University of Southampton

Faculty of Environmental and Life Sciences

School of Biological Sciences

An Investigation of Overlooked Complexities Affecting UK Vitamin C Security and the Potential for Local Crops to Address Insecurities: A Case of UK Strawberries

by

David William Fisher

ORCID ID: 0000-0002-7947-3366

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Abstract

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This thesis concerns a detailed assessment of the current state of vitamin C security in the United Kingdom. Vitamin C is essential for proper immune function, genetic regulation, and collagen synthesis, making its availability crucial for public health. Contemporary assessments indicate that the availability of vitamin C in the UK is heavily reliant on imported fruits and vegetables, leaving the country vulnerable to disruptions in global trade. However, there remains a gap in research regarding the accessibility, stability, and utilisation of vitamin C in the UK, which this thesis aimed to address. This thesis also asks to what extent could local food-based interventions contribute to increase vitamin C self-sufficiency.

Vitamin C accessibility, stability, and utilisation were examined through secondary analyses of nationally representative food purchase and dietary survey data. The risk of inadequate access to vitamin C was greatest in regions and households of lower socioeconomic status. Seasonal instabilities were also observed, with reduced household provisions and increased risk of inadequate intakes consistently occurring during the Autumn. Dose-response modelling showed that the vitamin C requirement to achieve adequate status was 73 mg/day, 1.8 times higher than the current recommended intake of 40 mg/day. Smokers and obese individuals were found to have attenuated utilisation of vitamin C, requiring intakes more than twice the amount compared to non-smokers and those of healthy weight.

The potential for local foods to enhance vitamin C self-sufficiency is explored through the case study of biofortified UK strawberries. An analytical survey of fresh strawberries available from UK grocery retailers found that vitamin C contents were 38% higher during the peak UK Summer production season (71 mg/100g) compared to the Winter when retailers were reliant on imported strawberries (52 mg/100g). Chapters 5 and 6 used experimental approaches to evaluate the potential to biofortify UK strawberries through agronomic interventions and selective breeding. Foliar fertiliser treatments were not found to influence vitamin C content, but did show positive effects on other parameters including selenium and berry sweetness. Selective breeding emerged as a promising strategy to increase vitamin C contents, with *Elsanta* (94 mg/100g) and *Vibrant* (91 mg/100g) strawberries containing 30-35% more vitamin C than the market leading genotype *Malling Centenary* (70 mg/100g).

The collective findings of this thesis highlight the need for targeted interventions to make vitamin C more accessible to population groups at risk of vitamin C insecurities. The case of biofortified strawberries highlights the potential for food-based interventions to enhance UK vitamin C security but emphasises the need for their integration into interdisciplinary strategies to effectively support the most vulnerable population groups.

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Research Thesis: Declaration of Authorship

Print name: David William Fisher

Title of thesis: An Investigation of Overlooked Complexities Affecting UK Vitamin C Security and the Potential for Local Crops to Address Insecurities: A Case of UK Strawberries.

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
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Definitions and Abbreviations

AC .... Total Antioxidant Capacity

AsA................................. Total Ascorbic Acid

CHL .... Chlorophyll Fluorescence

CoFID .... The Composition of Foods Integrated Dataset

DEFRA .... Department for Environment, Food, and Rural Affairs

EPIC-Norfolk .... European Prospective Investigation into Cancer - Norfolk

FAO .... Food and Agriculture Organization

FBS................................. Food Balance Sheets

FCD .... Food Composition Database

FE-NP .... Foliar Iron Oxide (Fe_3o_4) Nanoparticles Spray Treatment

FLAV .... Epidermal Flavanols

GAE .... Gallic Acid Equivalents

GBP .... Great British Pounds

HCP................................ Household Vitamin C Provisions

LRNI .... Lower Reference Nutrient Intake

NBI .... Nitrogen Balance Index

NDNS .... National Diet and Nutrition Survey

NHANES .... National Health and Nutrition Examination Survey

NRV .... Nutrient Reference Value

RNI .... Recommended Nutrient Intake

SEL .... Foliar Sodium Selenate Spray Treatment

TEAC............................... TROLOX Equivalent Antioxidant Capacity

TPC................................. Total Phenolic Content

TSS .... Total Soluble Solids

TTA .... Theoretical Titratable Acidity

WHO............................... World Health Organization

Chapter 1 General Introduction: Vitamin C Security in the UK Food System

> *Rather as a Doctor should have full knowledge of an illness before trying to devise a cure, so too is investigating the causes of current problems essential if we are to suggest a way to deal with the crises now confronting us all.*
>
> *Peter Frankopan, The Earth Transformed - An Untold History (2024)*

1.1 Background

As we enter the year 2025, the task of sustainably nourishing the global human population represents an increasingly urgent, yet daunting task. In 2023, the Food and Agriculture Organization (FAO) estimated that 2.3 billion people, or 29% of the global population, were experiencing moderate to severe levels of food insecurity - the inability to have stable access to sufficient, safe and nutritious food to meet their dietary needs and preferences for an active and healthy life (FAO et al., 2024). The impact of food insecurity is characterised by what is often termed the “double burden” of malnutrition (Popkin et al., 2020). The “double burden” refers to the co-existence of both under-nourishment (hunger and wasting) and over-nourishment (overweight and obesity). An estimated 713-757 million people were affected by under-nourishment or hunger in 2023, an increase of 1.6% of the global population compared to 2019 (FAO et al., 2024). The prevalence of overweight and obesity has also increased sharply over the past decade, estimated to have affected nearly 2.5 billion adults and 390 million children in 2022 (FAO et al., 2024; WHO, 2024). The resulting health costs of malnutrition are also beginning to become apparent, with recent work by Afshin et al. (2019) estimating that 11 million deaths and 255 million disability-adjusted life-years were attributable to poor diet. Whilst the scale and impacts of over- and under-nutrition are striking, there has been growing concern about a third, less studied burden of malnutrition (Ingram, 2020).

It is often argued that relative to the amount of resources directed towards calorific demands, there has been a lack of action to ensure widespread access to food of sufficient nutritional quality (Ingram, 2020). It has been estimated that over 2 billion people around the world are deficient in key micronutrients (FAO et al., 2020). Essential micronutrients are those vitamins and minerals that the human body only requires in “micro” quantities - mg or even µg each day - but that can only be acquired through dietary intake (Shergill-Bonner, 2017). Micronutrients are integral components in a broad spectrum of physiochemical processes, examples of which include proper immune – iron (Fe^{2+})-dependent demethylation of the H3K9 gene promotes B-cell proliferation (Jiang et al., 2019); cognitive function - synaptic zinc (Zn^{2+}) in the amygdala has been linked to learning and memory (Sensi et al., 2011; Medina et al., 2015); tissue development and repair - vitamin C acts as a co-factor for prolyl-hydroxylases in the synthesis of collogen (Traber and Stevens, 2012); regulation of hormones and neurotransmitters - vitamin B6-dependent dopa-decarboxylase is critical to the production of serotonin, dopamine, and norepinephrine (Parra, Stahl, and Hellmann, 2018); and regulation of gene expression - vitamin B9, in the form of 5-methyltetrahydrofolate (5-MTHF), acts as a methyl donor to homocysteine to facilitate histone demethylation (Maynard et al., 2024).

Whilst humans are not able to synthesise micronutrients *de-novo*, metabolic pathways are active in modifying ingested micronutrients to convert them into readily absorbable and biologically active forms. For example, folate (vitamin B9) can be ingested in different forms, but is converted in the gut and absorbed as 5-MTHF (Patanwala et al., 2014). The cellular conversion of 5-MTHF to 10-formyltetrahydrofolate, however, enables folate to also act as a glycine and 1-carbon donor in the synthesis of purine (Baggot and Tamura, 2015). Beyond their complex metabolic regulation, the essential nature of micronutrients means that ensuring that diets provide sufficient micronutrients is imperative to meeting the aspirations of food security.

Even in affluent countries such as the United Kingdom (UK), malnutrition and micronutrient deficiencies are prevalent but are often overlooked due to the overall surplus food supply and lack of visible hunger (Mensink et al., 2013). In reality, financial and time poverty have made healthy diets unattainable for many who instead adopt diets with excessive calorific content and limited nutritional value in relation to modern dietary guidelines (Bates et al., 2020; Jones et al., 2014; Kirkpatrick and Tarasuk, 2008). Poor, unbalanced, and energy-dense diets are direct drivers of increasing health and economic costs arising from conditions such as obesity, diabetes, and cardiovascular disease (Muka et al., 2015; Whicher et al., 2020; Willett et al., 2019). The SARS-CoV-2 pandemic further stressed the importance of micronutrients in human immune health, whilst also highlighting the prevalence and associated health problems of nutrient deficiencies in the UK (Al-Sabah et al., 2020; McAuliffe et al., 2020; Richardson and Lovegrove, 2021). Understanding the capacity of modern food systems - a contemporary way of thinking about how food is produced, distributed, marketed, and consumed (Fanzo et al., 2021; Figure 1.1) - to provide sufficient dietary micronutrients is urgently required if societies are to effectively tackle the growing burden of disease (FAO et al., 2024).

