily sugar intake should not exceed 90 g/day. This means that one serving (250 ml) of smoothie 901 would provide approximately 23% of a person's daily sugar intake. Furthermore, the sugar content in smoothie 901 was at the lower end of the range suggesting, from the health perspective that smoothie 901 may be better than the fruit-based drinks in terms of amount of sugar.

Therefore, from a nutritional perspective, it can be concluded that smoothie 901 may be superior to four of the most popular existing smoothies in the UK market in terms of ascorbate level, antioxidant capacity, sugar content and dietary fibre. Also, in context of phenolics it comes at an intermediate level. However, precise nutritional benefits, if any, are unknown.

### 3.16.1 DISCUSSION OF VARIATION IN NUTRIENT CONTENT OF SMOOTHIE 901 COMPONENTS

The first hypothesis was that nutrient levels in the fresh constituents will vary due to seasonality. The preliminary analysis of seasonal variation has been shown to have an effect on all nutritional components of the F&V and consequently on the smoothie. This hypothesis can thus be provisionally accepted. The nutritional composition of beetroot, red pepper, strawberry and orange juice were determined individually. There was variability in the vitamin C, antioxidant and phenolic values of the raw materials included in smoothie 901 throughout the four seasons. Laboratory results were carried out during the years 2010, 2011 and 2017 in each season. These variations between the seasons were expected as these have been shown to be affected by environmental seasonal changes. Also, the recent study of 2018 found an effect of organic farming on L-ascorbic acid which was reliant on cultivar and environment (Martí et al., 2018).

#### 3.16.1.1 Variation in Total Ascorbic Acid (TAA)

According to the laboratory results (Figure 3.3), it appeared that fresh red pepper was the richest source of vitamin C, more than in strawberry and orange juice. While beetroot was poor in vitamin C throughout the four seasons. The results showed that the highest level of ascorbic acid (140mg/100g) was found in red pepper in the winter (January-February), 80mg/100g in spring and the lowest TAA (31mg/100g) was found in the summer. It can be argued that higher temperatures during summer could decrease the amount of vitamin C in red pepper. However, generally the season to harvest red peppers is from August to October (late summer to mid-autumn). This can be compared to the estimated vitamin C value (126mg/100g) in capsicum raw red pepper from the study by McCance and Widdowson (2008). Thus, as observed in the present thesis, the data base value falls within the range.

A study by Deepa et al., in 2006 measured ascorbic acid in several cultivars of red sweet peppers, grown over two consecutive years. They found that ascorbate ranged from 48.23-192.63mg/100g in 2002 and from 75.26-175.46mg/100g in 2003. Again, the results reported in our thesis are consistent with these values which also demonstrate clearly the impact of cultivar and the year of harvest. Another study by Zhang et al in 2003 measured ascorbic acid content in methanol extracts using HPLC and showed that red pepper had a higher content of vitamin C (191.2 ±2.3mg/100g FW) as compared to green and yellow peppers. This value is slightly higher than the maximum observed in the present work.

The TAA values in the cooked beetroot used in the present study ranged from 0-1.6mg/100g and the highest value was again obtained in the winter. McCance and Widdowson in 2008 quoted higher values of 4-5 mg/100g in raw beetroot that has been boiled for 45min. These values are higher than those seen in our thesis. There are only a few studies in the literature that have analysed the vitamin C in beetroot. One recent study by Olumese et al., in 2016, investigated Nigerian Beetroot (Beta vulgaris) and found that vitamin C content was higher in beetroot juice (44.34± 2.8AAEmg/g) than in the raw beetroot (33.02 ± 1.33AAEmg/g). These values appear to be much higher than those reported here or in the data sheets. The values were determined using (DNPH) dinitophenyl hydrazine to measure vitamin C indirectly. Another study done in 2014 by Kazimierczak et al., showed that organic fresh beetroot had significantly higher amounts of vitamin C than conventional beetroots with values ranging from 15.36-20.83mg/100g, which is again much higher than observed in our study, but again reflecting the use of pre-processed beetroot in our case.

Strawberry TAA reached its highest level (34-35mgTAA/100g) during autumn and spring. This might be expected since the season for strawberries is late spring and early summer. In winter, the level of TAA was

28mgTAA/100g and the lowest was again found in the summer (15mgTAA/100g).

In general, the vitamin C level in strawberry ranged from 15-35mg TAA/100g. However, the vitamin C value quoted by McCance and Widdowson (2008) gives a higher value of 57mg TAA/100g. Similarly, the amount of TAA reported on all the strawberry packs was 70-77mg/100g despite the fact that the country of origin was different in different seasons (Holland, Egypt and locally from UK). According to Nuñez-Mancilla et al., in 2013 strawberries had a vitamin C content ranging from 47.09 to 56.37mg/100g. Again, these are higher than found in our study, but these authors pointed out that strawberries are delicate fruits and whilst rich sources of vitamin C, have a short post-harvest life during which vitamin C levels are likely to decline. The paper by Giampieri et al., in 2012 studied the composition and nutritional quality of strawberries and reported vitamin C contents of 58.8 mg/100g. This result is similar to that from McCance and Widdowson (2008) but these were probably obtained from field fresh strawberries rather than shop bought as in the present study.

In the orange juice, the laboratory results showed that total ascorbic acid ranged from 7, 10, 13mg TAA/100mL in spring, summer and autumn, respectively, with the level increasing in the winter to 22mg TAA/100g. However, according to McCance and Widdowson (2008) results of vitamin C amount in 10 samples of 8 premium brands and from concentrates including 'smooth' and 'with bits' orange juices provided a higher average value of

40mg/100mL. These differences might be due to different experimental methods used in the analysis of vitamin C, different type of juices, seasonal changes in the fruit, different production methods or the addition of ascorbic acid for preservative purposes and shelf life.

Generally, orange juice had a lower content of ascorbic acid as compared to red pepper and strawberry. One of the reasons may be that as a juice, vitamin C levels may have deteriorated due to either the juicing process or storage. Overall, there was no obvious general trend in ascorbate levels. A study by Terpstra (2005), found that freshly squeezed orange juice had the highest amount of vitamin C since oranges were picked fresh and were not stored, preserved or exposed to oxygen. They also found by comparing some orange juices that the 'boiled McCain juice' might have had more vitamin C because it was boiled for only two minutes. 'McCain Old South from frozen concentrate' ranked third in both experiments probably because the freezing process preserved vitamin C and possibly early maturing oranges were used. However, other juices such as C-plus and Kool-aid, as expected, had no vitamin C because they were orange flavoured.

Additionally, according to Kabasakalis (2000), who studied ascorbic acid content in commercial orange juice with respect to time and temperature of storage, showed that once stored in the refrigerator in open containers for 31 days, it lost 60 to 67% of its ascorbic acid. However, fresh orange juice lost ascorbic acid at a much slower rate of 7 to 13%.

