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**The effect of high-polyphenol sumac on food intake in younger and older adults, using
sensory and appetite analysis**

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Abstract

Ageing sees a decline in appetite and food intake with associated deficiencies in both macronutrients and micronutrients. Reduced food intake will eventually lead to malnutrition. Different factors may contribute to malnutrition, including the physiological condition of a person, such as smell or taste dysfunction. Research has investigated methods that help to increase food consumption in the older population, with some studies demonstrating the impact of added herbs and spices on improving the liking and palatability of food. Spices with high polyphenol and antioxidant activity exhibit various health benefits, including regulating blood glucose levels. Moreover, monosodium glutamate (MSG) has been added to different foods, resulting in flavour enhancement and increased appetite. Hence, it can be hypothesised that sumac, containing glutamic acid, in addition to its high antioxidant and polyphenol content as a potential natural flavour enhancer, might also increase appetite among older adults.

This PhD study aimed: (1) to determine the antioxidant activity (mol/ L), polyphenol content (mg GAE/g) and glutamic acid level (g glutamic acid/100g protein) of two forms of sumac (fresh and commercial) from four regions (Turkey, Palestine, Iran and the UK), in two colours (red and brown), using three solvents (water, acetone 80% and ethanol 80%); (2) to assess the acceptability and liking of different doses (0%, 0.25%, 0.5%, 0.75% and 1%) of sumac among free-living older and younger adults; (3) to evaluate food intake following the consumption of sumac (1%) in vegetable soup – during an *ad libitum* lunch, a main evening meal and an all-day – among free-living older adults , younger adults and older adults living in residential nursing homes. The food intake of older adults in residential nursing homes was assessed based on an *ad libitum* lunch and all-day data. Soup samples were compared based on different methods for the addition of sumac: sumac during (SSC) and after cooking (SSE), along with a control soup (SC) with no sumac added.

To evaluate the polyphenol content and antioxidant activity of the sumac samples, a Folin-Ciocalteu assay (FCR) and ferric ion reducing antioxidant power (FRAP) were used, respectively. L-glutamic acid was assessed using a Megazyme L-glutamic assay. Compusense (Compusense Inc., Ontario, Canada) software was used to assess the sensory evaluation attributes of free-living older adults (over 65 years old) and younger adults (18–35 years old) receiving different doses of sumac in butternut squash soup. Nutritics software was used to assess food intake following the addition of 0.37g of sumac to soup.

This study demonstrates that fresh brown sumac (FBS) had the highest levels of antioxidants (water: 14.1 ± 4.9 , acetone 80%: 14.2 ± 2.1 mol/L), polyphenols (water: 2.4 ± 1.3 , acetone 80%: 5.4 ± 5.3 and ethanol 80%: 3.4 ± 1.4 mg GAE/g) and L-glutamic acid (8.7 ± 0.2 g glutamic acid/100g protein). Following the FBS sample, commercial Iranian brown sumac (CIBS) had the next highest antioxidant (water: 9.1 ± 2.7 , acetone: 13.9 ± 2.1 and ethanol: 27.6 ± 6.3 mol/L), polyphenol (water: 1.5 ± 0.7 , acetone: 5.1 ± 4.8 and ethanol: 2.7 ± 0.6 mg GAE/g) and L-glutamic acid (5.2 ± 0.3 g glutamic acid/100g protein) levels. A positive correlation coefficient was observed between polyphenol and antioxidant activity in water ($r=0.66$, $p=0.001$), acetone 80% ($r=0.787$, $p=0.001$) and ethanol 80% ($r=0.388$, $p=0.001$).

Sensory evaluation results revealed that the intensity of the brown colour between the soup samples and control soup was greater in the younger adults group ($\chi^2(4) = 23.5$; $p=0.001$) than free-living older adults ($\chi^2(4) = 10.2$; $p=0.037$). Other attributes did not show any statistical differences; however, the highest dose of sumac in soup received the highest ranking for overall liking acceptance by both groups of adults compared with the other samples. This study found that during the *ad libitum* lunch, free-living older adults consumed more energy (kcal; $\chi^2(20) = 8.6$; $p=0.014$), protein (g; $\chi^2(20) = 7.4$; $p=0.025$), carbohydrate (g; $\chi^2(20) = 8.7$; $p=0.013$) and fat (g; $\chi^2(20) = 12.6$; $p=0.002$) in sumac added at the end (SSE), and sumac contributed to soup (SSC) sessions compared to control session (SC). The outcome of a pairwise comparison

confirmed that the two methods of adding sumac (SSE and SSC) compared with the control session (SC) had no impact on food intake ($p>0.05$). The evening meal outcome showed younger adults had a higher intake of protein in the SSC session compared with free-living older adults ($t(38) = 10.02$; $p=0.003$). In continuation to the assessment of both groups of adults, all-day protein intake was significantly higher in younger adults compared with free-living older adults in both SSE ($t(38) = 0.54$; $p=0.006$) and SSC ($t(38) = 1.72$; $p=0.004$) sessions. The influence of the addition of sumac following the SSE and SSC sessions among residential older adults demonstrated a borderline difference in the carbohydrate ($F(2,8) = 0.558$; $p=0.049$) intake of the *ad libitum* lunch. However, a pairwise comparison demonstrated no differences between test sessions ($p>0.05$). Thus, the overall findings of this thesis suggest that the addition of sumac to food may have a potential benefit for *ad libitum* lunch intake, leading to a more effective strategy for managing malnutrition and increasing healthy ageing, which can contribute to reductions in cost and concern for public health.

Conference abstract and Publication

Determination of the antioxidant activity and polyphenol content of different types of *Rhus coriaria Linn* (sumac) from different regions. Proceedings of the Nutrition Society. **76 (OCE4)**, E137.

List of Abbreviations

- MSG: Monosodium glutamate
- FCR: Folin-Ciocalteu reagent
- FRAP: Ferric ion reducing antioxidant power
- GAE: Gallic acid equivalent
- TAC: Total antioxidant activity
- SD: Standard deviation
- CTS: Commercial Turkish sumac
- CPS: Commercial Palestinian sumac
- CIRS: Commercial Iranian red sumac
- CIBS: Commercial Iranian brown sumac
- FRS: Fresh red sumac
- FBS: Fresh brown sumac
- SC: Control Soup
- SSE: Sumac added at the end soup
- SSC: Sumac added during cooking
- T: Texture
- T&F: Taste and flavour
- A: Appearance
- LS: Low dose (0.25%)
- MS: Medium dose (0.50%)
- HS: High dose (0.75%)
- TS: Total dose (1%)

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Chapter 1: Literature Review

1.1. Introduction to functional foods and nutraceuticals

The term "nutraceutical" was coined by Stephen DeFelice in 1989 and originates from two words: nutrition and pharmaceutical (Egharevba and Gamaniel, 2017). According to his description, a nutraceutical refers to a food or any part of a food that contributes to health, regardless of whether it acts as a prevention or treatment of illness (Cencic and Chingwaru, 2010; Egharevba and Gamaniel, 2017). A generally accepted definition of 'functional food' is lacking. However, the International Life Sciences Institute refers to food as being composed of beneficial components that contribute to health, other than fundamental nutritional advantage (International Life Sciences Institute, 1999).

Early records demonstrate that herbs and spices were traditionally used to enhance the taste of food, as well as for medicinal purposes (Kaefer and Milner, 2008). The majority of traditional dishes are well known for their signature herbs and spices and the health benefits they confer on the world (Kaefer and Milner, 2008; Abu-Reidah *et al.*, 2014; Asgarpanah and Saati, 2014). The terms 'herbs' and 'spices' are used interchangeably, although the word 'herbs' refers to green and leafy plants with seasoning for medicinal purposes, and the term 'spices' is applied to plants used for flavouring and as condiments. It has been demonstrated that spices enrich the taste and aroma of food, even though they are also recognised for their health benefits (Egharevba and Gamaniel, 2017).

It is a widely held view that phenolic compounds are secondary metabolites found naturally in many plants (Manach *et al.*, 2004). Current findings have reported that the primary sources of phenolic compounds are not limited to fruit and vegetables but also include herbs, spices and grains (Hollman, 2014). Epidemiological studies have also confirmed the association between a high level of antioxidant consumption and decreased rates of mortality and morbidity

(Devasagayam *et al.*, 2004). As a result, the enhancement of foods using natural antioxidants and polyphenols drew the researchers' attention (Bashash *et al.*, 2012). Many published studies describe the role of sumac in health and its vital role in treating various diseases (Abu-Reidah *et al.*, 2014; Romeo *et al.*, 2015). This spice is commonly used in Middle Eastern dishes for seasoning and flavouring; it grows in the wild in tropical regions (Zargham and Zargham, 2008).

This literature review attempts to highlight the physical characteristics, chemical composition, biological activities, sensory evaluation and potential health benefits of sumac. The influence of ageing on food intake among older adults, and the impact of food interventions using herbs and spices, is presented.

1.2. Sumac

1.2.1. Description, definition and morphological structure of sumac

Rhus coriaria Linn (generally known as sumac; the name originates from 'summaq', meaning 'dark red' in Arabic) is a spice that is most notably used in Iranian, Turkish and Middle Eastern foods, in either pure form or combined with other herbs (Za'tar) (Nasar-Abbas and Halkman, 2004). In these regions, sumac fruit is used for seasoning or flavouring (in crushed form). It is used as an appetiser, for souring food, condiments or drinks (with an astringent taste), on meat (kebabs) and in soups, salads and stuffed grape leaves (Özcan and Haciseferogullari, 2004).

Sumac has a tangy flavour, due to the presence of citric and malic acids that account for the sour taste of this spice (Bahar and Altug, 2009; Abu-Reidah *et al.*, 2014). Sumac has a long history of health benefits; it was used in folk medicine to treat different types of diseases (Sakhr and El Khatib, 2020). Prior studies have noted the vital usage of sumac in the leather industry for dyeing, due to the presence of tannins (Shabbir, 2012). The plant presents as a small shrub

or tree that is typically 3-4 metres in height, with small greenish-white flowers; the fruit has a red colour with one seed (Caliskan and Nur Dirim, 2016). A morphological analysis of sumac illustrates that this plant is composed of 9–15 leaflets, and the leaves are not seen in paripinnate form (Shabbir, 2012).

1.2.2. Historical usage of sumac

Sumac has long been used as a herbal medicine by Middle Eastern and Mediterranean practitioners of traditional medicine (Shabbir, 2012). Knowledge of the health benefits of sumac dates back to 40–49 A.D. when Pedanius Dioscorides (a Greek physician) mentioned the benefits of sumac, mainly as a diuretic and anti-flatulent, in his voluminous “Of Medicinal Matter” (“De Materia Medica”) (Norton, 2006). According to Palestinian herbal folk medicine, sumac was applied to prevent and improve many diseases, including diabetes, hypertension and anorexia (Abu-Reidah *et al.*, 2014).

A considerable amount of literature has been published on the potential health benefits of sumac, including its antimicrobial (Sağdic and Özcan, 2003; Fazeli *et al.*, 2007) and antioxidant activity (Bozan *et al.*, 2003; Kosar *et al.*, 2007). Additionally, anti-hyperlipidaemic activity and hypoglycaemic effects (Shafie *et al.*, 2011; Anwer *et al.*, 2013; Madihi *et al.*, 2013) are other potential benefits of sumac that have been reported.

1.2.3. Sumac species

The genus *Rhus* comprises 250 species in the Anacardiaceae family (Rayne and Mazza, 2007). Several studies have reported the health benefits of different species of sumac, including *Rhus typhina* Linn, also known as staghorn sumac. This species of sumac is mostly found in certain areas of China (Beijing, Hebei and Lanzhou) and grows across the east of North America. Staghorn sumac can grow under any geographical conditions (Kossah *et al.*, 2009). However,

most studies of sumac have focused on *Rhus coriaria Linn* (Kossah *et al.*, 2009). Wild *Rhus coriaria Linn* is mainly found in tropical and temperate regions, including Mediterranean coastlines from the Canary Islands to Iran and the Middle East (Rayne and Mazza, 2007; Abu-Reidah *et al.*, 2015). Sumac is inexpensive to harvest and has a shelf life of two to three years. This spice contains polyphenol compounds that play an essential role as a natural preservative in the food industry (Abu-Reidah *et al.*, 2014). The natural antibacterial properties of sumac have seen renewed interest in their usage in the food industry for food safety (Mahdavi *et al.*, 2018).

1.2.4. Chemical composition of sumac

Although much research has been carried out on the polyphenol and antioxidant activity of sumac, little attention was paid to the detailed chemical composition of this spice. Kossah *et al.* (2009) demonstrated that Syrian sumac contains moisture, protein, fat, fibre and ash. The same study also assessed this sumac for fatty acid composition and revealed that it contains both unsaturated fatty acids (69% of total fatty acids) and saturated fatty acids (31% of total fatty acids). A mineral assessment of Syrian sumac manifested that potassium was found in abundance, whilst manganese had the lowest concentration among other minerals in this study. Also, oleic acid (37%), linoleic acid (30%) and palmitic acids (27%) were shown to be substantial components of sumac. The same study demonstrated that Syrian sumac contains many essential and non-essential amino acids, including phenylalanine and glutamic acid, respectively (Kossah *et al.*, 2009).

1.3. Polyphenols

Functional food studies have shown the effectiveness of phenolic compounds in the human diet, with remarkable awareness and increasing interest among consumers (Bashash *et al.*, 2012). These compounds contribute to health by playing a role in cell proliferation, chronic

disease and metabolic regulation (Vázquez-Fresno *et al.*, 2019). Anti-inflammatory and antioxidant activities of phenolic compounds in managing and preventing diseases including cancer and non-communicable diseases have been reported (Vázquez-Fresno *et al.*, 2019). Appetite is composed of hunger and satiety sensations and can be activated by these compounds before, during and after eating episodes (Serna *et al.*, 2021). Following the consumption of food that contains polyphenols, appetite triggers the hypothalamus to initiate hunger by activating a series of neurotransmitters and hormones including ghrelin. Although the mechanism and metabolic pathway of these compounds in appetite have not yet been studied (Serna *et al.*, 2021), the antioxidant and anti-inflammatory properties of these compounds have been reported in increasing and regulating ghrelin (Akki *et al.*, 2021). The prevention of lipid oxidation by phenolic compounds via different pathways including the blockage of reactive oxygen species (ROS) and its termination has been described (Akki *et al.*, 2021). Therefore, the consumption of phenolic compounds may reduce these agents in cells and improve the secretion of ghrelin in balancing the energy haemostasis of cells (Akki *et al.*, 2021).

The antioxidant activity of curcumin, resveratrol and SRT1720 has been studied in mouse gastric mucosal cells (Mani, *et al.*, 2020). The results illustrate the reduction in oxidative stress, activated secretion of ghrelin via nuclear factor erythroid-derived-2-like 2 (Nrf2) (Mani, *et al.*, 2020). The effectiveness of polyphenol compounds for appetite was exemplified in work by Mohammadi *et al.* (2011). The result of their research revealed that the body weight of broiler chickens following the addition of sumac to their diet was higher at the end of their study compared with a control group ($p < 0.05$). It is evident that polyphenol compounds play a vital role in appetite. However, more studies are needed to understand their molecular interaction with the food matrix and appetite pathway.

To date, various structures of polyphenol molecules have been discovered in edible plants (Manach *et al.*, 2004). These well-known secondary metabolites are categorised into four main classes (phenolic acids, flavonoids, lignans and stilbenes), based on the number of phenol rings (Figure. 1.1) (Manach *et al.*, 2004). Phenolic acid and flavonoids are found ubiquitously in herbs and spices (Manach *et al.*, 2004).

1.3.1. Phenolic Acids

Phenolic acids come in two forms: hydroxybenzoic acid that is derived from benzoic acid and hydroxycinnamic acid that is derived from cinnamic acid (Manach *et al.*, 2004). It has been shown that the concentration of hydroxybenzoic acid is low in edible plants, except for black radishes, red fruits and onion (Shahidi and Naczki, 1995). However, the other form of phenolic acid, hydroxycinnamic acid, is more common. Hydroxycinnamic acid mainly comprises *p*-coumaric, caffeic, ferulic and sinapic acids in two forms: bound and free (Manach *et al.*, 2004). The presence of protocatechuic acid, *p*-OH-benzoic acid and vanillic acid have been reported in sumac (Sakhr and El khatib, 2020).

1.3.2. Flavonoids

Flavonoids are generally found in low concentrations in plants (almost 15-30 mg/kg of fresh weight). The health benefits of this polyphenol and its subclasses, including anthocyanins, flavanones and catechins, have been reported as reducing morbidity from cancer by preventing cell growth (Wang *et al.*, 2013) and the reduction of platelet activity and lowering blood pressure and enhancing cardiovascular health (Zhou *et al.*, 2016; Ardalani *et al.*, 2016a), and improving type 2 diabetes by protecting beta cells from toxicity resulting in managing glycaemia (Xia and Högger, 2015). The most common flavonoids are flavonols that present as quercetin and kaempferol. Previous studies have reported that sumac contains

gallic acid, flavonoids, anthocyanins and isoflavonoids (Ardalani *et al.*, 2016a; Sakhr and El khatib, 2020).

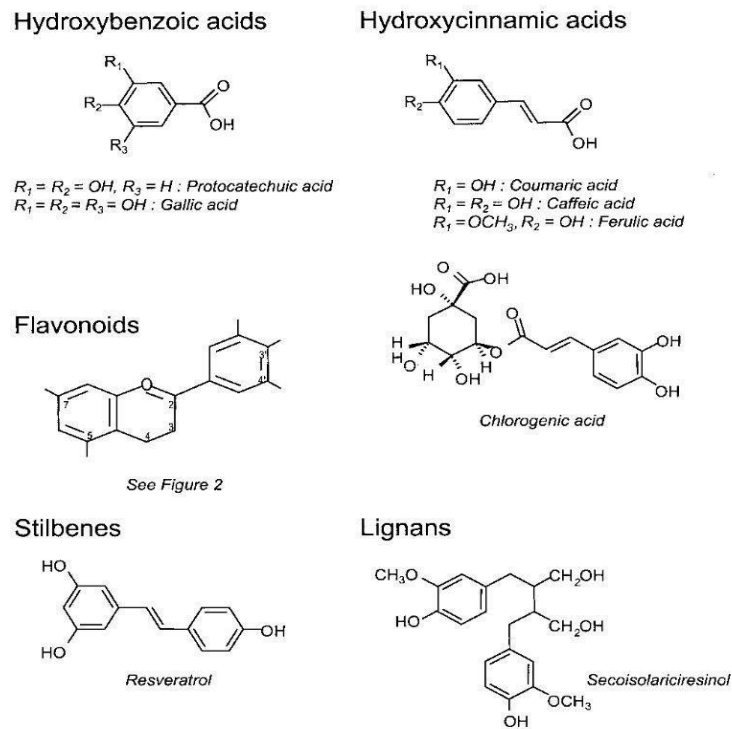


Figure1.1. Chemical structure of the main class of polyphenols

1.3.3. Polyphenol and antioxidant capacity of sumac

During the last decade, researchers in the food industry have been interested in natural polyphenol (Opara and Chohan, 2014). It is well understood that the consumption of synthetic antioxidants, including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), has toxic and carcinogenic effects (Talaz *et al.*, 2009). This finding has suggested the necessity of replacing synthetic compounds with natural sources (Bashash *et al.*, 2012). In support of this replacement, Bozkurt (2006) reported that water extraction of sumac fruit had higher antioxidant activity compared with BHT. Therefore, it is suggested that sumac can be used as a natural replacement for BHT to increase the quality of food. The medicinal and nutritional

properties of different parts of sumac, including the bark, flowers and seeds, have been studied by extracting them with different solvents, including water, ethanol, acetone and methanol (Abu-Reidah *et al.*, 2014). The literature demonstrates that various methods of extraction have been applied to sumac, including percolation, reflux and Soxhlet extraction at room temperature or with heat (Sakhr and El khatib, 2020).

The phenolic compound composition of ripe, oven-dried sumac fruit from the Hamedan-Iran region was assessed using high performance liquid chromatography with mass spectrometry (HPLC-MS) (Ardalani *et al.*, 2016a). Sumac was extracted with methanol (80%) and found to contain 191 phenolic compounds based on metabolic solubility. A continuation of the phytochemical assessment of the same sumac sample showed that hydrolysable tannin (41%) was higher compared to other components. The next abundant compound identified in the same sample was flavonoids (31%). However, some compounds, including diterpene (0.52%), were identified as having the lowest phenolic composition in the sumac sample from this region and 38 unidentified compounds. Previously, a methanolic extraction study using high performance liquid chromatography-mass spectrometry (HPLC-MS) revealed that sumac contains mainly gallic acid, followed by anthocyanin, which contains cyanidin, peonidin, pelargonidin, petunidin, delphinidin glucosides and coumarates (Shabir, 2012). A study assessed the phenolic compound composition of sumac using the Folin-Ciocalteu method and the results showed that methanol extraction of sumac yielded 151.7 mg/g of phenol, 65.31 mg/g of ethyl and 6.10 mg/g of water (Raodah *et al.*, 2014). Based on these findings, it can be speculated that sumac may possess high antioxidant and polyphenol activities.

1.4. Health benefits of sumac

It is well documented that polyphenol components play an essential role in preventing and managing various kinds of diseases caused by oxidative stress damage (Tawaha *et al.*, 2007).

On the other hand, the impact of lipid oxidation in food which causes a loss of nutritional value and leads to the development of various diseases including cancer, heart disease and stroke, is reported (Nikousaleh and Paraksh, 2016). Hence, evaluation of the impact of added sumac in the food and pharmaceutical industries is recommended.

1.4.1. Glycaemic properties of sumac

Diabetes is one of the most common and rapidly growing diseases in the Western world (Shidfar *et al.*, 2012). By 2045, the number of people diagnosed with diabetes around the world is estimated to rise by 40–45%, in comparison with 2017 (Lin *et al.*, 2020). Owing to the presence of different components in sumac, it has been suggested that this spice may contribute to regulating or treating the impact of metabolic disorders, including diabetes, and its consequence, hyperglycaemia. A growing body of research has assessed this property of sumac *in vitro* and *in vivo*. Shidfar *et al.* (2014) assessed the anti-diabetic activity of sumac in a double-blinded randomised controlled trial (RCT). Participants with type 2 diabetes (n=41; aged 20–60 years) were divided into two groups: sumac (n=22) and control (n=19). The results of a three-month intervention in the sumac group (3 g/day of sumac) revealed a significant reduction (~20 mg/dl) at $p=0.0001$ in serum glucose between the control and sumac groups. This could have occurred due to the abundance of gallic acid which has an antioxidant activity effect in the prevention of ROS. Gallic acid stimulates the production of peroxisome proliferator-activated receptor- γ (PPAR- γ), resulting in the prevention of hyperglycaemia and hypertriglyceridemia, also, a reduction in adipocytes size (Gandhi *et al.*, 2014). Hence, it is speculated, based on the metabolic role of gallic acid and its occurrence in sumac, that this may contribute to the management and prevention of type 2 diabetes.

The anti-diabetic effect of sumac was evaluated among participants with type 2 diabetes (n=41) who were randomly divided into two groups: control (n=19, 3 g/day of lactose) and sumac

(n=22, 3 g/day of sumac) (Rahideh *et al.*, 2014). The results of this analysis in both insulin and homeostatic model assessments of insulin resistance (HOMA-IR) showed a significant reduction ($t(20) = -0.9, p < 0.05$) at the end of the intervention period (three months) compared with the beginning of study. Studies have reported that sumac is composed of flavonoids that may interfere with β -cell function following oxidative damage to the cell; therefore, it can be hypothesised that the progression of insulin resistance is prevented (Graf *et al.*, 2005). However, malondialdehyde (MDA), an oxidative stress biomarker, in the same study showed a considerable decrease at the end of the study in the sumac group ($t(20) = -.07; p = 0.001$) but a significant increase in the control group ($t(17) = 0.08; p = 0.001$) after the intervention period. Flavonoids have anti-inflammatory and antioxidant activity, thus, they contribute to preventing diseases, including diabetes, by regulating various pathways that regulate insulin secretion and signalling, glucose uptake and carbohydrate digestion, resulting in an increase in β -cell proliferation (Graf *et al.*, 2005). Hence, it can be proposed that the hydroxyl group activity of a flavonoid that is present in sumac plays a role in lipid oxidation-reduction, resulting in diabetes management. According to these findings, it is suggested that daily consumption of sumac may help to alleviate the development of metabolic diseases. This promising finding may contribute to developing potential treatments in the pharmaceutical industry.

1.4.2. Cardiovascular risk factor properties of sumac

Non-communicable diseases increase various risk factors including high blood pressure, or hypertension conditions which are manageable and, in some cases, preventable. The treatment of hypertension is important and may reduce the risk of the development of many morbidity and mortality cases including metabolic syndrome, stroke, myocardial infarction coronary and peripheral arterial disease (PAD) (Zhang *et al.*, 2013). According to NICE (2019) guidelines, clinical hypertension is divided into three stages: stage 1 (140/90 mm); stage 2 (160/100 mm); and stage 3 (severe blood pressure, 180/110 mm or over). Hypertension can be caused by

genetic factors and/or environmental factors, including obesity, the excessive consumption of sodium chloride, physical inactivity and ageing (National Institute for Health and Care Excellence, 2019).

The influence of sumac consumption in the reduction of systolic blood pressure has been studied (Ardalani *et al.*, 2016b). This work was carried out with 80 participants (n=39 female, n=41 male) over eight weeks. Participants were randomly divided into two groups: a control group (average age ~57 years) comprising men (n=21) and women (n=20), and a sumac group (average age ~59 years) that included 21 men and 18 women. The sumac group received two capsules (500 mg/day of sumac) with captopril (25 mg/day), and the control group were provided with two capsules of starch (500 mg/day) with captopril (25 mg/day). The results of the study showed a significant reduction ($p < 0.05$) among the sumac group (30.1/12.6 mmHg; $p = 0.03$) compared with the control group (12.3/8.9; $p = 0.04$). However, no previous study has investigated the impact of sumac on hypertension. Hence, the mechanism of interaction of sumac in lowering blood pressure has not yet been determined. It could, however, be argued that the positive outcome of the recent study is due to a flavonoid compound, known as quercetin. The influence of quercetin in the vasodilation of vascular smooth muscles has been reported (Perez-Vizcaino, *et al.*, 2009). Thus, it can be hypothesised that sumac may play a role in reducing blood pressure following the dilation of the blood vessels.

1.4.3. Antioxidant properties of sumac

Researchers have reported that the human body has several defense systems that play a role in protecting the organs from free radicals (Ulewicz-Magulska and Wesolowski, 2019). Dysfunctional performance in this system may lead to life-threatening diseases including cancer and cardiovascular diseases (Shidfar *et al.*, 2014). Although some research has been carried out on the antioxidant levels of sumac *in vitro*, only one study has thus far attempted to

investigate the impact of sumac on blood in human studies. Shidfar *et al.* (2014), as discussed earlier, compared total antioxidant activity (TAC) in participants with type 2 diabetes (n= 41), who were randomly allocated to either a sumac (3 g/day) group or a control group (3 g/day lactose). Their results showed an increase of 0.8 $\mu\text{mol/L}$ ($p=0.001$) at the end of the intervention in comparison with the baseline in the sumac group. Comparing the results between this group and a control group after a three-month intervention showed a significant reduction ($p=0.026$) in blood TAC in the sumac group (Shidfar *et al.*, 2014). This could be due to the presence of quercetin in sumac. Previous work on the impact of this flavonoid's derivatives unravelled its antioxidant activity following the suppression of lipid peroxidation (Li *et al.*, 2016).

1.5. Umami and glutamic acids and their impact on appetite

Glutamate and nucleotides play an essential role in the taste of food, in addition to its palatability (Yamaguchi and Ninomya, 2000). Umami taste is enhanced following the breakdown of amino acids into free nucleotides, including glutamate, during the cooking process. It is now well established that free glutamate (in the form of L-glutamic acid) may bind to G-protein receptors, including the metabotropic glutamate receptors GPCR, mGluR4 and TR1/TR3, which are present in taste buds (Li *et al.*, 2002; Nelson *et al.*, 2002; Chaudhari *et al.*, 2009). As the result of the interaction of $G_{\beta\gamma}$ (Chaudhari and Roper, 2010), a subunit of GPCR, with mGluR4, it breaks down and activates phospholipase C and IP3 which may cause a reflux of calcium ions (Ca^{++}) into the cytosol. Ca^{++} may bind to transient receptor potential channel 5 (TRPM5) and activate the transport of sodium into wells, resulting in depolarisation (Kinnamon, 2009). Alternatively, Ca^{++} may activate the pannexins (Pax1) path where extracellular ATP stimulates sensory nerve fibres and generates presynaptic cells (Chaudhari *et al.*, 2009). Then, the brain's taste receptors are triggered following serotonin (5-HT) and norepinephrine (NE) release (Chaudhari *et al.*, 2009). It is noted that umami substances activate the taste buds and generate a signal in the primary and orbitofrontal gustative cortex, resulting

in activation of the gut-brain path (de Araújo *et al.*, 2003). This finding, while preliminary, suggests that glutamic acid, as an important component that is present in sumac, could influence appetite via activation of this path.

The connection between the presence of free glutamic acids and levels of acceptability and palatability of food has previously been investigated (Yamaguchi and Ninomiya, 2000). Studies on flavours that contain odours and non-volatile components have shown, following their addition to food, and subsequent extraction of these components, that taste and somatosensory may be stimulated (Schiffman, 2000). A previous study demonstrated that the addition of monosodium glutamate (MSG) into low-energy soup showed a higher perception of the pleasantness (100 g; $p < 0.005$) attribute in MSG soup compared with a control soup (Yeomans *et al.*, 2008). On the other hand, the role that sodium chloride plays as a food ingredient is not limited to improving taste and texture (Anderson *et al.*, 2015); it also contributes to preventing the bitterness of food by enhancing its sweetness (Keast and Breslin, 2002) at lower concentrations (Keast and Breslin 2003). However, the findings indicate that salt consumption has an adverse impact on health; on the other hand, citric acid's enhancement of sourness (Veldhuizen *et al.*, 2018) and the addition of amino acids and MSG to improve the taste and palatability of food (Jinap and Hajeb, 2010) have been reported. Considering all this evidence, it seems that selected herbs and spices may contain alternative food additive ingredients that allow manufacturers to offer low-salt food with no adverse impact on taste or acceptability.

According to Anwer *et al.* (2013), sumac is composed of organic acids (mainly malic acid) that are responsible for its acidic and lemony taste. There is data on the sensory characteristics of sumac published by Bahar and Altug (2009). They concluded that sumac has an acidic aroma, with dried lemon balm and astringency characteristics, following an assessment of water extracted, supercritical carbon dioxide extracted and ground sumac. It can thus be suggested

that the addition of higher doses of sumac may enhance a tangy flavour in food. The benefits of spices and herbs added to soup were shown by Ghawi *et al.* (2014). This study demonstrated that the consumption of low-salt tomato soup combined with a blend of herbs and spices improved its acceptability to participants (n=160; 35-60 years old) as a less-salty food in three consecutive test sessions. Hence, adding herbs and spices, including sumac which also contains glutamic acid (Kossah *et al.*, 2010), to older adults' food could be considered by researchers and manufacturers to enhance appetite, palatability and food intake, along with a reduction in salt intake.

1.6. Sensory evaluation in older adults

Sensory evaluation is the most widely accepted scientific technique and instrumental method in food product development (Singh-Ackbarali and Maharaj, 2014) in the determination of human responses to taste and flavour using the five senses (sight, hearing, smell, taste and touch) (Lawless and Heymann, 2010). This method is used to characterise and improve food quality by trained persons (Beinner *et al.*, 2010). The importance of selecting an effective herb or spice as a complementary food ingredient to enhance the palatability and appetising nature of foods has been emphasised (Ghawi *et al.*, 2014). Reduction in taste and smell perceptions are, however, a primary concern among older adults and may impact on the accuracy of sensory results. This finding was observed in a study that showed the olfactory stimuli among this group were less sensitive (Methven *et al.*, 2016).

Different methodologies have been used for older adults' sensory assessments. Various factors can affect the outcomes of these tests including memory recall, cognitive issues and visual impairment (Methven *et al.*, 2016). Therefore, the importance of the effectiveness of these tests for healthy and frail older adults has been emphasised in the literature (Cavazzana, *et al.*, 2018). The discrimination threshold, identification threshold, detection threshold and suprathreshold

are used in older adults who have lost their senses due to ageing. However, perceived measurements and hedonic methods are the most used tests (Doty and Kamath, 2014).