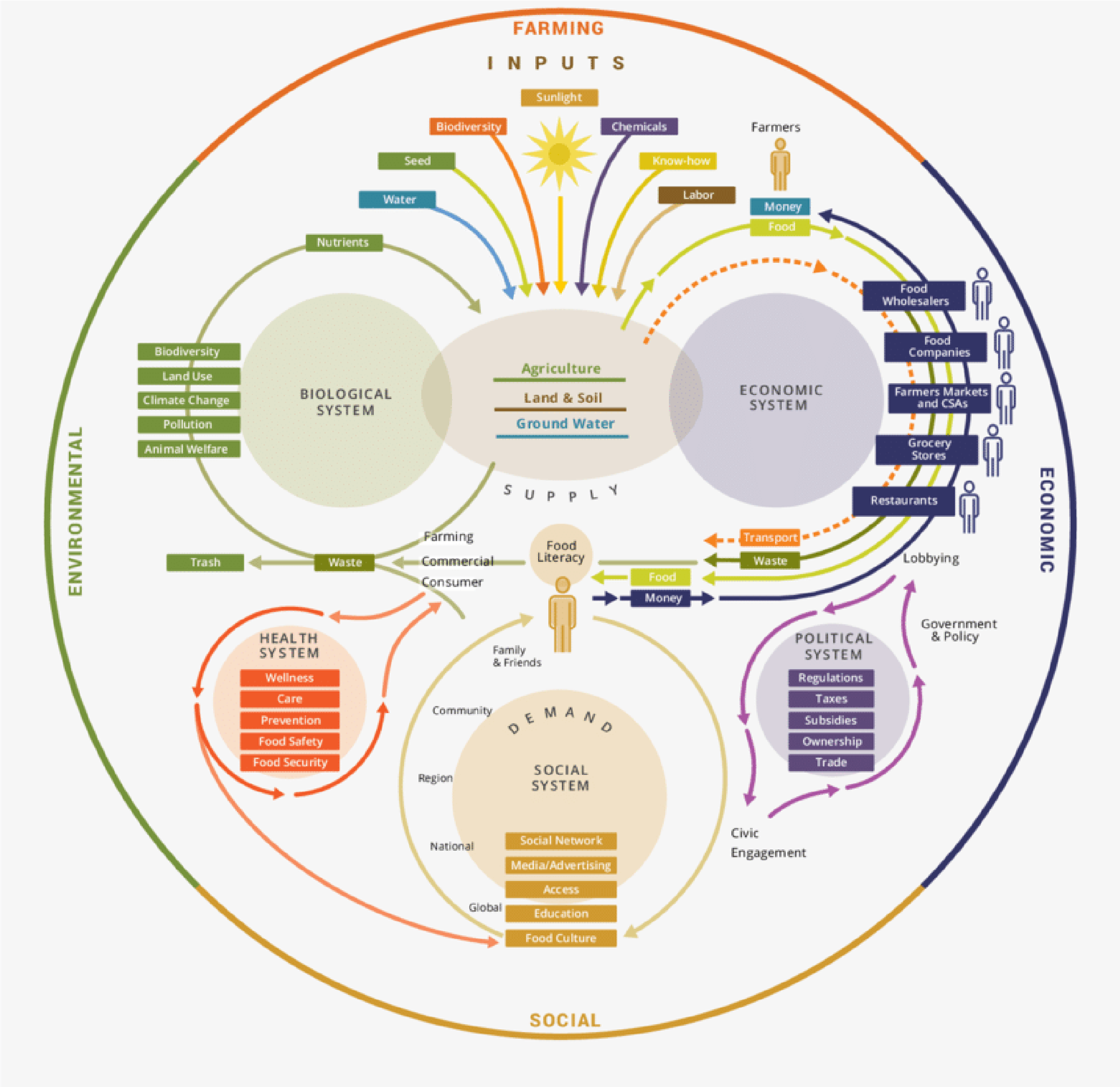


Figure 1.1 Concept map of a modern food system. The map highlights different inputs, processes, and outcomes of a food system (circles and labels) and how each element overlaps and connects with other elements (directional arrows). Figure originally created and published by Zhang et al. (2019).

Vitamin C is a micronutrient that has been shaping world history since long before its discovery in 1928 (Svirbely and Szent-Györgyi, 1932). Most famously, extended sea voyages during the age of exploration were blighted by a disease known as scurvy. Scurvy is a severe illness caused by the depletion of vitamin C stores, characterised by internal bleeding, deterioration of gums and skin, malformed hairs, confusion, fatigue and depression (Hodges et al., 1971). Famously, Sir George Anson’s circumnavigation of the globe in 1740 returned to England with just 10% of its initial 2000-strong crew, the vast majority of deaths having been attributed to scurvy (Kinlin and Weinstein, 2023; Bartholomew, 2002). Vitamin C is now known to function as a potent antioxidant in addition to acting as an enzymatic co-factor in the biosynthesis of norepinephrine, vasopressin, carnitine, and collagen, as well as in histone demethylation and regulation of DNA structure (Carr et al., 2015; Padayatty and Levine, 2016). Vitamin C has also been shown to play a key role in the absorption and metabolism of non-haem iron, which is of particular interest in a world where iron deficiencies are so common (Lane et al., 2013; Lane and Lawen, 2008; Lane and Richardson, 2014).

Vitamin C is not regarded as a micronutrient of high priority relative to other micronutrients such as iron, vitamin A, and zinc, for which the prevalence of deficiencies is much higher (Bailey et al., 2015; Bates et al., 2020; Han et al., 2022). Vitamin C intakes as low as 10 mg/day, equivalent to 25% of the UK recommended nutrient intake (RNI) or 1/4 of an easy peeler orange, have been suggested to be sufficient to prevent the onset of scurvy (Hodges et al., 1971; Public Health England, 2016). The current incidence of scurvy in the UK population is ~1/100,000 based on hospital admission data (NHS Digital, 2025). Despite this, there are reports of an increasing prevalence of scurvy in European populations in recent years (Assad et al., 2025; Bates et al., 2019). Assad et al. (2025) found that the number of scurvy cases in hospitalised children in France increased by 34% after the SARS-CoV-2 pandemic, affecting 0.12/100,00 children per month in 2024. In the UK context, Bates et al. (2019) found that the number of adult women with blood plasma vitamin C levels below the threshold associated with scurvy increased by 6% between 2008 and 2019. Notably, however, the rates of individuals with blood biomarkers below the severe deficiency threshold in other population groups were too low to perform statistical time-series analysis (Bates et al., 2019).

Whilst the prevalence of scurvy remains relatively low, the absence of clinical symptoms does not mean that an individual is not vitamin C deficient. Indeed, moderate vitamin C deficiencies manifest in a condition known as hypovitaminosis C, characterised by symptoms including fatigue, depression, irritability, and increased incidence and duration of infections (Hemilä, 2017; Rowe and Carr, 2020). Moderate vitamin C deficiencies have also been associated with increased risk of cardiovascular disease and specific cancers, lending credence to the importance of maintaining good vitamin C status to improve long-term health outcomes (Granger and Eck, 2018). Despite the negative health outcomes associated with hypovitaminosis C, no progress has been made in the UK toward understanding the prevalence or societal cost of moderate vitamin C deficiencies. All of this is to say, vitamin C security is a more complex case than a binary assessment of scurvy prevalence.

The necessary components required to make a full assessment of vitamin C security can be considered in the context of the four pillars of food security (FAO, 2008); how much vitamin C is available, how much of the available vitamin C is accessible, how stable is people’s access to sufficient vitamin C and, ultimately, how much of the available vitamin C is utilised? No single study has examined UK vitamin C security in this scope, meaning that an informed and concerted assessment has not yet been made. As such, this review will begin by examining the case of UK vitamin C security in relation to the four pillars: drawing attention to strengths, weaknesses, and gaps in our current understanding of the UK food system's capacity to support a state of vitamin C security.

1.2 The Current State of Vitamin C Security in the UK

1.2.1 Availability

The first pillar supporting the security of any nutrient, availability, refers to whether nutrient supplies within a food system are sufficient to support population requirements. Recent work by Macdiarmid et al. (2018) pioneered a method that enabled the FAO's food balance sheets (FBS) to be converted from food supply quantities to micronutrient quantities. The conversion subsequently enabled Macdiarmid et al. (2018) to estimate the quantity and sources of the UK's micronutrient supplies and how they have changed over time.

Concerning vitamin C, the analysis revealed that total supplies had increased between 1961 and 2010, from 90 to 150 mg/capita/day (Macdiarmid et al., 2018). The availability of 150 mg/capita/day in 2010 was equivalent to 375% of the UK RNI of 40 mg/capita/day (Macdiarmid et al., 2018). The availability of vitamin C, therefore, was in substantial excess relative to the proposed UK requirements. Poppy et al. (2022) built upon the analysis of Macdiarmid et al. (2018), using the same methodology to extend the time series up to 2016. Despite a fall in vitamin C availability of ~30 mg/capita/day between 2010 and 2016, vitamin C availability in 2016 was still equivalent to 300% of the UK's requirements (Poppy et al., 2022).