#### 3.16.1.2 Variation in Total Anti Oxidant Capacity (TAC)

As with the ascorbate, seasonal variations in TAC were also observed. Looking at the laboratory results for TAC (Figure 3.4), beetroot had the highest TAC among the "901" components which ranged from 450 in the winter to 1907µmolesTE /100g in the autumn. The red beetroot has been ranked among the 10 vegetables with the highest antioxidant capacity (Carrillo et al., 2017). The paper by Wootton-Beard et al., (2011) analysed the TPC in 23 commercial vegetable juices using the FRAP, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot +}$) assays. They reported that beetroot had TAC of 1.17mMTE/mL which is much higher than in our present study. Another study by Wootton-Beard et al., (2011) found that beetroot juice had a high TAC of 697.9 ± 1.6 µmol/70 mL using FRAP method. It is difficult to compare these observations directly to our results, but this could equate to around 997 µmol/100g, which is within the range observed in our work. Similarly, another study (Pellegrini et al., 2003) reported a TAC value of 15.31mmol $Fe^{2+}$/Kg FW in beetroot. This could equate to 1531 µmolesTE /100g, which is within the range observed in the present study. It should be noted that a different standard was used in this case for the FRAP assay. Furthermore, another recent study by Carrillo et al., in 2017 found that using certain extraction procedures would underestimate TPC in beetroot. They recommended two novel approaches, QUENCHER (QUick, Easy, New, CHEap and Reproducible) and GAR (global antioxidant response), as these resulted in greater values of TPC in beetroot. However, this study reveals that the traditional extraction-based method appears beneficial to screen changes in the “bioavailable” antioxidant potential.

Red pepper had similar TPC values to beetroot and ranged from 450 in the winter to 1359μmoles /100g in the autumn. There are few comparable reports of antioxidant activity in red peppers. Red bell peppers have been reported to contain 20.98mmol $Fe^{2+}$/Kg FW (Pellegrini et al., 2003). This employed the FRAP assay but utilised a different standard to that used in this present study. A direct comparison cannot be made but this could equate to around 2098μmoles /100g. This is higher than the values found in the present study, but this is probably due to the different standard employed.

Strawberry had a relatively stable TAC starting from the highest value during spring (937μmoles/100g), followed by autumn (893μmoles/100g) and summer (870μmoles/100g). The lowest level of TAC was found in winter (465μmoles/100g). The paper by Pellegrini et al., (2003) reported TAC in wild strawberry as 28mmol $Fe^{2+}$/Kg FW and in cultivated strawberry as 22.74mmol $Fe^{2+}$/Kg FW. These equate to 2800 and 2274 μmoles/100g, respectively and are higher than those reported in our present work. Again, there were different standards used in each case and the reported values are likely for field fresh strawberries rather than shop bought.

The orange juice had the lowest amount of TAC. It was highest in the winter (310μmoles/100mL) and lowest in the autumn (117μmoles/100mL).

The ingredients used in smoothie 901 has significant antioxidant activities. However, the vegetable with the highest antioxidant capacity as found by

Pellegrini et al., (2003) was spinach with 26.94mmol $Fe^{2+}$/Kg FW. Whereas berries had the highest amount of TAC among the measured fruits i.e. blackberry, redcurrant, raspberry (51.53, 44.86, 43mmol $Fe^{2+}$/Kg FW respectively).

A study by Song et al., in 2010 determined the cellular antioxidant activity (CAA) of 27 vegetables commonly consumed in the United States. They found that beetroot, broccoli and red pepper had the highest CAA values, whereas cucumber had the lowest. On the other hand, in 2012 Wang et al., found fruit and fruit juices to be the primary contributors to total dietary antioxidant capacity (from foods/foods and supplements), followed by tea, vegetable and vegetable juices. Carlsen and his colleges (2010) published an inclusive Antioxidant Food Database which analysed 3100 different foods, beverages, spices, herbs and supplements used worldwide, using a modified version of FRAP assay. The study confirmed that plant-based foods contribute significantly more TAC into the human diet than non-plant foods.

#### 3.16.1.3 Variation in Total Phenolics Content (TPC)

Regarding the TPC, the laboratory results (Figure 3.5) showed that orange juice had a very low level of phenols throughout the four seasons ranging from 15mg GAE/100g in the autumn to 45mg GAE/100g in the winter and 23-25mg GAE/100g in summer and spring, respectively. This was probably expected because orange has no strong pigmentation and as juice does not contain the peel, the richest source of phenolics in most fruit. According to a study, processing and storage can affect the phenolic content of plant foods (Bravo, 1998). However, in a study by Gil-Izquierdo et al., (2001), found that homemade squeezed navel orange juice comprised 839mg phenolics/L, including flavanones, flavones and hydroxycinnamic acid derivatives but commercial pasteurized orange juices comprised only 81−200mg/L soluble flavanones. In addition, a recent study by Davies et al., (2017) reported that phenolic composition in orange juice included phenolic acids, mainly ferulic and gallic acid, and flavanones. These compounds can be oxidized to quinones by polyphenol oxidases (PPO) present in vegetable tissues.

Strawberry had the highest level of phenols in spring and winter 194-180mg GAE/100g, respectively. But lowest TPC in the summer (82mg GAE/100g) and medium levels in the autumn (152mg GAE/100g). The author Bravo, (1998) stated that berries, in general, are categorised by their high anthocyanin content. In particular, they found that the TPC in strawberries ranged from 38-218mg/100g FW, values that are consistent with those found in our present study. This data is in contrast with both TAA and TAC levels of our study, which was lower than those reported in the literature. This may suggest that TPC, in contrast to TAA and TAC, is relatively constant during the post-harvest storage of strawberry.

The paper by Fernández-Lara et al., in 2015 analysed the phenolic composition of four strawberry cultivars -Nikte, Zamorana, Jacona and Pakal. They found 24 monomeric phenolics, including 15 anthocyanins, 5 phenolic acids, 1 flavanol and 4 flavonols. They showed that the highest phenolic potential was mainly due to their higher content of anthocyanins, while Pakal was richer in phenolic acids.

Red pepper showed very low TPC in spring and winter, 42 and 47mg GAE/100g, respectively, and reached its peak in the summer (146mg GAE/100g) and had intermediate levels in the autumn (106mg GAE/100g). A study (Marinova, Ribarova and Atanassova, 2005) which determined TPC, using Folin−Ciocalteu assay and total flavonoids in Bulgarian F&V, found that TPC in red peppers was 173mg GAE/100g. This is quite similar to the maximum value found in the present study.

The beetroot had its minimum content in the spring (78mg GAE/100g) and reached its highest TPC in the summer (144mg GAE/100g) followed by autumn (140mg GAE/100g) with intermediate levels in the winter (110mg GAE/100g). A study analysed TPC in beetroot using the Folin−Ciocalteu method and expressed as gallic acid equivalents (GAE) (Kujala et al., 2000). They reported a TPC of 150 mgGAE/100g DW. This was on a dry weight basis, but is very similar to our value based on fresh weight. They also found that the TPC was higher in the peel than in the crown and was lowest in the flesh. In another study, the TAC activity of a range of plant extracts were found to be associated significantly and positively with TPC (Kaur and Kapoor, 2002).