Literature highlights the applicability of threshold tests for older adults due to competence in the perception of taste and flavour (Methven *et al.*, 2016). In threshold testing, older adults can recognise these stimuli at their lowest concentration. The utilisation of alternative forced-choice (AFC), 2-AFC or 3-AFC, for assessing older adult threshold detection, has been recommended (Zabernigg *et al.*, 2010). The advantages of these methods are simplicity in use and a reduction in the exhaustion levels of individuals during tests. However, a disadvantage of these techniques is that they have been reported as being subjective in terms of ability in flavour detection depending on the level of concentration. However, it is suggested that the average results of this method may be used to minimise errors. In the recognition threshold step, they have to establish the stimuli. It is easy to use and can be used for those who have a visual impairment. However, it relies on memory (Methven *et al.*, 2016). In order to recognise the stimuli, a series of pictures can be used to support the participants. It is inconvenient due to the differences in recognition of the provided concentration. Each older adult has a different threshold level, therefore, providing a concentration that can be perceived is another challenge (Doty and Kamath, 2014).

The visual analogue scale (VAS) is a linear scale which provides an uncomplicated method for assessing older adults' liking and preference of hedonic testing (Methven *et al.*, 2016). The widespread usage of this method suggests that it is a simple and uncomplicated technique for use among younger adults, healthy older adults and older adults with medical conditions (Kennedy *et al.*, 2010). One of the disadvantages of this method is its difficulty for older adults with a visual impairment. The results of these tests could be considered for personalising the dosage of added spices and improving food intake among older adults.

In order to find the optimal concentration level of hedonic testing “Just about Right (JAR)” test scales have been used (Methven *et al.*, 2016). This produces a liking intensity among older adults and can be useful when there is a loss of perception due to ageing, so that the JAR concentration can be found for a product (Methven *et al.*, 2016). However, the disadvantage of hedonic tests is increasing the number of participants to find accurate and unbiased results which can be challenging for recruiting (Methven *et al.*, 2016).

It was reported that sour and salty threshold detection increases with ageing, whereas bitter and sweet perception does not (Methven *et al.*, 2012). The threshold for salty taste among adults (aged 40–75 years) was evaluated following the application of direct contact of sodium chloride to tongues of healthy adults (n=600) and adults diagnosed with gastric cancer (n=300) (Yang *et al.*, 2011). Their results revealed a direct relation between threshold sensitivity compared with the control group. Moreover, the threshold sensitivity was regulated by administration of doses of salt. Hence, the outcome of this study would be beneficial in meeting the food dietary requirements for adults who have chronic diseases. A study on adults 20–90 years old revealed a higher salt intake for adults over 70 years, and a bitter taste was intensified in those over 60 years old, while sour perception was amplified for males over 60 years and females over 80. The same study reported that age had no influence on the reception of a sweet taste (Yamauchi *et al.*, 2002). Various factors have a positive influence on the palatability of food, including taste, colour and smell (Wijnhoven *et al.*, 2015).

A study assessed the perception of sour, sweet, bitter, salty and umami tastes among adults (n=205, aged 19–79 years old) (Puputti *et al.*, 2019). Their results revealed that, with an average concentration, a sour sample was identified as a bitter taste rather than sour, and an umami taste was identified as salt. The explanation for this could be the perception of colour and the misidentification of taste and flavour. It was revealed that the intensity of colour correlated with perceptions of higher intensity levels of taste and flavour (Calvo *et al.*, 2001;

Spence *et al.*, 2010). Another reason for the contrast could be the interchangeable usage of taste and flavour definitions for describing soups. While taste defines the comprehension of sour, bitter, sweet and salty, flavour indicates the identification of compounds by retronasal olfactory attributes, including citrusy, fruity and meaty (Spence *et al.*, 2015).

Spices are defined as aromatic vegetables that are mainly used for seasoning and refer to the different parts of plants (including flowers) which contain volatile oils (Embuscado, 2015). Research has shown the contribution of herbs and spices in the prevention of chronic diseases (Vázquez-Fresno *et al.*, 2019). The benefits of using flavouring agents, including herbs and spices, to reduce salt intake resulting in a reduction in cardiovascular diseases, are noteworthy (Anderson *et al.*, 2015). The daily intake of herbs and spices varies globally; however, an interest in increasing their consumption is reported due to the desire for ethnic and cultural foods (Williams, 2006).

1.7. Nutritional status in older adults

The term ‘older adult’ refers to people aged 65 years and over. According to the Office for National Statistics, the older adult population (85 years and over) in the UK in mid-2019 was 1,647,271, and 10,727,690 for those aged 65–84 years (Office for National Statistics, 2020). The proportion of the population who are 65 years and above is expected to increase to 20% by 2026 (Office for National Statistics, 2017). The latest results on the census showed that the older adults over 65 years old were 19% of the total UK population in 2019 (Office for National Statistics, 2020).

Malnutrition may be influenced by various conditions, including psychological, physiological and biological alterations to the gastrointestinal tract, social life changes and nutritional intake alterations (Malafarina *et al.*, 2003; Ahmed and Haboubi, 2010). These factors influence food intake in older adults (Shlisky *et al.*, 2017). It has been shown that as a result of ageing, some

diseases (such as dysphagia) and certain medications, including lovastatin and acetazolamide (more commonly taken by the older adult age group), may cause alterations in sensory perception, resulting in decreased sensitivity to taste and smell (Withers *et al.*, 2013; Doty and Kamath, 2014). These alterations are associated with an increased health risk (including malnutrition) consequently reduction in nutrient intake, food choice changes and immunity disorders (Methven *et al.*, 2016).

The literature emphasises the importance of older adults consuming food with sufficient energy in order to maintain their immune system levels and muscle mass to counter a reduction in physical activity (Baum *et al.*, 2016). According to the UK dietary reference nutrient intake, it is stated that an adult's daily protein requirement is 0.75 g/kg body weight (Department of Health, 1991). An increase in daily protein intake for healthy adults is recommended to almost 1.0 g/kg of body weight, and for those who are either malnourished or at risk of malnutrition, the amount should increase by half a gram (Deutz *et al.*, 2014). Epidemiological studies have shown that around one-third of older adults in residential care homes in Europe do not consume the required daily amount of protein (0.7 g/kg bodyweight) (Tieland *et al.*, 2012b).

Although differences of opinion still exist, there appears to be a consensus that the term malnutrition refers to a deficiency and/or imbalance in macronutrients and micronutrients, as a consequence of ageing or other conditions (including post-surgical recovery and physical impairment) in all populations, especially adults over 65 years of age (Ahmed and Haboubi, 2010; NICE, 2012; Engelheart and Brummer, 2018). Therefore, malnutrition can be defined as an imbalance (under- or over-) nutrition in terms of energy, protein and other micronutrient status that could adversely affect health and function (Field and Duizer, 2016). Malnutrition and its complications are a growing public health concern in the UK and around the world; notably, the growth in the older adult population has been highlighted globally (Engelheart and Brummer, 2018). However, malnutrition is a common condition that has a considerable impact

on an individual's nutritional health status. An association between malnutrition and reductions in levels of functional status, increasing numbers of hospital admissions, greater bone mass reduction, immune system malfunctions and, ultimately, mortality has been reported (Chapman, 2006).

The prolonged improvement period among older adults indicates the importance of the management of food and energy intake to prevent malnutrition and its complications. It has been shown that older adults, compared to younger adults, require a longer period to regain body weight and satisfy nutritional requirements. A study compared the impact of the recovery period from malnutrition in older adults (Nieuwenhuizen *et al.*, 2010). Their results demonstrated that older adults had reduced their energy intake following six-month recovery period by 1,000 kcal per day and were compared with results obtained for younger adults obtained at the end of study; only 64% of the weight loss at the baseline was regained.

Blood tests, including serum proteins, serum albumin and the measurement of vitamins and minerals, a malnutrition universal screening tool (MUST) and mini-nutritional assessment (MNA) (Robinson, 2018) are the most commonly used tests to evaluate nutritional status among the older adult population. In addition, the literature reports the application of food intake measurement techniques, including 24-h dietary recall, food records and telephone interviews. Although these methods are cost-effective and easy to administer, there are some disadvantages to using them. In general, dietary recall is time-consuming for the individual and there is a risk of over- or under-reporting, underestimating food portions and unsatisfactory recollection (Robert and Lee, 2007).

Previous research comparing the nutritional status of free-living older adults and a residential population found that residential adults had a lower intake of vitamins A, B and C, in addition to lower intakes of iron and thiamine, when compared to Dutch recommended dietary

allowances (RDAs), whereas intake levels were higher among free-living older adults (Löwik *et al.*, 2013). In 1988, Sahyoun *et al.* assessed the nutritional status of older adults (mean: 80.7 years of age) living in a care residence and found there were low levels of zinc in both males and females. Other important findings of this study were the levels of albumin, pre-albumin and transferrin in the residential group, which were lower compared to a free-living adult group. These biomarkers of protein status can be considered for screening and the nutritional assessment of older adults, mainly for those at higher risk. Various epidemiological studies have reported that the nutritional status of residential older adults is lower than that of free-living older adults. An aetiology of these deficiencies varies, including sarcopenia, long-term hospitalisation, psychological factors (for instance, loneliness), gastric emptying and satiety level. Therefore, the importance of nutritional assessment should be considered along with unravelling predictor factors for the prevention and managing of anorexia of ageing.

Various studies have reported the relevance of malnutrition in residential care homes (Saletti *et al.*, 2000; Garber *et al.*, 2003). One explanation for this could be the lack of regular malnutrition assessments and the risk of overlooking individualised nutritional requirements, which have been reported extensively. Alterations to the management of malnutrition have been recommended, using various techniques, including staff training with nutritional assessment methods. Some studies have reported an improvement in food and energy intake in residential older adults following the training of care staff (Riviere *et al.*, 2001; Lorefält *et al.*, 2012). In general, therefore, it seems that nutritional assessment as a fundamental method could be carried out by healthcare professionals to manage the individual status of residential older adults and reduce public health expenses.

1.8. Appetite in older adults

Balancing energy intake and expenditure is vital for individual health. However, the lack of such regulation may cause a negative energy balance, leading to weight loss and malnutrition (Roberts and Rosenberg, 2006). Satiating and satiety are key components of appetite, where the term satiation generally refers to inhibition in eating as a result of postprandial suppression and satiety can be defined as a feeling of repletion that prevents further consumption of food (Benelam, 2009). The main aspects of satiety involve two phases. The first phase begins immediately after the consumption of food when a series of hormonal signals are sent to the hypothalamus, and the second phase points to the level of stored energy in the body (Benelam, 2009). Appetite regulation is orchestrated by a series of mechanisms subsequent to consumption of food, digestion and absorption. Researchers have shown that satiety can be controlled through gastric and intestinal mechanisms and hormonal signalling as a consequence of the absorption of food from the gastrointestinal tract. The gastric mechanism is initiated when food comes into contact with the stomach via the secretion of gastric content, including gastrin-releasing peptides (GRP) (Ritter, 2004). Following the passage of food into the small intestine, the mechanism of satiation commences (Ritter, 2004). Therefore, the gut-brain receptors (vagal nuclei and the vagus nerve) are stimulated, releasing peptides which have orexigenic (including ghrelin) and anorexigenic (including CCK and gastric leptin) effects, resulting in appetite and satiety, respectively.

Cholecystokinin (CCK), a gut hormone, plays a vital role in satiation, following obstruction of the stomach and slowing down the emptying process (Camilleri, 2015). As a result of ageing, hormone secretion levels alter by increasing the satiety hormones (CCK and PYY) and reducing the hunger hormone (ghrelin) including those that control satiety. Subsequently, weight loss and malnutrition occur (Pilgrim *et al.*, 2015) which are more common among

residential care and hospitalised older adults, and especially women (Malafarnia *et al.*, 2013). A comparison between younger and older adults revealed that higher satiety may be due to higher levels of anorexigenic cholecystokinin (CKK) hormone, observed in younger adults (MacIntosh *et al.*, 2001), and glucagon-like-peptide 1 (GLP 1) which was greater postprandially among older adults (Di Francesco, *et al.*, 2010). Furthermore, the reduction in appetite among older adults was highlighted due to an increase in the concentration of leptin (Whitelock and Ensaff, 2018). To minimise the development of, or prevent and decelerate, malnutrition, a variety of methods have been considered. Increasing the intake of energy is recommended as a first strategy in older adults. Hence, the importance of implications in the development of various methods including the addition of different types of food (Van der Meij *et al.*, 2015), altering the process of cooking (Sergi *et al.* 2017) and reducing salt intake (Goncalves *et al.*, 2014) in order to improve nutrition and health among the older population is required.

1.9. Conclusion

In summary, this literature review has shown the value and health benefits of sumac, which may contribute to an individual's health by increasing palatability, enhancing food intake and lowering salt consumption. It was also illustrated that in addition to antioxidants and polyphenol components, sumac contains citric acid (Abu-Shanab *et al.*, 2005) and glutamic acid (Kossah *et al.*, 2009) components. The influence of glutamic acid in the enhancement of taste and palatability of food is noted. Thus, it can be suggested that sumac as an additive (Shabbir, 2012) could be considered for improving food intake.

1.10. Aims and novelty of this PhD

Traditionally, sumac was used for medicinal and culinary purposes due to its relatively high polyphenol content. Researchers have reported the beneficial effects of sumac *in vivo* (human

and animal studies) and demonstrated the polyphenol content and antioxidant activities *in vitro*. Although some research has been carried out on sumac, gaps exist in our understanding. Previous studies have reported the polyphenol content and antioxidant activity of sumac from the same regions and compared different species of sumac (Kossah *et al.*, 2009; Bashash *et al.*, 2012). Sumac is a source of gallic acid (Bozan *et al.*, 2003) and hydrolysable tannins (Ardalani *et al.*, 2016a), and the benefits of these compounds for the prevention and management of the aetiology of many diseases, including cardiovascular diseases and type 2 diabetes, have been reported (Sakhr and El Khatib, 2020). Comparing sumac from different regions and different varieties may identify a type with higher levels of the above components. Hence, the body of this thesis seeks to explore the usage of sumac as an additive in the food and pharmaceutical industries to increase health benefits, including food intake.

However, no previous study has shown the level of L-glutamic acid using photometric methods, the polyphenol and antioxidant content of fresh and commercial dried sumac of the same species, from different regions in two different colours. Moreover, no report was found on the sensory evaluation of sumac *in vivo* and the impact of the addition of this spice on food intake among older adults. Although sumac is beneficial for some health conditions, uncertainty remains about the relationship between the consumption of sumac, appetite and food intake. Ageing alters threshold perceptions of the taste and flavour of food; moreover, malnutrition among older adults has been reported extensively. Hence, it can be suggested that the addition of herbs and spices in combination with low salt to free-living adults' food and residential care home food may improve the taste and flavour of food, resulting in better appetite and higher intake. Therefore, this PhD seeks to evaluate several aspects of sumac to enhance food intake among older adults.

Thus, in study 1, the antioxidant activity, polyphenol content and L-glutamic acid level of different types (fresh and commercial dried) of sumac from four different regions, Turkey,

Palestine, Iran and the UK, were evaluated (Chapter 2). And both qualitative and quantitative methods (study 2) were used to identify the level of liking and intensity attributes of different doses of sumac using a sensory evaluation method (Chapter 3). The results of this study may indicate the optimal dose of sumac as an additive for food intake improvement. To the best of the author's knowledge, no previous study has investigated the impact of the addition of sumac on food intake. The present study used sumac as a food flavour enhancer (Chapter 4) to determine its impact on food intake in free-living older adults and younger adults (phase 1). Furthermore, a similar study (Chapter 4) was carried out to estimate food intake influence among residential older adults (phase 2).

Chapter 2: Determination of the total antioxidant activity, polyphenol content and glutamic acid content of sumac from different regions

2.1. Introduction

The popularity of using spices in food for their health benefits has been reported (Vázquez-Fresno *et al.*, 2019). Various species of sumac, including *Rhus glabra* Linn (known as smooth sumac) traditionally, it has been reported, were used by native Americans to cure bacterial diseases (Alam *et al.*, 2017). Sumac is a spice that has been used for culinary and medical purposes over centuries, most notably in Middle Eastern regions (Özcan and Haciseferogullari, 2004). Different parts of sumac, including the bark, leaves, seeds and flowers, were used for these purposes (Shabbir, 2012).

Phytochemical studies of different parts of sumac have shown that this spice contains a variety of phenolic compounds (including hydrolysable tannins, anthocyanin and delphinidin) (Rayne and Mazza, 2007; Capcarova *et al.*, 2010), organic acids (including citric acid and malic acid), fatty acids, essential oils, minerals and fibre (Zalacain *et al.*, 2003; Kosar *et al.*, 2007; Kossah *et al.*, 2009; Shabbir, 2012). The role of citric and malic acid compounds in developing a sour taste in various foods has been reported (Priecina and Karklina, 2015).

Recent evidence suggests that polyphenol compounds in the human diet are obtained from plants, including grains, herbs and spices, and have significant health benefits (Shahidi, 2009). It has been reported that there is a strong relationship between the consumption of food containing high levels of polyphenols and reductions in morbidity and mortality caused by an excess ROS (Devasagayam *et al.*, 2004). The antioxidant properties of phenolic compounds prevent the formation of ROS, resulting in the prevention of protein, lipid and nucleic acid oxidation (Alsamri, *et al.*, 2021). Although the levels of these compounds, including amino

acids, known as primary metabolites in plants, may vary, depending on defence mechanisms (Sempruch *et al.*, 2011), they can be used for their nutritional benefits (Jiang and Wang, 2006). Sumac is a tangy, lemony spice found in a variety of dishes, mainly in the Middle East region, in its pure form or combined with other herbs and spices (Nasar-Abbas and Halkman, 2004). A few studies have reported that sumac contains glutamic acid (Kossah *et al.*, 2009) and the benefits of glutamic acid in increasing appetite and food intake have been argued (Jinap and Hajeb, 2010; Ghawi *et al.*, 2014). It is likely, therefore, that the addition of sumac could have the benefits of increasing appetite and food intake.

Much attention has been paid to the analysis of the activity of polyphenol and antioxidants of sumac; however, limited data are published on L-glutamic acid. Furthermore, to the best of author's knowledge, no single study has compared the levels of polyphenols, antioxidants and L-glutamic acid in different varieties of sumac from different regions.

2.1.1. Aims and hypotheses

It was hypothesised that sumac from different regions, in different forms and colours, is responsible for various activities due to the availability of phenolic compounds. Furthermore, it can be suggested that there is a correlation between antioxidant activity and phenolic compounds in sumac, with the additional possibility of a correlation of the level of these activities with the quantity of L-glutamic acid in the same sumac sample. Thus, this study aimed to ascertain the total antioxidant activity, polyphenol content and L-glutamic acid levels of six varieties of sumac (Turkish, Palestinian, Iranian red, Iranian brown, fresh red UK, fresh brown Iran) in two colours (red and brown) and two forms of dried samples (fresh and commercial) using three different solvents (water, acetone 80% and ethanol 80%).

2.2. Methodology

2.2.1. Materials

All chemicals and reagents were purchased from Sigma Aldrich (Poole, UK) and the Megazyme Co. (Wicklow, Ireland). Turkish sumac was purchased from Buy Whole Foods Online Ltd and Palestinian sumac from YAFFA Ltd, both via the Amazon website. However, red (Mahan products) and brown Iranian sumac (Donya Company) were purchased from a local shop in Oxford (Figure 2.1).

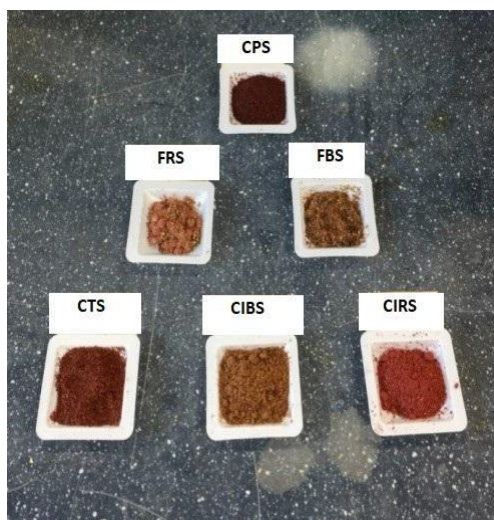


Figure 2.1. Different types of sumac based on origin and colour. CTS: commercial Turkish sumac; CPS: commercial Palestinian sumac; CIRS: commercial Iranian red sumac; CIBS: commercial Iranian brown sumac; FRS: fresh red sumac; FBS: fresh brown sumac

FRS was hand-picked by the researcher in London (Figure 2.2), and FBS was obtained from Kordestan, Iran.



Figure 2.2. Fresh red sumac (FRS) from London, UK

2.2.2. Sample preparation and extraction

First, the FRS and FBS were air-dried for four weeks, sheltered from bright light. Then, the samples were ground and stored away from the light, in airtight containers, for further assessment. Commercial varieties of sumac were obtained in powder form, ready for extraction. In order to extract commercial sumac (Turkish, Palestinian, Iranian red, Iranian brown) and non-commercial sumac (UK red and Iranian brown), 0.2g of each type (Figure 2.1) was weighed in triplicate using a set of scales (Sartorius, Type 1712), then the weights were recorded.

Next, each sumac sample was transferred into a clean amber bottle with a screw cap. Then, 4 ml of solvent was added to the bottle. For the determination of total polyphenol content and antioxidant activity, three solvents were used (distilled water, acetone 80% and ethanol 80%) and distilled water was used for the L-glutamic acid activity of the samples. The bottles were labelled, and the caps secured with parafilm to avoid oxidation. They were transferred to a shaking water bath and incubated for 2 hours at room temperature (Thondre *et al.*, 2011).

Then, all the samples were transferred to clean plastic test tubes. The extracted sumac samples were centrifuged at 3,000 rpm for 10 minutes using a benchtop centrifuge (Heraeus Instruments, Kendro Laboratory Products, D-37520 Osterode, Germany). The supernatant of each sumac was then immediately transferred to a clean labelled glass test tube. The samples were covered with foil to protect them from light and stored at -20°C until they were assessed.

2.2.3. Ferric ion reducing antioxidant power (FRAP) assay

The total antioxidant capacity (TAC) of each sample was assessed using a FRAP assay (Benzie and Strain, 1996). This method allowed the samples to demonstrate their antioxidant activity using the reduction of ferric tripyridyl triazine (Fe^{3+} TPTZ) to its ferrous form (Fe^{2+} TPTZ). FRAP reagent (300 mM) was prepared from three solutions: an acetate buffer (pH 3.6), 10mM of tripyridyl-s-triazine (TPTZ) and 20 mM of ferric chloride, in a ratio of 10:1:1. The acetate buffer was made in advance and kept in a fridge, whilst TPTZ and ferric chloride were prepared fresh on the day. 1.0 mL of distilled water was added to each tube and then incubated for 5 minutes in a water bath at 37°C. Then, 25 μL of each diluted sample or standard solution was added to it and mixed. Following this, 1.0 mL of freshly prepared FRAP reagent was added to each test tube and mixed. The test tubes were further incubated for 4 minutes in a water bath at 37°C. Finally, the antioxidant activity of the samples was measured at 593nm using a spectrophotometer (UV-1800-Shimadzu; Figure 2.3) with ferrous sulphate (0.001M) solution used as a standard (Benzie and Strain, 1996; Ryan *et al.*, 2011).

2.2.4. Total phenolic content (TPC)

The total phenolic content of each sumac extract was analysed using the method described by Coe *et al.* (2013). The method demonstrates the polyphenol content of a sample following the reduction of metal oxide and generates blue molybdenum-tungsten using Folin-Ciocalteu reagent (FCR). Extraction was carried out in water, acetone 80% and ethanol 80%. To each diluted extract (200 μL), 1.5 mL of diluted FCR (1:10 v/v with water) was added. Then, the mixtures were equilibrated for 5 minutes, before 1.5 mL of 60 g/l sodium carbonate solution was added. All mixtures were incubated for 90 minutes in the dark (wrapped in foil) at room temperature. The absorbance of the sumac samples was measured immediately, at 725 nm, using a spectrophotometer (UV-1800-Shimadzu; Figure 2.3), with gallic acid (1 mg/mL of each

solvent) used as a standard. The results were expressed as grams of gallic acid equivalent per gram of sumac sample (mg GAE/g) (Singleton and Rossi, 1965; Coe *et al.*, 2013).

2.2.5. Glutamic acid assay

L-glutamic acid was measured according to the procedure described for a Megazyme assay. The assay was used to quantify INT-formazan, which is stoichiometric with the amount of L-glutamic acid in the sample. However, some steps in the original procedure were modified in the extraction method in which 4 mL of water was added to 400 mg of the sample and incubated for 2 hours at room temperature, in a securely screwed bottle. Then, the sample was centrifuged at 3000xg for 10 minutes and frozen until the testing session. Before testing, the samples were defrosted at room temperature, and then the water-extracted sample was diluted (1:10 v/v distilled water). A preservative buffer (pH 8.6) was mixed in 0.20 mL of nicotinamide-adenine dinucleotide/iodonitrotetrazolium chloride (NAD⁺/INT), then 0.05 mL of diaphorase was added. The buffer was added to 0.10 mL of the diluted sample prior to the addition of 2.00 mL of distilled water. Then, the sample was mixed using a vortex. The absorbance of the sumac samples was measured twice at this point, with a 2-minute gap, using a spectrophotometer (UV-1800-Shimadzu; Figure 2.3) at 492 nm. The difference between absorbance greater than 0.010 indicated interference. Therefore, the sample was diluted with distilled water (1:10 v/v) and followed by the immediate addition of glutamate dehydrogenase (GIDH) in an interference removal procedure. The samples were processed in triplicate, and the results reported as mean and standard deviation (SD).



Figure 2.3. Spectrophotometer (UV-1800-Shimadzu)

2.2.6. Statistical analysis

All samples were tested for their antioxidant (mol/L), polyphenol (mg GAE/ g) and L-glutamic acid (g glutamic acid/100g protein) content. The tests were performed in triplicate, and the results presented as means \pm standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 23, USA). Data were analysed for normality with Shapiro-Wilk's tests. Based on the results, for normally distributed data, a parametric one-way analysis of variance (ANOVA) a post hoc test (using Tukey adjustment) was applied to determine significant differences within sumac samples and between solvents. The significance level was set to $p < 0.05$. For data not normally distributed, the results were analysed using a non-parametric Kruskal-Wallis test between sumac samples' types, and the significance level was set to $p < 0.05$. In order to assess the relationship between the antioxidant activity and polyphenol content of all the sumac samples, a Pearson correlation test was run.

2.3. Results

2.3.1. Total antioxidant activity of sumac samples

Table 2.1. shows the mean (\pm SD) antioxidant activity of each sumac sample (Turkish, Palestinian, Iranian red, Iranian brown, fresh red UK and fresh brown Iranian) extracted by

three solvents (water, acetone 80% and ethanol 80%). Significant differences were observed within and between sumac samples ($p < 0.05$).

The total antioxidant activity (FRAP value) of water-extracted samples ranged from 0.8 ± 0.1 (mol/L) to 14.1 ± 4.9 (mol/L). The results revealed that FBS extracted with water had the highest yield of antioxidant activity, followed by commercial Iranian brown sumac (CIBS), FRS, commercial Turkish sumac (CTS), commercial Iranian red sumac (CIRS) and commercial Palestinian sumac (CPS). Similar to the results for antioxidant activity extracted with water, FBS manifested the highest yield, and CPS the lowest, following extraction with acetone (80%). The total antioxidant activity of all sumac samples extracted with acetone (80%) ranged from 4.2 ± 0.3 (mol/L) to 14.2 ± 2.1 (mol/L). The results for the antioxidant activity of ethanol (80%) extraction for all sumac samples ranged from 9.1 ± 3.5 (mol/L) to 27.6 ± 6.3 (mol/L). Table 2.1 highlights that the antioxidant activity of CIBS was the highest, followed by FBS, CTS, FRS, CPS and finally CIRS. Data from this table reveal that ethanol (80%) sumac sample extraction showed higher antioxidant activity compared with the other two solvents.

Table 2.1. Total antioxidant activity (mol/L) of each type and form of sumac

Sumac type	Water	Acetone 80%	Ethanol 80%
CTS ^a	3.5 ^{**} ±0.2	10.4 ^{**} ±2.6	25.9 ^{**} ±7.8
CPS ^b	0.8 ^{**} ±0.1	4.2 ^{**a} ±0.3	12.9 ^{**a} ±2.9
CIRS ^c	1.2 ^{**} ±0.4	4.3 ^{**a} ±0.7	9.1 ^{**a} ±3.5
CIBS ^d	9.1 ^{**a,b,c} ±2.7	13.9 ^{**a,b,c} ±2.1	27.6 ^{**b,c*} ±6.3
FRS ^e	7.3 ^{***a,b,c} ±3.1	10.8 ^{***b,c,d} ±1.5	14.4 ^{***d} ±5.9
FBS ^f	14.1 ^{**a,b,c,d,e*} ±4.9	14.2 ^{**a,b,c,e*} ±2.1	27.4 ^{**b,c,e} ±8.6

Data are expressed as mean ±SD of triplicate measurements. CTS: commercial Turkish sumac; CPS: commercial Palestinian sumac; CIRS: commercial Iranian red sumac; CIBS: commercial Iranian brown sumac; FRS: fresh red sumac; FBS: fresh brown sumac.

The superscripts (a,b,c,d,e,f) of sumac types are significantly different at $p < 0.05$.

Column values with no superscript are not significantly different ($p > 0.05$).

* = was significantly higher than the other samples (one-way ANOVA test, at $p < 0.05$)

**= was significantly higher between solvents at $p = 0.001$

***= was significantly higher between solvents at $p = 0.003$

2.3.2. Total phenolic content of sumac samples

The results, as shown in Table 2.2, significant differences within sumac samples for the solvents used (water, acetone 80% and ethanol 80%) at $p < 0.05$. The polyphenol content results for the water extraction samples ranged from 0.1 ± 0.1 (mg GAE/g) to 2.4 ± 1.3 (mg GAE/g). The mean values of CIBS and FBS were higher from the other samples ($p < 0.05$). The polyphenol activity of the acetone (80%) extracted samples ranged from 1.7 ± 0.1 (mg GAE/g) to 5.4 ± 5.3 (mg GAE/g). There was a greater difference between FBS and all the other samples (except CIBS) at $p < 0.05$. The polyphenol content following acetone 80% extraction for the sumac sample showed that the CTS sample had higher differences from CPS, and CIRS at $p = 0.001$ and was statistically less than FBS at $p = 0.001$, and from FRS at $p = 0.007$.

The results for polyphenol activity following ethanol (80%) extraction showed that the total phenolic content ranged from 0.4±0.2 (mg GAE/g) to 3.4±1.4 (mg GAE/g). FBS only indicated differences from two sumac samples (CPS and CIRS) at $p<0.05$. FBS had the highest level of polyphenol content in comparison to the other samples (in all three solvents).

Table 2.2. Polyphenol content (mg GAE/g) of each type and form of sumac

Sumac type	Water	Acetone 80%	Ethanol 80%
CTS ^a	1.4 ^{**} ±0.4	3.5 ^{**} ±0.9	2.4 ^{**} ±0.5
CPS ^b	0.1 ^{**a} ±0.1	1.7 ^{**a} ±0.1	0.5 ^{**a} ±0.3
CIRS ^c	0.4 ^{**a} ±0.2	1.8 ^{**a} ±1.8	0.4 ^{**a} ±0.2
CIBS ^d	1.5 ^{**a,b,c} ±0.7	5.1 ^{**a,b,c} ±4.8	2.7 ^{**b,c} ±0.6
FRS ^e	1.01 ^{**b,c,d} ±0.5	4.5 ^{***a,b,c} ±4.5	2.1 ^{***} ±0.8
FBS ^f	2.4 ^{***a,b,c,d,e*} ±1.3	5.4 ^{***a,b,c,d*} ±5.3	3.4 ^{***b,c*} ±1.4

Data are presented as mean ±SD of triplicate measurements. GAE: gallic acid equivalent.

CTS: commercial Turkish sumac; CPS: commercial Palestinian sumac; CIRS: commercial Iranian red sumac; CIBS: commercial Iranian brown sumac; FRS: fresh red sumac; FBS: fresh brown sumac.

The superscripts (a,b,c,d,e,f) of sumac types are significantly different at $p<0.05$.

Column values with no superscript are not significantly different ($p>0.05$).

*=was significantly higher than other samples (one-way ANOVA test, at $p<0.05$)

**=was significantly different between solvents (Kruskal-Wallis test, at $p<0.05$)

***=was significantly different between solvents (Kruskal-Wallis test, at $p<0.05$)

2.3.3. Correlation between total polyphenol content and antioxidant activity

The correlation between antioxidant activity and total phenolic content for all the sumac samples extracted with three solvents (water, acetone 80% and ethanol 80%) was assessed using Pearson correlation (Table 2.3). The overall outcomes of these relationships showed significant ($p<0.01$), strong and positive correlations between the polyphenol content and

antioxidant activity of the sumac samples in all solvents: water, $r=0.813$; acetone (80%), $r=0.887$; and ethanol (80%), $r=0.623$.