Both studies noted similar patterns in the UK's increasing reliance on imported fruits and vegetables as its primary source of vitamin C. Poppy et al. (2022) showed that 85% of the UK's available vitamin C came from fresh fruits and vegetables in 2016. Poppy et al. (2022) also found that domestic production of vitamin C decreased from 54 mg/capita/day in 1961 (135% of population requirements) to 37 mg/capita/day in 2016 (93% of population requirements). Simultaneously, the contribution of imported fruits and vegetables had increased from 36 mg/capita/day in 1961 (90% of population requirements) to 72 mg/capita/day in 2016 (180% of population requirements) (Poppy et al., 2022; Figure 1.2).

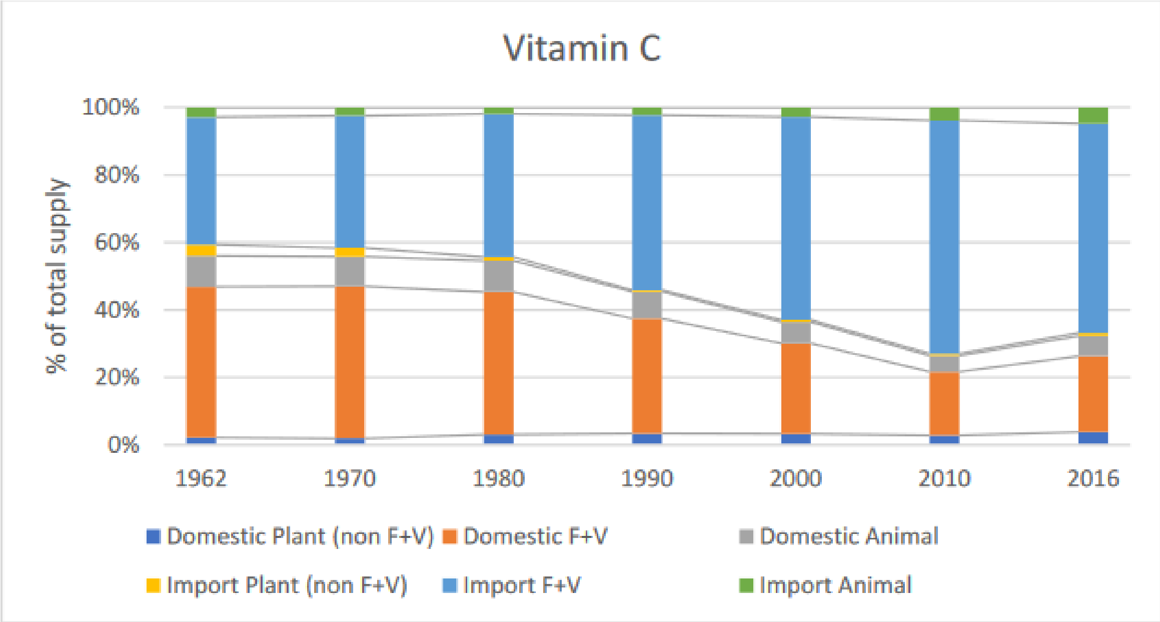


Figure 1.2 Stacked bar graph illustrating changes in the contribution of domestic produce and imports of different food sources towards UK vitamin C supplies between 1962 and 2016. The figure has been reproduced from Poppy et al. (2022).

The increase in the contribution of imported fruits and vegetables to vitamin C supplies is consistent with the changing preferences of UK consumers toward more exotic fruits and vegetables (Scheelbeek et al., 2020). Indeed, Poppy et al. (2022) conducted further analyses of HMRC trade data (Food Standards Agency, 2024; HMRC, 2022) to break down the FBS “fruit and vegetable” category into individual commodities. They identified the top 10 imported fruits and vegetables contributing to UK vitamin C supplies, finding that bananas, apples, oranges, and tomatoes made up 51% of total vitamin C supplies (Poppy et al., 2022). Imported bananas alone made up 24% of total vitamin C supplies or 44% of the UK population’s requirements (Poppy et al., 2022).

The accuracy of the supply quantities presented by Macdiarmid et al. (2018) and Poppy et al. (2022) is largely dependent on the accuracy of the food composition data used to convert quantities of foods to quantities of micronutrients (Matthews, 2022). Both Macdiarmid et al. (2018) and Poppy et al. (2022) utilised the Composition of Foods Integrated Dataset (CoFID) to convert supply volumes - kilograms or tonnes of food - into quantities of vitamin C (Public Health England, 2015; Roe et al., 2015). CoFID is a particularly good food composition database, especially for UK-focused analyses, due to the comprehensive nutrient analysis that has been conducted for 2890 foods commonly consumed in the UK (Roe et al., 2015; Traka et al., 2020). There are, however, some key limitations to be considered when working with the

CoFID, mainly relating to the often-limited scale of sampling for each food commodity (Roe et al., 2015). The reference values for bananas, for example, are based on just 11 bunches of bananas collected in November 2011. Whilst the UK has remained reliant on Cavendish bananas since 2011, the CoFID sampling does not account for seasonal differences in nutritional composition, or changes related to climate and farming practices since 2011 (Phillips et al., 2018). For other commodities where multiple varieties are available to consumers, such as strawberries or tomatoes, limited seasonal sampling could also mask variations arising from genotypic differences (Phillips et al., 2018). These examples of under-sampling in key commodities could limit the accuracy and reliability of CoFID reference values and should be considered when scaling them to vast quantities of food supplies (McLaren et al., 2021; Roe et al., 2015).

A key conclusion of both studies is that, concerningly, over time, the UK's self-sufficiency for maintaining sufficient availability of vitamin C has declined and is now more reliant on imports. The UK's current reliance on imported fruits and vegetables makes the UK food system more susceptible to supply chain shocks that could threaten the stability of the UK's vitamin C supply. In 2013, for example, an estimated 32% of the UK's fruits and vegetables originated from regions vulnerable to climate shocks (Scheelbeek et al., 2020). Poppy et al. (2022) similarly showed that, in 2017, fruit and vegetable imports from climate vulnerable regions (including Spain, Ecuador, The Dominican Republic, Costa Rica, Columbia, and South Africa) contributed 45% of the UK's total imported vitamin C. Beyond climatic concerns, *Fusarium* wilt, caused by the pathogen *Fusarium oxysporum* F., is continuing to spread rapidly and devastate banana crops around the world (Ploetz, 2015). Recent evidence also suggests that interspecific gene transfer is leading to increased pathogenicity in other *Fusarium* species that cause other diseases such as banana leaf blight (Cui et al., 2021). As a dominant source of UK vitamin C, future disruptions to the production and trade of bananas could introduce further instability for UK vitamin C supplies. However, whilst supply chain shocks represent a considerable conceptual threat to UK vitamin C availability, Poppy et al. (2022) propose that the large surplus of vitamin C, relative to population requirements, means that UK vitamin C supplies can still be considered secure.

1.2.2 Accessibility

Beyond the scope of the work of Macdiarmid et al. (2018) and Poppy et al. (2022), information as to the accessibility and distribution of UK vitamin C supplies is currently limited. It is, however, possible to make some inferences about access to vitamin C from broader studies of UK food security.

As previously discussed, the work of Macdiarmid et al. (2018) and Poppy et al. (2022) provides an assessment of available vitamin C up to and including 2016. Since 2016, the UK food system has weathered a series of unprecedented challenges; international trading relationships have been re-formed as part of Brexit negotiations, persistent political unrest, food supply disruptions due to climate shocks, the SARS-CoV-2 pandemic, wars in Eastern Europe and the Levant, and the subsequent cost-of-living crisis which followed (Michael Archer, 2024).

The whirlwind of events in the past eight years has had a marked impact on the number of food-insecure households in the UK (Broadbent et al., 2023; Pörtner et al., 2022; The Food Foundation, 2020, 2017). Koltai et al. (2021) found that the number of people reporting difficulty accessing nutritious food rose by 13% at the start of the SARS-CoV-2 pandemic, an increase which has been attributed to a combination of inconsistent availability of food due to supply chain disruptions and stockpiling, isolation policies preventing people getting to food outlets, and increasing financial inaccessibility (The Food Foundation, 2021; Brown et al., 2022). More recent work by The Food Foundation identified 9 million people who reported experiencing household food insecurity in 2023, with increasing socioeconomic hardships again being identified as a key driver (The Food Foundation, 2023). Concurrently, purchases of fruit decreased by 57% and purchases of vegetables decreased by 42% in food-insecure households (The Food Foundation, 2023). Given that fruits and vegetables have been found to comprise at least 85% of UK vitamin C supplies, current trends in household food insecurity could well be having an adverse impact on people’s ability to access sufficient vitamin C. To directly study food insecurities in the context of access to vitamin C, it will be important to find datasets that enable up-to-date assessments of the amount of vitamin C that is reaching UK households.