Vegetables were found to have greater phenolic content and consequently higher antioxidant activity. This is perhaps expected as both assays are to some extent based on antioxidant principles. Any differences between our results and those in the literature could be partially explained by differences between cultivars, as shown in some previous work (Pincemail et al., 2012 and Holland, 2008). The cultivars used in the present study were not defined.

Many other factors might influence the nutrient content of fruit and vegetables, such as harvest time, culture conditions and environmental factors like poor climate, unexpected bouts of drought or excessive rainfall. The source of origin, which varied for each ingredient during each period of analysis, is also likely to have an effect on the nutritional composition, i.e. strawberry was grown in Derbyshire during summer and autumn, in Kent during spring and in Egypt during winter. Furthermore, transportation of F&V from their source could have affected their nutritional composition, i.e. red pepper was sourced from Holland. Several studies have shown great variability in the quality and texture of crops grown in different locations and seasons (Knee et al., 1989; Sams 1999). It is well established that there is enormous variability among F&V due to factors of pre-harvesting, during harvesting and the post-harvesting stage. In addition, different experimental analysis of determining nutrients and shelf life in another significant factor.

## 3.17 DISCUSSION OF THE RETENTION OF NUTRIENTS DURING PRODUCTION OF SMOOTHIE 901

The hypothesis to be tested was that the designed vegetable-based smoothie will retain the nutritional value of the fresh constituents. The recovery of TAA in the smoothie was equal to 73, 90 and 94%, respectively over the three consecutive weeks. In general, this showed good retention of the ascorbate in the home-made smoothie 901. The recovery of TAC in the 901 smoothie over the three weeks was 86, 85 and 66%, respectively. Again, this demonstrates reasonable retention of antioxidant capacity in the home-made smoothie 901. The recovery of TPC on the three weeks was 93, 77 and 82%, respectively. This again shows the acceptable recovery of TPC in the smoothie. Thus, in terms of these three nutritional parameters, the hypothesis can be accepted. This is probably to be expected since the smoothie contained the whole ingredients and there was no opportunity for nutrients to be lost by juicing or other processes. However, it does demonstrate there were no adverse interactions brought about by the combination of ingredients in the smoothie.

### 3.17.1 Discussion Retention of Sugars in Smoothie 901

As expected, beetroot had the maximum amount of sucrose and had little glucose and fructose. In contrast, strawberry and pepper had mostly equal amounts of glucose and fructose with very little sucrose. The orange juice presented very stable sugar levels with almost equivalent amounts of glucose, fructose and sucrose. Regarding the recovery of total sugar, it was 100%, 92% and 126% for weeks 1, 2 and 3, respectively. This might suggest that the values for the individual ingredients were indeed correct. However, observing the recovery of each sugar separately, recoveries of glucose and fructose were greater than expected and the recovery of sucrose was lower. This suggests that sucrose, which mainly came from beetroot, was being converted to glucose and fructose by enzyme action during the preparation of the smoothie. In general, plants contain invertase enzymes which are located in several parts of the fruit. Invertase is responsible for sucrose hydrolysis (Luchese et al., 2017) and converts the disaccharide sucrose into the hexose monomers glucose and fructose (Roitsch and González, 2004). Thus, beetroot has a high amount of sucrose, and it is highly unlikely that beetroot will contain high levels of invertase enzyme. However, the other ingredients, in particular, the strawberry (Ranwala et al., 1992) do have significant amounts of this enzyme. Thus, when the ingredients are mixed, the acidic pH which is optimal pH for invertase activity and the temperature will favour the activity of the enzyme (Ranwala et al., 1992).

There is an obvious link between the loss of sucrose and increases in glucose and fructose as the sucrose is probably being catalysed into glucose and fructose by the enzyme invertase. Invertase activity requires a low pH for its activity thus, is favoured by the low pH in the smoothie. Moreover, fructose is perceived by the human tongue as being sweeter than either glucose or sucrose. Thus, the production of more fructose may be beneficial in that it might make the smoothie taste sweeter and be more acceptable to the consumer.

Another discussion point is whether this change in sugar composition has any nutritional impact. Glucose, fructose and sucrose are all dietary sugars, and can all be utilised by the body. Thus, a change in profile is unlikely to have any major nutritional significance. In general, smoothie consumption has increased at a rapid pace due to its convenience and popularity among the population. The production processes include extraction, blending, pasteurising and canning or filling, all of which would possibly result in loss of ascorbate. According to Nagy, (1980) retention of vitamin C was greater in canned juice than in bottled juice due to the reduced activity of the tinplate. However, there is generally a lack of data around the retention of F&V nutrients within smoothies in the literature.

## 3.18 DISCUSSION ON THE STABILITY OF NUTRIENTS IN SMOOTHIE 901 DURING STORAGE

The hypothesis to be tested was that the designed smoothie will be nutritionally stable in storage at 4°C for 5 days. This hypothesis can only be accepted for antioxidants including phenolic content, but cannot be accepted for vitamin C and sugars. Numerous studies have shown that storage has an impact on the quality and nutrient levels of fresh fruits and also their by-products including dried fruits and juice (Gil et al., 2006). However, there are relatively few studies concerning the stability of nutrients in a combined F&V smoothie. Only smoothies that have been commercially treated have been tested. For instance, the work of Castillejo et al., in 2016 analysed a green fresh vegetable smoothie, consisting of cucumber (77.2%), broccoli (12%) and spinach (6%), using two thermal treatments to reduce microbial load and preserve quality (the results will be mentioned later in the specified discussion parts for general comparison and wider knowledge). The results here would be of relevance to home-made smoothie's application to commercial practice and would obviously need to consider and include responses to treatments.

The level of ascorbic acid in 901 smoothie was high when analysed on the day of preparation of the smoothie (28-30mg/100mL). However, as expected, the total ascorbic acid level decreased with the increasing storage time at 4°C. On day 5, the smoothie 901 had lost around 82% of its TAA having only 5mg/100mL. In the study of Castillejo et al., in 2016 the loss in total vitamin C in the green vegetable smoothie were reported after a short time at high temperature (45 s at 90 °C) and mild thermal (3 min at 80°C) treatments. Samples were then stored in the dark at 5 and 15 °C. The samples stored at 15 °C had significantly less vitamin C than the sample stored at 5 °C.

In addition, Kabasakalis in 2000, found that commercial fruit juice ascorbic acid content ranged from (2.4 to 43 mg/100 mL). Then storage in closed containers at room temperature for 4 months caused a loss in ascorbic acid ranging from 29 to 41%. However, open containers of commercial fruit juice, stored outside the refrigerator for 10 days, lost 12.5% of their ascorbic acid content, whereas when refrigerated for the same period the ascorbic acid lost around 9%. It is widely believed that enzymes including ascorbic acid oxidase and peroxidase were probably responsible for the loss of ascorbate since these have been implicated in the metabolism of ascorbate in plants (Smirnoff, 1996). Therefore, it is highly recommended to consume the smoothie 901 on the day of preparation (homemade) when high vitamin C intake is needed.