Table 2.3. Correlation coefficients of FRAP and FCR for different types of sumac extraction (water, acetone 80%, ethanol 80%)

Sample extraction	FCR vs FRAP (r)
Water	0.813*
Acetone (80%)	0.887*
Ethanol (80%)	0.623*

Ferric reducing antioxidant power assay (FRAP); Folin-Ciocalteu assay (FCR); r: Pearson correlation coefficient.

* Statistically significant at $p<0.01$.

2.3.4. L-glutamic acid assay

The results for L-glutamic acid in sumac samples were initially assessed as g/100g and ranged from 1.1 to 8.7 g/100g (Table 2.4.). The results are presented as g glutamic acid/100g of protein for each sample (Aremu *et al.*, 2011). A 6.75 g sumac contains 1 g protein (Sakhr and El Khatib, 2020); therefore, the average g glutamic acid/100g protein of each sample was calculated, as shown below:

$$\frac{\text{average of absorbance} \times 6.75}{100} = (\text{g/g protein})$$

$$(\text{g/g protein}) \times 100 = \text{g glutamic acid/100g protein}$$

Table 2.4. L-glutamic acid (g glutamic acid/100 g protein) of water-extracted sumac samples

Sumac type	Average of absorbance	Total L-glutamic acid
	Mean \pm SD	(g glutamic acid/100g protein)
CTS ^a	0.7 \pm 0.1 ^a	4.6
CPS ^b	0.2 \pm 0.1 ^{ab}	1.1
CIRS ^c	0.5 \pm 0.2	4.3
CIBS ^d	0.8 \pm 0.3 ^b	5.2
FRS ^e	0.7 \pm 0.2	4.5
FBS ^f	1.3 \pm 0.2*	8.7

Data expressed as mean \pm SD. Means followed by the same superscripts are significantly different and * mean is significantly greater than other samples ($p < 0.05$). One-way ANOVA test: CTS: commercial Turkish sumac; CPS: commercial Palestinian sumac; CIRS: commercial Iranian red sumac; CIBS: commercial Iranian brown sumac; FRS: fresh red sumac; FBS: fresh brown sumac.

2.4. Discussion

The six sumac samples used in this study were represented by two principle types (fresh and commercial) and two colours (red and brown) from four different regions (Turkey, Palestine, Iran and the UK). To the best of our knowledge, no previous study has compared the polyphenol, antioxidant activity and L-glutamic acid levels of different types and colours of sumac from these four regions. In the current study, the fruits of all the sumac samples were assessed for polyphenol content, antioxidant activity and L-glutamic acid after extraction with water, acetone 80% and ethanol 80% using FCR and FRAP assays; water for L-glutamic acid. The solvents chosen for this study were consistent with edibility in this PhD study and for future reference in the food industry. The results of the present study revealed that ethanol (80%) and acetone (80%) were the most effective solvents for the determination of antioxidant activity and polyphenol content, respectively. The FBS sample showed higher polyphenol activity in the results of extraction with all three solvents (water, ethanol 80% and acetone

80%). The same sumac sample (FBS) showed the highest antioxidant activity after extraction with water and acetone 80%. The polyphenol activity of these samples demonstrated that CIBS had higher antioxidant activity following extraction with ethanol 80% compared with the other samples. In a concurrent study, FBS showed a higher level of L-glutamic acid in contrast with the other samples.

2.4.1. Polyphenols and antioxidants

Various research has reported the polyphenol and antioxidant activity of different parts of sumac (leaves, seeds, flowers) using different solvents, mainly ethanol, methanol and distilled water (Abu-Reidah *et al.*, 2014). Additionally, primary methods have included FRAP, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging and thiobarbituric acid (TBA) assays mostly being used to analyse this activity (Abu-Reidah *et al.*, 2014). In the current PhD, considering the ensuing studies, the flowers of sumac in pure and ground form were used. Several studies have highlighted that an increase in the use of foods rich in natural antioxidants results in enhanced health benefits (Bashash *et al.*, 2012). It has been reported that gallic acid is the most abundant phenolic compound in sumac (Bozan *et al.*, 2003), and hydrolysable tannins and flavonoids that are responsible for antioxidant activity were found to be the most predominant content of this spice (Ardalani *et al.*, 2016a). Together, these studies indicate that the level of antioxidant activity of sumac is considerable, hence, it could be a promising bioactive product in clinical research and the food industry. In phytochemical assessments, a variety of methods with different conditions have been used for analysing the polyphenol compounds and antioxidant activity levels of sumac.

Previously, different solvents, including water, acetone, ethanol and methanol in different concentrations (0%-100%) for assessment of the polyphenol and antioxidant activities of sumac, were investigated (Abu-Reidah *et al.*, 2014). The effectiveness of ethanol 80% was

reported following the analysis of sumac with extraction concentration ranging from 0% to 100% (Kossah *et al.*, 2010). In contrast to the findings of this investigation, the study by Aliakbarlu *et al.* (2014) revealed that water was the most effective solvent for the extraction of sumac compared with ten other samples, including turmeric, fennel and cardamom. Based on the diversity of these findings, in the current study, water, acetone 80% and ethanol 80% were used to obtain robust and comparable results.

The literature highlights different methods for the validation of polyphenol and antioxidant content following the preparation and extraction of samples (Khoddami *et al.*, 2013). To evaluate polyphenol compounds, methods including spectrophotometry, gas chromatography (GC) and HPLC, and using FRAP, DPPH and *N,N*-dimethyl-*p*-phenylenediamine (DMPD) assays, are reported for antioxidant assessment. Antioxidant properties of sumac are attributed to the level of polyphenols.

To evaluate the potential health benefits of sumac, a Folin assay was considered in this study. Various reports demonstrate the interaction of the polyphenol compounds of plants with the redox complex of FCR assay, forming a blue chromophore compound which can be measured by spectrophotometry (Blainski *et al.*, 2013). The diversity of analytical procedures including reference substances, wavelength and reaction time which can influence the outcomes of analysis, have been reported (Badarinath, *et al.*, 2012). However, no optimal procedure for the analysis of polyphenol and the antioxidants of sumac has been defined. In this study, spectrophotometric analysis of polyphenols using Folin-Ciocalteu assay was used as the standard method by transferring the electron and forming blue compounds (Coe *et al.*, 2013). Although it is easy to use, can be reproduced, is rapid and has a low cost, it has some limitations. The interference and inhibition of oxidation following the alkali sample present in the assay may produce inaccurate results. The phenolic compounds also have a different kinetic

reaction and molecular masses which may influence phenolic compound outcomes (Blainski *et al.*, 2013).

The antioxidant activity of phenolic compounds has been reported in plants. The phenolic compounds that are responsible for antioxidant activity are hydroxybenzoic and hydroxycinnamic derivatives (Manach *et al.*, 2004) which donate hydrogen or electrons resulting in neutralising ROS and reduction of metal transition (Devasagayam *et al.*, 2004). The FRAP assay is not expensive and is fast and reliable. In this method, colourless Fe^{3+} -TPTZ is reduced to dark blue Fe^{2+} -TPTZ following its interaction with potential antioxidant compounds. The advantages of this method are its simplicity and low cost. However, this method cannot be used in the identification of glutathione and protein which requires hydrogen transfer (Badarinath *et al.*, 2012). In this concurrent study, the FRAP assay, due to its accuracy and simplicity, was used to measure the antioxidant levels of sumac samples by reducing ferric ions to complex ferrous compounds.

The present study's results demonstrate that water extraction samples ranged from 0.1 to 2.4 (mg GAE/g). This outcome contradicts the results of Bashash *et al.* (2012), who found that the water extraction yield ranged from 0.811 to 2.453 GAE/100g. The researchers compared the phenolic compounds of brown sumac fruit, brown sumac powder and red sumac powder extracted with four different solvents (ethanol, methanol, distilled water and an ethanol-methanol mixture). The contradictory results from those findings and the results of the current study may be explained by the different extraction method, reflux, that was used in their study. The inconsistencies between the extraction method of Bashash *et al.*, (2012), reflux extraction, and the current study may be explained by the fact that reflux is a less effective method, due to the concentration level of samples, which remains constant in the solvent.

In an analysis of the phenolic content of sumac seeds (mg/g) in various solvents (water, ethanol and methanol with 25%, 50% and 75% concentrations using 2,2,1-diphenyl-1-picrylhydrazyl (DPPH) assay), Al-Muwaly *et al.* (2013) found that methanol, in comparison with ethanol and water, had significantly ($p < 0.05$) greater activity for this assessment. The results of a recent study also confirmed that the level of antioxidant activity in extracted samples increased with higher concentrations of sumac. Additionally, this study showed that methanol (in comparison to water and ethanol), which has a higher polarity, was able to extract more phenolic compounds from sumac seeds. In contrast to Al-Muwaly *et al.* (2013) and Bashash *et al.* (2012), it is somewhat surprising that Bursal and Köksal's (2011) study found that water had higher polarity, compared with ethanol, to solubilise the phenolic compounds of sumac. The discrepancies in these results, compared with the present study of polyphenols could be attributed to various factors, including sunlight exposure, geographic area, analysis method, storage and plantation method. Although some research has been carried out on sumac, few studies have attempted to investigate the impact of geography on antioxidant and polyphenol content. To determine the effect of geographical conditions on chemical composition, Kossah *et al.*, (2009) compared two Syrian and Chinese sumacs. Their results showed higher moisture content for Syrian sumac (12%) compared with Chinese (7%) sumac, and higher protein (4%), fat (12%), ash (5%) and fibre (33%) content for Chinese sumac (Kossah *et al.* 2009). However, Syrian sumac demonstrated less protein (2%), fat (8%), ash (3%) and fibre (22%) content, compared with Chinese sumac. In an investigation of the chemical composition of sumac from Turkey, Özcan and Haciseferogullari (2004) found moisture was at a level of 1%, protein 3%, fat 15%, ash 2% and fibre 15%. The inconsistencies between the results of sumac from different regions could be explained by the different environmental growing conditions. Mazaheri Tehrani *et al.* (2017) compared the physicochemical properties of sumac from three different regions of Iran (Ferdows, Gonabad and Zoshk). The results of their study revealed the pH, ash,

protein, fat and moisture (%) were in similar ranges. In addition, the antioxidant activity of the same samples demonstrated a similar range of activity. However, their results, compared with previous reports by Kossah *et al.* (2009) and Özcan and Haciseferogullari, (2004), emphasise the influence of environmental factors on the nutritional components of sumac. It can therefore be assumed that Iranian sumac contains more malic acid and citric acid compared with Syrian, Turkish and Chinese varieties, which represents a more acidic flavour in food.

The positive correlation between the antioxidant activities of herbs and spices with phenolic compound levels has been identified (Cai *et al.*, 2004; Shan *et al.*, 2005). Therefore, this correlation suggests a major contribution of predominantly phenolic compounds to the antioxidant activity of plants (Tawaha *et al.*, 2007). There is a lack of findings on a correlation between the antioxidant activity and polyphenol content of *Rhus coriaria* Linn fruits in the literature. The outcome of the current work indicated a correlation between the antioxidant activity and polyphenol content of all the sumac samples following extraction with water, acetone 80% and ethanol 80%. Similarly, Aliakbarlou *et al.* (2014) found a strong positive correlation between antioxidant activity and polyphenol content. Another study illustrated that antioxidant activity is positively related to the polyphenol content of *Rhus typhina* Linn stems (Liu *et al.*, 2019).

Environmental factors influence the phytochemical components of plants, resulting in different antioxidant activity and polyphenol content. These environmental factors, which have an impact on flavonoid biosynthesis, include differences in latitude, temperature, soil type, water, development process, nutritional status and plant growth patterns (Idris *et al.*, 2018). Based on the findings of the current study, FBS showed the highest levels of polyphenol content and antioxidant activity compared with all other samples (except that the antioxidant activity of ethanol (80%) for CIBS was slightly higher).

2.4.2. L-glutamic acid

In this study, the level of L-glutamic acid of sumac samples chosen from several regions and in various forms was assessed based on the recognition of its role in the improvement of taste and flavour in food (Wang *et al.*, 2013). It has been found that non-essential amino acids (including glutamates) play a role as umami substances to enhance the taste of food by activating the chemical detecting components of taste buds (Mouristen, 2012). Herbs and spices contain significant concentrations of free glutamic acids (Aremu *et al.*, 2011); and, in some parts of the world, they have been used to improve taste and other health benefits, including higher food intake (Kivilompolo *et al.*, 2007; Park, 2011). To date, few studies have examined the level of L-glutamic acid in sumac. The amino acid composition of two types of sumac (Syrian and Chinese) was reported previously by Kossah *et al.* (2010). The results of this research showed that the level of glutamic acid in Chinese sumac (0.8 ± 0.40 g glutamic acid/100g protein) was significantly ($p < 0.05$) higher than Syrian sumac (0.2 ± 0.15 g glutamic acid/100g protein). The comparison between their results with the current study findings revealed that the sumac samples that were evaluated in this study had a higher quantity of glutamic acid compared with Chinese and Syrian sumac. Based on these results of the aforementioned study it can be concluded that geographical location, environmental factors and growing conditions may have an impact on the sumac from these regions.

There are different methods available to validate amino acid levels in herbs and spices, and one of the most common methods that have been used to assess free amino acid (L-glutamic acid) components in plant tissue is reverse-phase chromatography with pre-column derivatisation (Sochor *et al.*, 2011); however, no single method has been developed for this assessment. It has been demonstrated that a spectrophotometric method, in comparison to HPLC, is cost-effective and easier to use (El-Gindy *et al.*, 2001). The accuracy of a photometric method using a Megazyme L-glutamic acid kit was previously reported for foods. Pedraza *et al.* (2007) used

various methods for assessing L-glutamic acid levels in foods and their results confirmed that the L-glutamic acid content of food was highly soluble and clearly detected without interference from other compounds. Thus, it can be speculated that an Megazyme L-glutamic acid kit is an accurate, reliable and uncomplicated technique to use for evaluation in the current study. In the present study, the level of L-glutamic acid was determined by measuring absorbance at 492nm for each sample using a spectrophotometer. The results for L-glutamic acid levels in the sumac samples in the current study were higher than earlier findings. This divergence in findings may have occurred due to the different techniques that were used for processing (Brewer, 2011), extraction and analysis, storage and light exposure in addition to geographical and climate variability (Sochor *et al.*, 2011). Furthermore, chemical composition based on origin (Kossah *et al.*, 2009), nutritional status, soil type, pH, latitude and temperature could be a reason for these differences (Fredes *et al.*, 2014). However, the current study is limited by the lack of a similar assessment method, photometric, for a comparison of evaluation of L-glutamic acid in sumac. Therefore, further investigation is required to evaluate the accuracy of this method.

Further research is required to evaluate the results of this study and establish a general method for these assessments. Further, various doses of sumac have been used in assessing the polyphenol and antioxidant activity of this spice. Thus, additional assessment is required to evaluate the exact amount of sumac in contribution to food intake. The lack of study of all active polyphenol compounds in sumac is evident in the literature. Hence, in the foreseeable future, the identification and characterisation of all compounds of sumac are suggested to provide a better understanding of the role of these components in food intake.

The results of this study show that FBS, followed by CIBS, FRS and CTS samples, contain higher levels of polyphenol, antioxidant and L-glutamic acid compared to other samples. The findings of this study have an important implications for using sumac as a flavour enhancer in

the food industry due to the presence of glutamate acid which plays an important role in food palatability and acceptability. A small dose of sumac was considered for this study. Therefore, the effectiveness of sumac could not be validated as it was hypothesised. However, the estimation of effective doses of sumac is needed to validate the impact of this spice on health and appetite due to the presence of L-glutamic acid which secretes immunoglobulin A and saliva.

2.5. Conclusion

The present study aimed to determine the antioxidant, polyphenol and L-glutamic acid levels of sumac from different regions in two different colours (red and brown) and two forms (fresh and commercial). The most prominent finding to emerge from this study is the high level of antioxidant, polyphenol and L-glutamic acid activity of sumac. This finding raises intriguing questions for future nutritional studies regarding the correlation between the results for antioxidants, polyphenols and L-glutamic acids in each sample. The findings of this work highlight the association in both FBS, which showed the highest activity, and CPS, which demonstrated the lowest activity, similarly, between all three assays (polyphenol, antioxidant and L- glutamic). The discrepancy outcomes indicate that sumac samples may be influenced by geographical and environmental factors; therefore, a reduction in biological properties could be speculated for sumac samples with lower polyphenol, antioxidant and L-glutamic acid activity levels. Based on the findings of the present study, FBS and CIBS were shown to be more promising samples to pursue the aims of this PhD study. Taking health and safety factors into account for human studies, a CIBS sample was chosen for the next studies, sensory evaluation and food intake assessment.

Chapter 3: Sensory evaluation of sumac in butternut squash soup among free-living older adults (>65 years) and younger adults (18–35 years)

3.1. Introduction

Recently, the use of herbs and spices in food products has received global attention. Thus, the evaluation of liking and the acceptability of adding plant-derived products in food are important. Sensory evaluation is a multidisciplinary science, and, over the decades, it has provided a variety of methods related to the analysis of food product attributes (Lestringant *et al.*, 2019). Evidence suggests that sensory evaluation is the most precise method for measuring human responses to the perceived attributes of food, by using our five senses: sight, hearing, smell, taste and touch (Tuorila and Monteleone, 2009; Lawless and Heymann, 2010). Among the various sensory evaluation methods, sensory descriptive analysis and acceptability tests are the ones most used by food researchers (Yang and Lee, 2019).

Epidemiological studies indicate that various factors, including physiological, environmental and psychological, may have an impact on human senses, resulting in food choice alterations (Barclay and Brand-Miller, 2011; Olesen *et al.*, 2012). A study found that the food choice process may be affected by culture, individual liking factors and age (Hardcastle *et al.*, 2015). Ultimately, the taste of food plays an important role in consumer purchases and food intake (Biloukha and Utermohlen, 2001). Much research has been undertaken to evaluate possible explanations of changes in food choices. In this regard, ageing has an impact on human sensory functions, including taste and smell, compared to the younger generation. However, ageing is not the only factor that influences sensory activity, other factors including genetics, the environment and immune system dysfunction may show similar results (Hummel *et al.*, 2011). Age-related sensory loss has become an important topic in nutrition in relation to increasing energy intake and the palatability of food (Davenport, 2004; Field and Duizer, 2016). Previous

studies have demonstrated the purpose of adding herbs and spices to food, it not only improves food safety and reduces salt intake, but also imparts taste, flavour and colour due to the presence of chemical compounds including esters and terpenes (Little *et al.*, 2003; Rayne, 2011; Mitchell *et al.*, 2013; Ghawi *et al.*, 2014; Duncan *et al.*, 2017). Sumac has antibacterial activity (Aliakbarlu, *et al.*, 2014) and an improvement in the quality of broiler chicken following the addition of sumac has been noted (Mohammadi *et al.*, 2011). Thus, it can be speculated that the addition of sumac, which can be used as a natural product in food, may increase food safety. The adverse effect of salt intake on health is well documented and replacing this condiment with MSG has drawn the attention of researchers and food manufacturers. Natural additives, including sumac, could be an optimal strategy for the reduction or replacement of salt with no adverse impact on the acceptability of food.

As mentioned in Chapter 1, sumac has an acidic aroma (Bahar and Altug, 2009) and contains glutamic acid compounds (Kossah *et al.*, 2009), which can be considered for sensory appraisal following their addition to food. Although a little research has been carried out on the sensory characteristics of sumac, to the best of author's knowledge, no single study has investigated the sensory attributes of this spice in food. The literature has highlighted the importance of sensory perception in the palatability and liking of food, resulting in changes in eating behaviour. Therefore, this study aimed to evaluate the degree of five attributes (flavour, aroma, texture, acceptance, appearance) and four intensity attributes (salty, lemony, brown and red colours) of different doses of sumac in butternut squash soup, among both younger and free-living older adults. From the results presented in Chapter 2, commercial Iranian brown sumac (CIBS), with the highest levels of polyphenol, antioxidant activity and L-glutamic acid, was chosen for a sensory evaluation test. Thus, this study was intended to evaluate and compare the sensory characteristics of each test soup within and between younger and free-living older adult groups in order to determine the most acceptable dose of sumac among both groups of adults.

It is believed that older adults' threshold perceptions of taste and flavour are less sensitive than younger adults due to the impact of ageing. Hence, it was first hypothesised that free-living older adults in comparison with younger adults may accept higher doses of sumac and, second, increasing the dose of sumac in soup would have no adverse effect on the levels of liking and acceptability of soup samples by participants in both groups.

3.2. Methods and Materials

3.2.1. Sample preparation

Iranian commercial brown sumac, Donya, was chosen for this study based on the higher levels of polyphenol, antioxidant and L-glutamic acid which are present in comparison with other samples. Butternut squash from South Africa, Brazil or Spain regions and 25% less salt vegetable stock cubes (Knorr) were purchased from local supermarkets. The soup was prepared weekly in the Oxford Brookes Centre for Nutrition and Health (OxBCNH) kitchen, using a modified recipe from BBC Good Food.

To prepare the soup, butternut squash was steamed, then peeled and deseeded. Then, water was added to the chopped butternut squash along with a low-salt vegetable stock (Table 3.1). As the butternut squash pieces became tender, and after thorough cooking, the soup was puréed using a handheld blender.

Table 3.1. Ingredients of the test soup

Ingredient	Amount
Butternut squash	500 ±10 (g)
Vegetable stock (less salt)	4.5 ±0.2 (g)
Water	1 L

G: gram. L: Litre

The soup was cooled and for each portion a 100 ml was transferred into a zipped sealed bag (Bacofoil Zipper). The soup was frozen for a week in order to maintain its nutrients for the entire study and, on the test day, two hours before the start, the soup was taken out, defrosted at room temperature and warmed on a medium heat for 20 minutes. Each participant received 100ml of soup in a 4 oz (~118 ml) polystyrene pot container. The doses of sumac were added to the containers before the warmed soups were poured into containers (Fig. 3.1). The soup samples were prepared 10 minutes before the test started and kept at room temperature with the lids on. The sumac was mixed well with the soup by stirring, ahead of serving.

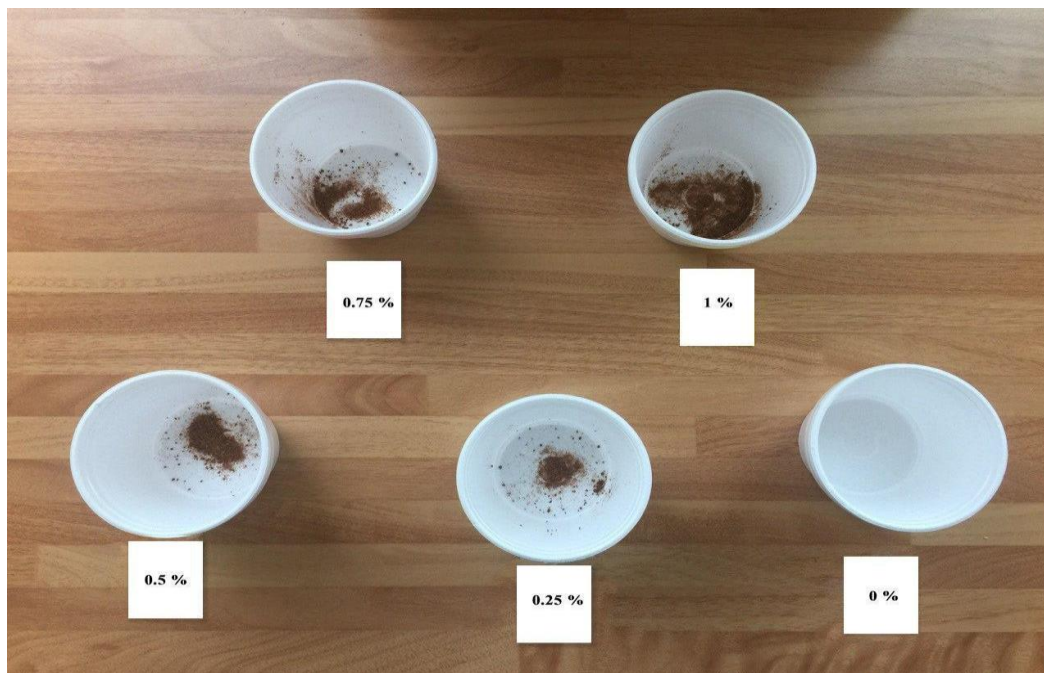


Figure 3.1. Four respective doses of sumac (0.25%, 0.5%, 0.75% and 1%) and a control sample (SC, 0%) prepared for sensory evaluation tests.

A total of five samples, to which the participants were blinded, were tested during two separate visits, with at least a two-day wash-out period in-between test days. The participants were randomly provided with three samples in the first session and two samples in the second session. The test comprised of a control soup (SC) without sumac and four different doses of

sumac: 0.25% (low-dose soup, LS), 0.50% (medium-dose soup, MS), 0.75% (high-dose soup, HS) and 1% (total dose soup, TS) (Figure. 3.2).



Figure 3.2. 100ml servings of butternut squash soup, with Four respective doses of sumac (0.25%, 0.5%, 0.75% and 1%) and a control sample (SC, 0%), prepared for a sensory evaluation test

Samples were coded with three-digit random numbers from the Compusense software package (Compusense Inc, Ontario, Canada) website. A glass of water served at room temperature and three crackers (each 8g, Jacob's, UK) were provided to the participants to cleanse their palate between each sample. All sessions were carried out in individual sensory booths at normal room temperature (21°C) (Figure 3.3) in the lab of OxBCNH, which has three separate booths lit by artificial light.



Figure 3.3. Sensory evaluation test set-up in an individual sensory booth

3.2.2. Study design and participant recruitment

The study was a randomised, crossover repeated measures design. A total of 40 participants were recruited for this study and separated into two groups. The older adult group comprised 20 participants (11 females and 9 males) aged over 65 years old, and the younger adult group also had 20 participants (13 females and 7 males) aged 18-35 years old. In the current study, the sample size was assessed using G*power (Version 3.1.9.7) which indicated the medium effect size of $d=0.5$, α of 0.05 and power of $(1-\beta)$ 0.80, so a total of 35 participants were required in order to identify a differences with minimum of 0.6 to 1.00 on a 9-point hedonic scale (Gacula and Rutenbeck, 2006). However, a total of 40 participants, equally divided into two groups, were recruited.

Participants could take part in this study if they met the following criteria:

- Either or an older adult over 65 years of age, a younger adult 18–35 years old
- No allergies to vegetables, herbs or spices (including tomatoes and celery)
- Non-smoker

- No cold or hay fever on the test day
- No diseases that might affect taste and smell perception (including high cholesterol and acute or local chronic inflammatory nasal diseases)
- No medication that might affect sensory taste, including antibiotics and anti-inflammatory agents (Appendix 1)
- Ability to understand information in English
- Able to attend, stand and sit for up to one hour
- Functional ability including travelling to a neighbourhood should be at a normal level.

Ethical approval for the current study was granted by the University Research Ethics Committee (UREC) of Oxford Brookes University (Registration No.: 161059) (Appendix 2). A study poster (Appendix 3) was used to recruit participants, by placing it around Oxford Brookes University campus (including notice boards and the sports centre), the online University Research activity group and the online Brookes Society for Retired Staff. In addition, a study advertisement for older adult participants was posted at venues for the fifty-plus community: a yoga venue, the daily info online, a table tennis group for the over-50s, a community centre for the over-60s, a community minibus, 'community clue' and voluntary action and placement teams in social work and social media.

Following their agreement to participate in the current study, volunteers received a participant information sheet (PIS) (Appendix 4) via email, mail or in person. When they attended the first test session, they completed a consent form (Appendix 5) followed by a health questionnaire (Appendix 6). Demographic data for both groups of participants were collected using a health questionnaire. Information about their weight (kg) and height (cm) was self-reported. Older adult participants were also asked to complete a functional ability health questionnaire (Appendix 7).

Furthermore, based on the functional ability health questionnaire (Appendix 7), free-living older adults were asked to rate the levels of four different categories of their performance on a scale from zero to three. They were asked to rate their shopping performance, paying attention and understanding levels, and their capabilities in terms of travelling, reading and following instructions. On this scale zero was defined as “normal”, one as “having difficulty but does it by themselves”, two as “requires assistance” and three as “dependent”. Participants received log-in information for a Compusense sensory test the day before the first session and they were trained on how to use this software by an iPad prior to their session. They were instructed not to eat or drink (except water) for one hour before the test commenced.

3.2.3. Data collection

The study was carried out using a 9-point hedonic scale method for measuring degrees of liking, whereby numerical values are assigned for verbal classification (Wichchukit and O’Mahony, 2015). On a descriptive scale, 1 is assigned to dislike extremely, 9 to like extremely, and 5 to neither dislike nor like (Appendix 8). The intensity scale is designed to show 1 as extremely light and 9 as extremely dark for both red and brown colour, whereas 5 illustrates neutral intensity (Appendix 9). On the same scale, 1 describes an extremely low intensity of a salty and lemony flavour, 5 shows as “just right” and 9 extremely high. A hedonic test was designed using the Compusense software package (Compusense Inc, Ontario, Canada) with an iPad provided to each participant for this purpose. In each session, participants were asked to evaluate the liking attributes of acceptance, flavour, aroma, appearance and texture of soups on a 9-point hedonic scale. In addition, on a similar scale, they assessed the intensity of the lemony flavour, salty flavour, brown colour and red colour of each tested soup. Furthermore, a second set of questions validated their perceptions of liking and disliking each sample in open text comments (for instance, the participants were asked if they liked or disliked any attributes of the soup samples).

3.2.4. Statistical analysis

Data were recorded and organised in Microsoft Excel 2010. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS, version 23, USA). A non-parametric Friedman test was used to evaluate the significance of differences in attributes among younger and older adult groups, while a Wilcoxon test was run to determine differences via pairwise comparisons. Furthermore, comparisons between the two groups of adults (younger and older) were applied using a non-parametric Kruskal-Wallis test, and an independent T-test was used to compare significant differences between the groups (younger and free-living older adults). Statistical significance was set at $p < 0.05$.

Analyses of overview comments on the most liked and disliked perceptions of the samples by both groups of adults were classified manually by optimising similarities of attributes. Hence, results were generated in three categories: texture (T), taste and flavour (T&F) and appearance (A), based on the consistency shown by both groups for their like and dislike comments on the samples. The results were presented as percentages (%) of liking and disliking of each attribute by both older and younger adults.

3.3. Results

3.3.1. Demographic characteristics of the participants

Baseline characteristics of age, weight and height of each participant were assessed (Table 3.2). The results of this analysis revealed, understandably, a significantly higher difference between the ages of both groups of adults, $t(38) = -32.6$, $p = 0.001$ with an effect of $d = 10.4$. However, no difference was observed in the comparison between the weight and height of older and younger adults ($p > 0.05$).

Table 3.2. Baseline characteristics of free-living older adults (>65 years) and younger adults (18-35 years old)

Characteristics	Older Adults	Younger Adults	<i>p</i> -value
	Mean ±SD	Mean ±SD	
Age (years)	71.3 ±4.3	27.5 ±4.2	0.001 ^a
Weight (kg)	69.9 ±11.4	68.3 ±10.9	0.654 ^a
Height (cm)	170.9 ±13.9	165.3 ±9.9	0.155 ^b

All data are displayed as mean ± SD. Significant difference at $p < 0.05$ compared between both groups. T-test^a and Wilcoxon^b test were used on baseline characteristics between older and younger adults..

According to the functional health questionnaire results, all participants rated their shopping performance (including for clothes and groceries), paying attention to and understanding the level of books or television shows, and travelling outside their neighbourhood (including taking a bus or driving) as normal levels of ability to function in these tasks independently. However, 15% of the participants were scored ‘having some difficulty but able to do reading unaided and following the instructions on appliances or gadgets.

3.3.2. Sensory evaluation: Hedonic test

3.3.2.1. Free-living Older Adults

The mean scores for the liking and intensity attributes of all the soup samples rated by free-living older adults are presented in Figure 3.4. The MS sample received the most liked mean score for its appearance, compared with the other samples, at 7.1. Free-living older adults rated the LS sample as their most preferred flavour, at 6.8. Furthermore, the TS samples scored the highest for aroma (6.5). In addition, this group scored the HS sample highest for texture and the TS sample highest for acceptance (6.9). According to the intensity scale, free-living older adults found that the SC sample had the highest intensity of red colouration (4.9). They reported that the intensity of the brown colour for LS and MS were similar, at 4.4. Continuing with the intensity of attributes, they scored the MS samples for their intensity of salty and lemony flavour similarly, at 3.6.