Another important consideration is that contemporary assessments of vitamin C security have been made at the level of national supplies at annual intervals. Regional, sub-regional, and even communal hotspots of food insecurity have previously been observed in the UK (The Food Foundation, 2023). Furthermore, fruits and vegetables also follow seasonal patterns in both production and consumer preference (Spence, 2021; Toorang et al., 2013). What this means is that macro-scale analyses run the risk of masking regional disparities and seasonal instabilities that could limit an individual’s access to vitamin C. To assume that vitamin C supplies remain stable throughout the year and are equitably distributed across the population seemingly represents potential oversights that require further investigation.

1.2.3 Utilisation

1.2.3.1 Vitamin C Status in the UK Population

The fourth pillar of food security, utilisation, refers to the understanding of how nutrient supplies and intakes ultimately translate into biological status. The state of vitamin C intakes and status in the UK population has previously been investigated using national cohort studies. Two of the key diet-related cohort studies in the UK are the National Diet and Nutrition Survey (NDNS) (Bates et al., 2020, 2019) and the European Prospective Investigation of Cancer - Norfolk (EPIC-Norfolk) (Day et al., 1999).

The NDNS is an ongoing programme that began in 2008 and has collected dietary intake and nutritional biomarker data from a combined cohort of 15465 people. Participants join and leave the survey on a rolling basis but are selected to maintain a cohort that is representative of the UK's national demography. The EPIC-Norfolk cohort was studied between 1993 and 1997 and comprised 22277 British men and women living in Norfolk. Whilst larger, the EPIC-Norfolk cohort only includes adults aged between 45-75 years. Considering also that the cohort was studied nearly 30 years ago, the EPIC-Norfolk cohort is considerably less representative of the UK's demography than the NDNS. The amount of data collected, however, makes EPIC-Norfolk a particularly useful study to investigate associations between dietary intakes and nutritional status.

The NDNS and EPIC-Norfolk utilised food recall diaries and food frequency questionnaires, respectively, to estimate the quantities and frequencies of food intakes. Intake data in such studies are, therefore, subject to inaccuracies from recall errors and selective reporting but this is considered a necessary trade-off when collecting human data at such a scale (Henríquez-Sánchez et al., 2009). The reported foods consumed are converted to vitamin C intakes using food composition data in the same manner as already discussed in this review (Section 1.2.1).

Vitamin C status is reported based on plasma ascorbic acid (PAA) concentrations, which have been ratified as an effective biomarker of overall vitamin C stores in the body (European Food Safety Authority, 2013). The following PAA concentration thresholds will be considered for this review; <11 μM - severe deficiency; 11-22.9 μM - hypovitaminosis C; 23-49.9 μM - inadequate; >50 μM - adequate (European Food Standards Agency, 2013; German Nutrition Society, 2015; Rowe and Carr, 2020). Adequate vitamin C status associated with PAA >50 μM has been further supported by recent evidence that 50 μM is the renal threshold for vitamin C under normal physiological conditions, indicating that circulating vitamin C is surplus to requirement at this plasma concentration (Ebenuwa et al., 2022).

At the population level, reporting on vitamin C intakes and status from the NDNS is limited, if not completely excluded, with more emphasis placed on nutrients for which there is a greater prevalence of severe deficiencies (Bates et al., 2020). The most recent information in official publications suggested that the mean PAA was “well above” the threshold for severe vitamin C deficiency of <11 μM; (Bates et al., 2019). As previously discussed, however, the same report did note an increase of 6% in the prevalence of adult women with PAA <11 μM between 2008 and 2017 (Bates et al., 2019). The overall prevalence of vitamin C deficiencies in the UK population was estimated to be around 10% in 2017 (Bates et al., 2019).

There have been an extensive number of studies on the vitamin C status of the EPIC-Norfolk cohort and how it relates to chronic illnesses and other morbidities. As a baseline, the median vitamin C intake of the EPIC-Norfolk cohort has been reported as 76 mg/day and the median PAA as 53 μM (Carr et al., 2023). Interestingly, Carr et al. (2023) also found that 65 mg/day was the intake requirement for 50% of the EPIC-Norfolk cohort to reach an adequate vitamin C status, which is 62.5% greater than the current UK RNI.

Similarly to studies on vitamin C supplies then, vitamin C intakes and biological status appear to be sufficient to support the conclusion that the UK is vitamin C secure. However, it is again in assessing the limitations of those studies and understanding how well they represent the vitamin C status of various sub-populations that insecurities begin to become apparent.

1.2.3.2 Vitamin C Status in Relation to Health, Lifestyle, and Social Factors

In a study of 1018 men and women from south London, Ness et al. (1999) observed a mean PAA of 45.9 and 36.0 μM in women and men, respectively. The reported PAA values were 13% and 15% lower than those observed for women and men in the EPIC-Norfolk cohort and are within the inadequate range (Carr et al., 2024; Ness et al., 1999). The lower vitamin C status of men compared to women has since been widely reported and is thought to be due to increased body weight (Carr et al., 2022; Carr and Lykkesfeldt, 2023a). That said, awareness of the increased requirements of men is not reflected in current UK dietary recommendations and more needs to be done to ensure that men can access a diet that fulfils their vitamin C requirements.

Ness et al. (1999) also observed that South Asian and Black African-Caribbean participants had PAA values as much as 30% lower than in White participants living in London, England - 52.4 μM vs 37.3 μM in White and South Asian women, respectively. Similar differences have been observed in the PAA of White, Black African-Caribbean, and South Asian children in the UK (Donin et al., 2016). Such differences indicate that conclusions about national vitamin C status must be made using populations that represent the UK’s broad cultural diversity (Ness et al., 1999). Indeed, given that the study by Ness et al. (1999) was conducted at a similar time to the

EPIC-Norfolk study, the differences in PAA between the two populations are reflective of the fact that geography, and its associated demography, likely influences vitamin C status within sub-populations.

Donin et al. (2016) also highlighted the relationship between socioeconomic status and PAA, with children of parents in managerial occupations having the highest PAA. Earlier work using the low-income diet and nutrition survey (LIDNS; Tedstone, 2008) found that 25% of men (n = 433) and 16% of women (n = 876) from low-income households had PAA <11 μM (Mosdøl et al., 2008). The proportions of men and women with PAA indicative of hypovitaminosis C or vitamin C inadequacy were 46.3% and 34.6% respectively (Mosdøl et al., 2008). Whilst the LIDNS is a smaller survey compared to NDNS or EPIC-Norfolk, the importance of socio-economic status has also been identified as a predictor of PAA in the EPIC-Norfolk cohort (Shohaimi et al., 2004). As previously discussed, economic hardships are increasingly prevalent in the UK in the wake of the SARS-CoV-2 pandemic and subsequent cost of living crisis (Section 1.2.2; Broadbent et al., 2023; Koltai et al., 2021). Facilitating low-income households to access healthy foods, and by extension vitamin C, therefore remains a pressing issue.

In a series of recent publications, the impact of body weight and body mass index (BMI) on vitamin C requirements has been investigated using the US National Health and Nutrition Examination Survey (NHANES) and the EPIC-Norfolk cohorts (Carr et al., 2024, 2022; Carr and Lykkesfeldt, 2023a). The research, led by Anitra Carr, has shown strong inverse correlations between body weight and PAA. It has been estimated that relative to a healthy individual weighing 70 Kg, every 10 Kg increase in body weight requires an extra 20 mg/day to be consumed to maintain adequate vitamin C status (Carr et al., 2024, 2022)

The number of overweight and obese adults in the UK has increased from 53% in 1993 to 64% in 2022 (Stiebahl, 2025). Evidence suggests that rates of overweight and obesity are higher in more deprived parts of the wider population, exposing these populations to a double threat of increasing vitamin C requirements and decreasing access to vitamin C (Burgoine et al., 2017; Cronin et al., 2022; Stamatakis et al., 2010).

Vitamin C status has also been found to be worse in type-2 diabetics than in healthy individuals (Carr et al., 2023; Harding et al., 2008). Harding et al. (2008) found that the PAA of individuals in the EPIC-Norfolk cohort who developed type-2 diabetes during the study was 43 μM (inadequate) compared to 54 μM (adequate) in individuals who did not develop diabetes. Moreover, individuals in the top quintile for PAA were 62% less likely to develop diabetes than individuals in the bottom quintile (Harding et al., 2008). Inverse correlations between PAA and insulin resistance have also been observed in other cohorts of adults and young children (Donin et al., 2016; Wilson et al., 2017). It is also suggested that type-2 diabetes confers increased vitamin C requirements due to a combination of oxidative stress, inhibition of vitamin C absorption through glucose transporters, and increased incidence of renal leakages (Wilson et al., 2017; Ebenuwa., 2022).

Whicher et al. (2020) recently outlined the looming health crisis that type-2 diabetes presents in the UK. Incidences of type-2 diabetes have doubled since 2000 to now affect an estimated 13 million people (Whicher et al., 2020). Sub-populations including individuals of South Asian and Black African-Caribbean ethnicity, and individuals who are overweight or obese, are at increased risk of developing type-2 diabetes (Whicher et al., 2020). As already discussed, vitamin C status in these population groups is commonly inadequate and appears to further correlate with the development of type-2 diabetes, a disease which also confers increased biological requirements (Donin et al., 2016; Wilson et al., 2017; Ebenuwa et al., 2022).