The antioxidant activity in smoothie 901 showed a stable level over 5 days of storage at 4°C with no statistical difference between 1, 3 or 5 days. The values ranged from 1064 µmoles/100mL on the day of preparation and increased to 1092µmoles/100mL on the third day which then decreased slightly (1039µmoles/100mL) on day 5. This result may have been unexpected given that ascorbate, which is thought to be a contributor to TAC in fruit, was shown to decrease significantly over storage. This might indicate that for smoothie 901 ascorbate is in fact not a major contributor to TAC. The study by Castillejo et al., in 2016 reported a TAC of 234.2 ± 20.3 mg Trolox equivalent $kg^{-1}$ fw, which is approximately 5-fold higher than in the smoothie 901. However, levels of TAC in this green smoothie also remained constant during the short time–high temperature and mild thermal treatments and were better preserved during storage in thermally-treated samples.

TPC stability in smoothie 901 showed a similar pattern as TAC. There were no significant differences in phenolic levels between days 1, 3 or 5 in the smoothie throughout the 5 days of storage at 4°C. The TPC on the day of preparation averaged 88mg/100mL, on day 3 it increased to 95mg/100mL. It then decreases again on day 5 and reached 75mg/100mL.

It was noted that a majority of previous studies testing antioxidant capacity were done via scavenging of the radical 2, 2-diphenyl-1-picryhydrazyl (DPPH) assay (Donzález-tejedor et al., 2017 and Hurtado et al., 2017). Furthermore, in the study of Bahorun et al., in 2004, it was found that there were strong correlations between antioxidant capacity using Trolox equivalent antioxidant capacity (TEAC) and the TPC and total flavonoids amount. However, contents of vitamin C showed poor correlation with TEAC values, whereas no correlation was detected with FRAP values. Likewise, the study of Pantelidis et al., in 2007 found that ascorbic acid level was negatively correlated with FRAP.

The preservation of phenolic compounds could have a great impact on the quality of F&V as it contributes to the enzyme browning action and nutritional status of the product in terms of potential antioxidant capacity. Although there were no significant losses in either TAC and TPC during storage, both had declined slightly after 5 days. The potential reasons for this were similar to those for ascorbate. The loss of phenolics could be via enzymatic and non-enzymatic oxidation reactions. However, according to the recent study of Castillejo et al., in 2016, short time–high temperature and mild thermal treatments of their green vegetable smoothie, increased the TPC by 36% (151.1 ± 4.04 mg $kg^{-1}$fw).

There were very small variations in sugar levels (glucose, fructose and sucrose) in the smoothie 901 but that was expected and may reflect the variation in sugar levels of the individual smoothie ingredients. Glucose and fructose were present at similar levels of 4 and 4.4g /100mL respectively. They both declined slightly, but not significantly, over 3 and 5 days of storage. But the fructose seemed to be more stable of the two. The average amount of sucrose in smoothie 901 was less than glucose and fructose on day 1, which is equal to only 1g/100mL and declined steeply to reach an average of

0.8g/100mL on day 5. In general, by day 5 of storage there seemed to be a decline in the glucose and sucrose content. This may reflect potential microbial contamination in smoothie 901. Therefore, it is best to consume the fresh homemade smoothie 901 before reaching 5 days at 4$^{o}$C.

Most of the studies reported in the literature about the stability of fruit products are related to improved methods of nutrient preservation. A recent study by Hurtado et al., in 2017, used high-pressure processing (HPP), which is non-thermal pasteurisation to retain the properties of some smoothie products. HPP was carried out at 10°C for 5 min. For red fruit-based smoothies, they found that the nutritional losses over storage (at least 14 days at 4 °C) were not as much as was expected This was perhaps due to the high natural antioxidant level provided by the red fruits. There were acceptable retention levels of vitamin C and total phenols. Also, the content of sugar present was not affected by any of the HPP treatments. However, the mild HPP treatment did not change the sensory and nutritional properties of the smoothies.

## 3.19 DISCUSSION OF pH IN SMOOTHIES

In food production, lower pH levels may increase the shelf life of the smoothie (Walkling-Ribeiro et al., 2010). In the present research smoothie 901 had a pH of 4 and statistically it was stable during storage from 1 to 5 days at 4$^{o}$C. A study by Blacker et al., in 2011, presented levels of pH in a range of common fruit smoothies in the UK. The mean pH value for the Innocent smoothies were 3.5 for

Strawberry and Banana, 3.38 for Cranberry and Raspberry, 3.49 for Blueberry and Blackberry, 3.35 for Mango and Passion Fruit and 3.37 for Tropicana Smooth Orange Juice. Therefore, it can be said that fruit smoothies and orange juice are more acidic than smoothie 901. This is probably the result of the relative amounts of vegetables in each case. In addition, the pH of the green fresh vegetable smoothie analysed in the study of Castillejo et al., in 2016 was found to be at pH $4.49 \pm 0.01$. This was similar to the pH of smoothie 901.

Furthermore, a study by Tahmassebi et al., in 2014 measured pH and titratable acidity of Innocent smoothies. The strawberries and bananas had pH of 3.73, mangoes and passion fruit with pH 3.59, and also Diet Coke had pH 2.95. They also assessed their impact on enamel slabs. They found that the smoothies tested were all acidic and had high titratable acidity. They also showed a significant erosion of enamel *in vitro*.

## 3.20 DISCUSSION OF THE RHEOLOGICAL ANALYSIS OF SMOOTHIES AND THE EFFECT OF ENZYMES

The hypothesis to be tested was that the 'treatment with cell wall degrading enzymes will reduce the viscosity of the 901 and 134 smoothies'. This hypothesis can be accepted since the results of the treatment with a pectinase enzyme cocktail reduced the viscosity of smoothie 901 which was then comparable to Innocent smoothie viscosity. Moreover, there was no significant difference between the viscosity of the smoothie 901 after 3 hrs of treatment with pectinase enzyme. According to the results of the tasting session of the present research, the high viscosity of 901 smoothie was an undesirable criterion coming from the inclusion of fibre-rich vegetables in a smoothie. Additionally, it may be considered as a barrier for commercial production mainly because a very viscous F&V combination may be considered as a purée or maybe as baby food. Therefore, the application of cell wall degrading industrial enzymes including pectinase and cellulase may be the key to improve the texture of the smoothies.

Plant cell walls are components of dietary fibre, the major cell wall components of F&V are cellulose, hemicelluloses and pectin (Buren, 1979).