As with the younger adults group, a Friedman test was run to evaluate statistical differences in the liking and intensity attributes among free-living older adults. The results showed no significant differences ($p>0.05$) (Fig. 3.5) for any of the liking attributes or the intensity of a lemony flavour, salty flavour or red colour. However, the addition of sumac to soup samples demonstrated a positive intensity of brown colour compared with the SC sample at $p<0.037$ ($\chi^2(4) = 10.2$; 2-tailed) for all soup samples among free-living older adults. Despite an overall positive difference, a pairwise comparison showed no statistical changes between the soup samples with the SC sample which revealed the statistical difference ($p>0.05$).

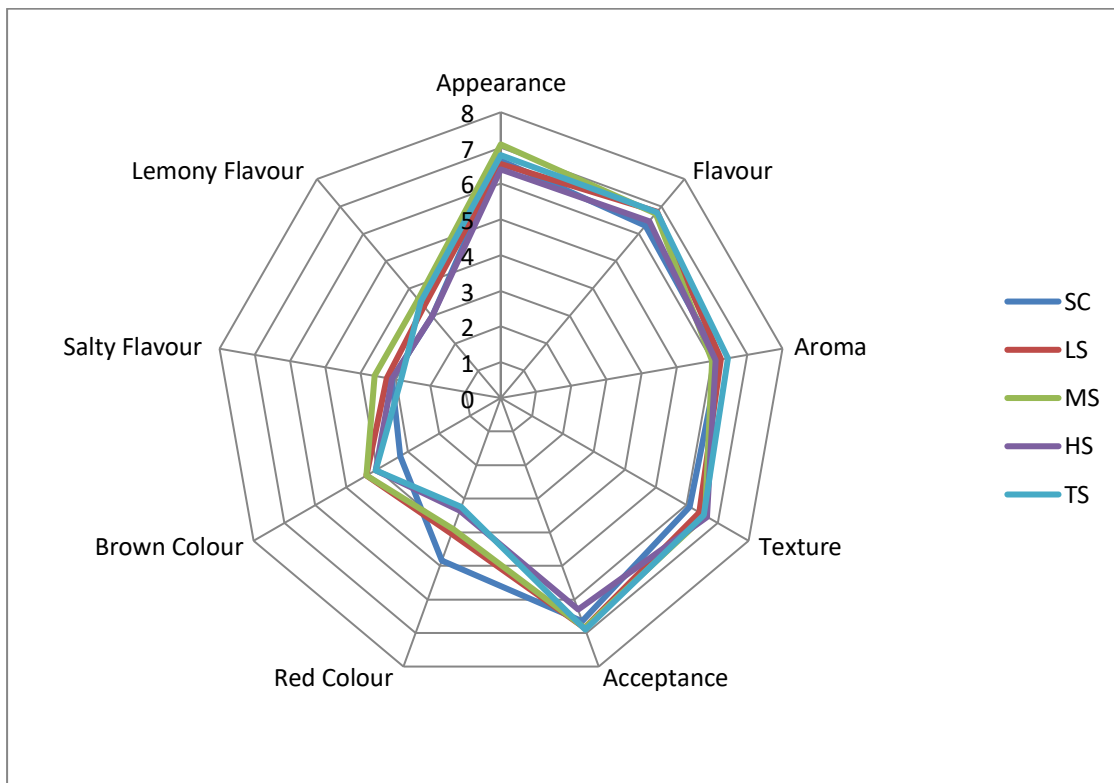


Figure 3.4. Spider chart for the sensory profile of butternut squash soup, with added sumac, for older adults (over 65 years). The liking and intensity attributes were ranked from 1 to 9. SC: control soup; LS (0.25%): low-dose sumac; MS (0.5%): medium-dose sumac; HS (0.75%): high-dose sumac; and TS (1%): total dose sumac.

3.3.2.2. Younger adults

The liking attributes' (acceptance, texture, aroma, flavour, appearance) and intensity attributes' (lemony flavour, salty flavour, brown colour, red colour) mean scores of the younger adults

group are presented in Figure 3.5. As the figure shows, the MS sample received the most liked mean score, of 6.9, for its appearance. However, the HS sample received the highest mean for liking both its flavour and aroma attributes, at 7.2 and 6.6, respectively. The highest mean value for liking the texture attribute was ranked for the SC sample, at 6.9, and the TS sample was ranked as the highest acceptance sample. On an intensity scale, the HS sample was rated as having the highest intensity of red colouration (4.1), and the MS samples had the highest intensity of brown colouration (5.4). In addition, the LS sample had the greatest intensity in terms of a salty flavour and the TS sample ranked 4.1 for the highest intensity of a lemony flavour among younger adults.

A Friedman test was successful in identifying a statistical difference for the intensity of the brown colour of soup samples compared to a control soup (SC) among younger adults ($\chi^2(4) = 23.5$; $p = 0.001$; 2-tailed). Pairwise comparisons revealed a large effect between the SC samples with LS and HS ($T = -1.6$, $r = -0.5$; $p = 0.012$), SC with TS ($T = -1.7$, $r = 0.5$; $p = 0.006$) and SC with MS ($T = -1.9$, $r = -0.6$; $p = 0.001$).

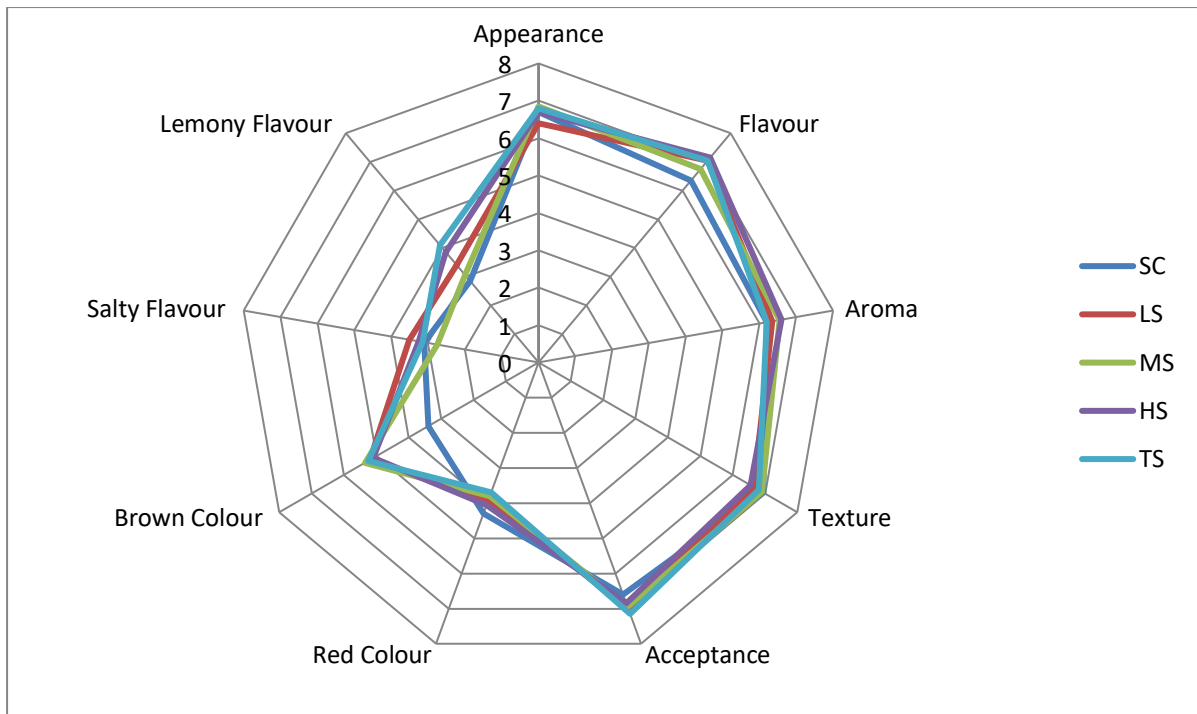


Figure 3.5. Spider chart for the sensory profile of butternut squash soup, with added sumac, for younger adults (18–35 years old). Liking and intensity were ranked from 1 to 9. SC: control soup; LS (0.25%): low-dose sumac; MS (0.5%): medium-dose sumac; HS (0.75%): high-dose sumac; and TS (1%): total dose sumac.

Overall, analysis of the effect of different doses of sumac in soup samples compared with a SC sample showed no impact on liking and intensity for any attributes between older and younger adults, except for the brown colour of soup TS, at $p=0.028$, $t(38) = 2.3$ and $d=1.3$.

Table 3.3. Sensory profiles of all attributes for all soup samples for free-living older adults (> 65 years old) and younger adults (18-35 years old)

Attribute	Group	SC	LS	MS	HS	TS
Appearance	O	6.7 ±1.8	6.6 ±1.4	7.1 ±1.1	6.4 ±1.4	6.8 ±1.3
	Y	6.7 ±1.6	6.4 ±1.3	6.9 ±1.4	6.7 ±1.3	6.8 ±1.2
	<i>p</i> -value	0.923 ^a	0.616 ^a	0.433 ^a	0.486 ^b	1.000 ^b
Flavour	O	6.3 ±1.6	6.8 ±1.4	6.8 ±1.1	6.5 ±1.5	6.7 ±1.3
	Y	6.4 ±1.9	7.1 ±1.5	6.8 ±1.7	7.2 ±1.5	7.1 ±1.5
	<i>p</i> -value	0.782 ^a	0.598 ^a	0.674 ^a	0.104 ^a	0.299 ^a
Aroma	O	6.3 ±1.1	6.3 ±1.1	6.1 ±1.3	6.1 ±1.3	6.5 ±1.3
	Y	6.2 ±1.4	6.4 ±1.1	6.6 ±1.1	6.6 ±1.4	6.2 ±1.4
	<i>p</i> -value	0.834 ^a	0.845 ^a	0.327 ^a	0.223 ^a	0.636 ^a
Texture	O	6.1 ±1.5	6.4 ±0.9	6.6 ±1.1	6.7 ±1.2	6.6 ±1.3
	Y	6.9 ±1.6	6.7 ±1.1	6.9 ±1.4	6.6 ±1.3	6.8 ±1.5
	<i>p</i> -value	0.074 ^a	0.514 ^a	0.469 ^a	0.645 ^a	0.570 ^b
Acceptance	O	6.7 ±1.3	6.9 ±1.3	6.9 ±1.1	6.0 ±1.8	6.9 ±1.5
	Y	6.6 ±1.6	6.9 ±1.3	6.9 ±1.5	6.9 ±1.6	7.2 ±1.3
	<i>p</i> -value	0.944 ^a	0.842 ^a	0.525 ^a	0.308 ^a	0.748 ^a
Red Colour	O	4.9 ±1.8	4.1 ±1.9	3.9 ±1.7	3.4 ±1.8	3.3 ±1.8
	Y	4.3 ±2.2	3.9 ±1.9	3.8 ±1.9	4.1 ±1.8	3.7 ±1.8
	<i>p</i> -value	0.485 ^a	0.812 ^b	0.681 ^b	0.227 ^b	0.428 ^b
Brown Colour	O	3.3 ±1.6	4.4 ±1.7	4.4 ±1.8	4.1 ±1.5	4.1 ±1.5
	Y	3.4 ±2.1	5.2 ±1.9	5.4 ±1.5	5.1 ±1.7	5.3 ±1.8
	<i>p</i> -value	0.799 ^b	0.151 ^b	0.058 ^b	0.059 ^a	0.028 ^b
Salty Intensity	O	3.1 ±1.6	3.3 ±1.7	3.6 ±2.1	3.1 ±1.8	2.9 ±1.6
	Y	3.1 ±1.7	3.5 ±1.6	2.8 ±1.6	3.3 ±1.4	3.2 ±1.9
	<i>p</i> -value	1.000 ^b	0.550 ^a	0.247 ^a	0.563 ^a	0.741 ^a
Lemony Intensity	O	3.0 ±1.9	3.4 ±1.8	3.6 ±1.9	3.0 ±1.9	3.6 ±2.1
	Y	2.9 ±1.4	3.4 ±1.6	3.1 ±1.4	3.9 ±1.9	4.1 ±1.9
	<i>p</i> -value	0.934 ^a	0.804 ^a	0.332 ^a	0.142 ^a	0.305 ^a

All data are displayed as mean ± SD. Significant difference at $p < 0.05$ compared between both groups of adults on attributes of each sample. Independent T-test^b and Kruskal-Wallis^a test were used to analyse the differences between groups. SC: control soup; LS (0.25%): low-dose sumac; MS (0.5%): medium-dose sumac; HS (0.75%): high-dose sumac; and TS (1%): total dose sumac. Y: Younger Adults (18–35 years old); O: Older Adults (over 65 years).

3.3.3. Qualitative data

The taste and flavour (T&F) attribute was the one most liked by participants in both groups of adults (Fig. 3.6). In this regard, younger adults liked the T&F of the HS sample the most, whereas free-living older adults liked the MS sample the most. Interestingly, younger adults liked the texture of the SC and TS samples the most. However, free-living older adults liked the texture of the MS sample the most. Interestingly, none of the free-living older adults liked the appearance of the SC sample, while younger adults liked this sample, alongside the LS sample, the most.

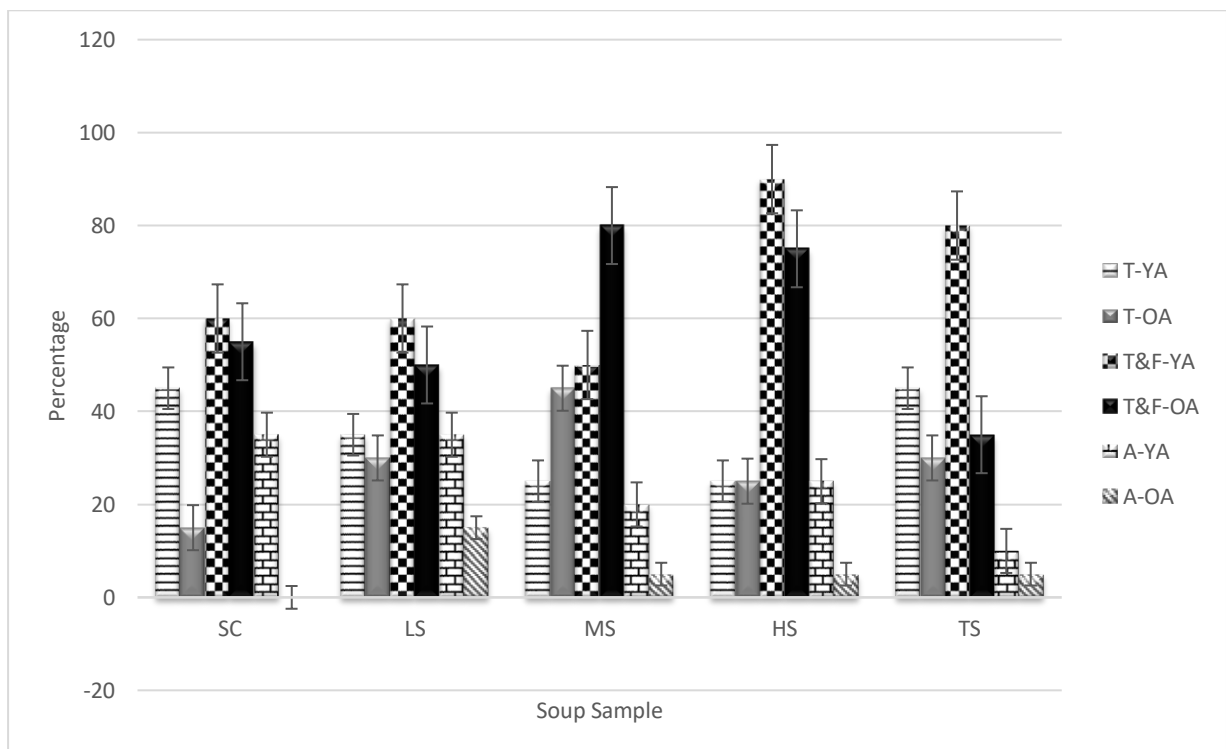


Figure 3.6. Percentages of liking attributes for test soups. All data are displayed as mean \pm SD. SC: control soup; LS (0.25%): low-dose sumac; MS (0.5%): medium-dose sumac; HS (0.75%): high-dose sumac; and TS (1%): total dose sumac. Y: Younger Adults; O: Older Adults; T: Texture; T&F: Taste & Flavour; A: Appearance.

In continuation of the qualitative results, participants reported disliking some attributes of each sample (Fig. 3.7). The outcome for texture evaluation showed that the HS sample received the

highest level of disliking (35%) among younger adults, whereas the SC and LS samples had the highest levels of disliking (20%) among free-living older adults. Younger adults disliked the SC soup sample mostly for its T&F attribute, followed by TS, MS, LS and HS, while free-living older adults disliked the T&F attribute of the HS soup sample, followed by SC, MS, TS and LS. Moreover, appearance made less contribution to disliking among both adult groups, as both MS and HS samples scored 15%, and SC, LS and TS each scored 5%.

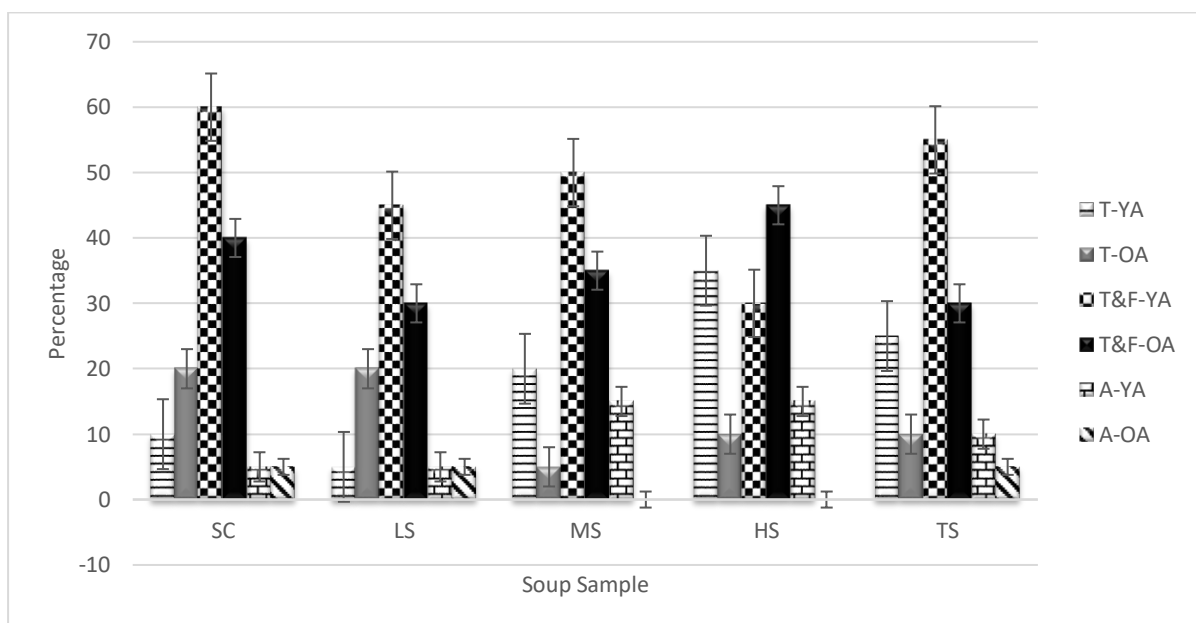


Figure 3.7. Percentages for disliking the attributes of test soups. All data are displayed as mean \pm SD. SC: control soup; LS (0.25%): low-dose sumac; MS (0.5%): medium-dose sumac; HS (0.75%): high-dose sumac; and TS (1%): total dose sumac. Y: Younger Adults; O: Older Adults; T: Texture; T&F: Taste & Flavour; A: Appearance.

3.4. Discussion

Adding herbs and spices is counted as a strategy for increasing the palatability and liking of less-flavoured and reduced salt foods, including vegetables, resulting in increasing the impact of health benefits (Bouhlal *et al.*, 2013; Ghawi *et al.*, 2014) and an increase in nutrient consumption, including energy and fat (Peters *et al.*, 2014; Polsky *et al.*, 2014). The current study explored the liking of different doses of sumac added to butternut squash soups. The

outcome of this study was a greater intensity of brown colour on the sumac sample compared to the control soup for both groups of adults ($p < 0.05$). Moreover, the highest dose of added sumac in soup (TS) was ranked as the most acceptable by both groups of adults.

3.4.1. Sensory evaluation: Hedonic test

The quantitative results showed that the different doses of sumac in soup compared with a control soup were unable to reveal differences between and within the groups of older and younger adults for any attributes. Hence, this implies that there were no preferences between sumac doses compared with a control sample for the participants. The findings of the current study are consistent with the results of Ghawi *et al.* (2014), in which no differences were observed in immediate improvements to liking the soups which had herbs and spices added. Furthermore, the importance of the roles of taste, age and gender have been highlighted for seasoned food (Hiza *et al.*, 2013); however, these factors had no influence on the results for SC and other samples in the present study.

A study assessed the impact of seasoning vegetables with various herbs and spices (0.27–0.42g) including salt, cinnamon, garlic powder, onion powder, dill, black pepper and dried parsley (Manero *et al.*, 2017). The results showed no differences between seasoned and unseasoned vegetables for liking, with higher interest among individuals who do not consume vegetables regularly for the seasoned test food ($p > 0.05$). In comparison with the concurrent study, despite the use of different spices, the addition of sumac without lessening the acceptability of or having any negative impact on food can be considered for further studies of flavour enhancement of foods. A study evaluated the acceptability of adding five doses (0–2 g) of MSG to food (mashed potatoes, spinach, ground beef) among adults: older and younger (Essed *et al.*, 2009). Their results revealed that 0.5% MSG was the most acceptable dose in mashed potato. This finding contradicts the results of the present work, which used sumac ranging between 0–1%, where it was found that the highest dose (0.25 g) of sumac was the one most acceptable to

both groups of adults. This could be explained based on personal preferences for the concentration of additives, which habitually range from 1–5% (Vázquez-Fresno, *et al.*, 2019). Previous studies have shown that a reduction in salt has an impact on liking the taste, texture and appearance of food, compared with a control (Doyle and Glass, 2010; Liem *et al.*, 2011). However, adding herbs and spices, due to their volatile aroma and flavour compounds, to enhance a salty taste in food is recommended (Batenburg and van der Velen, 2011). The perception of a salty and lemony flavour for sumac in MS samples (Figure 3.4) can be explained based on the fact that the volatile compounds present in sumac may intensify these attributes in this soup, while an intensity perception was not observed with higher dose samples, LS and TS; it seems possible that the taste sensitivity threshold of individuals may play a role in this recognition. It has been demonstrated that the threshold level is subjective, and not only age-related; however, this may be influenced by various factors including gender and background. Comparison work on salt threshold between the genders revealed that males had a higher recognition threshold for salt than women (Mitsuhashi *et al.*, 2008). Moreover, a meta-analysis of 25 studies demonstrated that threshold detection is reduced by ageing for bitter, salty, sour and sweet tastes (Methven *et al.*, 2012).

It is well documented that an increase in the level of spices added to food products may increase the liking and acceptance of aroma and taste; however, this may reduce the liking of colour and texture (Wilczyńska *et al.*, 2017). Food perception can alter following physiological changes caused by ageing (Field and Duizer, 2016). In the current study, free-living older adults indicated that they liked the aroma of the TS sample most, whereas younger adults preferred the HS sample. This finding supports previous work which showed that free-living older adults, compared to a younger adult group, are less sensitive in their perception of samples' (citric acid, caffeine, salt, sucrose, MSG) taste (Puputti *et al.*, 2019). Therefore, ageing could be a major factor in physiological changes to the olfactory system, which is affected by ageing.

Sumac in the present work was unable to demonstrate its sour and lemony properties in soup. This could occur due to the method of cooking and interaction of vegetable low-salt stock ingredients with soup (Opara and Chohan, 2014). Based on the variety of the chemical structure of aroma, including acids, alcohol and esters, various factors participate in the release of aroma from spices in foods. Moreover, multisensory perception, for instance, olfactory, gustatory and oral-somatosensory, play an important role in food flavour perception. The impact of viscosity on flavour recognition and the importance of organoleptic in food preferences have been reported previously (Bult *et al.*, 2007). Therefore, volatile compounds may interact with the food matrix via the saliva and nasopharynx pathway and, hence, flavour release following olfactory activation. However, the interaction of sumac in the food matrix is still unknown. This mechanism includes binding and partition releases based on the physicochemical properties of sumac. The flavour compounds of sumac may interact differently with different types of food, including hydrophobic, hydrogen, covalent and physical binding. A study of the physiochemical structure of sumac and its interaction in various foods revealed the impact of flavour intensity, resulting in enhanced food intake (Naknean and Meenune, 2010). Determination of the interaction of sumac in the matrix is inconclusive unless more varieties of food matrices are studied. A future study is required to confirm that under conditions of a higher concentration of volatile compounds of sumac in soup, it can retain the flavour of sumac. On the other hand, it has been demonstrated that protein and fat can retain volatile compounds more than carbohydrates. Therefore, it can be hypothesised that food type may play an important role in releasing the flavour of sumac and increasing the palatability and consumption of food (Ammari and Schroen, 2018).

In the current study, doses of sumac changed the intensity of the soup colour and both groups of adults reported the MS soup sample as having the most likeable appearance and the highest intensity of brown colour. Free-living older adults have identified the intensity of the brown

colour of the LS sample to be similar to MS. Butternut squash, due to the presence of carotenoids, has a dominant orange colour (Conti *et al.*, 2015) and sumac contains hydroxyphenyl pyranoanthocyanin compounds, which are responsible for colour pigments (Dabas, 2016). Moreover, the importance of colour as a key factor in food choice, before taste and flavour, is emphasised (Hutchings, 2003). The literature highlights the impact of colour and its relationship with flavour in foods (Spence, 2015). This fact was demonstrated in a study in which participants were asked to distinguish the flavour of solutions which were coloured relevant or irrelevant, or were colourless. Their results revealed the impact of colour hints on misperceptions of flavour (Zampini, *et al.*, 2007). Hence, butternut squash with added sumac was chosen for this study to provide further information for future research on using natural colourants in the enhancement of the palpability of food.

3.4.2. Qualitative data

In this study, the overall acceptability and liking attributes of the sample showed no differences between the groups of adults. These results are in agreement with Ghawi *et al.* (2014), who reported that the liking attribute was on the basis of participants' preferences. It has been suggested that the addition of herbs and spices to food should be evaluated based on individual preferences (Hollis and Henry, 2007) due to the impact of the level of polyphenol compounds on results for bitter and astringent flavours in products. Therefore, based on individual taste perception, the liking and acceptability of products may alter (Ghawi *et al.*, 2014). However, the results of the current study manifested that the highest value of sumac was accepted by both groups of adults. The number of selected doses in the current study can be justified by the acceptability of sumac by both groups of adults, although the chosen dose in the present work is less than the dose suggested by Douglas (2012). The literature demonstrates that sumac contains high levels of phenolic compounds that are responsible for bitterness (Delcour *et al.*, 1984; Shafiei *et al.*, 2011). Furthermore, it has been recommended that an administrable dose

of a plant-based polyphenol compound determines the acceptability of bitterness extracts in food (Rodgers *et al.*, 2005). Thus, it can be concluded that the low dose of added sumac in the present work may be responsible for the acceptability of TS samples.

In addition, the habitual consumption of polyphenol compounds may differ among individuals (Opara and Chohan, 2014). Various factors may alter the intake of these components, including the health benefits, age, culture, affordability, gender and genetics. The literature emphasises various health benefits of polyphenol compounds; however, there are some incompatibilities with marketing and consumer studies on optimal doses of phenolic compounds added to food. This was reflected in the health benefits of Brussels sprouts in a cancer study due to the presence of glucosinolate compounds (Fahey *et al.*, 1998). Regardless of the health benefits of these polyphenol compounds, the development of bitterness (including astringent and bitter taste) of these compounds in food and their results on the level of consumption of products increases food industry concerns (Fenwick *et al.*, 1990; Drewnowski and Gomez-Carneros, C, 2000). Furthermore, the level of fungiform taste papillae for the perception of a bitter taste correlates with ageing. This may result from a reduction in the density of papillae, which results in higher perceptions of a bitter taste from the presence of propylthiourea (Fekete *et al.*, 2017). Therefore, this parameter could explain the sweetness or tasteless perceptions reported by free-living older adults in this study. The relation between the genotypes of a bitter taste and phenotypes was previously studied and this revealed an association between PROP taster status and both TAS2R38 genotype and fungiform papillae density (FPD). The researchers found that participants who showed higher propylthiourea sensitivity had mainly TAS2R38 genotypes and higher fungiform papillae density (Shen *et al.*, 2016). A higher density of fungiform taste papillae increased the perception of bitter taste by participants. This may confirm that ageing had an effect on taste and flavour perception (Methven *et al.*, 2012).

In the current study, the findings were not statistically different ($p>0.05$), with the exception of the intensity of brown colouration with sample soups. There are several possible explanations for the inconsistency of these results. One explanation could be the small differences between doses so that it was difficult to differentiate the taste and flavour of the samples (Methven *et al.*, 2016). Another reason for that may be the number of participants in a sensory test which plays an important role, as they are considered to be an analytical instrument that is required for precise results (Singh-Ackbarali and Maharaj, 2014). A smaller number of individuals may reduce the power of a study (Methven *et al.*, 2016), therefore a large group of untrained panellists is required in order to obtain more accurate results; however, this number can be reduced with trained individuals (Lestringant *et al.*, 2019). To obtain significance in a hedonic test with a power of 90% among older adults, a study requires 112 participants, but this number can be difficult to achieve in a clinical study (Cardello *et al.*, 2008). The number of panellists also depends on the complexity of the tested product (Mammasse and Schlich, 2014), and this may vary in different tests (Hough *et al.*, 2006; Gacula and Rutenbeck, 2006) and cannot be defined for any study (Mammasse and Schlich, 2014). An accurate sample size plays a role in obtaining valid and conclusive results and minimising type I and type II errors (Faber and Fonseca, 2014). The application of a hedonic test to a healthy older adults' population has been recommended due to it being a precise method (Dermiki *et al.*, 2013). Therefore, based on the current study outcomes, increasing the number of participants and higher doses of sumac should be applied in a future study to achieve a robust outcome. Lastly, further studies require validating the reproducibility of the method that is used in the assessment of liking and acceptability of doses of sumac among free-living older adults and younger adults. Future outcomes could confirm that the highest dose of sumac (1%) is the most acceptable dose among and between groups.

3.5. Conclusion

In the present study, overall, different doses of sumac did not affect perceptions of liking and the intensity of different doses of sumac, with the exception of the intensity of a brown colour ($p < 0.05$). The present study, to the best of author's knowledge, is the first attempt to compare the impact of different doses of sumac in a sensory evaluation by free-living older and younger adults. The findings of the current study confirm that the TS sample was the sample most preferred by both groups of adults. However, the results rejected the hypothesis of a difference between adult groups for the liking and intensity of attributes of different doses of sumac in soup samples. Thus, it seems reasonable to conclude that the TS sample soup (0.25 g per 100 mL) can be considered for further study in this PhD thesis, and this could encourage researchers and food manufacturers to use sumac which had no adverse impact on acceptability in both groups of adults.

Chapter 4: Effect on appetite and food intake following the addition of sumac to soups among free-living older and younger adults using two cooking methods

4.1. Introduction

A report from the United Nations Statistics Division showed a potential population rise for those over 60 years of age by up to 38% around the world by 2050 (United Nations, 2017). The increase in the older adult population is in direct proportion to a decline in health status; therefore, an improvement in the later quality of individuals' lives is required (World Health Organisation, 2016). Ageing, disease and medication have all been found to be major factors that influence the reduction of taste and smell senses (Schiffman, 2000). An association between ageing and malnutrition has previously been reported (Ahmed and Haboubi, 2010). The relationships between ageing with loss of lean tissue (Boutari and Mantzoros, 2017) and weight loss with reduction of daily energy intake (Zhu *et al.*, 2010) are more significant than energy expenditure reduction (Speakman and Westerterp, 2010).

Physiological anorexia of ageing is defined as appetite reduction resulting in decreased energy intake (Lee *et al.*, 2008; Saha *et al.*, 2010). It has been reported that a reduction in physical activity and diminished lean-tissue energy expenditure play a role in appetite alteration (Salas-Salvadó *et al.*, 2011; Appleton *et al.*, 2016). This is not limited to physiological alterations, as it has been shown that medication and disease have an impact on taste and smell receptors (de Boer *et al.*, 2013). Gustatory dysfunction may result in a series of difficulties, including oral health and perception of taste and smell (Sergi *et al.*, 2017).