Further factors that increase an individual's vitamin C requirements, and have been reviewed in detail elsewhere, include old age and smoking status (Bates et al., 1999; Carr and Zawari, 2023). Within the population studies of PAA discussed thus far, regardless of size or demographic background, population groups for whom vitamin C status is inadequate have been identified. The presented evidence shows that cultural and socioeconomic factors can limit an individual's access to vitamin C-rich foods, subsequently increasing their risk of inadequate vitamin C status. Furthermore, there is evidence that selected health and lifestyle factors can directly limit an individual's utilisation of dietary vitamin C, similarly increasing their risk of poor status. As such, there remain several factors that limit the efficiency with which available vitamin C supplies translate into accessible vitamin C intakes, and finally into utilised body stores of vitamin C. Thus, to conclude that the UK is vitamin C secure based on supplies alone is insufficient.

1.2.4 UK Dietary Recommendations

The thresholds by which security has been judged represent a further overlooked element of contemporary assessments of UK vitamin C security. Concerning vitamin C recommendations, the UK is one of only two countries in the world to adopt an RNI of 40 mg/day which, coincidentally, is also the lowest RNI published by any regional authority (Carr and Lykkesfeldt, 2021).

Here it is important to reaffirm that food security aspires to facilitate individuals to live "a healthy life", not merely to avoid disease. Many countries have re-evaluated vitamin C status within their population and have subsequently adopted substantially higher RNI's to support positive health aspirations. The EU, for example, use an RNI of 110g mg/day for men and 95 mg/day for women (European Food Standards Agency, 2013). Germany, Austria, and

Switzerland have published further guidance for smokers (155 mg/day - men, 135 mg/day - women) and pregnant or lactating women (105 mg/day - pregnant, 125 mg/day - lactating) (German Nutrition Society, 2015). The US, China, Japan, and Nordic countries have similarly re-evaluated their RNI's such that they are between 70 mg/day to 110 mg/day (Carr and Lykkesfeldt, 2021; Table 1.1). The Japanese recommendation also explicitly states that the RNI's have been determined "*from cardiovascular disease prevention effects and antioxidative effects, not from intake sufficient enough to avoid scurvy*." (Ministry of Health and Welfare, 2019). In contrast to the contemporary positive health aspirations of other countries, the current UK RNI remains at a level designed to prevent deficiencies (Department of Health, 1991).

Table 1.1 Recommended Nutrient Intakes (RNI) for vitamin C from selected global authorities.

Country / Authority	RNI – Men (mg/day)	RNI – Women (mg/day)
European Union[1,2]	110	95
Japan[3]	100	100
United States[4]	90	75
Denmark, Finland, Iceland, Norway, Sweden[5]	75	75
United Kingdom[6]	40	40

[1] European Food Standards Agency (2013)
[2] German Nutrition Society (2015)
[3] Ministry of Health, Labour and Welfare (2019)
[4] Institute of Medicine (2000)
[5] Nordic Council of Ministers (2014)
[6] Department of Health (1991)

The UK's adoption of a 40 mg/day RNI was partly based on evidence derived from depletion studies that were conducted in white males during the 20th century (Department of Health, 1991). Studies referenced in the Department of Health report indicated that 10 mg/day of vitamin C was sufficient to prevent clinical signs of scurvy (Krebs, 1953). The Lower Recommended Nutrient Intake (LRNI) was, therefore, set at 10 mg/day. The panel also examined dose-response curves that indicated 40 mg/day was the intake at which most people exhibited measurable concentrations of PAA. Circulating PAA was considered as being available to transfer between tissues and support necessary biochemical functions and, thus, was a suitable RNI that would conservatively mitigate the prevalence of scurvy (Department of Health). The presence of circulating vitamin C was also seen as an indication that an RNI of 40 mg/day was sufficient to overcome any potential limitations related to bioavailability of vitamin

C. Vitamin C's bioavailability is often assumed to be more than 80-90% (Melse-Boonastra, 2020; Lykkesfeldt & Tveden-Nyborg, 2019; European Food Standards Agency, 2013), but research into whether this assumption is true in varied food matrices or in different gut environments is currently lacking (Andaç Öztürk & Yaman, 2022).

How a vitamin C intake of 40 mg/day relates to contemporary PAA thresholds and biological status has not been examined in detail. Carr et al. (2024) did, however, show that only 35% of the EPIC-Norfolk cohort could achieve adequate vitamin C status with an intake of 40 mg/day. As previously discussed, the average required intake to reach adequate PAA was 65 mg/day, whereas an intake of 100 mg/day, in-accordance with the more aspirational RNI used in the EU, would have been sufficient for 70-75% of the cohort to achieve adequate PAA. Having contextualised the UK's RNI, it is seemingly the case that 40 mg/day may be effective to prevent scurvy but is unlikely to support good health for most individuals. Based on the global perspective on vitamin C requirements, higher and more granular recommendations for different population groups may be necessary to support the broader aspirations of food security. It will also be important to re-consider the security of UK vitamin C availability in the context of potentially increased population requirements.

1.3 The Importance of Local Fruits and Vegetables in Securing Future UK Vitamin C Supplies

Due to the myriad of interacting biological, economic, social, and political factors at play within modern food systems (Fanzo et al., 2021), there will be no single action capable of addressing all of the potential vitamin C insecurities discussed in this review. As is commonly discussed in relation to food systems, an interdisciplinary problem requires interdisciplinary solutions (Poppy and Baverstock, 2019). Rather, what will be required is the development of smaller, modular interventions within relevant disciplines that can work harmoniously toward the aspiration of a vitamin C-secure UK food system.

Having explored the current understanding of UK vitamin C security in the context of the four pillars, conceptual threats to the availability and stability of vitamin C supplies have been the most well-established. As discussed in section 1.2.1, the UK is increasingly reliant on imported fruits and vegetables to meet the vitamin C demands of its population (Macdiarmid et al., 2018; Poppy et al., 2022). To mitigate the potential impacts of disruptions in international trade, increasing domestic production of vitamin C would appear to be an intuitive strategy to increase the security of the UK's vitamin C supplies.

From an agricultural perspective, two potential approaches to increasing domestic vitamin C production are to: 1) increase the overall production of vitamin C-dense crops; and 2) increase the amount of vitamin C in crops for which the UK is already highly self-sufficient. Conceptually, both approaches would lead to increases in domestic vitamin C production, either on their own or in combination with each other. The effectiveness of any such approach would, however, be dependent on the crop to which it was applied.

1.4 Strawberries as a Case for Increasing Domestic Vitamin C Production

Strawberry (*Fragaria x ananassa* Duch.) is already one of the most widely consumed fruits in the UK. The Department for Environment and Rural Affairs (DEFRA) horticultural statistics show that strawberry is the most valuable horticultural crop grown in the UK, valued at an estimated £421 million in 2023 (DEFRA, 2023). For perspective, apple, pear, and plum production combined was worth an estimated £293 million in 2023 (DEFRA, 2023). UK consumption of strawberries was estimated to exceed 168,000 tonnes in 2015, of which 68% were produced in the UK (Pelham, 2017). DEFRA statistics also show that self-sufficiency has remained stable between 64-75% since 2015 (DEFRA, 2023; The Food Foundation, 2017). There is, however, a lack of reliable evidence to confirm whether the popularity of strawberries is equally common across different social and cultural groups.

Strawberries contain a diverse array of health-promoting compounds, the principal among which are vitamin C and folate - vitamin B9 (Giampieri et al., 2015). CoFID reference values indicate that an 80g serving of strawberries (around 4-5 average-sized berries) would provide 46 mg of vitamin C (115% of RNI) and 48.8 µg of folate (24.4% of RNI) (Public Health England, 2016, 2015). Furthermore, the vitamin C content in strawberries exceeds that of all other fruits within the top 10 imported contributors to vitamin C supplies presented by Poppy et al. (2022). Compared to commonly cited sources of vitamin C such as oranges and pineapples, strawberries have 1.5 and 2.5 times more vitamin C per serving, respectively (Public Health England, 2015; Figure 1.3).