Cellulose is the main cell wall constituent in plant cell walls of F&V. The general structure of cellulose is an unbranched linear chain of glucose monomers with β-1, 4 glucosidic linkages. Cellulose is an insoluble substance which is not digested to any extent by the enzymes of the human gastrointestinal system (Dhingra et al., 2012). Hemicelluloses are cell wall polysaccharides and are variable in structure. The major hemicellulose found in fruit is xyloglucan, which also has a backbone of glucose units with β-1, 4 glucosidic linkages, but differs from cellulose in that they are smaller in size, consist of several other sugars and are typically branched. An example of a major hemicelluloses in the cereals is the arabinoxylans, which have a xylose backbone and with branching.  They mostly contain xylose and some galactose, mannose, arabinose and other sugars (Dhingra et al., 2012).

Pectin is a complex group of polysaccharides which are water-soluble due to the ionic charges on their galacturonic acid residues and exhibit gelling behaviour. Pectin can be entirely metabolised by colonic bacteria (Dhingra et al., 2012). Pectins have many diverse structures depending on the source. Consequently, pectin solutions can have different physical characteristics depending on its content. Major factors influencing the gelling properties of pectin include the degree of methoxylation of the galacturonic acid residues and degree of polymerisation. The pectins in fruits vary in their methoxyl content and hence in their jellying power (Ranganna, 1986). The pectin in beet is also highly acetylated, which prevents jelly formation (Ranganna, 1986).

The properties that pectin brings to food products, in terms of firmness of texture and to viscosity/jelly formation, is partly due to its molecular weight and charge. Pectin contains carboxyl groups, on the galacturonic acid residues, which when ionized contribute to the flow behaviour of pectin solutions because electrostatic repulsion between them cause lengthening of the chain. This influence can be reduced by the addition of either salt or acid (Ranganna, 1986). However, since smoothies cannot have added salt or acid because of health consequences and consumer taste acceptability, the action of degradative enzymes may be used to reduce the viscosity in the designed smoothie.

In the present study, the addition of either a pectinase or cellulose cocktail successfully reduced the viscosity of the vegetable containing smoothies. This was expected as these enzymes are designed to break down the fibre constituents. A major reduction in viscosity was observed when the two enzyme cocktails were added together and reduced the viscosity of the smoothie to a level similar to that of the commercial Innocent (strawberry and banana) smoothie. However, this reduction in viscosity may have been too excessive and the use of a single enzyme cocktail may prove to be appropriate. In case of treatment with pectinase, there appeared to be no or very little further reduction in viscosity of the treated smoothie 901 after about one hour of treatment. This may indicate that by this time the enzymes had reached their limit of digestion. Therefore, the application of cell wall degrading industrial enzymes including pectinase and cellulase may be the key to improve the texture of smoothies for commercial practice.

Pectinase enzyme cocktails are already used in the fruit industries. This enzyme can break down the complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. The role of acidic pectinases in minimising the cloudiness and bitterness of fruit juices is well recognised (Kashyap et al., 2001). Similarly, cellulase enzyme cocktails are also used in food production, brewing and wine making, animal feed, textile and laundry industries, pulp and paper industries as well as in agriculture (Bhat, 2000).

Brito et al., in 2012 used a mixture of commercial cell wall enzymes which contained balanced proportions of pectinases and cellulases with other different secondary activities to hydrolyse the main polysaccharides in fully ripe table mango fruit. The result of their research showed that the rheological properties of mango purées could be controlled simply by altering the concentration of the enzyme solution and incubation time. The consistency of the mango puree could be significantly reduced in minutes using low quantities of enzymes (150 $\mu l.L^{-1}$) and would be suitable for the needs of food production.

## 3.21 CONCLUSION OF THE SMOOTHIES NUTRITIONAL ANALYSIS

Some actual nutritional contents of the “new 901 smoothie recipe” were determined (vitamin C, antioxidant activity, phenolic content, sugar and fibre) and compared with commercially available smoothies. The nutritional analysis demonstrated that 901 smoothie contained higher vitamin C, antioxidant capacity, phenols and fibre than the nearest commercially equivalent smoothies.

## 3.22 CONCLUSION MEASURING VARIATION NUTRIENTS IN INDIVIDUAL INGREDIENTS OF SMOOTHIE 901

The nutrient levels recorded in our experiments during 2011, 2012 and 2017 for the 901 smoothie preparations are shown in (Table 3.9) it showed a range of values that may be related to seasonal variation in the nutritional content of the ingredients. Moreover, the nutritional composition of the smoothie was likely to vary with season.

## 3.23 CONCLUSION RETENTION NUTRIENTS DURING PRODUCTION AND STABILITY DURING STORAGE OF SMOOTHIE 901

A preliminary study was undertaken to assess variation in the nutritional composition of the raw ingredients and the retention and stability of these in the "smoothie". The major findings that can be drawn from both sections 3.17 and 3.18:

The 'home-made' smoothie retained the nutritional value of its fresh F&V constituents. Comparable levels of sugar, fibre and vitamin C were found in both.

TAA in the 901 smoothie showed a sharp decline during 3 and 5 days of storage at 4°. The TAA was not stable. TAC levels in the 901 smoothie showed a slight increase at day 3 of storage but in general TAC appeared to be stable over 5 days of storage at 4°C. The TPC levels in the 901 smoothie showed a slight increase on day 3 of storage followed by a slight decrease on day 5. However, in general, TPC appeared to be stable between day 1 and 3 or 5 days during storage at 4°C.

Glucose and fructose showed stability in the 901 smoothie between 1 to 5 days. However, levels of sucrose were not stable in the 901 smoothie between 3 and 5 days of storage. The potential barrier that might prevent the commercial production of a vegetable-rich smoothie is the pH level. However, the pH in 901 smoothie was stable during 1 to 5 days of storage at 4°C.

## 3.24 CONCLUSION OF RHEOLOGICAL ANALYSIS OF SMOOTHIES AND THE EFFECT OF ENZYMES

The texture (mouthfeel and high viscosity) was, as expected, identified as the major contributor in consumer acceptance. A preliminary study was thus undertaken to assess the potential to use plant cell wall degrading enzyme cocktails to improve the textural properties of the products.

One problem with the vegetable-based smoothies was the high viscosity and adverse mouthfeel/texture. Treatment with a pectinase enzyme mix was able to reduce the viscosity to a value similar to that of a commercial smoothie. The enzyme action will definitely affect the levels of fibre in the smoothie. The plant cell wall material forms a major part of the dietary fibre. However, the enzyme cocktails used in this study contains enzymes that attack all three major components of the cell wall i.e. cellulose, hemicellulose and pectin. In general, pectin is the most readily degraded polymer and is thus likely to be the most affected. It is also probably the most important in terms of fibre. Cellulose is most resistant to degradation. It is probable that the enzyme treatment will not degrade all of the polymers. Indeed, the final viscosity as determined by the experiments was above that of a simple water solution suggesting that some of the fibre remains.

The two enzyme cocktails used in the experiments were chosen because they were readily available in the laboratory at that time (they were being used in a bioenergy study). It was felt that they can be representative of other enzyme cocktails and thus suitable for this essentially “proof of concept” study.