Reducing the factors that have an impact on weight loss, lean body tissue and hospitalisation periods are considered by public health organisations (Thibault *et al.*, 2011). Thus, improving food and energy intake can be suggested as the first strategy. Traditionally, salt has been added to food to enhance its taste. However, dietary sodium intake has become a worldwide wellness

concern, due to its adverse impact on health, as it contributes to the development of diseases, including cardiovascular disease and hypertension (Hayabuchi *et al.*, 2020). Evidence suggests that food additives that contain sodium play various roles in food products, including flavour improvement, texture maintenance and preservation (Henney *et al.*, 2010). The replacement of salt with an alternative additive, including MSG, has been recommended for the reduction and prevention of individual morbidity and mortality (Jinap *et al.*, 2016). Recent work has demonstrated that MSG contains 12.28g of sodium per 100g, whereas the sodium content in 100g salt is 39.34g; therefore, MSG, compared with salt, has one-third of the sodium (Maluly *et al.*, 2013). It has been reported that MSG might increase the secretion of saliva from two sources: gastric juice (Zolotarev *et al.*, 2009) and the parotid gland (Hodson and Linden, 2006) by reflecting taste signals in the brain through taste buds and/or gastric receptors.

As a result of a reduction in older adults' physical capacity, including masticatory function, individuals may develop malnutrition (Soojeong and Nami, 2015). It is well understood that the process of ageing causes deterioration in taste and smell perception. The impairment of these two functions leads to appetite loss, monotony in food perception and reduction in food intake (Alves and Dantas, 2014). Several studies showed that saliva plays a pivotal role in gustatory function (Sasano *et al.*, 2014; Sergi *et al.*, 2017).

Research on polyphenol (Al-Marazeeq *et al.*, 2019) and antioxidant activity (Mazaheri Tehrani, *et al.*, 2017) of sumac and the impact of L-glutamic acid on increasing food intake (Masic and Yeomans, 2014) have been conducted. Taking such research into account, it is possible that sumac, as a spice that contains high levels of polyphenols, antioxidants and L-glutamic acid, may contribute to enhancing taste and flavour, resulting in increased food intake among older adults. Therefore, commercial Iranian brown sumac (CIBS) was chosen for this study, based on the results achieved in previous chapters (Chapters 2 and 3).

The aims of the current study were:

1. Phase 1: to analyse food intake following the consumption of sumac in butternut squash soup (added at the end (SSE) and added during cooking (SSC)) among and between free-living older adults (over 65 years of age) and younger adults (18–35 years of age) compared with a control soup (SC) session. It was hypothesised that added sumac would increase energy and macronutrients intake in both groups following SSE and SSC sessions compared with SC sessions in an *ad libitum* lunch, an evening meal and all-day food intake. Further to this, it was speculated the method of adding sumac to soup (SSE and SSC) would have an impact on the level of food consumption.
2. Phase 2: To evaluate the influence of sumac added to mushroom soup (SSE and SSC) compared with a control soup (SC) on food intake among residential older adults (over 65 years old) in a care home. It was hypothesised that the consumption of mushroom soup that contained sumac would improve food intake compared to the SC sample. It was postulated that the addition of sumac in SSE and SSC sessions would demonstrate an improvement in energy and other macronutrients intake in an *ad libitum* lunch and all-day intake compared with an SC session. Moreover, it was theorised that two methods of adding sumac (SSE and SSC) could play a role in improving food intake among this population.

4.2. Methodology

4.2.1. Participants

This study was carried out in two phases. Phase 1 involved two groups: free-living older adults (over 65 years of age) and younger adults (18–35 years old). In phase 2, the over 65 years participants from a residential care home were recruited.

4.2.1.1. Phase 1

Advertisements for the study for free-living older adults (Appendix 10) and younger adults (Appendix 11) were posted on notice boards in different buildings at Oxford Brookes University, in the Oxford Brookes University research activity group, in personal Twitter and LinkedIn accounts, in daily info and also via personal contact with participants from the sensory evaluation study in Chapter 3 (who verbally indicated their interest in participating in further studies); additional sites were used to advertise for free-living older adults, including the over-50s walking activity on the Oxford Council website and the Oxford Brookes Society for retired staff.

A total of 40 healthy male and female participants were recruited from the above avenues. Each group had 20 participants. In the free-living older adults' group, 11 healthy females and nine healthy males took part in the study; in the younger adult's group, 15 females and five males participated. Dietary intake was analysed based on food consumed in an *ad libitum* lunch, evening meal and all-day meal. In this study, G*power (v. 3.1.9.7) indicated that for a two-tailed test, a medium effect of $d=0.5$, α of 0.05 and a power of $(1-\beta)$ 0.95, a sample size of 16 was required in each group in order to increase an intake of 45 kcal (Flood and Rolls, 2007).

A participant information sheet (PIS) was sent to interested free-living older adults (Appendix 12) and younger adults (Appendix 13) via email or post. The participant inclusion criteria were as follows:

- Free-living older adults, aged 65 years or over
- Younger adults, aged 18–35 years
- No allergies to herbs or spices
- No allergies to any types of vegetables (including celery and tomatoes)
- Non-smoker

- Does not have a cold or hay fever (on test day)
- No disease that may affect taste and smell sensation (including local chronic or acute inflammatory nasal disease and high cholesterol)
- No medication that may affect sensory taste, such as antibiotics, antihistamines and decongestants, anti-inflammatory agents, muscle relaxants (Bromley, 2000) (Appendix 1)
- Ability to read and understand information in English
- Ability to attend, stand and sit for up to one hour
- A normal level of capability and functional activity (i.e., travelling outside the neighbourhood, shopping alone)
- Not diagnosed with dementia.

Participants received a consent form (Appendix 5) via email or post. A signed consent form was returned prior to the first test session. All participants completed a health questionnaire (Appendix 6); additionally, older adult participants filled in a functional ability health questionnaire (Appendix 7) (Hall *et al.*, 2011). The age (years), weight (kg) and height (cm) data presented in Table 4.4 were self-reported by the participants.

Ethical approval for the study was granted by the University Research Ethics Committee (UREC) of Oxford Brookes University on 13 March 2018 (Registration No.: 181174- Phase 1) (Appendix 14a and 14b).

4.2.2.2. Phase 2

Nursing homes based in Oxfordshire were contacted via email or letter (Appendix 15). A follow-up email was sent after two weeks in case of no response. In agreement with the nursing home an advertisement (Appendix 16) of the study was emailed and/or posted to the manager of the nursing home. Volunteers were provided with a PIS (Appendix 17) following the

agreement of St Luke's Hospital care home's manager's contribution. On expressing interest, volunteers signed a consent form (Appendix 5). A health questionnaire (Appendix 6) was completed with the help of a nurse. Food intake was analysed based on the amount of consumption in an *ad libitum* lunch and an all-day meal. In this study, G*power (v. 3.1.9.7) indicated that, for a one-tailed test, a large effect of $d=0.8$, for a significant of α of 0.05 and a power of $(1-\beta)$ 0.8, a sample size of 12 was required in order to increase an intake by 174 kcal (Leslie *et al.*, 2012). Due to challenges in recruiting and drop out, only five participants (three females and two males) could take part in the study and met the following criteria:

- Adult, aged 65 years or over
- No allergies to herbs or spices
- No allergies to vegetables, including celery and tomatoes
- Non-smoker
- Does not have a cold or hay fever (on test day)
- No disease that could affect taste and smell sensation (including local chronic or acute inflammatory nasal disease, high cholesterol, head injury, stroke or exposure to radiation therapy for head or neck cancer)
- No medication that might affect sensory taste, such as antibiotics, antihistamines and decongestants, anti-inflammatory agents, muscle relaxants (Bromley, 2000) (Appendix 1)
- Ability to read and understand information in English.

Ethical approval for the study was granted by the University Research Ethics Committee (UREC) of Oxford Brookes University on 14 November 2018 (Registration No.: 181174-Phase 2) (Appendix 18).

4.2.2. Study Design

Figure 4.1 shows the study design for both phases of this study.

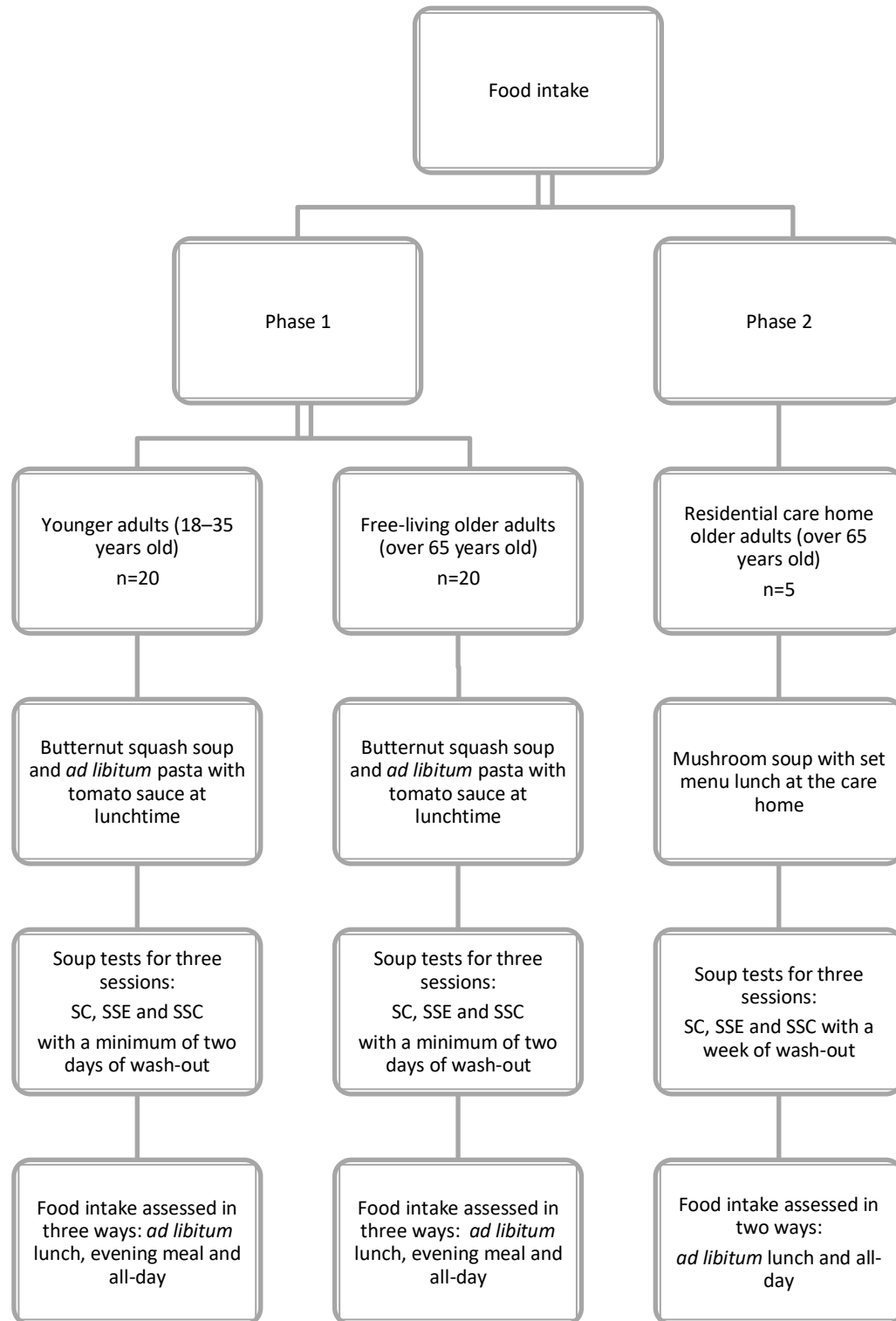


Figure 4.1. Food intake flowchart for both phases of the study

4.2.2.1. Phase 1 Protocol

This study was a randomised, crossover repeated measures design. The participants were randomly allocated to different groups for each session using Research Randomizer (Urbaniak and Plous, 2013). All 40 participants were asked to attend the Oxford Brookes Centre for Nutrition and Health (OxBCNH) for three test sessions, with at least two days wash-out in-between each session. The participants were contacted a day before their first test session (by email, text, post or telephone call), received the standard breakfast (86–247 kcal) menu and preparation instructions for the test day. Based on these instructions, they were asked to keep their breakfast intake consistent with the same quantity for all three sessions.

Breakfast on the morning of the test was recommended (per serving) to be:

- 1 cup of tea or coffee

And any one of the following:

- Two slices of toast with spread
- Weetabix with milk in a small bowl, serving size 2 biscuits (37g)
- Cornflakes with milk in a small bowl, serving size 1 cup (23g)
- A breakfast cereal bar

In addition, they were asked not to eat or drink (except water) one hour prior to the test. In the first test session, participants were asked to complete a 24-hour dietary intake form (Appendix 19) to determine baseline energy and nutrient intake (from lunchtime the day before, up to the test session). Following each test session, participants were provided with a 12-hours dietary intake form (Appendix 20) in order to record all foods and/or drinks consumed subsequent to their test session up to midnight.

The soup (Figure. 4.2) was served between 12:00 and 14:00 during each session. Participants were asked to consume the entire serving of each session. A period of 10 minutes was allocated between serving the soup and *ad libitum* lunch. The weight of the food and water were recorded before and after consumption. However, the pasta (180 g) and water (340 ml) were consumed at their desired level. Each session lasted for about half an hour and all sessions in this study were conducted in individual booths.



Figure 4.2. Soup samples: Control soup (SC), soup with sumac added at the end (SSE) and soup with sumac added during cooking (SSC).

4.2.2.2. Phase 2 Protocol

Unlike in the previous phase, mushroom soup, due to its higher acceptability and liking among nursing home older adult participants, was provided. Participants in this group received their soup at 12:30 on the test day. Approximately 10 minutes later, an *ad libitum* lunch (Appendix 21) which was prepared by the nursing home chef, was served. Similar to lunch, dinner was prepared and served at 18:00. Similar to phase 1, participants were instructed to consume their soup fully and no restriction was applied to their food intake. Foods were weighed by the

researcher, before and after consumption at both lunchtime and dinnertime, using Salter 1150 electronic kitchen scales.

4.2.3. Test Food and *ad libitum* lunch

4.2.3.1. Phase 1

In this study, the participants were given a test food (butternut squash soup, 150g) and an *ad libitum* lunch (pasta with tomato sauce, 180g). All the ingredients used in this study were purchased in the United Kingdom (UK). Iranian brown sumac, Donya, was purchased from a local shop in Oxford. Furthermore, ingredients including butternut squash (from either South Africa, Brazil or Spain), 25% reduced salt vegetable stock cubes (Knorr), pasta (Napolina fusilli), vegetable oil (Tesco brand) and tomato sauce (Dolmio Bolognese Original) were all purchased for the current study from local supermarkets, including Tesco and Waitrose.

4.2.3.2. Phase 2

In this phase of the study, Iranian brown sumac (Donya) was provided to the nursing home. Mushroom soup (180g) and meal ingredients were purchased by the nursing home on a daily/weekly basis. The soups were prepared by the nursing home chef and served to the participants.

4.2.4. Soup preparation

4.2.4.1. Phase 1

Participants were given 150g of butternut squash soup in each session: a control (SC), sumac added at the end of cooking (SSE), or sumac added during cooking (SSC). The soup was prepared on a weekly basis in the OxBCNH kitchen, using a similar method to that used in Section 3.3.1 in individual portions and stored in a freezer. On the test day, two hours before the participants arrived, the soup was taken out of the freezer and defrosted at room

temperature. For each participant, 1% sumac was added to 150g of soup, either at the end of cooking (SSE) and mixed well before being served, or during cooking (SSC), and the soup was heated for 30 minutes. In the SC session, 150g of soup, without added sumac, was served to the participants. The ingredients of the butternut squash are as previously specified in Chapter 3 (section 3.3.1, Table 3.2).

4.2.4.2. Phase 2

Similar to the previous phase, participants attended all three sessions and consumed their soup before their lunch in the nursing home. This group of participants received 180g of freshly made mushroom soup on the test day. Identical to phase 1, SSE and SSC soups contained 1% of sumac, and SC was consumed without any sumac. Table 4.1 presents the ingredients of the mushroom soup.

Table 4.1. Ingredients of mushroom soup for five participants

Ingredient	Amount (g)
Mushrooms	500
Onion	110
Salt	0.5
Pepper	0.7
Vegetable stock	28
Whipping cream	65
Mixed herbs:	
Basil	
Marjoram	
Oregano	0.7
Rosemary	
Sage	
Thyme	

4.2.5. *Ad libitum* lunch preparation

4.2.5.1. Phase 1

First, dried pasta was weighed (100 ± 0.5 g) for each participant, then added to a pot of boiling water with one teaspoon of vegetable oil and cooked for 10 minutes. Then, 60g of tomato sauce was added to the pasta and mixed. The pasta was weighed (180 ± 1 g) and served along with a glass of water (340 mL) at room temperature (Peter *et al.*, 2014). Participants were instructed to eat as much pasta and drink as much water as they desired.

4.2.5.2. Phase 2

The participants received their food based on their choice from the daily menu (Appendix 21) of the nursing home. Their menu included three choices including the main course and dessert.

4.2.6. Nutritional composition analysis

4.2.6.1. Nutritional analysis of test food, *ad libitum* lunch and food record in phase 1

To calculate the energy and macronutrient intake from completed dietary intakes, Nutritics software version 5.022 (Nutritics Ltd, Dublin, Ireland) was used (Table 4.2.). The energy and macronutrients of many recorded foods are pre-defined in this software; however, some foods and/or brands were not listed. Therefore, each ingredient was added to the software individually. The energy and macronutrient profiles of pasta served (180g) in each test session were evaluated using Nutritics software (Table 4.2).

Table 4.2. Nutritional composition of butternut squash soups with 1% sumac per portion (150g)

Nutrient	Pasta (180g)	Soup (150g)	
	SC/SSE/SSC	SC	SSE/SSC
Energy (kcal)	227	56	58
Carbohydrate (g)	37.8	12.1	12.3
Sugar (g)	4.2	6.6	6.6
Protein (g)	6.1	1.6	1.7
Fat (g)	5.7	0.2	0.2
Saturated fat (g)	0.5	0	0

Data were calculated using Nutritics software. SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.2.6.2. Nutritional composition analysis of soups in phase 2

The energy and macronutrients of mushroom soup were calculated using Nutritics software (Table 4.3). For each participant, the soup, lunch and dinner consumed during each test session were entered into Nutritics software to estimate the total energy and macronutrient content. For this phase, the *ad libitum* lunch and all-day food intake were used to assess the impact of both methods of added sumac on food intake among residential care home participants.

Table 4.3. Nutritional composition of mushroom soup with 1% sumac per portion (180g)

Soup Nutrient	SC	SSE/SSC
Energy (kcal)	89	91
Carbohydrate (g)	3.8	4
Sugar (g)	3.1	3.1
Protein (g)	2.3	2.4
Fat (g)	7.2	7.3
Saturated fat (g)	4.1	4.1

Data were calculated using Nutritics software. SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.2.7. Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) v. 25 (Chicago, IL, USA). All data are presented as means \pm standard deviation (SD). Statistical significance was set at $p < 0.05$. The normality of the data was assessed using a Shapiro-Wilk's test. An independent T-test was used to compare demographic data between both groups of adults (older and younger) in phase 1. A parametric repeated measurement ANOVA and non-parametric Friedman tests assessed the differences between the test sessions in energy and macronutrient contents of the *ad libitum* lunch, evening meal and all-day, applied to normally and not normally distributed data, respectively. At the baseline in phase 1, data collected for

the 24-hour dietary intake were analysed using a T-test for normally distributed data and a Mann-Whitney test for results not normally distributed within the two groups of adults. In phase 1, the energy and macronutrients intakes of each test session were compared within and between each group of adults based on the methods used for the addition of sumac. To analyse the impact of adding sumac (SSE and SSC) to energy and nutrient intakes within free-living older and younger adult groups, repeated measures ANOVA was applied for normally distributed data. A non-parametric Friedman test was run on data which were not normally distributed. Multiple pairwise comparisons were made using a Kruskal-Wallis H post hoc test. A comparison of food intake between free-living older adults and younger adults was conducted utilising a parametric independent T-test for normally distributed data and a Mann-Whitney test for data not normally distributed.

In the phase 2 study, the food consumed by residential older adults during the *ad libitum* lunch and all-day were measured to compare the impact of adding sumac using two methods (SSE and SSC). A Shapiro-Wilk's test was used to test the normal distribution of data. Thereafter, repeated measures ANOVA and Friedman tests were run to evaluate the results of the *ad libitum* lunch and all-day meal intake between the test days for normally and not normally distributed data, respectively.

4.3. Results

4.3.1. Phase 1

4.3.1.1. Participants' demographic data

In total, 40 participants, 20 free-living older adult participants and 20 younger adult participants, volunteered to take part in this study. The free-living older adult participants were aged from 65 to 84 years, and the younger adults from 20 to 35 years. A total of 20 participants

in each group completed the study, with no attrition. Table 4.4 shows the demographic characteristics (means \pm SD) for both groups of participants. On average, the age of older adults was greater than that of younger adults, with a large effect size of $d=8.9$ ($t(38) = -26.8$, $p=0.001$). However, no significant differences ($p>0.05$) were observed in the weight and height of the two groups of adults.

Table 4.4. Demographic characteristics of free-living older adults and younger adults in phase 1

Classification	Free-living Older adults Mean \pmSD	Younger adults Mean \pmSD	<i>p</i>-value
Age (years)	72.1 \pm 5.6	26.4 \pm 5.1	0.001
Weight (kg)	72.5 \pm 10.5	76.9 \pm 33.1	0.581
Height (cm)	169.8 \pm 9.5	156.7 \pm 35.4	0.123

Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test, statistically significant at $p<0.05$. An independent T-test was used to analyse the data between free-living older adults and younger adults.

4.3.1.2. Food Intake

Participants were instructed to consume their soup fully, however, on average 1–2 g of soup was left over after each test session due to misinterpretation of the instructions. There were no differences observed between the consumed soup and pasta among each group of adults ($p>0.05$). Table 4.5 shows the means \pm SD results for the amount (g) of soup and pasta consumed on each test day for both groups of adults.

Table 4.5. Amount (mean \pm SD) of food consumed, soup and pasta (g), in each test session (SC, SSE, SSC) by participants in phase 1

Food consumed	Group	SC (g)	SSE (g)	SSC (g)	<i>p</i> -value
Test soup	O	149.9 \pm 0.8	148.2 \pm 8.7	148.5 \pm 6	0.907
	Y	150.0 \pm 0.0	150.0 \pm 0.0	150.0 \pm 0.0	-
	O	146.8 \pm 57.8	145.8 \pm 64.3	168.6 \pm 33.6	0.075
Pasta	Y	173.9 \pm 19.2	171.4 \pm 27.4	176.8 \pm 13.9	0.494

Data are displayed as mean \pm SD. Statistically significant at $p < 0.05$. A Shapiro-Wilk's test was used for normality. A Friedman test was used to analyse the data between the test sessions within adult groups. O: older adults; Y: younger adults; SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.3.1.3. Energy and nutritional profile of baseline dietary intake

The energy and macronutrient intake at the baseline were calculated based on 24-hour dietary recall (Table 4.6). There was a medium difference observed in 24-h dietary intake between the male free-living older adults and the females on the consumption of carbohydrates (g; $z = -2.35$, $r = -0.53$ and $p = 0.016$). Younger male participants consumed higher amounts of protein (g; $z = -2.57$, $r = -0.57$ $p = 0.008$) and fat (g; $z = -2.66$, $r = -0.59$ $p = 0.005$) than females in the same group.

Table 4.6. 24-hour energy and macronutrients intake of free-living older adults (>65 years old) and younger adults (18-35 years old), phase 1

Nutrient	Group	Female	Male	Total	<i>p</i> -value
Energy (kcal)	O	1462.3 ±523.6	1869.2 ±965.9	1645.4 ±761.8	0.230 ^b
	Y	1630.1 ±878.5	2484.8 ±1043.6	1843.8 ±970.6	0.088 ^a
Carbohydrate (g)	O	138.3 ±81.7	186.6 ±56.6	160.1±73.9	0.016^b
	Y	198.2 ±90.1	219.7 ±114.2	203.6 ±93.9	0.445 ^b
Sugar (g)	O	61.7 ±29.8	77.7 ±41.5	68.9 ±35.5	0.370 ^b
	Y	75.7 ±61.0	74.2 ±38.1	75.3 ±55.2	0.497 ^a
Protein (g)	O	65.7 ±26.4	89.6 ±76.0	76.4 ±54.3	0.603 ^b
	Y	71.3 ±86.5	121.2 ±48.3	83.8 ±80.6	0.008^b
Fat (g)	O	68.1 ±34.5	76.5 ±66.5	71.9 ±50.1	0.941 ^b
	Y	61.3 ±36.3	117.6 ±38.4	75.4 ±43.7	0.005^b
Saturated fat (g)	O	25.8 ±12.8	28.6 ±33.9	27.1 ±23.9	0.295 ^b
	Y	26.5 ±18.8	37.1 ±21.7	29.2 ±19.5	0.230 ^b
Alcohol (g)	O	3.1 ±10.3	9.7 ±15.7	6.1 ±6.1	0.272 ^a
	Y	-	8.5 ±19.1	2.1 ±9.5	0.083 ^a

Data are shown as mean ±SD and tested for normality using a Shapiro-Wilk's test. O: free-living older adults; Y: younger adults. Data were calculated using Nutritics software. T-test^a and Mann-Whitney test^b were used to analyse the data between female and male within adults groups, statistically significant at *p*<0.05.

4.3.1.4. Energy and nutritional profile of an *ad libitum* lunch within free-living older and younger adults

The soup samples were randomly provided to the participants in each session. Considering the elimination of orosensory impact, an *ad libitum* lunch comprising pasta and water was served ten minutes after soup consumption (Barbara *et al.*, 2000). Despite the fact that the soup sample had no statistical impact on pasta intake within both groups of adults (Table 4.5.), free-living older adults showed a higher intake of energy and some macronutrients during the *ad libitum* lunch (Table 4.7). The results of a Friedman test revealed there were a large effect size between the SSE and SSC sessions with the SC session for energy (kcal, $\chi^2(20) = 8.6; p = 0.014$), protein (g, $\chi^2(20) = 7.4; p = 0.025$), carbohydrates (g, $\chi^2(20) = 8.7; p = 0.013$) and fat (g, $\chi^2(20) = 12.6; p = 0.002$) with higher indications in the SSC session. Comparison of the multiple tests showed that the method of addition of sumac (SSE and SSC) had no impact on the intake. It showed that the SSE and SSC in compared with SC session had a very small size effect on energy (SC-SSE: $T = 0.05, r = 0.007$; SSE-SSC: $T = -0.07, r = -0.11$; SC-SSC: $T = -0.06, r = -0.01$); protein (SC-SSE: $T = -0.17, r = -0.12$; SC-SSC: $T = -0.5, r = -0.35$; SSC-SSE: $T = -0.325, r = -0.22$); carbohydrate (SC-SSE: $T = 0.001, r = 0.001$; SC-SSC and SSE-SSC: $T = -0.675, r = -0.47$) and fat (SC-SSE: $T = -0.025, r = -0.017$; SC-SSC: $T = -0.725, r = -0.51$; SSE-SSC: $T = -0.7, r = -0.5$).

Table 4.7. *Ad libitum* lunch energy and macronutrients intake on three test days (control, sumac added at the end and sumac added during cooking) within free-living older adults (>65 years old) and younger adults (18-35 years old, phase 1)

Nutrients	Group	SC	SSE	SSC	<i>p</i> -value
Energy (kcal)	O	185.3 ±16.3	195.3 ±15.4	201.3±14.1	0.014^a
	Y	219.3 ±5.4	216.2 ±7.7	222.9 ±3.9	0.494 ^b
Protein (g)	O	5.1 ±2.1	5.2 ±1.9	5.4 ±1.7	0.025^a
	Y	5.9 ±0.7	5.8 ±0.9	6.1 ±0.5	0.807 ^b
Carbohydrate (g)	O	30.8 ±12.1	32.5 ±11.5	33.5 ±10.6	0.013^a
	Y	36.5 ±4.1	35.9 ±5.8	37.1 ±3.1	0.494 ^b
Fat (g)	O	4.7 ±1.8	4.9 ±1.7	5.1 ±1.6	0.002^a
	Y	5.5 ±0.6	5.4 ±0.9	5.6 ±0.4	0.441 ^b
Saturated fat (g)	O	0.4 ±0.2	0.4 ±0.2	0.4 ±0.1	0.058 ^a
	Y	0.5 ±0.1	0.5 ±0.8	0.5 ±0.1	0.441 ^b
Sugar (g)	O	3.4 ±1.4	3.6 ±1.3	3.7 ±1.2	0.161 ^a
	Y	4.1 ±0.5	3.9 ±0.7	4.1 ±0.3	0.494 ^b

Data are shown as mean ±SD and tested for normality using a Shapiro-Wilk's test, statistically significant at $p < 0.05$. Repeated measures ANOVA^a and Friedman^b tests were used to analyse the data between the test sessions within adult groups. O: older adults; Y: younger adults; SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.3.1.5 Energy and nutritional profile of the evening meal within free-living older and younger adults

The energy (kcal) and macronutrients (g) of the evening meal consumed by free-living older adults and younger adults showed no differences between the SSE and SSC sessions and the SC session (Table 4.8).

Table 4.8. Evening meal energy and macronutrients intake between the test sessions (control, sumac added at the end and sumac added during cooking) within free-living older adults (>65 years old) and younger adults (18-35 years old), phase 1

Nutrient	Group	SC	SSE	SSC	<i>p</i>-value
Energy (kcal)	O	1336.3 ±700.6	1413.9 ±526.1	1238.7 ±466.9	0.949
	Y	1301.4±712.8	1455.6 ±1099.9	1372.6 ±759.4	0.538
Protein (g)	O	51.7 ±23.9	60.2 ±28.3	49.9 ±16.5	0.603
	Y	92.9 ±41.3	64.7 ±42.9	64.9 ±38.9	0.334
Carbohydrate (g)	O	137.6 ±93.9	142.2 ±70	131.6 ±65.6	0.786
	Y	132.5 ±73.6	167.1 ±161.7	141.2 ±103	0.316
Fat (g)	O	56.1 ±37.1	59.8 ±25.6	57.3 ±40.9	0.675
	Y	56.9 ±39.9	57.7 ±46.5	60.1 ±35.2	0.767
Saturated fat (g)	O	21.1 ±2.9	22.9 ±2.6	18.9 ±2.1	0.353
	Y	21.1 ±17.7	20.6 ±26.2	21.5±16.4	0.308
Sugar (g)	O	71.6 ±67.3	71.7 ±39.7	59.7 ±30.6	0.711
	Y	60.2±34.6	94.9 ±124.2	57.9 ±50.8	0.711
Alcohol (g)	O	12.8 ±24.9	7.6 ±12.9	6.7 ±14.3	0.401
	Y	0.9 ±2.9	1.3 ±5.7	0	0.074

Data are shown as mean ±SD and tested for normality using a Shapiro-Wilk's test. Statistically significant at $p<0.05$. Repeated measures ANOVA and Friedman tests were used to analyse the data between the test sessions among adult groups. O: older Adults; Y: younger Adults; SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.3.1.6. Energy and nutritional profiles of all-day food intake within free-living older and younger adults

The *ad libitum* lunch and all subsequent food that were consumed by both groups of adults formed the all-day food intake (Table 4.9). The analysis of these data showed that younger adults consumed more alcohol ($p=0.030$). However, pairwise comparisons rejected the impact of the method of addition of sumac on alcohol intake among younger adults. Furthermore, this evaluation indicates that added sumac (SSE and SSC) compared with SC had no impact on all-day food intake within both groups of adults ($p>0.05$).

Table 4.9. All-day energy and macronutrients intake between the test sessions (control, sumac added at the end and sumac added during cooking) within free-living older adults (>65 years old) and younger adults (18-35 years old), phase 1

Nutrient	Group	SC	SSE	SSC	<i>p</i> -value
Energy (kcal)	O	1521.7 ±716.8	1587.9 ±467.9	1442.9 ±470.5	0.247
	Y	1547.4 ±685.1	1690.7 ±1115.2	1595.1 ±764.4	0.638
Protein (g)	O	56.7 ±24.3	56.8±21.3	55.5 ±16.9	0.739
	Y	70.1 ±8.9	71.7 ±9.8	71.6 ±8.6	0.317
Carbohydrate (g)	O	168.5 ±96.5	173.1 ±69.2	165.8 ±65.1	0.861
	Y	171.2 ±70.4	205.4 ±164.1	178.3 ±103.7	0.259
Fat (g)	O	60.7 ±37.2	65.7 ±26.9	62.4 ±41.1	0.638
	Y	63.1 ±38.9	63.7 ±46.8	66.2 ±34.6	0.705
Saturated fat (g)	O	21.5 ±13.3	23.6 ±11.9	19.4 ±9.6	0.369
	Y	21.7 ±17.6	21.3 ±26.1	21.6 ±16.4	0.350
Sugar (g)	O	74.9 ±67.6	75.4 ±39.5	63.6 ±30.8	0.705
	Y	64.4 ±34.4	99.1 ±124.2	61.6 ±50.9	0.705
Alcohol (g)	O	12.8 ±24.9	7.2 ±12.1	6.7 ±14.4	0.311
	Y	2.2 ±5.9	1.3 ±5.8	0	0.030

Data are shown as mean ±SD and tested for normality using a Shapiro-Wilk's test, statistically significant at $p < 0.05$. Repeated measures ANOVA and Friedman tests were used to analyse the data between the test sessions within adult groups. O: older Adults; Y: younger adults; SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.3.1.7. Energy and nutritional profiles of an *ad libitum* lunch between free-living older and younger adult groups

The macronutrient intakes of *ad libitum* lunches in the test sessions (SC, SSE and SSC) between free-living older adults and younger adults were compared (Figure. 4.3). The results revealed that the addition of sumac had no impact on macronutrient (g) intake between free-living older adults and younger adults ($p>0.05$).