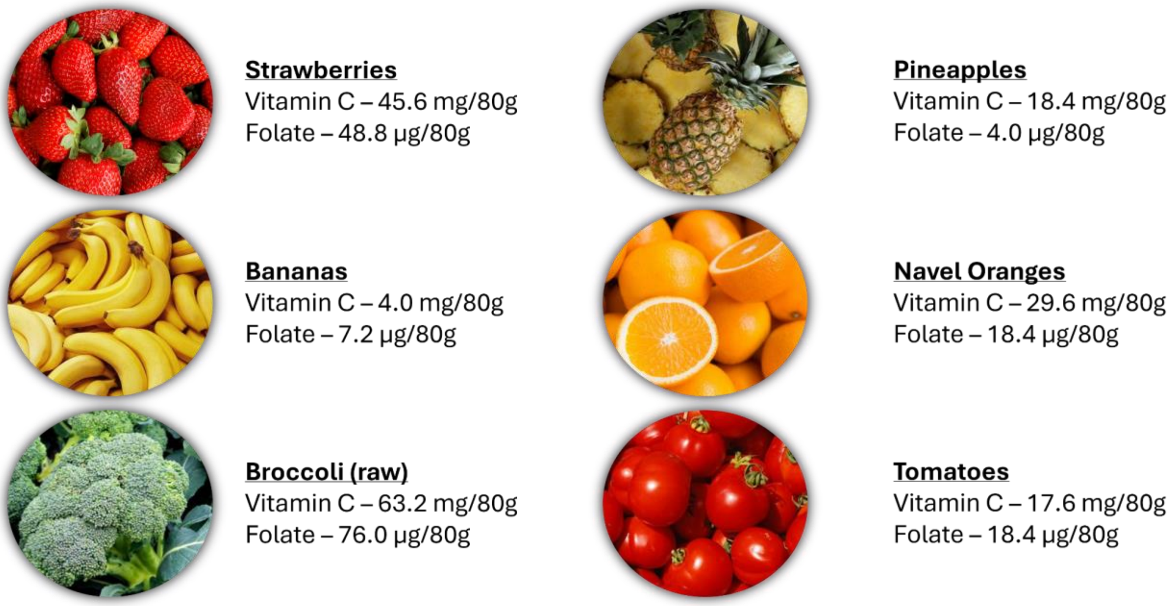


Figure 1.3 Pictorial representation of the vitamin C and folate content of strawberries in relation to five other popular fruits and vegetables. The five other fruits and vegetables were selected from the top 10 contributors to UK vitamin C imports, as identified by Poppy et al. (2022). Nutrient values are presented as mg (vitamin C) or µg (folate) per a standard 80-gram portion.

Broccoli was the only vegetable in Poppy et al.'s (2022) top 10 that exceeded the vitamin C density of strawberries, with a reported 63 mg/serving (Public Health England, 2015; Figure 1.3). A key consideration between strawberries and broccoli, however, is that strawberries are most often consumed fresh whereas broccoli is commonly cooked. Cooking can have detrimental impacts on vitamin C content, with multiple studies reporting a reduction of ~50% in broccoli's vitamin C content when boiled due to vitamin C's sensitivity to heat and leeching when cooked in water (Lee et al., 2018; Public Health England, 2015). Greater retention of vitamin C can be achieved using other cooking methods, including steaming and microwaving. Indeed, Lee et al. (2022) found that the vitamin C content of steamed and microwaved broccoli samples were 11% and 13% greater than in their fresh samples, attributed to lower cooking temperatures, shorter cooking time, and reduced contact with water. Despite some cooking methods being better than others in relation to vitamin C retention in broccoli, it remains unclear what proportion of broccoli is cooked using different methods within UK households, necessitating several assumptions to be made when considering broccoli's contribution to vitamin C supplies and intakes. Not needing to be cooked, therefore, further benefits strawberries as a domestic source of vitamin C.

Strawberries also contain a diverse array of non-essential phytochemicals, primarily composed of flavonoids, anthocyanins, tannins and phenolic acids (Giampieri et al., 2015). Whilst not an essential component of human diets, secondary plant metabolites such as those that are abundant in strawberries are known to exert positive health effects (Afrin et al., 2016). Indeed, vitamin C and other potent antioxidants present in strawberries have been identified as having cardioprotective, neuroprotective, anti-inflammatory, anti-diabetic, anti-carcinogenic and obesity-suppressive effects (see Afrin et al. (2016) for review). The biochemical mechanisms through which strawberry phytochemicals exert these positive health effects are compound dependent, but include neutralising reactive oxygen species, facilitating improved insulin signalling, and reducing inflammatory signalling molecules such as interleukin-6 and C-reactive protein (Afrin et al., 2016), Such secondary metabolites have also been associated with positive health responses to respiratory infections such as SARS-CoV-2, again attributed to their role in T-cell function and reduction of pro-oxidants (McAuliffe et al., 2020).

In terms of vitamin C density, self-sufficiency, market value and popularity, strawberries exhibit the necessary qualities for increasing domestic vitamin C production. As will be discussed in subsequent sections, in strawberry there is also the conceptual room for both increases in domestic production as well as increases in nutrient density. As such, strawberries have been selected as a case study to explore agricultural approaches to improving UK vitamin C self-sufficiency.

1.4.1 Strawberry Production in the UK

In a recent report, The Food Foundation considered strawberry as a "Channel Hopper" - a crop for which climatic conditions limit year-round production and so rely on European imports in the off-season (The Food Foundation, 2017). In contrast to this assessment, there is a growing body of research and development targeting the year-round production of UK strawberries (Pelham, 2017). Even as recently as the early 1990s, the UK strawberry season covered just six weeks during June and July (Calleja et al., 2012). With the advent of protected cropping systems, the UK strawberry season has extended and now runs from March to November. Moreover, in contrast to traditional June-bearing strawberry varieties which set a single flush of flowers in the autumn that mature in the following summer, many new strawberry varieties are everbearers, meaning that they continually produce new flowers and fruit providing the environment is warm and light enough. The introduction of everbearers and breeding efforts targeted at increasing crop yields has led to an increase in overall productivity in UK strawberries (Calleja et al., 2012). Together, novel farming methods and increasingly productive varieties have led to an increase in UK strawberry production by 243% between 1999 and 2019, reaching 144 thousand tonnes with an estimated £357million to the UK economy (Calleja et al., 2012; DEFRA, 2023; Figure 1.4).

Since 2019, there has been a moderate regression in domestic strawberry production. DEFRA statistics show that strawberry production in 2023 was 26% lower than in 2019 (DEFRA, 2023;

Figure 1.4). The value of UK strawberries has, however, increased over the same period (DEFRA, 2023). The decrease in local production could be a result of increased costs for recruiting seasonal labourers from Europe post-Brexit (Pelham, 2017; The Food Foundation, 2017), and increased energy and fertiliser costs since the start of the war in Ukraine (Sutherland et al., 2022). These challenges were reaffirmed in a recent survey of UK strawberry growers, that reported farmers planning to reduce horticultural production as returns from supermarkets were insufficient to cover the increased costs of production (British Berry Growers, 2023). There is, therefore, uncertainty regarding further growth in UK strawberry production in future years. As such, efforts to increase the vitamin C density of UK strawberries may be of more immediate impact than increases in overall production.

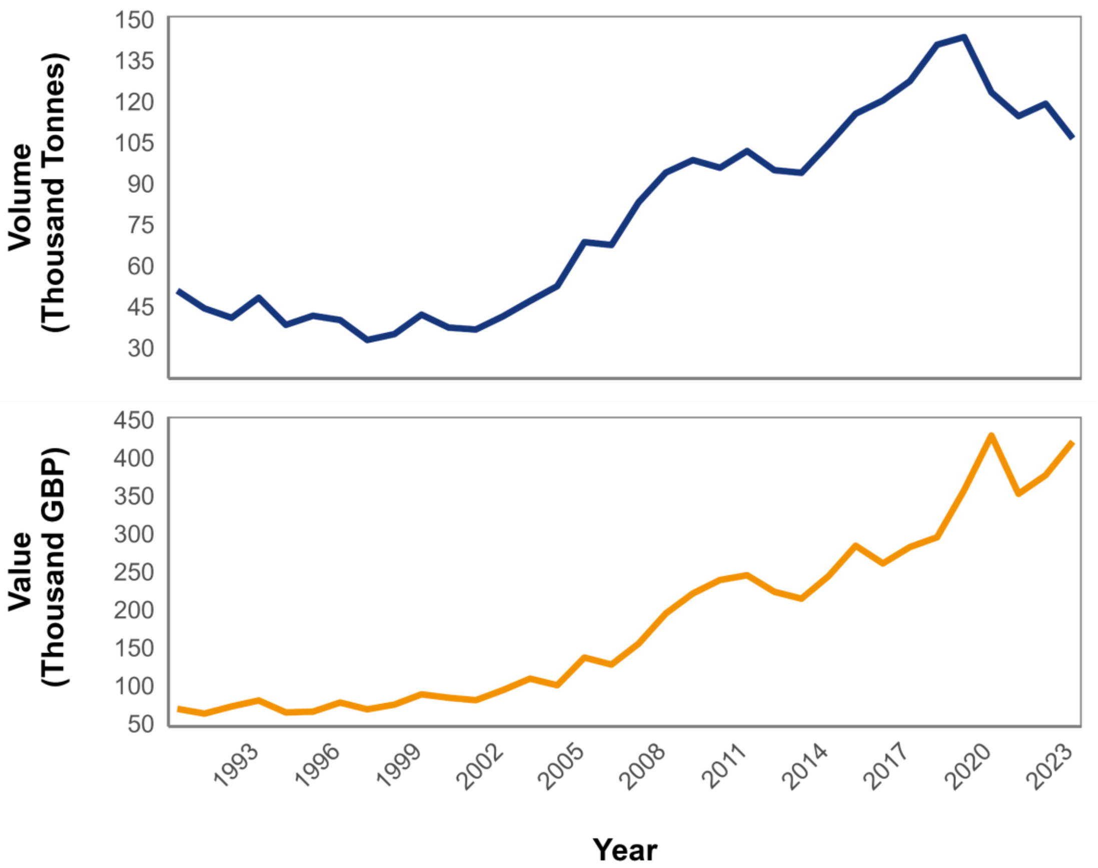


Figure 1.4 Time-series plots illustrating the growth of UK strawberry production between 1990 and 2023. The top panel illustrates changes in the volume of strawberries produced in the UK over time (blue line). The bottom panel illustrates changes in the market value of strawberries produced in the UK over time (Orange line). The plots are a visual representation of the data available from DEFRA (2023).