Similar enzyme cocktails are commercially available that are classed as GRAS (generally regarded as safe) and can be used in food preparation. These were not available in the laboratory at the time of the present investigation.

# CHAPTER 4 OVERALL SUMMARY

The ultimate aim was to assess whether it would be feasible to produce mixed fruit and vegetable "drinks" with improved nutritional characteristics either commercially or via homemade recipes.

The major findings that can be drawn from this research are as follows:

- University students failed to meet the 5 A DAY recommendation in the study undertaken. This indicates lower than recommended intake of antioxidants such as vitamin C and polyphenols in both the UK and KSA.
- In KSA more awareness is needed about smoothies being a part of F&V intake.
- Smoothie is a popular drink in both countries but was more preferred within the UK sample. Besides both samples consider a smoothie drink as part of F&V consumption and they consume it occasionally.
- From the questionnaire responses received, a novel vegetable combined with fruit-based smoothie would be acceptable.
- The nutritional analysis confirmed that "901 smoothie" had higher TAA, TAC, TPC and fibre than the nearest commercially available equivalent smoothies.
- The nutritional compositions of the 'home-made' smoothie were likely to vary with season, but "901 smoothie" retained the nutritional value of its fresh F&V ingredients.

- The TAC, TPC, glucose and fructose levels in the 901 smoothie appear to be stable for upto 5 days during storage at 4°C. But, TAA and sucrose levels were not stable in the 901 smoothie between 1 to 5 days of storage at 4°C.
- From the commercial perspective, the most important factor was the flavour. However, texture and price were also considered as significant factors.
- The potential barriers that might prevent the commercial production of a vegetable-rich smoothie were pH and consumer acceptability.
- Treatment of 901 smoothie with enzymes decreased the viscosity, which was then comparable to the commercial smoothie.

This project sought to encourage and increase the consumption of vegetables by designing a vegetable-containing smoothie that had a higher nutritional value and also met consumer preferences and acceptance. The project focused on the challenges around the production of "smoothies" with improved nutritional value by the introduction of mixed fruit and vegetable-based drinks.

The results suggest that smoothies could provide one option for increasing F&V consumption. This is a convenient way to help individuals consume the recommended number of portions of F&Vs, especially amongst students, whose lack of time can be an obstacle to maintaining a healthy diet.

## 4.1 LIMITATIONS OF THE STUDY

The present work makes a valuable contribution to nutrition research. However, the number of subjects, both responding to the questionnaire and in the tasting sessions was small. Use of a power calculation in future work, based on the present work data would improve reliability. The fact that all the subjects in this study were students is also a limitation and a broader sample would be required to reflect accurate opinions of the general public. A randomised control study of adult subjects in both the UK and KSA would be more appropriate. The panel members in the tasting session were untrained. It could be that a more sophisticated tasting session using trained panels would give better results. The use of a trained panel, in particular for sensory tests for flavour, along with appropriate viscosity testing for measuring texture would improve the study design.

Similarly, the numbers of replicates of the raw ingredients to investigate potential seasonal variation were also quite small. This does serve to demonstrate a variation, but the exact nature and extent of that variation across a wider range of sources of the material should be carried out. The smoothie 901 was chosen for the detailed study due to its acceptance by the consumers of this study. It contained beetroot and red pepper as the "vegetable" components, but green leafy vegetables are potentially healthier ingredients according to a number of studies. Thus, further analysis of those smoothies incorporating green leafy vegetables would be advisable. Whilst the nutrients measured in this study are nutritionally important constituents of fruit and vegetables, the number was limited and assays such as the TAC and TPC give only relative amounts of active components and may not be related directly to nutritional benefit. The fibre content in the smoothie 901 was only estimated in this study and could be explored further by a more detailed analysis of soluble and insoluble fibre content. The enzyme cocktails employed in this study were not food grade.

## 4.2 FUTURE WORK AND RECOMMENDATIONS

Given the limitations to the study, there are a number of potential areas for further work. Identifying smoothies that would appeal to a majority of consumers would be important. Thus, the survey work should be expanded from students to the general public as this would provide a broader insight into smoothie acceptability and compliance. This could also serve to obtain a wider insight into F&V consumption within the Gulf region in particular. Based on the studies in the literature review there is an urgent need for nutrition education programmes and FBDG policies to develop the nutritional knowledge, especially in the case of F&V consumption, among the AGR population, particularly in KSA. This is important to ensure healthy diets and to raise awareness about the diet-related diseases and their related risk factors due to the high prevalence of these diseases. Moreover, if started from an early age (good nutrition with high F&V intake), would make it easier to eventually increase intake in adults, thus helping to reduce obesity and chronic disease risks. Therefore, it can be recommended for the KSA population to implement an effective F&V scheme in schools.

More effort into the production of smoothies containing green leafy vegetables, such as spinach and parsley, should be pursued as these may be nutritionally significant. Also, it would be interesting for future research to determine their consumer acceptability. This will involve research into methods to improve their flavour and texture characteristics. In the case of viscosity, nutritional analysis of smoothies treated with enzymes should be conducted to establish any nutritional impact of the added enzymes. In particular, their potential impact on fibre since they would be expected to reduce this nutritional parameter. Suitable food grade enzyme cocktails should be found and tested. Tasting sessions with trained panels for the treated smoothie with lower viscosity, would also be interesting.

Research into the commercial viability of vegetable-based smoothies or beverages is required as profitability underpins any sustainable business. In regard to commercial production shelf life is a key consideration. A high-temperature heat treatment that preserves nutritional quality during storage would likely be employed to enhance product stability and shelf-life.

The texture is likely to be a major concern for consumer acceptability of any smoothie with a high vegetable content. The preliminary studies in the present thesis demonstrated that treatment with plant cell wall degrading enzyme cocktails may represent one potential approach to solve this problem. However, this is unlikely to be a realistic approach for "homemade" smoothies but could have potential commercial application. The use of these enzymes in the beverages industry is already well established, for instance, they are used for controlling cloudiness in fruit juices. It was originally proposed to investigate any type of impact of these enzymatic treatments on the nutritional composition of the smoothie, but time did not allow us to investigate further. Thus, further work in this area would be required. Also, the effect of the nutritional value of enzymatically-treated smoothie especially the actual impact on fibre and sugar would need a more detailed study.

The nutritional aspects of product development for functional/super drinks, for example, drinks with high antioxidant content could also be further investigated. It would be beneficial to find ways of enhancing the antioxidant and phenol levels in smoothies, such as adding ingredients such as green tea extract. In addition, it may also be possible to add seed extract into smoothies to increase TPC, as shown in some recent work by Xi et al., in 2017, to increase flavonoid content from the seeds. Also, green extracts from algae such as spirulina may be added for major health benefits. Furthermore, a dairy component in the form of yoghurt can be added for protein enhancement. This may also impact the texture. It would be interesting for future research to determine the nutritional composition of such modified smoothies.