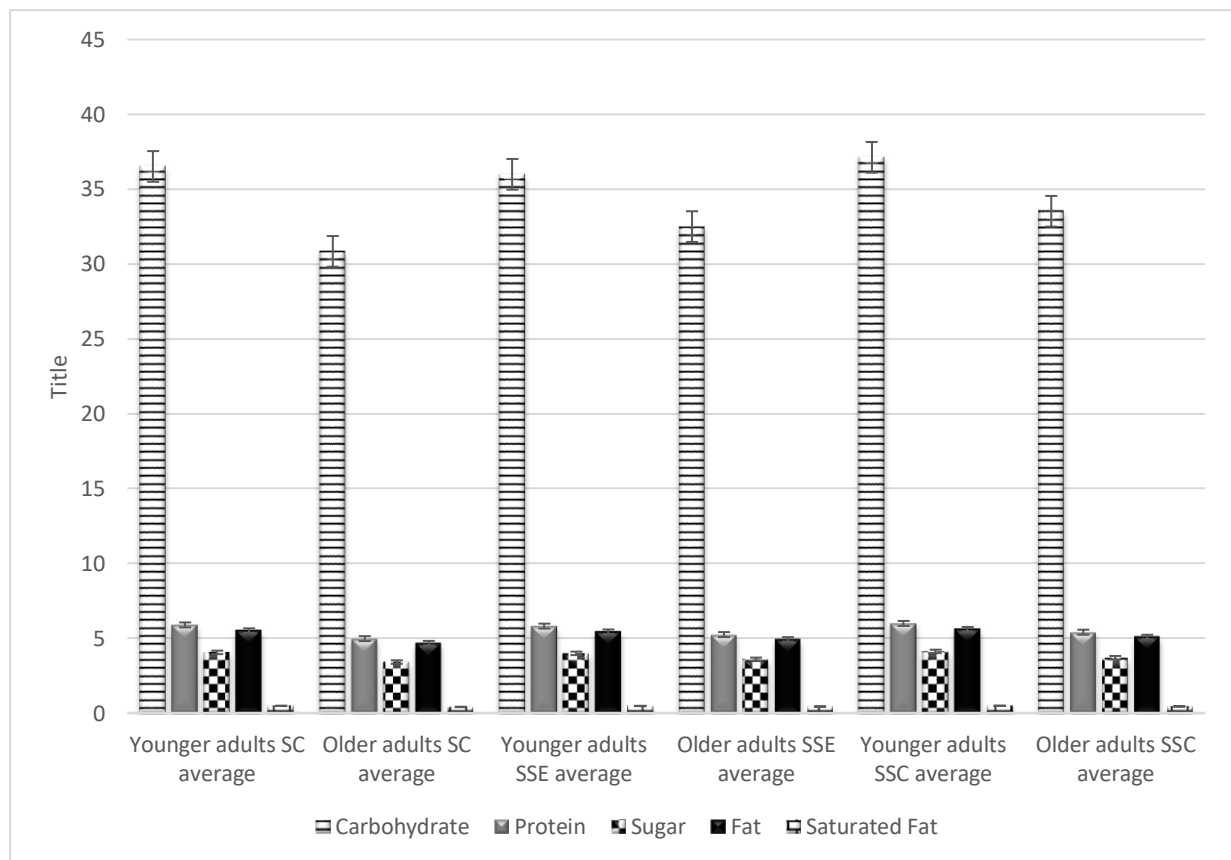


Figure 4.3. *Ad libitum* macronutrient intake between free-living older adults and younger adults among the test sessions; SC (Control soup), SSE (Sumac added at the end) and SSC (Sumac added during cooking). Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test. T-tests and Mann-Whitney tests were used to analyse the data. Significant difference at $p<0.05$.

The energy intakes of *ad libitum* lunches in the SC, SSE and SSC test sessions between free-living older adults and younger adults were compared (Figure. 4.4). Similar to the

macronutrient (g) findings, energy (kcal) intake showed no difference following the *ad libitum* lunch between free-living older adults and younger adults ($p>0.05$).

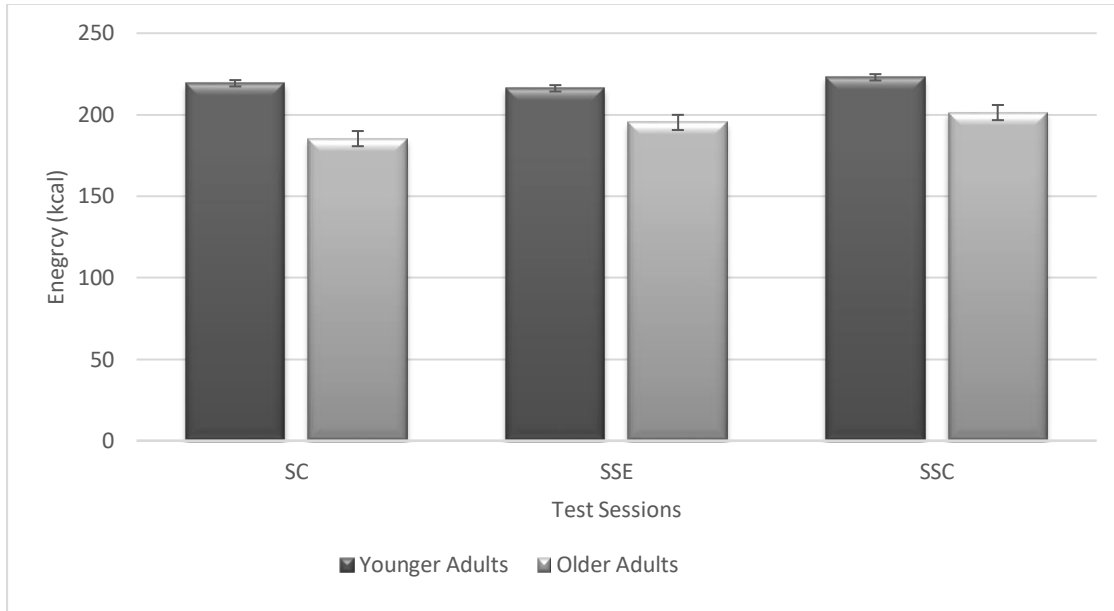


Figure 4.4. *Ad libitum* energy intake between free-living older adults and younger adults among the test sessions; SC (Control soup), SSE (Sumac added at the end) and SSC (Sumac added during cooking). Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test. T-tests and Mann-Whitney tests were used to analyse the data. Significant difference at $p<0.05$.

4.3.1.8. Energy and nutritional profiles of the evening meal between free-living older and younger adults

The macronutrient intakes of evening meals following each session (SC, SSE and SSC) were compared between free-living older adults and younger adults. The results of these comparisons demonstrated a higher intake of protein ($t(38) = 10, d = 0.9; p = 0.003$) among younger adults following the SSC session compared with free-living older adults. However, no other differences were found ($p>0.05$). Figure 4.5 shows the results of this analysis.

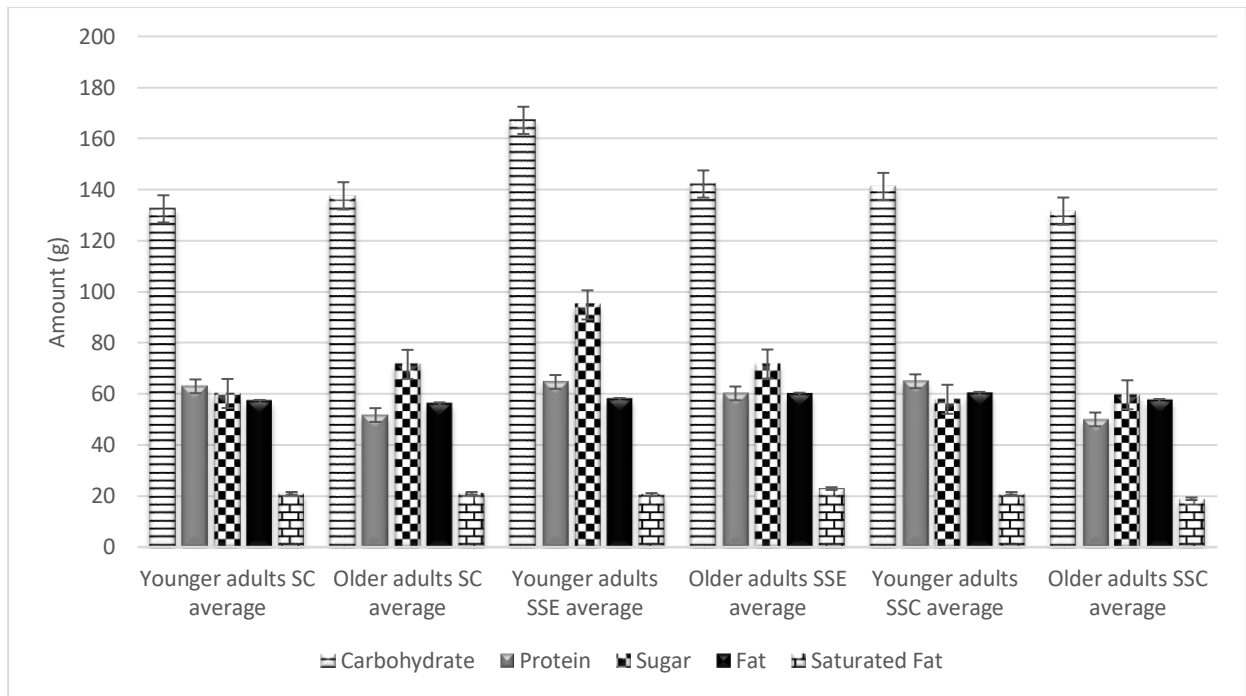


Figure 4.5. Evening meal nutrient intake between free-living older adults and younger adults among the test sessions; SC (control soup), SSE (sumac added at the end) and SSC (sumac added during cooking). Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test. T-tests and Mann-Whitney tests were used to analyse the data. Significant difference at $p < 0.05$.

In continuation of the assessment of the evening meal, the energy (kcal) intakes of test sessions between free-living older adults and younger adults were compared (Figure 4.6). However, the addition of sumac had no influence on the consumption of energy (kcal) between free-living older adults and younger adults ($p > 0.05$).

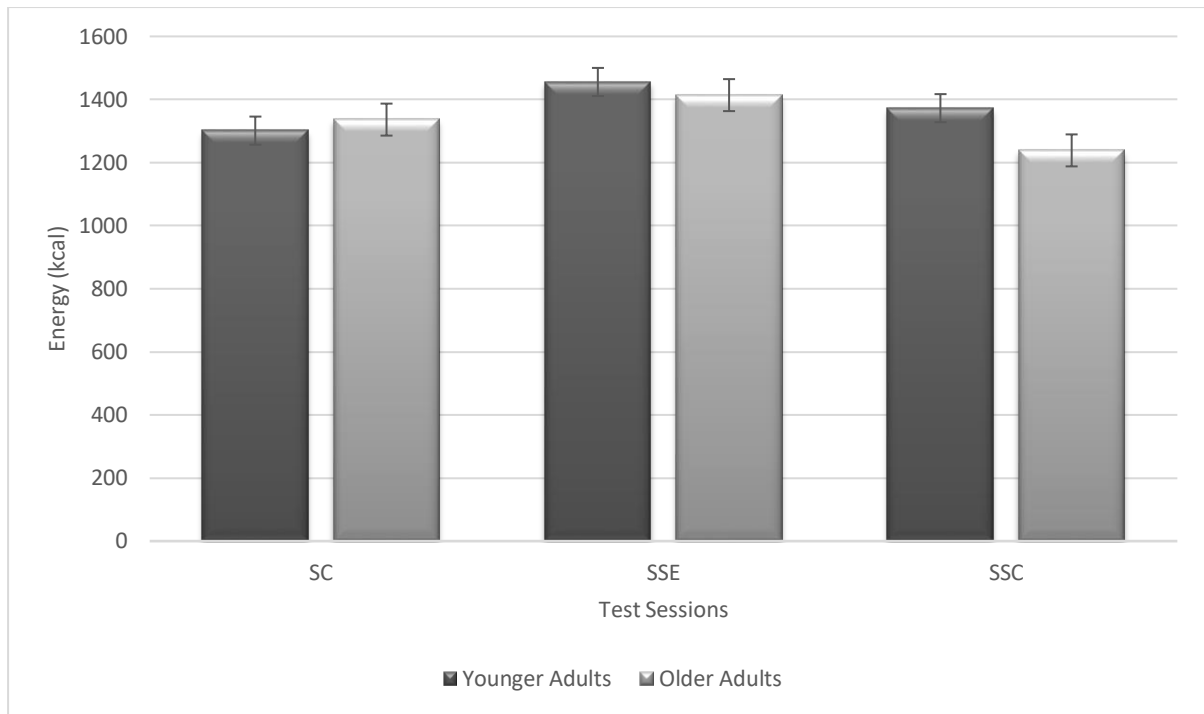


Figure 4.6. Evening meal energy intake between free-living older adults and younger adults among the test sessions; SC (control soup), SSE (sumac added at the end) and SSC (sumac added during cooking). Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test. T-tests and Mann-Whitney were used to analyse the data. Significant difference at $p < 0.05$.

4.3.1.9. Energy and nutritional profiles of the all-day meal between free-living older and younger adults

The all-day consumption of food, the total for the *ad libitum* lunch and evening meal, was compared between free-living older adults and younger adults for macronutrient intake (Figure 4.7). The findings of this comparison showed a medium effect size of protein (g) intake by the younger adults group in the SSE session ($t(38) = 0.5$, $d = 0.3$; $p = 0.006$) and SSC session ($t(38) = 1.7$, $d = 2.3$; $p = 0.004$) than free-living older adults. The differences in other macronutrients between both groups were not statistically significant ($p > 0.05$).

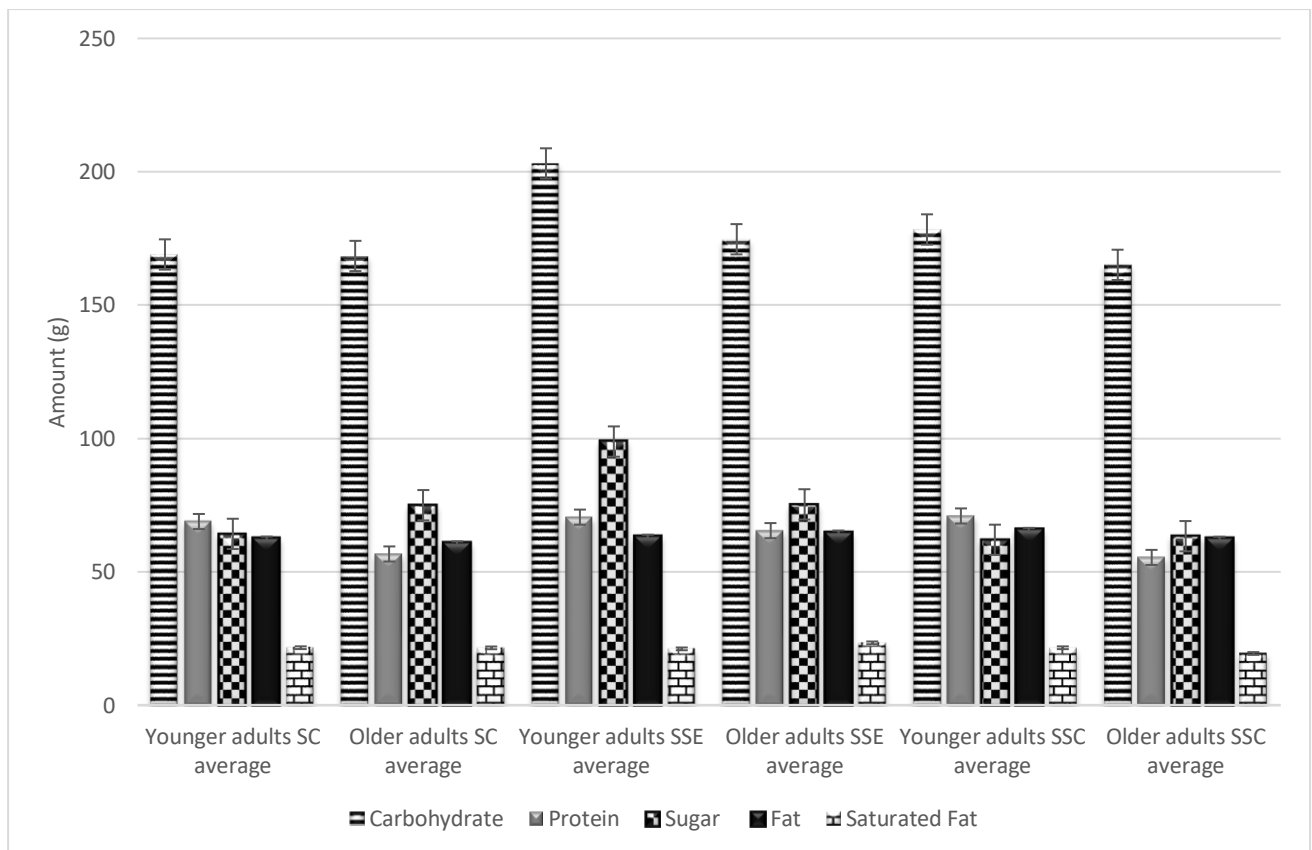


Figure 4.7. All-day nutrient intake between free-living older adults and younger adults among the test sessions; SC (control soup), SSE (sumac added at the end) and SSC (sumac added during cooking). Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test. T-tests and Mann-Whitney tests were used to analyse the data. Significant difference at $p < 0.05$.

The *ad libitum* lunch and evening meal formed the all-day energy intake (kcal). Figure 4.8 illustrates a comparison of energy (kcal) intake between free-living older and younger adults. The outcome of this comparison showed that added sumac (SSE and SSC) did not change the energy (kcal) intake of all-day consumption between both groups of adults ($p > 0.05$).

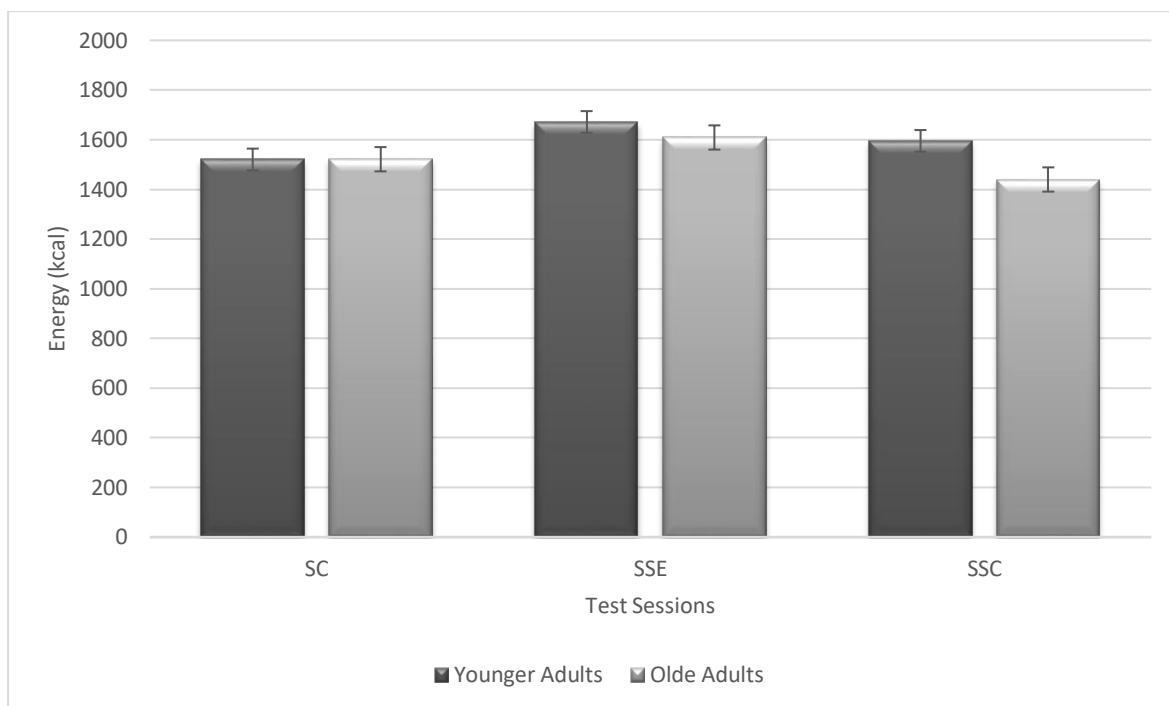


Figure 4.8. All-day energy between free-living older adults and younger adults among the test sessions; SC (control soup), SSE (sumac added at the end) and SSC (sumac added during cooking). Data are shown as mean \pm SD and tested for normality using a Shapiro-Wilk's test. T-tests and Mann-Whitney tests were used to analyse the data. Significant difference at $p < 0.05$.

4.3.2. Phase 2

4.3.2. 1. Participants' demographic data

A total of five residential care home older adults volunteers (3 females, 2 males >65 years old) participated in this study (Table 4.10). The participants were aged between 86 and 94 years.

Table 4.10. Characteristic of residential older adults, phase 2

Classification	Female Mean \pm SD	Male Mean \pm SD	Total Mean \pm SD
Age (years)	93.1 \pm 3	86.5 \pm 0.7	90.4 \pm 4.2
Height (cm)	163.7 \pm 7.2	175.0 \pm 7.1	168.2 \pm 8.8
Weight (kg)	57.9 \pm 8.1	70.1 \pm 7.1	62.7 \pm 9.4

Data are displayed as mean \pm SD.

4.3.2.2. Test food

Participants were provided with soups that were randomly allocated in the session and were instructed to consume all the test samples. Despite this instruction, not all participants did so. The residential older adult participants consumed the soups, on average, 67.0 ± 54.7 (g) to 139.0 ± 40.5 (g) over three test sessions and no differences ($p > 0.05$) were observed between sessions. However, the highest amount of consumed soup (g) was observed in the SSE session, followed by SSC and SC.

4.3.2.3 Energy and nutritional profile of *ad libitum* lunch

Residential older adults received a test soup prior to their lunch. Energy and macronutrient intake from the *ad libitum* lunch were assessed and the results are shown in Table 4.11. A borderline difference was observed for the carbohydrate ($F(2,8) = 0.6$; $p = 0.049$) intake of the *ad libitum* lunch between the test samples (SC, SSE and SSC); a pairwise comparison between test sessions revealed the method of adding sumac compared with the control group had no impact ($p > 0.05$). No other significant differences were observed following the analysis of energy and other macronutrients ($p > 0.05$).

Table 4.11. *Ad libitum* energy and macronutrients intake between the test sessions (control, sumac added at end and sumac added during cooking) within residential older adults, phase 2

Nutrient	SC	SSE	SSC	<i>p</i> -value
Energy (kcal)	548.8 ±24.5	616.2 ±287.3	422.6 ±235.6	0.819
Protein (g)	39.3 ±10.8	17.9 ±8.1	14.7 ±9.1	0.058
Carbohydrate (g)	55.3 ±4.1	69.9 ±20.6	47.2 ±11.4	0.049
Fat (g)	18.8 ±1.2	29.4 ±17.7	19.5 ±11.7	0.392
Saturated fat (g)	9.7 ±0.8	11.3 ±7.8	8.2 ±4.4	0.190
Sugar (g)	25.9 ±7.3	56.5 ±49.9	19.7 ±17.3	0.247

Data were tested for normality using a Shapiro-Wilk's test, statistically significant at $p < 0.05$. Repeated measures ANOVA and Friedman tests were used to analyse data between the test sessions on nutrient intake. SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.3.2.4. Energy and nutritional profile of all-day food intake

Table 4.12 shows the all-day energy and nutritional intake (*ad libitum* lunch in combination with evening meal) among residential care home older adults. However, the addition of sumac (SSE and SSC) had no effect on energy and other macronutrients of all-day intake among phase 2 participants ($p>0.05$).

Table 4.12. All-day energy and macronutrients intake between the test sessions (control, sumac added at end and sumac added during cooking) within residential older adults, phase 2

Nutrient	SC	SSE	SSC	<i>p</i> -value
Energy (kcal)	931.2±107.4	1197.4 ±405.9	840.8 ±237.9	0.224
Protein (g)	56.4 ±14.5	48.6 ±25.4	34.5 ±21.6	0.431
Carbohydrate (g)	94.7 ±19.1	119.8 ±45.1	77.6 ±31.7	0.549
Fat (g)	36.1 ±1.1	57.5 ±23.1	43.2 ±6.1	0.120
Saturated fat (g)	17.6 ±2.1	17.3 ±5.1	19.5 ±3.3	0.114
Sugar (g)	43.7 ±5.1	70.9 ±19.8	27.5 ±8.7	0.360

Data were tested for normality using a Shapiro-Wilk's test, statistically significant at $p<0.05$. Repeated measures ANOVA and Friedman tests were used to analyse data between the test sessions on nutrient intake. SC: control soup; SSE: sumac added at the end; SSC: sumac added during cooking.

4.4. Discussion

The current study was designed to determine the effect of added sumac on food intake. The study comprised two phases: Phase 1 participants were free-living older adults (over 65 years) and younger adults (18–35 years); in phase 2 participants were residential older adults (over 65 years). Based on previous studies, the highest dose of Iranian commercial brown sumac (0.37

g) was added to butternut squash and mushroom soups for phase 1 and phase 2 studies, respectively. In phase 1, the outcome for free-living older adults following the *ad libitum* lunch showed a higher intake of energy, protein, carbohydrate and fat following the SSE and SSC sessions and the SC session ($p<0.05$). A comparison of data between free-living older adults and younger adults revealed a higher intake of protein (g, $p=0.003$) by younger adults following SSC sessions. The findings for all-day assessment also showed a higher intake of protein (g) by younger adults compared with free-living older adults following SSE and SSC sessions ($p<0.05$).

The findings of this study confirm a higher intake of energy, protein, carbohydrate and fat during the *ad libitum* lunch among free-living older adults following the addition of sumac to soup despite the intake of the pasta was not statistically different. A positive influence of additive seasoning and sauce on older adults' food to enhance energy, protein and fat intake was reported previously (Best and Appleton, 2011). Free-living older adults (65–91 years old) participants in their study consumed similar types of food, including chicken, mashed potato and two vegetables over three sessions with either sauce or seasoning, or without sauce or seasoning. A variety of optional seasonings including sun-dried tomatoes and basil peri-peri marinade, lime and coriander peri-peri marinade and Cajun, and different kinds of sauces, were used, including onion, creamy mushroom, basil and tomato gravy. Their results showed energy and other macronutrients (protein, carbohydrate and fat) were higher following the addition of seasoning and flavoured sauce in contrast with food without these additives. Furthermore, no differences between the food with sauce and food with seasoning on consumption were reported. The outcome of their study was in accord with the findings of the current study, which showed an increase in energy, carbohydrate, protein and fat intake following the addition of sumac in an *ad libitum* lunch among free-living older adults, although the consumed pasta showed no statistical difference between the sessions. The impact of the *ad libitum* lunch intake

can be explained by the presence of L-glutamic acid in sumac. Via the activation of umami subunits and coupling with G-protein, appetite may be stimulated. On the other hand, the rapid omission of polyphenols and their weak absorption into the body's circulation are well documented (Hollman, 2014). The results for the *ad libitum* lunch compared with the evening meal and all-day may be due to the rapid elimination from the body's circulation of the polyphenol compounds that are present in sumac. However, this finding requires further assessment to unravel the bioavailability of these compounds following consumption.

The association between ageing and physiological impairments is highlighted by results showing a decline in food intake (Methven *et al.*, 2016). It has been reported that older adults consume less energy than younger adults (Giezenaar *et al.*, 2016). In agreement with previous studies, this study showed less energy and macronutrient intake among free-living older adults as compared to younger adults. This shows that the addition of herbs and spices may compensate for the chemosensory impairment of older adult participants as the energy, carbohydrate, protein and fat intake in the *ad libitum* lunch of these groups increased with the addition of sumac, whereas the younger adults showed no changes. It is well understood that sumac contains various volatile compounds that are released via the nasal route and trigger olfactory receptors. Following the consumption of food flavoured with herbs and spices, the sensory receptors located in the mouth are stimulated and enhance the food intake. Thus, it can be speculated that the addition of these compounds to older adults' food increases its palatability, resulting in greater food consumption.

The impact of MSG added to soup was assessed among a total of 40 participants (10 males, 30 females) of the older adults' group (aged 65–88 years) over eight sessions (Dermiki *et al.*, 2015). The sessions comprised six familiarisation and two pre- and post-allocation sessions. Their results revealed a significant difference between a control soup and soup with added MSG with a large effective size ($F(1,34) = 6.3; p=0.017$). In line with the results of the current

study, their outcome revealed an improvement in an *ad libitum* lunch although they used a different method. Therefore, comparing the methods applied in the current study with Dermiki *et al.* (2015), it can be speculated that familiarisation had no impact on food intake among older adults. However, an adverse impact on food intake may subsequently be observed among older adults with repeated consumption (Zandstra *et al.*, 2000).

The literature emphasises the importance of sample size for the accuracy of results in human clinical studies. Therefore, in the current study, for phase 1, the sample size calculation to achieve 95% power was 16. However, participants were over-recruited for this study to compensate for participants dropping out, and to minimise the misinterpretation of data due to the self-reporting of dietary intake. Previously, a food intake study was conducted among a group of 40 older adults, with an intervention group (n=20) and a control group (n=20) (Dermiki *et al.*, 2015). A randomised clinical trial on the impact of herbs and spices recruited a similar-sized group of participants (n=40) (Anderson *et al.*, 2015). However, their results contrasted with the current study, and this can be explained by the different sample size that was used in each group and the provision of intervention food in various dishes. Hence, the step of assessing the sample size is recommended in order to design an accurate study.

A lack of energy and low food intake is commonly demonstrated among care home residents, leading to an increased risk of malnutrition. However, the benefits of adding flavour to increase palatability and reduce salt intake have been reported (Nelson *et al.*, 2016). Therefore, this phase of the study set out to evaluate the impact of sumac added to mushroom soup (using two methods of addition) among residential care home older adults (over 65 years) in a nursing home, aiming to improve their energy and food intake and consequently develop a better health status. The impact of added sumac on mushroom soup was assessed in an *ad libitum* lunch and all-day food intake among residential older adults in phase 2. Results from these assessments showed no changes ($p>0.05$) in food intake following the consumption of sumac in soup,

except for a borderline difference in carbohydrate intake during the *ad libitum* lunch ($p=0.049$). However, a comparison between test sessions had no effect on carbohydrate intake among residential older adults. With a small sample size ($n=5$) caution must be applied, although finding was near borderline, but this could occur due to type I error. Various factors including food type (soup), number of sessions, inflexibility in the timing of soup consumption and clashes with other activity plans minimised the number of recruited participants.

The importance of an adequate intake of protein for older adults' health and the prevention of malnutrition is highlighted in epidemiological studies (Baum *et al.*, 2016). It has been reported that 35% of residents in care homes in Europe consume less protein than is recommended (0.7 g/kg of their body weight per day) (Tieland *et al.*, 2012a). The consumption of 1.0–1.2 g protein/kg of body weight per day by healthy older adults, increasing to 1.2–1.5 g/kg for malnourished older adults, has been suggested (Deutz *et al.*, 2014). In the current study, a higher intake of protein was observed in an evening meal following an SSE session by residential care home older adults, although the meal content varied between sessions and among participants, which were limited by sample size. Therefore, it can be hypothesised that the SSE session had a greater impact on protein intake compared with SSC and SC. However, this requires more investigation to reveal the impact and bioavailability of added sumac and protein intake on a standard all-day meal. This investigation may lead to enhancing protein intake and, hence, serum protein and malnutrition management in the early stages among older adults. Protein intake in the evening meal and the all-day meal for both groups of adults showed positive changes. Previously, the impact of the addition of chicken gravy as a flavoured sauce to food was assessed and the results revealed an increase in protein uptake on the test day, despite the participants not fully consuming the meal (Appleton, 2018). This may imply the influence of a minimum intake of flavoured sauce on post-conditioning food intake, as observed in the current study. Based on the findings in the current study with Appleton's (2018) work, it can be

speculated that the post-absorption of sumac due to the presence of phenolic compounds may play a role in protein intake.