That said, advances in strawberry growing systems are driving an increased capacity for year-round domestic production of strawberries (Van Delm et al., 2016). Off-season production of strawberries is still, however, very much in the research and development phase (Madhavi et al., 2023). Between 2010 and 2014, the average cost of locally produced strawberries in Belgium was €7.2/kg in February (Van Delm et al., 2016). By comparison, the cost of Belgian-grown strawberries in July was only €2.3/kg (Van Delm et al., 2016). Van Delm et al. (2016) also showed that average strawberry production in January and February over the same period amounted to just 32 tonnes, compared with the 9532 tonnes produced in May (Van Delm et al., 2016). The establishment of the necessary infrastructure and the energy required to power the facilities are undoubtedly driving the higher seasonal costs (Adams et al., 2021). As a result, large-scale domestic production of off-season strawberries is not likely to impact Winter consumption of vitamin C until prices can be reduced. As technologies continue to develop, however, increased strawberry production and self-sufficiency in the UK seems like an inevitability, rather than an impossibility.

1.4.2 Opportunities to Biofortify UK Strawberries

Increasing the amount of vitamin C in UK-grown strawberries offers a complementary and direct approach to increasing domestic vitamin C production. The process of enhancing the nutritional quality of food crops, known as biofortification, can be achieved through several approaches that will be discussed in the subsequent sections of this review.

1.4.2.1 Conventional Breeding

Classical plant breeding is perhaps the most conventional method currently discussed concerning crop biofortification. Plant breeding programmes rely on the presence of heritable phenotypic variation in a targeted trait. In the context of biofortifying strawberries with enhanced vitamin C, a conventional breeding approach would, therefore, require knowledge of variable accumulation of vitamin C in the fruits of different genotypes.

Genotypic variation within the Marche Polytechnic University strawberry breeding program (Ancona, Italy) has been shown to account for as much as 3-fold differences in vitamin C content (~30 mg/100g - lowest, ~90 mg/100g - highest; Figure 1.5) (Mezzetti et al., 2016). In a Korean study, the vitamin C content of 14 genotypes varied between 57 mg/100g and 108 mg/100g (Kim et al., 2015). Such levels of variation in vitamin C content and other nutritional traits are consistent across the literature (Giampieri et al., 2015; Pincemail et al., 2012; Tulipani et al., 2008). There is, therefore, a conceptual possibility to more than double the vitamin C content of strawberries through selective breeding.

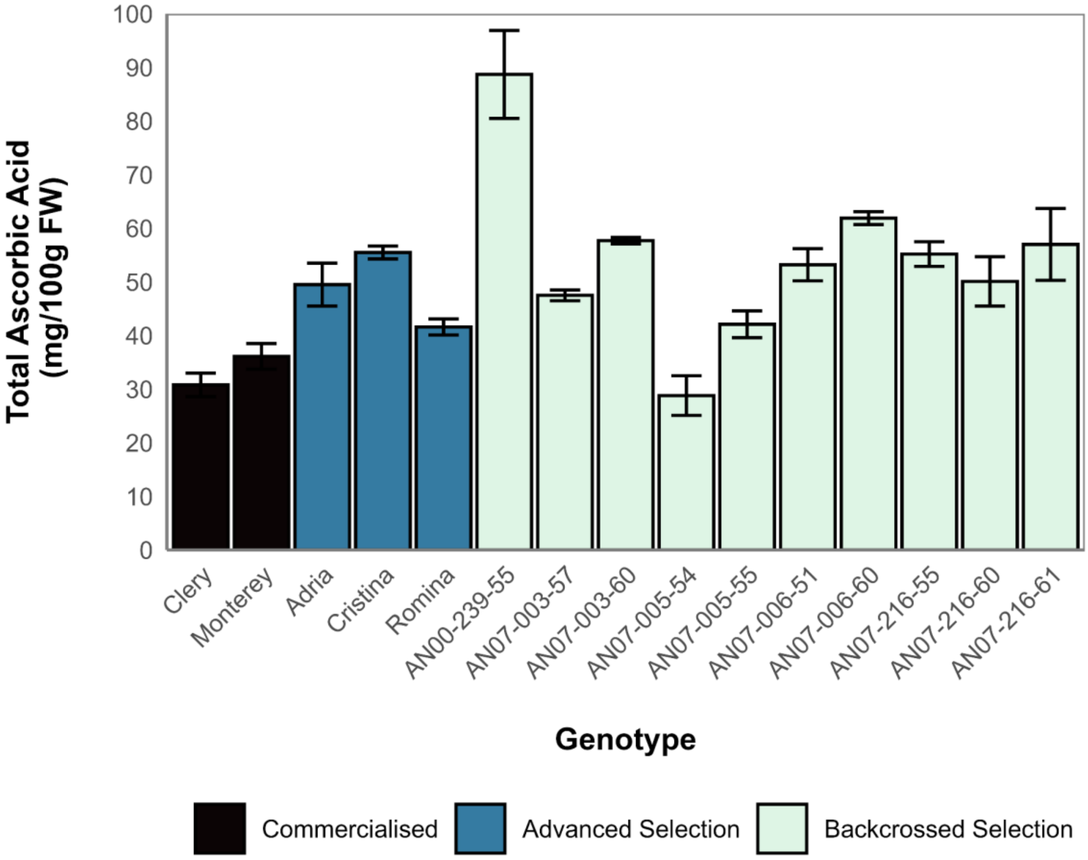


Figure 1.5 Bar graph illustrating the vitamin C content of 15 strawberry genotypes from the Marche Polytechnic strawberry breeding programme. The visualisation is adapted directly from tabular data published by Mezzetti et al. (2016). Berry vitamin C content was measured as total ascorbic acid content per 100g fresh weight (FW). Genotypes have been ordered from left to right and colour coded based on their breeding status. including commercialised (black bars), advanced breeding selections (blue bars), and backcrossed selections from the breeding programme (mint-green bars).

It is noteworthy, however, that both of the referenced studies used “domestic” genotypes that were specific to the regions in which the studies were conducted (Kim et al., 2015; Mezzetti et al., 2016; Sarıdaş, 2021). Similar studies of vitamin C variability in domestic UK strawberry genotypes are limited. Atkinson et al. (2006) conducted a study on ellagic acid variability in 17 UK-relevant genotypes, but only reported the vitamin C content for two (*Elsanta* and *Flamenco*). It will, therefore, be necessary to assess variation in UK-specific strawberry genotypes to establish the potential of UK breeding programmes to target increases in vitamin C content.

A further aspect to be considered when interpreting studies of genotypic variation in strawberries is the impact of interactions between genotype, environment, and cultivation practices. Cervantes et al. (2020) found vitamin C content to vary between seasons by as much as 90% within the same genotype cultivated under the same conditions. However, the extent of the inter-seasonal variability was not equal amongst all genotypes. Whilst the vitamin C content of *Primoris* strawberries varied by 90% between seasons, no statistically significant inter-seasonal variation was found in *Sabrina* strawberries, for which vitamin C content varied by a maximum of 56% (Cervantes et al., 2020). Pincemail et al. (2012) have also highlighted that harvest time and cultivation conditions can exert genotype-dependent effects on vitamin C accumulation in strawberries. It will be important, therefore, to conduct future studies of genotypic variation in UK-relevant genotypes over multiple seasons to account for environmental variability (Capocasa et al., 2008; Cervantes et al., 2020). Furthermore, UK-specific studies should utilise cultivation practices that are representative of those commonly used by commercial farms (Calleja et al., 2012; Pelham, 2017). Utilising commercial growing conditions would ensure that the findings can be more directly applied to UK growing systems.

Whilst conceptually promising, conventional breeding is also limited by its speed and financial cost. Commentary from those involved in the Marche Polytechnic breeding program indicates that targeting enhanced nutritional quality is becoming a more common theme in strawberry breeding (Mezzetti et al., 2018). The primary focus, however, is still set on developing cultivars that yield well and are popular with consumers as these are the key traits that make a genotype profitable (Mezzetti et al., 2018). In addition, personal communications with strawberry breeders at Niab indicated that it takes at least 8 years for a promising seedling to reach commercial markets. Considering the time it may take to build a case for biofortifying strawberries, in addition to the time it would take to develop biofortified cultivars, the benefits of selective breeding on domestic vitamin C production would not be realised for at least a decade.