This research will assist nutrition authorities, governments and commercial concerns to identify the potential for the production of smoothies, either commercially or homemade, that may enhance the intake of F&V and provide nutritional benefit. However, the potential health benefits of such smoothies should also be further investigated. Clinical intervention studies involving smoothie consumption would be interesting for future research, as these could determine the impact of consumption on the individuals' diet. Also, the role of specific antioxidants and phenolics in *vivo* should be investigated. These animal models and human studies are more appropriate but also more expensive and time-consuming, perhaps the participants can be monitored for one month using a placebo (coloured water), and then for another with smoothies in their diet. Also, more market research analysis on smoothie sales and profit in the KSA is recommended.

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# APPENDIX-1

I am a PhD student at The University of Nottingham, studying smoothie consumption and preferences.
I would appreciate it if you could complete all questions as honestly as possible. The questionnaire is 5 pages and will not take more than 10 minutes.
If you need any help please do not hesitate to ask me or contact me by e-mail: sbxbd@nottingham.ac.uk

***Section 1: General Questions:***

1. Gender? (Please tick)
    - Female
    - Male

2. Age? (Please tick)
    - 18-22 years
    - 23-25 years
    - 26-35 years
    - 36-50 years
    - 51-70 years
    - 71 and older

3. Course code..............................................

4. Year..........................................................

5. Tick according to your personal preference your reasons, when making drinks choices (purchasing non-alcoholic drinks):

| Attributes | Not at all important | Some importance | Very important |
|---|---|---|---|
| **a. Health benefits** | | | |
| **b. Cost effectiveness** | | | |
| **c. Convenience** | | | |
| **d. Personal preference** | | | |
| **e. Others. Please specify:** | | | |

***Section 2: This part deals with fruits and fruits intake:***

6. What are your 3 favourite fruits? *Please bear in mind Tomato is a fruit.*
   ………………………………………………………………
   ………………………………………………………………
   ………………………………………………………………
7. Please list the fruit you consumed yesterday and how many?
   ……………………………………………………………………
   ……………………………………………………………………
   ……………………………………………………………………
8. Have you ever tried a smoothie product? (A smoothie is a mix of blended fruit or vegetable with a thick consistency)
   - o Yes
   - o No
9. Do you like smoothies?
   - o Yes
   - o No
10. How often do you drink smoothies?
    - o Twice a day
    - o Every day
    - o 3 times a week
    - o Twice a week
    - o Once a week
    - o Once a month
    - o Very occasionally
    - o Never

11. Tick according to your personal importance, reasons for drinking smoothies*:*

| **Attributes** | **Not at all important** | **Some importance** | **Very important** |
|---|---|---|---|
| **a. I like the taste** | | | |
| **b. Filling as a snack** | | | |
| **c. Perceived health benefits (including Antioxidant, Detox and Reduce incidence of diseases)** | | | |
| **d. Helps me meet the 5 A DAY recommendation** | | | |
| **e. Replacement for fruit & vegetables:** | | | |

| | | | |
|---|---|---|---|
| **f. Replacement for other hot or cold drinks; like water, coffee, tea or fizzy drinks.** | | | |
| **g. Others. Please specify:** | | | |

12. Do you consider smoothies (not from concentrate) as a part of the 5 A DAY recommendation?
    - Yes
    - No
    - Don't know

13. Tick according to your personal preference, fruit you would find most appealing in a homemade smoothie or one prepared in a smoothie bar? *Please bear in mind Tomato is a fruit.*

| **Fruits** | **Not at all appealing** | **Appeals a little** | **Appeals a lot** |
|---|---|---|---|
| **a. Apple** | | | |
| **b. Banana** | | | |
| **c. Kiwi** | | | |
| **d. Lemon** | | | |
| **e. Melon** | | | |
| **f. Orange** | | | |
| **g. Pear** | | | |
| **h. Pineapple** | | | |
| **i. Strawberry** | | | |
| **j. Tomato** | | | |
| **k. Others. Please specify:** | | | |

14. The smoothies on the market may contain some of the following additions, which would you find it most appealing to your fruit smoothie:

| **Additions** | **Not at all appealing** | **Appeals a little** | **Appeals a lot** |
|---|---|---|---|
| **a. Milk** | | | |
| **b. Semi skimmed milk** | | | |

| | | | |
|---|---|---|---|
| c. Yoghurt | | | |
| d. Low fat yoghurt | | | |
| e. Probiotic yoghurt | | | |
| f. Soya milk | | | |
| g. Oats | | | |
| h. Nuts | | | |
| i. Honey | | | |
| j. Nothing | | | |
| k. Others. Please specify: | | | |

15. Would you prefer:
    - Pure smoothie (100% fruit)
    - Smoothie with additions

## *Section 3: This part deals with vegetables and vegetables intake:*

16. What are your 3 favourite vegetables? *Please bear in mind that potatoes do not count as vegetables.*
    ..........................................................................
    ..........................................................................
    ..........................................................................

17. Please list the vegetables you consumed yesterday and how many?
    ................................................................................
    ................................................................................
    ................................................................................

18. Tick according to, how often do you consume the following vegetable products?

| Products | Daily | 3 times a week | Twice a week | Weekly | Monthly | Occasionally | Never |
|---|---|---|---|---|---|---|---|
| a. Ready prepared salad. (ready to eat) | | | | | | | |
| b. Ready prepared vegetables on a tray. For example: cauliflower with cheese. (needs to be warmed) | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **c. Vegetable drink. For example: tomato juice, carrot juice or V8 juice. (ready to drink)** | | | | | | | |
| **d. Vegetable soup. (needs to be warmed)** | | | | | | | |

19. Tick according to your preference, vegetables you would prefer in a vegetable based smoothie?

| Vegetables | Do not prefer | Prefer a little | Prefer a lot |
|---|---|---|---|
| **a. Beetroot** | | | |
| **b. Broccoli** | | | |
| **c. Cabbage** | | | |
| **d. Carrot** | | | |
| **e. Cauliflower** | | | |
| **f. Celery** | | | |
| **g. Green pepper** | | | |
| **h. Lettuce** | | | |
| **i. Parsnip** | | | |
| **j. Radish** | | | |
| **k. Red pepper** | | | |
| **l. Spinach** | | | |
| **m. Spring greens** | | | |
| **n. Sweet potato** | | | |
| **o. Turnip** | | | |
| **p. Others. Please specify:** | | | |

20. Would you find a 100% vegetable smoothie acceptable
    - Yes
    - I might give it a try
    - No. If No, why not? Please specify....................................
21. Would you find a smoothie containing vegetables combined with fruit acceptable?
    - Yes
    - I might give it a try
    - No. If No, why not? Pleases specify..................................
22. If 1 fruit and 2 vegetables were mixed in a smoothie, what might be your favourite combination? (For example Apple, carrot and beetroot) Please write down your own combination:

    Fruit: ..........................................................................

    Vegetable 1: ..................................................................