An intervention work conducted on the effectiveness of food flavour using chicken, beef bouillon, turkey and lemon butter among residential older adults showed an improvement in food consumption compared with the baseline (Mathey *et al.*, 2001). The results showed a positive ($p < 0.05$) improvement in energy (133 ± 367 kJ), carbohydrate (3 ± 9 g) and total fat (2 ± 7 g) intake at the end of the study (16-weeks), whereas protein intake remained stable. Whilst a similar positive change was observed in carbohydrate intake in an *ad libitum* lunch in the current study among residential older adults, no other changes were detected for energy or other macronutrients. This discrepancy could be due to a higher dose (1.0 ± 0.2 g) of flavour enhancer sprinkled per dish and a longer intervention period (4 months), compared with the present study. Unlike Mathey *et al.* (2001), who added a flavour enhancer over the entire food including vegetables and carbohydrate, in the current study sumac was only added to soup. In the present study, the proportions of energy and other nutrients in participants' meals were not homogenous compared with their study. Hence, the differences in the methodology used in the current study compared with their study could explain the rejection of hypotheses in this phase.

A study in a residential home ($n=67$) revealed a higher intake of energy (321 kcal; $p=0.0001$) following a nine-month intervention, with increases in body weight (Lorefält and Wilhelmsson, 2012). Further to this, they provided malnourished participants with various appetisers including soup, eggs and individual snacks (such as smoothies and bread at lunchtime), whereas well-nourished participants received yoghurt and fruits to consume between meals, which were personalised based on the outcomes of mini-nutritional assessments (MNAs) and served at the participants' preferred times for eating (Lorefält and Wilhelmsson, 2012). Their findings showed a positive correlation between the intervention period and improvement in the acceptance of flavoured food. They also assessed the hunger level of their participants at the

start points. It is therefore likely that such a connection exists between the acceptance of flavoured food and hunger level assessment via food intake, which was lacking in this study. Nutritional assessment at the baseline could determine participants' requirements, thus justifying an optimal dose aiming to improve appetite. The results of hunger level assessment could be beneficial to evaluate the *ad libitum* lunch intake level among participants.

A smaller appetite and/ or different preferences for food intake during the day were reported by participants for various reasons including the ambient temperature. Previously, the impact of ambient temperature was assessed on food intake within two hours (the first hour was allocated to the participants' office work, and in the second hour test food was consumed) of exposure to room temperature (26–27°C) compared with a control group (19–20°C) in a randomised control trial (Bernhard *et al.*, 2015). Their results revealed that participants in the control group consumed more amount (g) than those in the heat-condition group. It seems possible that the participants' appetite in the current work was affected by heat during the day for those who completed the study during the summer, as it was reported.

The impact of the addition of MSG to soup was assessed among 32 adults (5 males, 27 females) to evaluate food intake between a control group and an MSG group (Yeomans *et al.*, 2008). An increase in appetite was observed following full consumption (200g) of a flavoured (MSG) sample (Yeomans *et al.*, 2008). Their results contrast with the current study's findings for the younger adults' group, which showed no change in appetite following soup consumption. A possible explanation for this is using methods that implied familiarisation of the participants with the enhancer during training sessions and the higher dose of MSG that they used compared with the current study. In addition, their participants were instructed to fast overnight and were provided with a set menu of breakfast that was consumed three hours before their test. Another difference in method that they used compared with the present study was food abstinence

(except for drinking water) prior to the test session for three hours in Yeomans *et al.* (2008), whereas in the current study the fasting time was limited to one hour.

Food intake was evaluated following a combination of herbs and spices with low salt added to a legume meal among younger adults (18–35 years old) (Dougkas *et al.*, 2019). Their results showed no changes in energy intake between five samples: control, low salt, only herbs, and with and without herbs and spices ($p>0.05$). In agreement with their results, in the current study sumac consumption had no effect on energy and macronutrient intake between the test sessions by younger adults. One explanation for this could be the addition of low doses in Dougkas *et al.*'s (2019) study. However, the importance of the oral administration of a specific amount of spice with the purpose of increasing food intake was reported (Opara, 2019) following the application of 3–20 g (Westerterp-Plantenga *et al.*, 2005; Gregersen *et al.*, 2013).

The influence of a soup sample as a preload starter compared with a control session (in which no soup was served) on reduction of energy intake was assessed among 60 adults (18–45 years old) (Flood and Rolls, 2007). The participants received similar soups in the form of separate vegetables with broth, chunky vegetable soup, chunky puréed vegetable soup and puréed vegetable soup. It was hypothesised that the consumption of soup as a starter to a meal could reduce food and energy intake. However, their results proved their hypothesis in which it was compared with a control session ($p=0.0001$). Despite the different methods of assessment between their study and this study, it can be suggested that although the portion of pasta was small and it was adjusted for older adults this could be adequate for younger adults in the present study in which this group consumed the entire pasta following each session. However, more studies should assess the impact of added sumac in different portion sizes of *ad libitum* lunch in both free-living older adults and younger adults.

The scope of this study was limited in terms of some divergence in the protocols that were applied in phase 1 and phase 2. In phase 1, the dietary intake was self-reported which is a challenging method in terms of accuracy of consumed portion. However, in phase 2 food intake was weighed by the researcher. The 24h dietary intake before the first session was used in phase 1 but excluded from the phase 2 protocol due to limited access to the nursing home. Participants in phase 1 were instructed to record the entire food intake which they consumed following their test session until midnight. In contrast, food intake during the *ad libitum* lunch and evening meal of phase 2 residential care home older adults were only recorded. The reason for that was limited access to the nursing home and respect for the personal space of staff and participants. Carrying out nutrition research in a nursing home was challenging. This can be explained first due to the time-consuming recruiting process. The number of participants plays an important role in the accuracy of results. Second, completing consent forms for participants and filling in the health questionnaires was time-consuming for the nursing home manager. Further to this, the restriction for the researcher to have a face-to-face conversation with volunteers was another challenge. However, the nursing home managers found this demand time- and cost-consuming. Adapting the research with each nursing home protocol may have an effect on the process of study and the data collected when participants are chosen from a different nursing home. Therefore, based on these challenges for recruiting participants, implementation of a control group along sumac group in a nursing home was excluded in this study.

Diverse types of soup were implemented between phase 1 and phase 2. In phase 1, based on a sensory evaluation study, a similar soup, butternut squash, was used. Despite the initial procedure of study design in phase 2, the type of soup was changed to mushroom soup based on higher acceptability by participants. Participants of both phases of the study were instructed to consume their soup fully. However, phase 2 participants, unlike in phase 1, did not follow

the instruction due to misunderstanding the study protocol and the portion size of soup samples which was served in the nursing home. This could explain why the phase 2 results, unlike phase 1, were inconclusive.

The wash out periods between the sessions differed between the phase 1 and phase 2. In phase 1 due to flexibility of participants' attendance to the centre a minimum of two-day was applied. Based on the similarity of the daily food menu, the study was completed on a specific day of week, so the wash out week was applied between the test sessions. Additionally, the different types of soup due to the dissimilarity of the umami profiles in the presence of sumac may contribute to different results. The sample size calculation for phase 2 led to recruiting fewer participants in this phase compared with phase 1. The study was limited by the lack of information on hunger levels prior to the test sessions. Therefore, the measurement of hunger level is recommended for the interpretation of *ad libitum* food intake results. It is well understood that a sedentary lifestyle leads to a reduction in gastric emptying and directly influences food intake. The results of this study revealed the lack of a power assessment for sumac doses at the beginning of study that may have magnified taste perception responses. Moreover, the study was conducted among a well-nourished, educated and white population in Oxford which was not heterogeneous. The lack of diversity in the social backgrounds of participants in the current study diminishes the generalisability of its findings. Thus, further heterogeneous research with a bigger sample size among residential older adults with the possibility of adding higher doses of sumac is required to establish the effectiveness of adding this spice to food intake among this population. Further work is required to establish the effect of sumac on food intake among older adults. Several questions remain unanswered by this study which can be addressed in the future. These include the effect of sumac in a larger sample size in the nursing home study based on the outcome of this pilot study. The similarity of methods including the type of soup, standard breakfast and *ad libitum* lunch between free-

living and nursing home adults are required for future studies. Based on the limitations on the method in phase 2, it is recommended to establish a study to evaluate the influence of sumac between the similar and dissimilar foods in energy and macronutrients methods among nursing home older adults.

4.5. Conclusion

This study set out to evaluate the effect of sumac added to soup on food intake, first, among free-living older adults and younger adults and, second, among care home residents. The study has shown that the addition of sumac had an effect on energy, protein, carbohydrate and fat intake during the *ad libitum* lunch among free-living older adults. The analysis of data between the free-living older adults and younger adults showed an increased intake of protein consumption by the younger group. Residential care home older adults showed a borderline effect of carbohydrate intake at *ad libitum* lunch. The results of this work indicate that sumac's addition to food can be an efficacious method for improving food intake among older adults during an *ad libitum* meal. However, additional studies could assess the impact of added sumac with larger sample sizes, longer intervention study times and higher doses of sumac. Hence, the outcome of this study can be seen as a promising approach in response to global health concerns over malnutrition in older adults, resulting in improvements to health and reductions in the cost of national health.

Chapter 5: General discussion and conclusion

The past decade has seen rapid growth in the older adult population, and it is estimated by the United Nations (2019) that one in six adults will be over 65 years old by 2050. This could exponentially increase the concerns over fulfilling nutritional requirements due to physiological and pathological changes (Ahmed and Haboubi, 2010). A study showed that around 30% of older adults have experienced anorexia of ageing which is more common among women, residential and hospitalised older adults (Malafarnia *et al.*, 2013). Loss of appetite may lead to inadequate intake of energy, macronutrients and micronutrients, resulting in succumbing to non-communicable diseases, malnutrition and longer hospitalisation (Volkert, 2002; Joyce *et al.*, 2005). Preventing and managing older adults' appetite is a public health interest and can be achieved first by understanding the causes of a poor diet (Pilgrim *et al.*, 2015).

Impairment in taste sense which enhances the enjoyment of food, and smell sense that triggers appetite resulted in a reduction of food intake (Nieuwenhuizen *et al.*, 2010). However, increasing the appetite can be influenced by a signal from food, which is known as the hedonic system (Pilgrim *et al.*, 2015). The importance of the addition of natural flavouring products has been considered in the improvement of the sensory properties of older adult food, and consequently food intake (Vázquez-Fresno, *et al.*, 2019), despite the irreversible chemosensory results of ageing (Sergi *et al.*, 2017). Nutritional studies have indicated that polyphenol compounds, including phenolic acid and flavonoids that are present in herbs and spices are major contributors to enhancing the colour, taste, flavour and preservation of food resulting in a reduction in salt intake and increased appetite (Spencer *et al.*, 2008; Anderson *et al.*, 2015; Duncan *et al.*, 2017). The literature highlights the historical use of sumac as a food additive in a variety of Middle Eastern dishes (Nikousaleh and Prakash, 2016). Thus, it can be concluded

that more studies involving the addition of sumac are required to investigate its effect on the enhancement of food intake in older adults.

This thesis comprises three novel studies. An *in vitro* study was devised to assess the antioxidant, polyphenol and L-glutamic acid contents of sumac (Chapter 2), with *in vivo* studies being the first to conduct a sensory evaluation (Chapter 3) of sumac; then, sumac was used as a flavour enhancer to assess food intake in two phases (Chapter 4). Phase 1 demonstrated the impact of the addition of sumac on food intake among free-living older adults, younger adults and a comparison between these two groups. Phase 2 applied a similar assessment to care home residents.

5.1. Summary of thesis aims and findings

The primary objective of the current PhD study was to assess the impact of food intake on residential older adults (Chapter 4, phase 2), following the addition of sumac to mushroom soup using two different methods. The second aim was to evaluate the same influence among free-living older and younger adults of butternut squash soup (Chapter 4, phase 1). Drawing upon these two aims, the work illustrated in Chapter 3 conducted a sensory examination of the liking and intensity attributes of different doses of sumac in butternut squash soup among free-living older and younger adults. Chapter 2 was designed to evaluate the antioxidant activity, polyphenol content and L-glutamic acid level of sumac from various sumac samples.

The evaluation of antioxidant, polyphenol and L-glutamic acid of sumac samples showed higher levels of these activities for fresh brown sumac, followed by commercial Iranian brown sumac. Although fresh brown sumac had a higher activity, the safety of the addition of this sample was considered for *in vivo* studies. The sensory evaluation of different doses of commercial brown sumac revealed higher acceptability and liking of the highest dose in comparison to other samples by both groups of adults. The intensity of the brown colour of the

sumac soup sample compared with the control group was identified by both groups of participants. Based on these results, the highest dose of commercial Iranian brown sumac was implemented in both phases of the food intake studies in Chapter 4.

The *ad libitum* lunch showed a higher intake of energy (kcal), protein (g), carbohydrate(g) and fat (g) among free-living older adults in phase 1. The protein intake was also higher in younger adults compared with free-living older adults in the evening meal SSC sessions. In addition, younger adults consumed more protein than free-living older adults in the SSE and SSC sessions on a whole-day assessment. The residential care home older adults consumed more carbohydrate (g) following *ad libitum* lunch with a higher intake in the SSE session.

5.2. Implications, applications and future prospects of this thesis

The outcome of this PhD study may contribute to understanding and developing a strategy for the enhancement of food intake and a reduction in salt intake following the addition of spices to older adults' food. The results show that the addition of sumac may have an immediate effect on food intake among older adults.

The findings of the *in vitro* study (Chapter 2) indicated that sumac contains high levels of antioxidants, polyphenol and L-glutamic acids. This is the first study that compared the activity of six varieties of sumac using three different solvents. An advantage of this was the opportunity to choose a type of sumac with the highest level of activity for *in vivo* studies. The application of the Megazyme kit was a first attempt in the assessment of the L-glutamic acid in sumac samples. Therefore, the findings for L-glutamic acid suggested the use of this method in future research on spices in foods as it is faster and easier than HPLC. The findings from this work may contribute to unravelling the mechanism of sumac in appetite and food consequently can be used in the food and pharmaceutical industries.

The second study (Chapter 3) was designed to evaluate the sensory attributes of four doses of sumac. The evidence of this work confirms previous findings on the non-toxicity effect of sumac suggesting the addition of this spice in the food industry. Although the findings reveal the acceptability and liking of doses, the outcome and insignificant comparison between adult groups suggested the possibility of assessment of low doses of sumac. Therefore, the finding adds to a growing body of literature on the effectiveness of additive doses of spice on food intake. This would gain insights into the preparation of added sumac to low salt food without lessening the liking. Thus, these findings cannot only be used in treating malnutrition but also in managing and preventing non-communicable diseases.

The main part of this study was composed of two phases (Chapter 4). The findings from phase 1 study make several contributions to food intake and appetite studies. First, they support previous research in which the addition of spices resulted in the enhancement of *ad libitum* food intake. Second, it confirmed the previous research on a lower intake of energy and macronutrients by older adults compared with younger adults. This work also provides a new understanding of additional methods, although the results were not statistically significant, but more food was consumed following the SSE session. Therefore, this finding can be applied in other studies which will use other spices for food enhancement.

Phase 2 of this study was completed among residential older adults. The outcome of this study was inconclusive, but this offers some insights into the influence of added sumac to residential care home older adults' soup. Although the study was based on small groups (n=5), it indicated that partial consumption of soup may interfere with *ad libitum* results. This will serve as a basis for future work in the field of appetite study in residential older adults. It extended our knowledge regarding the impact of small sample sizes on results and contributes to existing information on the difficulties in recruiting residential older adults. This work may improve the strategies and guidelines and the importance of research on nursing home adults.

This study may extend our information on the influence of higher polyphenol, antioxidant and L-glutamic acid content on human health and food intake, suggesting that sumac can be used as a natural additive to enhance food colour, taste, flavour and quality. Sumac is a shelf life spice and a low-cost plant that can grow under various geographical conditions, including drought and rainfall. Therefore, these findings have a number of important implications for future research and the food industry, including the production and supply of ready-to-cook and ready-to-eat foods with added sumac to increase food intake and replace salt for targeted age groups. Hence, further research on sumac can expand our knowledge of managing and/ or preventing malnutrition, thus facilitating a healthier population in the later stages of life.

5.3. Strengths and limitations

This study contributes to existing knowledge on sumac's health benefits. The findings provide additional evidence in relation to polyphenol, antioxidant and L-glutamic acid levels for appetite improvement.

The first study (Chapter 2) was designed to evaluate commercial and fresh, red and brown sumac from four different regions. The generalisability of these results is subject to certain limitations. First, there was no access to commercial forms of brown sumac from other regions, similar to Iranian sumac. Moreover, it was not possible to obtain fresh sumac from areas other than the UK and Iran. Therefore, this limitation results in less generalisability for polyphenol, antioxidant and L-glutamic acid content, and selecting sumac with the highest concentrations of polyphenol compounds. Second, due to the time limitation on assessing L-glutamic acid, only an easy and reliable Megazyme assay was used. In addition, the lack of mention of this assessment in the literature on sumac caused the interpretation of results to be cautious. Thirdly, using different methods for these assessments was time-consuming. Therefore, the comparison

of results was lacking. The study has a few strengths. First, the antioxidant and polyphenol activity of the samples were assessed using three different solvents. Second, a variety of sumac were used in this study. Thirdly, for the first time, the L-glutamic acid in sumac was evaluated by Megazyme assay.

This sensory evaluation of sumac is the first study (to the best of the author's knowledge) to evaluate the liking and intensity of this spice in butternut squash soup. The interpretation of results was limited due to a lack of previous work on the sensory evaluation of sumac in food. According to this and the limitation of time, only four doses of sumac were compared with the control soup and the outcome may imply that the sample dose was small. Another limitation of this study was that only one type of food, butternut squash soup, was used for the sensory evaluation of sumac. Although the results showed the higher intensity of the brown colour of the sumac sample, the samples did not reveal any other differences within the adults group for other attributes. However, using a qualitative assessment contributed to the findings for selecting the highest dose of sumac for food intake studies. Another aspect of this study was the possibility of using higher levels of polyphenol, antioxidant activity and L-glutamic acid of sumac, from a previous study (Chapter 2), to be assessed by two groups of adults: free-living older adults and younger adults.

The phase 1 study was limited in several ways. First, dietary intake was self-reported. In addition, the estimation of food consumption could be misinterpreted in terms of container sizes (such as cups, bowls etc.). This may have led to the misreporting of food intake by some participants. Second, the study included a single 24-hour dietary intake period, which may have affected its accuracy for standard daily energy intake. Due to the difficulties in the recruitment and cooperation of volunteers, the 24-hour dietary intake data were limited to only one day before the test. Thirdly, the current study was undertaken from July to November 2018. The participants noted and described the effect of hot weather on their appetite and food intake

during the day in the summer compared with those who completed the tests in the autumn. Therefore, it can be speculated that higher temperatures during the day may have suppressed the appetite of participants. Fourthly, the participants were provided with a standardised breakfast and were asked not to eat for one hour before their test. However, no other restrictions were imposed, such as fasting overnight and between breakfast and for an hour before their test. Assessing their satiety level prior to the test would be a beneficial element in the evaluation of the amount of sumac required for an effective impact on food intake. Lastly, the pasta portion size was adjusted for older adults. Therefore, the interpretation of the *ad libitum* lunch intake by younger adults could be inaccurate. For the first time, addition of sumac into soup was assessed on food intake among two groups of adults, so it had a few important aspects. First, two methods of adding sumac were used. Second, the effect of the addition was assessed on *ad libitum* lunch, an evening meal and all-day food intake. Another important aspect of this study was the populations that took part.

The phase 2 of the study may serve as a base for future study design by considering its limitations. This study, for the first time, used the addition of sumac, using two methods, among residential care home older adults to analyse food intake in Oxford. However, the small sample size (n=5) was a major limitation of this study as some participants had to withdraw from the study for various reasons including physiotherapy slots that clashed with their lunchtime. Some participants preferred not to consume soup as a starter or at lunchtime. The allotment test also included the number and time of sessions and was another factor that caused some participants to drop out of the study. Despite attendance at the same test session, the participants had a choice for their meal. The foods were different in energy and macronutrients profiles. Further to this, there was a lack of any record of food/ snack intake between and after meals. Lastly, participants did not receive a standard breakfast similar to phase 1. Due to the limitation of time and the desire of participants to change the soup, the study could not assess the sensory

evaluation of mushroom soup against butternut squash. However, the study used two methods of addition of sumac. The study was also able to evaluate the effect of sumac on an *ad libitum* lunch and an evening meal. Finally, the acceptability and willingness of the participants in this group to sumac, which was a novel spice for them, helped to complete this research.

5.4. Recommendations for future research

The methods that were used in the current study are reproducible for a future research. It is recommended to evaluate L-glutamic using other methods including HPLC for the same sumac samples in order to compare the effectiveness of methods. It would be interesting to assess polyphenol, antioxidants and L-glutamic acid using various sumacs and different methods to establish the level of these activities. More research is required to identify the chemical composition of sumac for a better understanding of the impact of its addition to foods.

Future research should assess the effect of sumac added in butternut squash soup using a similar method with a higher dose of sumac in the same and different foods. Different sumac from different regions can be used to evaluate sensory attributes. The feasibility of using Compusense software is recommended for older adults with cognitive issues in sensory evaluation study compared to pen and paper methods.

More research is needed to investigate the therapeutic benefits of sumac for the anorexia of ageing. It would be useful if the levels of appetite hormones (including leptin and ghrelin) were assessed in blood tests before and after the consumption of sumac. In addition, the effect of different doses of this spice on appetite hormone levels could be evaluated. This would eliminate the possibility of other factors that might have an effect on appetite, leading to robust outcomes in terms of the influence of sumac on appetite and diet. Study of the long-term effects of this spice on food intake is recommended with first, provided food for the all-day and second,

with a similar process using bigger portions of pasta served during an *ad libitum* lunch. Further sensory studies need to be carried out to validate the acceptability and liking of higher doses of sumac.

A number of possible future studies are recommended to explore the effect of added sumac among residential care home older adults. For that research, it is recommended first, to repeat this work with a larger sample size; second, to ensure that sumac sample was fully consumed; and lastly, to provide equal and controlled foods which are similar in energy and macronutrients to all participants. It would be interesting if the study could be compared control and sumac groups within a residential care home for older adults.

5.5. General conclusion

The non-toxic nature of sumac, along with other health benefits of this spice, is emphasised in the literature. Furthermore, the antimicrobial properties of sumac due to the presence of polyphenol compounds as natural agents may increase the shelf life of food products. Hence, it can be suggested that sumac, as a natural additive that contains L-glutamic acid, is a promising replacement for salt, aiming to enhance food intake and improve the taste and flavour of food.

The findings of this study enhance our understanding of the effect of added sumac to *ad libitum* lunch food intake among older adults. The current results add to the growing body of literature in the field of appetite following the addition of spices to support the improvement of food intake and appetite regulation among older adults. The insights gained from this study may contribute to the rapidly evolving industry of functional foods.

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Appendices

Appendix 1: List of medications

Drugs to lower cholesterol or lipids in blood	Drugs to fight infectious diseases	Drugs to treat cancer	Drugs for arthritis and pain	Drugs for hypertension and heart disease	Muscle relaxants and drugs for the treatment of Parkinson's disease	Drugs to improve mood or treat epilepsy	Drugs for asthma and breathing problems
Clofibrate Fluvastatin sodium Gemfibrozil Lovastatin Pravastatin sodium Antihistamines Chlorpheniramine maleate Loratadine Terfenadine and pseudoephedrine Cholestyramine	Ampicillin Ciprofloxacin Clarithromycin Ofloxacin Streptomycin Tetracyclines	Cisplatin Doxorubicin and methotrexate Vincristine sulfate	Auranofin Colchicine Dexamethasone Diclofenac potassium=diclofenac sodium Dimethyl sulfoxide Gold Hydrocortisone D-penicillamine and penicillamine	Acetazolamide Adenosine Amiloride Benazepril HCl and hydrochlorothiazide Betaxolol HCl Captopril Clonidine Diltiazem Enalapril Ethacrynic acid Nifedipine Propranolol Spironolactone	Baclofen Dantrolene sodium Levodopa	Amitriptyline HCl Carbamazepine Clomipramine HCl Clozapine Desipramine HCl Doxepin HCl Fluoxetine HCl Imipramine Lithium carbonate Phenytoin Trifluoperazine	Albuterol sulfate Cromolyn sodium Flunisolide Metaproterenol sulfate Terbutaline sulfate

Representative medications or treatments that alter taste or smell (Schiffman and Graham, 2000)

Appendix 2: UREC Ethical Approval for a Sensory Evaluation Study



Dr Helen Lightowler
Director of Studies
Department of Sport and Health Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

4 January 2017

Dear Dr Lightowler

UREC Registration No: 161059
Sensory evaluation of different doses of sumac in vegetable soup among healthy adults

Thank you for the email of 16 December 2016 outlining the response to the points raised in my previous letter about the PhD study of your research student Nasim Soleymani Majd and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 4 January 2019. If you need the approval to be extended please do contact me nearer the time of expiry.

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

Yours sincerely

A handwritten signature in blue ink, appearing to read "SQ", with a long horizontal flourish extending to the right.

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc: Sangeetha Thondre and Shelly Coe, Supervisory Team
Nasim Soleymani Majd, Research Student
Anne Delextrat, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator



www.brookes.ac.uk

Appendix 3: Sensory evaluation study advertisement

Taste and Flavour Perception of Sumac Spice

Nasim Soleymani Majd
PhD Researcher



- This study involves two 2 hours visits at the Functional Food Centre of Oxford Brookes University
- You will be trained on how to perform the sensory test in your first visit and will be asked to:

Taste vegetable soup with different doses of tangy sumac spice

- Evaluate appearance and flavor of each soup sample
- Assess the intensity of colour and flavour in each soup sample

We are looking for:

- Adults aged 18-35 years
- And adults, aged >65 years
- No allergy to herb, spices and vegetables including celery and tomato
- Non-smoker
- Do not have a cold or hay fever (on test day)
- Non-pregnant or lactating
- No disease or medication that may affect taste and smell sensation
- Ability to attend for two hours testing

*You will receive (£10)
Amazon voucher after
you finished the study*



Supervisors:
Dr. Helen Lightowler
Dr.Sangeetha Thondre
Dr. Shelly Coe

**If you would like to get involved and need more
information, please contact the researcher**

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Appendix 4: Participant Information Sheet for the sensory evaluation study

Participant Information Sheet

Sensory evaluation of different doses of sumac in vegetable soup among healthy adults

You are invited to take part in a research study that seeks to assess what is an acceptable amount of sumac spice in vegetable soup. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

Purpose of the study:

Rhus coriaria Linn, also known as sumac, is a spice with a tangy taste that is widely used in Turkish, Iranian and Middle Eastern foods for seasoning or flavouring, as an appetizer and for souring. In folk medicine, this spice has also been used to treat a number of different diseases. Moreover, recent studies have shown that sumac has high antioxidant activity and polyphenol content, which may contribute to reducing the risk of some diseases.

Taste and smell play a vital role in food preferences, nutrient intake and appetite, which can be affected by age and diseases. One of the major physiological changes in older adults (i.e. those aged 60 years and above) is a gradual decline in sensory perception, including smell and taste. This may increase the risk of poor health by reducing the enjoyment of food, followed by decreasing food and nutrient intake.

The aim of this study is to identify the most favourable dose of sumac in vegetable soup. These results may contribute to boosting our understanding of how this spice can increase the level of appetite among older adults.

Why have I been invited to participate?

You and 39 others are being invited to take part in this study, run at Oxford Brookes University, to help us determine the optimal amount of sumac in vegetable soup.

Inclusion criteria:

- Adult, aged 18–35 years
- Adult, aged >65 years
- No allergies to herbs and spices
- No allergies to vegetables, including celery and tomatoes

- Non-smoker
 - No cold or hay fever (on the test day)
 - Not pregnant or lactating
 - No disease that may affect taste and smell sensation (including local chronic or acute inflammatory nasal disease and high cholesterol)
 - No medication that may affect sensory taste, such as antibiotics, antihistamines and decongestants, anti-inflammatory agents, muscle relaxants (Bromley, 2000)
 - Ability to read and understand information in English
 - Ability of older adults to attend, stand and sit for up to two hours
-
- A normal capability level for functional activity (i.e. traveling outside the neighbourhood, shopping alone)

What will happen to me if I take part?

Should you agree and consent to participate in this study, you will be asked to complete a health questionnaire to ensure that you are indeed eligible to participate in the study.

Overall, the risk profile is low. However, there are some potential risks that may occur and that you should be aware of: choking, burning due to the temperature of the soup and other unexpected outcomes, such as allergy (e.g. skin rash). To date, no toxicity effect of sumac has been reported. Therefore, the chance of adverse/ unexpected outcomes that places the participant at any risk following this sensory evaluation is very small.

What the study involves

- You will be required to attend the Functional Food Centre (S404) for two test sessions with at least two days between each test.

Session 1:

- Visits will last for up to one hour
- Sign a consent form and complete a health questionnaire
- You will receive training for the purpose of recording your responses
- Three vegetable soups will be provided, each containing a different amount of sumac

- You will be asked to evaluate each sample by expressing your degree of like or dislike of five attributes (appearance, flavour, aroma, texture and acceptance) using a 9-hedonic point scoring system (9=like extremely; 1=dislike extremely)
- You will assess the strength of colours (red and brown) and flavours (salty and lemony) of the vegetable soup samples, using the intensity scale (light to dark and low to high)

Session 2:

- Identical to session one but,
- Two vegetable bowls of soup will be provided; each will contain a different amount of sumac.

In both sessions, cold water and crackers will be provided to you, to drink and cleanse your palate between samples.

Do I have to take part?

Your participation is entirely voluntary and you are free to withdraw from the study at any time.

How to prepare for the study

One hour before the test, no drinking (except water) and no eating.

Benefits of the study:

You will receive a £10 Amazon gift voucher on completion of the study.

Data protection and withdrawal:

Confidentiality of any information provided can only be protected within the limitations of the law.

All records will be coded and only available to the researchers involved in the study. Your name will never appear in any published work. The only information we will use from the health questionnaire is age and gender. All other data from the health questionnaires will be used only for screening.

All data from the study will be owned by Oxford Brookes University and will be retained and kept securely, in agreement with the University's policy on Academic Integrity, for a period of ten years.

You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied.

What happens if I want to take part?

If you would like to participate in this research study, you can do so by contacting the researcher at the phone number or email address given below. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

Participation is entirely voluntary. You are still free to withdraw at any time, without having to give a reason. If you are a student, by choosing to either take part or not take part in the study, or to withdraw at any time, this will have no impact on your grades, assessments or future studies. If you are a member of staff choosing to take part, or not to take part in the study or to withdraw at any time, this will have no impact on your employment status at the university. No details will be recorded for monitoring purposes.

Who is organising and funding the research?

I (Nasim Soleymani Majd) am conducting this research as a PhD researcher at Oxford Brookes University under the supervision of Dr Helen Lightowler, Dr Sangeetha Thondre and Dr Shelly Coe in the Department of Sport & Health Sciences. This study is self-funded.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be kept in a locked drawer at Oxford Brookes University; and if saved on a computer, it will be securely encrypted with a password. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after completion of this research project. Only researchers directly involved in the study will have access to personal data. The data will be statistically analysed in the United Kingdom under the supervision of Dr Helen Lightowler, Dr Sangeetha Thondre and Dr Shelly Coe at Oxford Brookes University.

What will happen to the results of this research study?

Ultimately, the results of the study will be written up as part of a PhD thesis. In addition, the results may be published in peer-reviewed journals and presented at meeting and conferences (either nationally or internationally). No participants will be identified in any publications.

Who has reviewed the study?

This study has been approved by the Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC at ethics@brookes.ac.uk

Contact for further information:

You can contact the researcher Nasim Soleymani Majd at any time if you have any questions or concerns:

Nasim Soleymani Majd 15119901@brookes.ac.uk Tel: 01865 483283

Supervisors:

Dr Helen Lightowler hlightowler@brookes.ac.uk Tel: 01865 483245

Dr Sangeetha Thondre pthondre@brookes.ac.uk Tel: 01865 483988

Dr Shelly Coe scoe@brookes.ac.uk Tel: 01865 483839

Thank you for taking your time to read this information sheet.