    Vegetable 2: ..................................................................

23. The smoothies on the market may contain some of the following additions, which would you find it most appealing to your vegetable and fruit smoothie:

| Additions | Not at all appealing | Appeals a little | Appeals a lot |
|---|---|---|---|
| **a. Milk** | | | |
| **b. Semi skimmed milk** | | | |
| **c. Yoghurt** | | | |
| **d. Low fat yoghurt** | | | |
| **e. Probiotic yoghurt** | | | |
| **f. Soya milk** | | | |
| **g. Oats** | | | |
| **h. Nuts** | | | |
| **i. Herbs; like mint or parsley** | | | |
| **j. Honey** | | | |
| **k. Nothing** | | | |

| | |
|---|---|
| l. | Others. Please specify: |

24. Tick according to your personal importance, what would encourage you to buy and consume a smoothie containing vegetables?

| Attributes | | Does not encourage me at all | Encourages me a little | Encourages me a lot |
|---|---|---|---|---|
| a. | Perceived health benefits (including Antioxidant, and Reduce incidence of diseases) | | | |
| b. | Helps me meet the 5 A DAY recommendation | | | |
| c. | Tasty | | | |
| d. | Low in price | | | |
| e. | Replacement for fruit & vegetables: | | | |
| f. | Nothing will encourage me. Please specify why: | | | |
| g. | Others. Please specify: | | | |

## *Section 4: Marketing:*

25. Please indicate the maximum price that you would be prepared to pay for a 250ml smoothie bottle (or 1 glass portion):

- £1.00 - £1.50
- £1.51 - £2.00
- £2.01- £2.50
- £2.51 - £3.00
- £3.01- £3.50

26. Please indicate the maximum price that you would be prepared to pay for a 1L. Smoothie bottle (or carton):
- £1.50 - £2.00
- £2.01 - £3.00
- £3.01 - £4.00
- £4.01 - £5.00

*Based on the information received from students completing these questionnaires, I will prepare combinations of suggesting smoothies. Tasting sessions will be held in the dietetic lab at the North lab in the Sutton Bonington campus. It will require maximum 15 minutes, on the date that will be confirmed (likely to be immediately after the exams).*

*If you are willing to participate please confirm your name, study course and your contact details by sending me an e-mail.*

*My e-mail is:* sbxbd@nottingham.ac.uk

Thank you for your time, I appreciate your help.

## APPENDIX-2 Novel Smoothie Evaluation Sheet

**Please taste the six smoothie samples in random order and answer the following questions for each sample. Please use the water provided to cleans your palate before tasting each sample.**

Sample: 348

1- **How acceptable is this sample:**

__ Like very much

__ Like slightly

__ Neither like nor dislike

__ Dislike slightly

__ Dislike very much

**Rate preference in term of:**

2- **Colour:**

Attractive ☐ Neither ☐ Unattractive ☐

3- **Flavour:**

Acceptable ☐ Neither ☐ Unacceptable ☐

4- **Mouth feel:**

Smooth ☐ Neither ☐ Very thick ☐

5- **If you do not like this sample please comment on why?**

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

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

6- **Would you buy a 250ml smoothie for £1.50 of this sample?**

Yes ☐ No ☐

Sample: 267

1- **How acceptable is this sample:**

__ Like very much

__ Like slightly

__ Neither like nor dislike

__ Dislike slightly

__ Dislike very much

**Rate preference in term of:**

2- **Colour:**

Attractive ☐ Neither ☐ Unattractive ☐

3- **Flavour:**

Acceptable ☐ Neither ☐ Unacceptable ☐

4- **Mouth feel:**

Smooth ☐ Neither ☐ Very thick ☐

5- **If you do not like this sample please comment on why?**

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

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

6- **Would you buy a 250ml smoothie for £1.50 of this sample?**

Yes ☐ No ☐

Sample: 551

1- **How acceptable is this sample:**

__ Like very much

__ Like slightly

__ Neither like nor dislike

__ Dislike slightly

__ Dislike very much

**Rate preference in term of:**

2- **Colour:**

Attractive ☐ Neither ☐ Unattractive ☐

3- **Flavour:**

Acceptable ☐ Neither ☐ Unacceptable ☐

4- **Mouth feel:**

Smooth ☐ Neither ☐ Very thick ☐

5- **If you do not like this sample please comment on why?**

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

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

6- **Would you buy a 250ml smoothie for £1.50 of this sample?**

Yes ☐ No ☐

Sample: 134

1- **How acceptable is this sample:**

__ Like very much

__ Like slightly

__ Neither like nor dislike

__ Dislike slightly

__ Dislike very much

**Rate preference in term of:**

2- **Colour:**

Attractive ☐ Neither ☐ Unattractive ☐

3- **Flavour:**

Acceptable ☐ Neither ☐ Unacceptable ☐

4- **Mouth feel:**

Smooth ☐ Neither ☐ Very thick ☐

5- **If you do not like this sample please comment on why?**

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

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6- **Would you buy a 250ml smoothie for £1.50 of this sample?**

Yes ☐ No ☐

**Please turn the page**

Sample: 729

1- **How acceptable is this sample:**

__ Like very much

__ Like slightly

__ Neither like nor dislike

__ Dislike slightly

__ Dislike very much

**Rate preference in term of:**

1- **Colour:**

Attractive ☐ Neither ☐ Unattractive ☐

2- **Flavour:**

Acceptable ☐ Neither ☐ Unacceptable ☐

3- **Mouth feel:**

Smooth ☐ Neither ☐ Very thick ☐

4- **If you do not like this sample please comment on why?**

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

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

5- **Would you buy a 250ml smoothie for £1.50 of this sample?**

Yes ☐ No ☐

Sample: 901

2- **How acceptable is this sample:**

__ Like very much

__ Like slightly

__ Neither like nor dislike

__ Dislike slightly

__ Dislike very much

**Rate preference in term of:**

1- **Colour:**

Attractive ☐ Neither ☐ Unattractive ☐

2- **Flavour:**

Acceptable ☐ Neither ☐ Unacceptable ☐

3- **Mouth feel:**

Smooth ☐ Neither ☐ Very thick ☐

4- **If you do not like this sample please comment on why?**

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

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

5- **Would you buy a 250ml smoothie for £1.50 of this sample?**

Yes ☐ No ☐

## Final Stage:

**7- Which sample do you like better?**

| 348 | 267 | 551 | 134 | 729 | 901 |
|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

8- **Would you buy a 250ml smoothie for £1.50 of this preferred sample regularly?**

Yes ☐ No ☐

9- **If no, please comment on why?**

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10- **Your age:**

- 18-22 years
- 23-25 years
- 26-35 years
- 36-50 years
- 51-70 years
- 71 and older

11- **Your nationality:**

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12- **Any comments:**

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**Thank you for your participation**

**Evaluation Sheet for Identify Liking and Preference, Including Taste, Colour and Texture of the Six Selected Vegetable–based Smoothies**