Appendix 5: Consent Form

Consent Form

Sensory evaluation of different doses of sumac in vegetable soup among healthy adults

Contacts:

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Supervisors:

Dr Helen Lightowler hlightowler@brookes.ac.uk Tel: 01865 483245

Dr Sangeetha Thondre pthondre@brookes.ac.uk Tel: 01865 483988

Dr Shelly Coe scoe@brookes.ac.uk Tel: 01865 483839

Functional Food Centre
Department of Sport and Health Sciences
Oxford Brookes University
Gipsy Lane Campus
Oxford OX3 0BP
Please INITIAL the boxes

1. I confirm that I have read and understand the information sheet for the above research project.
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.
3. I understand that my participation is voluntary and I am free to withdraw at any time, without giving a reason.
4. I understand that the confidentiality of information provided can only be protected within the limits of the law.
5. I agree to participate in this study

Name of Participant Date .../.../.....

(in block capitals)

Signature

Contact number: email:

Name of Researcher Date .../.../.....

(in block capitals)

Signature

Appendix 7: Functional ability questionnaire for older adults

Functional Ability Questionnaire in Older Adults

Subject Number:

• Sex: Male Female

• Age:

Please rate as appropriate:

The level of performance on each of the following tasks using this scale:

0: normal; 1: has difficulty but able to do unaided 2: requires assistance; 3= dependent

- Shopping alone for clothes, household necessities or groceries:
- Paying attention to and understanding a television show, book or magazine:
- traveling out to the neighbourhood (for example: take a bus or driving):
- Writing cheques, paying bills and keeping financial records:
- Trouble in reading and following the instructions of appliances or gadgets (e.g. computer, mobile, microwave):

Thank you for taking the time to answer this

Appendix 8: Sensory evaluation descriptive scale

Descriptive Scale:

Sample Code:

Date: / /

Appearance:

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Flavour:

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Aroma:

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Texture:

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Acceptance:

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Appendix 9: Sensory evaluation intensity scale

Intensity Scale:

Sample Code

Date: .../.../....

Please rate the appearance of red colour of the sample:

Light					Dark			
Extremely light	Very Light	Slightly Light	Light	Neutral	Dark	Slightly Dark	Very Dark	Extremely Dark

Please rate the appearance of brown colour of the sample:

Light					Dark			
Extremely light	Very Light	Slightly Light	Light	Neutral	Dark	Slightly Dark	Very Dark	Extremely Dark

Please rate the salty flavour:

Low					High			
Extremely Low	Very Low	Slightly Low	Low	Just Right	High	Slightly High	Very High	Extremely High

Please rate the lemony flavour:

Low					High			
Extremely Low	Very Low	Slightly Low	Low	Just Right	High	Slightly High	Very High	Extremely High

Appendix 10: Appetite study advertisement for free-living older adults, phase 1

The effect of consumption of sumac in vegetable soup on appetite and food intake among older adults

Nasim Soleymani Majd PhD Researcher

Sumac is a spice that was used to treat different type of disease in addition being a flavour commonly used in Middle East.
The aim of this study is to assess the impact of consumption of sumac in vegetable soup on appetite and food intake among older adults.

We are looking for:

- Adults, aged >65 years
- No allergy to herb, spices and vegetables including celery and tomato
- Non-smoker
- Does not have a cold or hay fever (on test day)
- Disease that may affect the taste and smell sensation
- No medication that may affect the sensory taste
- Ability to read and understand information in English
- Ability to attend, stand and sit for up to one hour
- A normal level of capability of functional activity (i.e. traveling out to the neighbourhood, shopping alone)
- Not diagnosed with Dementia



Supervisors:

Dr. Helen Lightowler
Dr. Sangeetha Thondre
Dr. Shelly Coe

You will receive (£20) M&S voucher after you finished the study

- This study involves three 1 hours visits at the Oxford Brookes Centre for Nutrition and Health (OxBCNH) of Oxford Brookes University
- You will be asked to:
 - Consume vegetable soup with or without added sumac spice
 - Followed by consuming the prepared pasta for lunch
 - Record your 12h dietary intake after each visit

If you would like to get involved and need more information, please contact the researcher

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Appendix 11: Appetite study advertisement for younger adults, phase 1

The effect of consumption of sumac in vegetable soup on appetite and food intake among young adults (aged 18-35 years old)

Nasim Soleymani Majd PhD Researcher

Sumac is a spice that was used to treat different type of disease in addition being a flavour commonly used in Middle East.

The aim of this study is to assess the impact of consumption of sumac in vegetable soup on appetite and food intake among young adults.

We are looking for:

- Adults, aged 18-35 years
- No allergy to herb, spices and vegetables including celery and tomato
- Non-smoker
- Does not have a cold or hay fever (on test day)
- Disease that may affect the taste and smell sensation
- No medication that may affect the sensory taste
- Ability to read and understand information in English



Supervisors:

Dr. Helen Lightowler
Dr. Sangeetha Thondre
Dr. Shelly Coe

You will receive (£10) Amazon voucher after you finished the study

- This study involves three 1 hours visits at the Oxford Brookes Centre for Nutrition and Health (OxBCNH) of Oxford Brookes University
- You will be asked to:
 - Consume vegetable soup with or without added sumac spice
 - Followed by consuming the prepared pasta for lunch
 - Record your 12h dietary intake after each visit

If you would like to get involved and need more information, please contact the researcher

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Appendix 12: Participant Information Sheet for phase 1 of the appetite study: Free-living older adults



Participant Information Sheet

The effect of consuming sumac in vegetable soup on appetite and food intake among older adults

You are invited to take part in a research study that seeks to evaluate the impact of sumac on appetite and food intake. This will be done by adding sumac to cooked soup and by adding sumac to soup from the beginning of the cooking process. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

Purpose of the study:

Sumac is a tangy spice that is widely used in Turkish, Iranian and Middle Eastern foods for seasoning or flavouring, as an appetizer and for souring. In folk medicine, this spice has also been used to treat a number of different diseases. Moreover, recent studies have shown that sumac has high levels of antioxidant activity and polyphenol content that may contribute to reducing the risk of some diseases. Taste and smell play a vital role in food preferences, nutrient intake and appetite, which can be affected by age and disease. One of the major physiological changes in older adults (i.e. those aged 60 years and above) is a gradual decline in sensory perception, including smell and taste. This may increase the risk of poor health by reducing the enjoyment of food – followed by decreased food and nutrient intake.

Inclusion criteria:

- Adults, aged >65 years
- No allergies to herb and spices
- No allergies to vegetables, including celery and tomatoes
- Non-smoker
- No cold or hay fever (on test day)
- No disease that may affect taste and smell sensation (including local chronic or acute inflammatory nasal disease, high cholesterol, head injury, stroke and exposure to radiation therapy for head and neck cancer)
- No medication that may affect sensory taste, such as antibiotics, antihistamines and decongestants, anti-inflammatory agents, muscle relaxants (Bromley, 2000)
- Ability to read and understand information in English
- Ability to attend, stand and sit for up to one hour
- A normal level of functional activity (i.e. traveling outside the neighbourhood, shopping alone)

What will happen to me if I take part?

Should you agree to participate in the study you will be asked to complete a health questionnaire to ensure that you are indeed eligible to do so. Thereafter your consent will be sought.

Overall, the risk of this study is low. However, there are some potential risks that may occur and that you should be aware of: choking, burning due to the temperature of the soup and other unexpected outcomes such as allergies (e.g. skin rash). To date, no toxicity effects of sumac have been reported. Therefore, the chance of adverse/ unexpected outcomes that places the participant at any risk following consumption of this soup is negligible.

What the study involves

- This study comprises of sessions with at least one-week between each test session.

Session 1:

- Complete a health questionnaire, sign a consent form
- During lunchtime you first will be asked to consume the soup provided and then your lunch prepared by the chef at your residential care home
- At teatime and dinnertime, you will be provided with food prepared by the chef based on the standard residential care-home menu

Session 2:

- During your lunchtime you will first be asked to consume the soup provided and then your lunch
- At teatime and dinnertime, you will be provided with food prepared by the chef based on the standard residential care-home menu

Session 3:

- Identical to session two

Do I have to take part?

Your participation is entirely voluntary and you are free to withdraw from the study at any time.

How to prepare for the study

You will be asked not to eat any food or drink (except water) from 11 a.m. on the test day. The study will consist of three non-consecutive test days that will be completed on the same day of the week. The test meal will be served between 12–1 p.m.

Benefits of the study:

You will receive a £20 M&S voucher on completion of the study.

Data protection and withdrawal:

Confidentiality of any information provided can only be protected within the limitations of the law.

What happens if I do want to take part?

If you would like to participate in this research study, you can do so by contacting the researcher at the phone number or email address given below. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. Participation is entirely voluntary. You are still free to withdraw at any time, without having to give a reason.

Who is organising and funding the research?

I (Nasim Soleymani Majd) am conducting this research as a PhD researcher at Oxford Brookes University under the supervision of Dr Helen Lightowler, Dr Sangeetha Thondre and Dr Shelly Coe in the Department of Sport, Health Sciences and Social work. This study is self-funded.

Who has reviewed the study?

This study has been approved by the Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC at ethics@brookes.ac.uk

Contact details for further information

You can contact the researcher Nasim Soleymani Majd at any time if you have any questions or concerns:

Nasim Soleymani Majd 15119901@brookes.ac.uk Tel: 01865 483283
Mob: 07507463202

Supervisors:

Dr Helen Lightowler hlightowler@brookes.ac.uk Tel: 01865 483245
Dr Sangeetha Thondre pthondre@brookes.ac.uk Tel: 01865 483988
Dr Shelly Coe scoe@brookes.ac.uk Tel: 01865 483839

Thank you for taking your time to read this information sheet

Appendix 13: Participant Information Sheet for phase 1 of the appetite study: younger adults



OXFORD
BROOKES
UNIVERSITY

Participant Information Sheet

The effect of consumption of sumac in vegetable soup on appetite and food intake among young adults aged 18-35 years

You are being invited to take part in a research study that seeks to evaluate the impact of sumac on appetite and food intake. This will be done by adding sumac to the finished cooked soup and by adding sumac to the soup from the beginning of cooking process. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

The purpose of the study:

Rhus coriaria Linn, also known as Sumac, is a spice with a tangy taste that is widely used in Turkish, Iranian and Middle Eastern foods for seasoning or flavouring, as an appetizer and for souring. In folk medicine, this spice has also been used to treat a number of different diseases. Moreover, recent studies have shown that sumac contains a high level of antioxidant activity and polyphenol content that may contribute to reducing the risk of some diseases.

Taste and smell play a vital role in food preference, nutrient intake and appetite that can be affected by age and disease. One of the major physiological changes in older adults (i.e. those aged 60 years and above) is the gradual decline in sensory perception including smell and taste. This may increase the risk of poor health by reducing the enjoyment of food followed by decreasing food and nutrient intake.

Why have I been invited to participate?

You and 19 others are being invited to take part in this study that is run at Oxford Brookes University, to help us determine the impact of added sumac in vegetable soup in comparison with free living older adults

Inclusion criteria:

- Adults, aged 18-35 years old
- No allergy to herb and spices
- No allergy to vegetables including celery and tomato
- Non-smoker
- Does not have a cold or hay fever (on test day)
- Disease that may affect the taste and smell sensation (including local chronic or acute inflammatory nasal disease and high cholesterol)
- No medication that may affect the sensory taste such as: antibiotics, antihistamines and decongestants, anti-inflammatory agents, and muscle relaxants (Bromley, 2000)
- Ability to read and understand information in English

What will happen to me if I take part?

Should you agree and consent to participate in this study, you will be asked to complete a health questionnaire to make sure that you are indeed eligible to participate in the study.

Overall the risk of this study is low. However, there are some potential risks that may occur and that you should be aware of: choking, burning due to the temperature of the soup and other unexpected outcomes such as allergy (i.e. skin rash). To date, no toxicity effect of sumac has yet been reported. Therefore, the chance of adverse/ unexpected outcomes that places the participant at any risk following the consumption of this soup is very small.

What the study involves

- You will be required to attend the Oxford Brookes Centre for Nutrition and Health (OxBCNH) for three sessions with at least two days (48 Hours) between each test session.

Session 1:

- Visit will last up to one hour
- Sign the consent form, complete a health questionnaire and 24 hours dietary intake
- You will be asked first to consume the provided soup and then a prepared pasta for lunch
- Also, you will be asked to record a 12 hours dietary intake after you have left the centre

Session 2:

- You will be asked first to consume the provided soup and then the prepared pasta for lunch
- Also, you will be asked to record 12 hours dietary intake after you have left the centre

Session 3:

- Identical to session two

Do I have to take part?

Your participation is entirely voluntary and you are free to withdraw from the study at any time.

How to prepare for the study?

You will be instructed what to eat for breakfast in advance and not to eat any food or drink (except water) one hour before your test. The test meal will be served between 12-2 pm and each participant will be tested at the same time of day for each session.

Benefits of the study:

You will receive a £10 amazon voucher on completion of the study.

Data protection and withdrawal:

Confidentiality of any information provided can only be protected within the limitations of the law.

All records will be coded and will only be available to the researchers involved in the study. Your name will never appear in any published work. The only information we will use from the health questionnaire is age and sex. All other data from the health questionnaires will only be used for screening.

All data from the study will be owned by Oxford Brookes University and will be retained and kept securely in agreement with the University's policy on Academic Integrity for a period of ten years.

You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied.

What happens if I do want to take part?

If you would like to participate in this research study, you can do so by contacting the researcher at the phone number or email address given below. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

Participation is entirely voluntary. You are still free to withdraw at any time, without having to give a reason. If you are a member of staff choosing to take part, or not to take part in the study or to withdraw at any time, will have no impact on your employment status at the university. No details will be recorded for monitoring purposes.

Who is organising and funding the research?

I (Nasim Soleymani Majd) am conducting this research as a PhD researcher at Oxford Brookes University under the supervision of Dr. Helen Lightowler, Dr. Sangeetha Thondre and Dr. Shelly Coe in the Department of Sport, Health Sciences and Social Work. This study has been self-funded.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely encrypted with a password. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. Only researchers directly involved in the study will have access to personal data. The data will be statistically analysed in the United Kingdom under the supervision of Dr. Helen Lightowler, Dr. Sangeetha Thondre and Dr. Shelly Coe at Oxford Brookes University.

What will happen to the results of this research study?

Ultimately, the results of the study will be written up as part of a PhD thesis. In addition, the results will be published in peer-reviewed journals and presented at meeting and conferences (either nationally or internationally). Participants will not be identified in any publications.

Who has reviewed the study?

This study has been approved by Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC on ethics@brookes.ac.uk

Contact for further information:

You can contact the researcher Nasim Soleymani Majd at any time if you have any questions or concerns:

Nasim Soleymani Majd 15119901@brookes.ac.uk Tel: 01865 483283
Mob: 07507463202

Supervisors:

Dr. Helen Lightowler hlightowler@brookes.ac.uk Tel: 01865 483245
Dr. Sangeetha Thondre pthondre@brookes.ac.uk Tel: 01865 483988
Dr. Shelly Coe scoe@brookes.ac.uk Tel: 01865 483839

Thank you for taking your time to read this information sheet

Appendix 14a: UREC Ethical Approval for a food intake study in phase 1: Free-living older adults



Dr Helen Lightowler
Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

13 March 2018

Dear Dr Lightowler

UREC Registration No: 181174

The effect of consumption of sumac in vegetable soup on appetite and food intake among older adults

Thank you for your email of 6 March 2018 outlining your response to the points raised in my previous letter about the PhD study of your research student Nasim Soleymani Majd and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study for the 'free-living' group of participants to begin.

The UREC approval period for the data collection phase of the study is two years from the date of this letter, so 13 March 2020. If you need the approval to be extended please do contact me nearer the time of expiry.

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

Yours sincerely

A handwritten signature in blue ink, appearing to read "S Quinton". The signature is fluid and cursive, with a long horizontal stroke at the end.

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Sangeetha Thondre and Shelly Coe, Supervisory Team
Nasim Soleymani Majd, Research Student
Roger Ramsbottom, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator

Appendix 14b: UREC Ethical Approval for a food intake study in phase 1: Younger adults



Dr Helen Lightowler
Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

13 March 2018

Dear Dr Lightowler

UREC Registration No: 181174
The effect of consumption of sumac in vegetable soup on appetite and food intake among older adults

Thank you for your email of 6 March 2018 outlining your response to the points raised in my previous letter about the PhD study of your research student Nasim Soleymani Majd and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study for the 'free-living' group of participants to begin.

The UREC approval period for the data collection phase of the study is two years from the date of this letter, so 13 March 2020. If you need the approval to be extended please do contact me nearer the time of expiry.

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

Yours sincerely

A handwritten signature in blue ink, appearing to read "S Quinton".

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Sangeetha Thonrdre and Shelly Coe, Supervisory Team
Nasim Soleymani Majd, Research Student
Roger Ramsbottom, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator

www.brookes.ac.uk

Appendix 15: Sample gatekeeper

Sample gatekeeper email

Dear XXX

I am a PhD student from Oxford Brookes University and I am interested in looking at the effect of consumption of two methods of cooking of sumac in vegetable soup on appetite and food intake among two groups of older adults.

I am emailing you because I am interested in recruiting adult free living volunteers >65 years and/ or older adults residents in your nursing home for the study as mentioned above.

I have attached a copy of the study advertisement to this email that requires your authorisation to put up this in your facility.

If you are willing to grant me permission to display the study advertisement in your facility/ organisation in order to facilitate recruitment for this research, please reply with your official confirmation so that I may have your permission for my records. If additional permission is needed from another source, please do let me know.

Please do not hesitate to contact me if you have any further questions or concerns

Thank you for your consideration of this request.

Yours sincerely,

Nasim Soleymani Majd, BSc, MSc

PhD Research Student

Oxford Brookes Centre for Nutrition and Health (OxBCNH) Oxford Brookes University

Telephone number: 01865 483283

Email address: 15119901@brookes.ac.uk

Supervisors:

Dr. Helen Lightowler Senior Lecturer in Human Nutrition

Dr. Sangeetha Thondre Senior Lecturer in Nutrition

Dr. Shelly Coe Lecturer in Nutrition

Appendix 16: Appetite study advertisement for residential older adults, phase 2

The effect of consumption of sumac in vegetable soup on appetite and food intake among older adults

Nasim Soleymani Majd
PhD Researcher

Sumac is a spice that was used to treat different types of diseases in addition to being commonly used as flavouring in the Middle East.

The aim of this study is to assess the impact of consumption of sumac in vegetable soup on appetite and food intake among older adults.



Supervisors:

Dr. Helen Lightowler
Dr. Sangeetha Thondre
Dr. Shelly Coe

We are looking for:

- Adults, aged >65 years
- No allergy to herb, spices and vegetables including celery and tomato
- Non-smoker
- Does not have a cold or hay fever (on test day)
- No disease that could affect taste and smell sensation
- No medication that may affect the sensory taste
- Ability to read and understand information in English

You will receive (£20) M&S voucher after you finished the study

- This study involves three visits at your residential centre
- You will be asked to:
 - Consume vegetable soup with or without added sumac spice
 - Followed by consuming the prepared food by your Chef for your lunch

If you would like to get involved and need more information, please contact the researcher

Nasim Soleymani Majd
15119901@brookes.ac.uk
07507463202

Nasim Soleymani Majd
15119901@brookes.ac.uk
07507463202

Nasim Soleymani Majd
15119901@brookes.ac.uk
07507463202

Nasim Soleymani Majd
15119901@brookes.ac.uk
07507463202

Nasim Soleymani Majd
15119901@brookes.ac.uk
07507463202

Appendix 17: Participant Information Sheet for phase 2 of the appetite study



Participant Information Sheet

The effect of consumption of sumac in vegetable soup on appetite and food intake among older adults

You are invited to take part in a research study that seeks to evaluate the impact of sumac on appetite and food intake. This will be done by adding sumac to finished cooked soup and by adding sumac to soup from the beginning of the cooking process. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

Purpose of the study:

Sumac is a tangy spice that is widely used in Turkish, Iranian and Middle Eastern foods for seasoning or flavouring, as an appetizer and for souring. In folk medicine, this spice has also been used to treat a number of different diseases. Moreover, recent studies have shown that sumac contains high levels of antioxidant activity and polyphenol content, which may contribute to reducing the risk of some diseases.

Taste and smell play a vital role in food preferences, nutrient intake and appetite, which can be affected by age and disease. One of the major physiological changes in older adults (i.e. those aged 60 years and above) is a gradual decline in sensory perception, including smell and taste. This may increase the risk of poor health by reducing the enjoyment of food – followed by decreased food and nutrient intake.

Inclusion criteria:

- Adults, aged >65 years
- No allergies to herb and spices
- No allergy to vegetables, including celery and tomatoes
- Non-smoker
- No cold or hay fever (on test day)
- No disease that could affect taste and smell sensation (including local chronic or acute inflammatory nasal disease, high cholesterol, head injury, stroke and exposure to radiation therapy for head and neck cancer)
- No medication that may affect sensory taste, such as antibiotics, antihistamines and decongestants, anti-inflammatory agents, muscle relaxants (Bromley, 2000)
- Ability to read and understand information in English.

What will happen to me if I take part?

Should you agree to participate in the study you will be asked to complete a health questionnaire and a functional ability questionnaire in older adults to ensure that you are indeed eligible to do so. Thereafter, your consent will be sought.

Overall, the risk of this study is low. However, there are some potential risks that may occur and that you should be aware of: choking, burning due to the temperature of the soup and other unexpected outcomes such as allergies (e.g. skin rash). To date, no toxicity effects of sumac have been reported. Therefore, the chance of adverse/ unexpected outcomes that places the participant at any risk following consumption of this soup is negligible.

What the study involves

- This study comprises three sessions with at least one week between each test session.

Session 1:

- Complete a health questionnaire and sign a consent form
- During lunchtime you first will be asked to consume the soup provided and then your lunch prepared by the chef at your residential care home
- At dinnertime, you will be provided with food prepared by the chef based on the standard residential care-home menu

Session 2:

- During your lunchtime you first will be asked to consume the soup provided and then your lunch
- At dinner time, you will be provided with food prepared by the chef based on the standard residential care-home menu

Session 3:

- Identical to session two

Do I have to take part?

Your participation is entirely voluntary and you are free to withdraw from the study at any time.

How to prepare for the study

You will be asked not to eat any food or drink (except water) from 11 a.m. on the test day. The study will consist of three non-consecutive test days that will be completed on the same day of the week. The test meal will be served between 12-1 p.m.

Benefits of the study:

You will receive a £20 M&S voucher on completion of the study.

Data protection and withdrawal:

Confidentiality of any information provided can only be protected within the limitations of the law.

What happens if I do want to take part?

If you would like to participate in this research study, you can do so by contacting the researcher at the phone number or email address given below. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. Participation is entirely voluntary. You are still free to withdraw at any time, without having to give a reason.

Who is organising and funding the research?

I (Nasim Soleymani Majd) am conducting this research as a PhD researcher at Oxford Brookes University under the supervision of Dr Helen Lightowler, Dr Sangeetha Thondre and Dr Shelly Coe in the Department of Sport, Health Sciences and Social work. This study is self-funded.

Who has reviewed the study?

This study has been approved by the Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC at ethics@brookes.ac.uk

Contact details for further information:

You can contact the researcher, Nasim Soleymani Majd, at any time if you have any questions or concerns:

Nasim Soleymani Majd	15119901@brookes.ac.uk	Tel: 01865 483283 Mob: 07507463202
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Supervisors:

Dr Helen Lightowler	hlightowler@brookes.ac.uk	Tel: 01865 483245
Dr Sangeetha Thondre	pthondre@brookes.ac.uk	Tel: 01865 483988
Dr Shelly Coe	scoe@brookes.ac.uk	Tel: 01865 483839

Thank you for taking your time to read this information sheet.

Appendix 18: UREC Ethical Approval for a food intake study in phase 2: Residential older adults



Dr Helen Lightowler
Director of Studies
Department of Sport, Health Sciences and Social Work
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

14 November 2018

Dear Dr Lightowler

UREC Registration No: 181174

**The effect of consumption of sumac in vegetable soup on appetite and food intake among older adults:
Phase two**

Thank you for the email of 30 October 2018 outlining the response to the points raised in my previous letter about the PhD study of your research student Nasim Soleymani Majd and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for phase two of the study to begin.

The UREC approval period for the data collection phase of the study is two years from the date of this letter, so 14 November 2020. If you need the approval to be extended please do contact me nearer the time of expiry.

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

Yours sincerely

A handwritten signature in blue ink, appearing to read "S Quinton".

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Sangeetha Thondre and Shelly Coe, Supervisory Team
Nasim Soleymani Majd, Research Student
Roger Ramsbottom / Anne Delextrat, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator

www.brookes.ac.uk

Appendix 19: Food diary intake for a 24 hours

FOOD DIARY

Subject ID: _____

Diary dates

Day 1: _____

We would like you to keep a diary of everything you eat and drink over 1 day

Please read carefully the instructions below before starting to fill in the diary. It is important to record everything you eat and drink, no matter how small the amount.

1. Each day is marked in 6 sections, beginning with the first thing in the morning and ending with bedtime. For each part of the day, write down all food and drink consumed. If nothing is eaten or drunk during a part of the day, draw a line through that section.
2. Give as much information as possible about the foods and drinks you eat. It is very useful if you include:
 - Brand name e.g. *Branston* pickle,
 - Food weight where known – often detailed on the packaging
 - How the food was cooked, e.g. baked, grilled, raw, etc.
 - Extra ingredients, e.g. teaspoon of grated parmesan on pasta or dressings/sauces added to salads
3. Estimating food weights:
 - Recording quantities of food is important.
 - Household measures may help;
 - A general recording of whether portions are small, medium or large is also helpful.

REMEMBER

Remembering later is often more difficult than we initially envisage, so it is often easier to try to record as you go through the day, where possible.

Please start each day on a new sheet

Date: 03/01/09		Day of the week: Saturday	
BEFORE BREAKFAST			
Food/Drink	Description and Preparation	Amount	
Water		250 ml	
BREAKFAST			
Food/Drink	Description and Preparation	Amount	
Cereal	Weetabix	2 biscuits	
Milk	Semi-skimmed	125 ml	
Sweetener		10 g	
Juice	Orange, unsweetened	200 ml	
MID-MORNING (between breakfast and lunch)			
Food/Drink	Description and Preparation	Amount	
Apple		120 g	
LUNCH			
Food/Drink	Description and Preparation	Amount	
Bread	Wholemeal, medium slice	2 slices	
Spread	Low-fat (40% fat)	14 g	
Tuna	Canned (in brine)	50 g	
Cucumber	Sliced	4 slices	
Tomato	Cherry tomatoes	6 small	
Drink	Fruit drink	250 ml	

TEA (between lunch and evening meal)		
Food/Drink	Description and Preparation	Amount
Yogurt	Low-fat yogurt drink	220 ml
EVENING MEAL		
Food/Drink	Description and Preparation	Amount
Chicken	Breast, grilled	130 g
Potatoes	New, boiled	6 potatoes
Peas	Frozen, boiled	1 tbsp
Sweetcorn	Frozen, boiled	1 tbsp
Apple crumble	Homemade	150 g
Custard	Made with skimmed milk	100 g
Water	Tap	500 ml
AFTER EVENING MEAL (between evening meal and bedtime)		
Food/Drink	Description and Preparation	Amount
Tea	With skimmed milk	2 mugs
Banana		1 medium
Water	Tap	250 ml

Date: <input type="text"/>		Day of the week: <input type="text"/>	
BEFORE BREAKFAST			
Food/Drink	Description and Preparation	Amount	
BREAKFAST			
Food/Drink	Description and Preparation	Amount	
MID-MORNING (between breakfast and lunch)			
Food/Drink	Description and Preparation	Amount	
LUNCH			
Food/Drink	Description and Preparation	Amount	

TEA (between lunch and evening meal)		
Food/Drink	Description and Preparation	Amount
EVENING MEAL		
Food/Drink	Description and Preparation	Amount
AFTER EVENING MEAL (between evening meal and bedtime)		
Food/Drink	Description and Preparation	Amount

Appendix 20: Food diary intake for a 12-hour period

FOOD DIARY

Subject ID: _____

Diary Dates: _____

We would like you to keep a diary of everything you eat and drink over next 12 hours.

Please read carefully the instructions below before starting to fill in the diary. It is important to record everything you eat and drink, no matter how small the amount.

- Each day is marked in 6 sections, beginning with the first thing in the morning and ending with bedtime. For each part of the day, write down all food and drink consumed. If nothing is eaten or drunk during a part of the day, draw a line through that section.
- Give as much information as possible about the foods and drinks you eat. It is very useful if you include:
 - Brand name e.g. *Branston* pickle,
 - Food weight where known – often detailed on the packaging
 - How the food was cooked, e.g. baked, grilled, raw, etc.
 - Extra ingredients, e.g. teaspoon of grated parmesan on pasta or dressings/sauces added to salads
- Estimating food weights:
 - Recording quantities of food is important.
 - Household measures may help;
 - A general recording of whether portions are small, medium or large is also helpful.

REMEMBER

Remembering later is often more difficult than we initially envisage, so it is often easier to try to record as you go through the day, where possible.

Please start each day on a new sheet

Date	03/01/09	Day of the week	Saturday
BEFORE BREAKFAST			
Food/Drink	Description and Preparation	Amount	
Water		250 ml	
BREAKFAST			
Food/Drink	Description and Preparation	Amount	
Cereal	Weetabix	2 biscuits	
Milk	Semi-skimmed	125 ml	
Sweetener		10 g	
Juice	Orange, unsweetened	200 ml	
MID-MORNING (between breakfast and lunch)			
Food/Drink	Description and Preparation	Amount	
Apple		120 g	
LUNCH			
Food/Drink	Description and Preparation	Amount	
Bread	Wholemeal, medium slice	2 slices	
Spread	Low-fat (40% fat)	14 g	
Tuna	Canned (in brine)	50 g	
Cucumber	Sliced	4 slices	
Tomato	Cherry tomatoes	6 small	
Drink	Fruit drink	250 ml	

TEA (between lunch and evening meal)		
Food/Drink	Description and Preparation	Amount
Yogurt	Low-fat yogurt drink	220 ml
EVENING MEAL		
Food/Drink	Description and Preparation	Amount
Chicken	Breast, grilled	130 g
Potatoes	New, boiled	6 potatoes
Peas	Frozen, boiled	1 tbsp
Sweetcorn	Frozen, boiled	1 tbsp
Apple crumble	Homemade	150 g
Custard	Made with skimmed milk	100 g
Water	Tap	500 ml
AFTER EVENING MEAL (between evening meal and bedtime)		
Food/Drink	Description and Preparation	Amount
Tea	With skimmed milk	2 mugs
Banana		1 medium
Water	Tap	250 ml

Date <input type="text"/>		Day of the week: <input type="text"/>
LUNCH		
Food/Drink	Description and Preparation	Amount
TEA (between lunch and evening meal)		
Food/Drink	Description and Preparation	Amount
EVENING MEAL		
Food/Drink	Description and Preparation	Amount

AFTER EVENING MEAL (between evening meal and bedtime)		
Food/Drink	Description and Preparation	Amount

Appendix 21: Nursing home food menu

[REDACTED]		04/07/2019	
Thursday		Thursday	
Name: [REDACTED]		Name: [REDACTED]	
Room No: [REDACTED]		Room No: [REDACTED]	
<input type="checkbox"/> Small <input checked="" type="checkbox"/> Standard <input type="checkbox"/> Large		<input type="checkbox"/> Small <input checked="" type="checkbox"/> Standard <input type="checkbox"/> Large	
<input type="checkbox"/> Soup of the Day		<input type="checkbox"/> Fresh Soup of the Day	
<input checked="" type="checkbox"/> Chicken Breast & Orange Glaze <input type="checkbox"/> Cheese Omelette <input type="checkbox"/> Smoked Salmon with Salad Served with: <input checked="" type="checkbox"/> Creamed Potato <input checked="" type="checkbox"/> Broccoli <input type="checkbox"/> Mashed Swede		<input type="checkbox"/> Quiche Lorraine <input type="checkbox"/> Pasta with Tomato Sauce & Pesto <input type="checkbox"/> Caesar Salad with Chicken Sandwiches: <input type="checkbox"/> White <input checked="" type="checkbox"/> Wholemeal <input type="checkbox"/> Egg Mayonnaise <input checked="" type="checkbox"/> Cheddar Cheese	
Desserts: <input type="checkbox"/> Chocolate & Orange Tart Ice Cream: <input checked="" type="checkbox"/> Vanilla <input type="checkbox"/> Chocolate <input type="checkbox"/> Strawberry <input checked="" type="checkbox"/> Fresh Pineapple Salad <input checked="" type="checkbox"/> Natural Yoghurt <input type="checkbox"/> Strawberry Yoghurt <input type="checkbox"/> Brie & Crackers		Desserts: <input type="checkbox"/> Banana Mousse <input checked="" type="checkbox"/> Stewed Apple <input checked="" type="checkbox"/> Natural Yoghurt <input type="checkbox"/> Strawberry Yoghurt <input checked="" type="checkbox"/> Banana <input type="checkbox"/> Apple <input type="checkbox"/> Satsuma <input type="checkbox"/> Cheddar & Crackers	