1.4.2.2 Agronomic Strategies

Another, potentially quicker route to biofortification is through the development of nutrition-smart agronomic practices - cultivation practices that lead to an increase in nutritional content without detriment to commercial yields or sensory qualities.

Central to the development and application of nutrition-smart practices is the understanding that vitamin C and other phytochemicals are as important to plant health as they are to human health (Paciolla et al., 2019). In plants, vitamin C is active in neutralising reactive oxygen species (ROS) (Kirova et al., 2021; Sun et al., 2016) and iron homoeostasis (Grillet et al., 2014; Hoang et al., 2021; Sun et al., 2016). Vitamin C is also tightly linked with photosynthesis and protection of the photosystems under times of stress (Awad et al., 2015; Saga et al., 2010; Tóth et al., 2011).

Due to the role of vitamin C in plant stress responses, a viable biofortification approach involves the deliberate application of mild stresses that elicit increases in vitamin C content, known as eustresses. Exposing plants to mild stresses that generate ROS, such as water deficit (Perin et al., 2019), or nutrient stress (Ramírez et al., 2013; Valentinuzzi et al., 2015), have been found to increase vitamin C accumulation without significant losses to yield. Perin et al. (2019), for example, observed an increase in vitamin C accumulation under mild drought stresses in *Camarosa* strawberries, although the relative values are not clear from their published manuscript. Valentinuzzi et al. (2015) grew *Elsanta* strawberry plants in a hydroponic system under iron and phosphorus deficits and observed increases in phenolic and antioxidant compounds with no negative impact on yield relative to control plants.

The application of mineral nanoparticles to fertiliser solutions has also been of increasing research interest as a way of increasing both yield and nutritional quality (Jahan et al., 2022; Kah et al., 2018). Calcium (Mogazy et al., 2022), iron (Mogazy et al., 2022; Mozafari et al., 2018a, 2018b), and selenium (Shahraki et al., 2022) nanoparticles have been found to increase strawberry tolerance to environmental stress through the activation of stress response pathways. Activation of these pathways has subsequently driven an increase in vitamin C and other nutritious compounds (Mogazy et al., 2022; Mozafari et al., 2018a, 2018b; Shahraki et al., 2022). Studies of the effects of nano-fertilisers on strawberry have largely been conducted in the context of treating plant diseases or mitigating the impacts of environmental stresses (Mogazy et al., 2022; Mozafari et al., 2018a, 2018b; Shahraki et al., 2022). Direct study of the effects of nano-fertilisers on healthy strawberry plants in a commercial setting has not been explored but, conceptually, could offer an opportunity to elicit increases in both yields and nutritional quality.

The sources of exploitable variation in vitamin C content presented in the previous two sub-sections do not constitute an exhaustive list. The purpose was to highlight that there are opportunities to achieve nutritionally meaningful increases in strawberry vitamin C content and how these relate to UK-specific studies. In doing so, it is clear that more evidence will be required to understand the viability of biofortification as a means of increasing the UK's domestic production of vitamin C.

1.5 Conclusion

In summary, the current understanding of UK vitamin C security can be separated into the pillars of food security that have, and those that have not, been studied in the context of vitamin C. Previously studied elements of UK vitamin C security have primarily focused on the pillar of availability and threats to the stability of national vitamin C supplies. The key findings have shown that overall vitamin C supplies greatly exceed UK dietary recommendations but are heavily reliant on increasingly unstable global supply chains.

In contrast, information concerning the pillars of accessibility, utilisation, and seasonal stability is currently limited with regards to vitamin C. Key questions are yet to be addressed; How equitably are vitamin C supplies distributed throughout the UK population? Does the availability and accessibility of vitamin C exhibit seasonal trends? And are current dietary recommendations sufficient to meet the vitamin C requirements of a healthy UK population? The datasets and approaches currently used to study vitamin C security are not detailed enough to address such questions.

Understanding how vitamin C relates to each of the four pillars and how those pillars link to one another will be paramount to developing a concerted assessment of UK vitamin C security. Such an assessment will be key to the development of targeted intervention strategies that safeguard UK vitamin C security, as well as facilitating effective evaluation of the impacts of proposed interventions. Building upon what is known about vitamin C availability, the UK's reliance on imported fruits and vegetables represents a tangible threat to the security of vitamin C supplies. As such, investigations into strategies that could strengthen vitamin C self-sufficiency have been discussed as a necessity to secure future vitamin C supplies.

Strawberry has been identified as a local source of vitamin C with the capacity for increases in domestic production and as a target for vitamin C biofortification. Research and development towards increasing the UK's capacity to produce year-round strawberries is already well underway. There remain, however, gaps in our understanding as to whether biofortification represents a viable approach to increasing domestic production of vitamin C. Within the proposed case study of strawberries, it will also be important to consider who, when, and how strawberries could benefit vitamin C security.

1.6 Aims of the Thesis

The purpose of the thesis presented herein was twofold: 1) To investigate overlooked vitamin C insecurities within the UK food system in the context of the pillars of accessibility, utilisation, and seasonal stability; 2) To use UK strawberries as a case study to explore the potential of biofortification as an approach to improving the UK's vitamin C self-sufficiency. To those ends, the specific aims addressed in the thesis were:

1. ***To investigate access to vitamin C supplies at the household level and to explore seasonal and regional trends that could produce unstable or inequitable access to sufficient vitamin C.***

2. ***To conduct a detailed analysis of vitamin C intakes and biological status in the UK population and to understand the prevalence and impact of known risk factors on an individual's vitamin C requirements*.**

3. ***To comprehensively describe the vitamin C content of UK-marketed fresh strawberries and explore potential seasonal instabilities at the crop level.***

4. ***To evaluate the effects of previously tested foliar nutrient fertilisation strategies to enhance vitamin C accumulation in UK strawberries grown under standard commercial conditions***.

5. ***To assess the vitamin C content of a range of popular UK strawberry genotypes grown under standard commercial conditions.***

Addressing these aims will contribute to a more granular picture of vitamin C security in the UK and offer insight into strategies that could help to strengthen UK vitamin C security. Furthermore, the body of research will develop a framework that could facilitate similar assessments of other micronutrients and crops, ultimately contributing to the complex task of achieving a state of food and nutrition security in the UK.

1.7 Structure of the Thesis

The thesis comprises a total of seven Chapters. Following this general introduction, Chapter 2 addresses the first aim of the thesis. The analysis in Chapter 2 utilises a household consumer panel dataset acquired in collaboration with Kantar World Panel. The dataset covers purchases of fresh fruits and vegetables between April 2017 and March 2022, a period encompassing the UK's departure from the EU, the SARS-CoV-2 pandemic, and the early stages of the ongoing war in Ukraine. Chapter 2 begins by exploring household vitamin C provisions through purchases of fresh fruits and vegetables, building an initial understanding of vitamin C accessibility in relation to the studies of vitamin C availability discussed in Chapter 1 (Section 1.2.1). Further analysis uses the purchase data to investigate seasonal instabilities in household access to vitamin C, as well as regional disparities driving inequitable access to vitamin C.

Chapter 3 addresses the second aim of the thesis, building further on Chapters 1 and 2 with a focus on the utilisation of vitamin C in the UK population. Chapter 3 presents analyses of the UK's National Diet and Nutrition Survey (NDNS). Analysis of PAA in the NDNS cohort was used to examine the proportions of the population that fall into the status categories of Severe Deficiency, Hypovitaminosis C, Inadequate, and Adequate (Section 1.2.3). Dose-response modelling was then used to study the relationship between vitamin C intakes and PAA to estimate the vitamin C requirements of the average UK citizen. Further regression analysis and predictive modelling based on the dose-response relationships were then used to investigate health, lifestyle, and social factors that can impair an individual's access and utilisation of vitamin C. Specifically, the impacts of household income, age, sex, body weight, and smoking status are investigated.

The third aim of the thesis is addressed in Chapter 4. Samples of fresh strawberries were collected twice a month from local retail markets over 12 months from 23 March 2023 to 01 March 2024. The study focuses on exploring seasonal trends in the vitamin C content of UK-marketed strawberries, whilst also documenting changes in sensory quality and price. The implications and benefits of seasonal nutrient reference values are discussed in the context of seasonal analyses of vitamin C security.

Together, Chapters 5 and 6 present a series of experiments designed to address the fourth and fifth aims of the thesis. Chapter 5 describes an experiment that evaluated the effects of foliar-applied iron nanoparticles and sodium selenate on the accumulation of vitamin C in strawberries. The experiment was novel in its approach by using two UK strawberry genotypes grown in a standard commercial cultivation system. Ultimately, Chapter 5 explores the potential of a relatively simple agronomic practice to improve the nutritional quality of strawberries without compromising marketable traits. In Chapter 6, the nutritional, agronomic and sensory qualities of seven UK strawberry genotypes were characterised in a standard commercial cultivation system. Chapter 6 provides insight into the genetic variation that could be exploited in selective breeding for increased vitamin C content in UK-grown strawberries.

The thesis concludes with a discussion of the major findings from the previous 6 chapters and their conceptual impact on contemporary assessments of UK vitamin C security. The discussion builds a novel view of UK vitamin C security as it is today, highlighting the importance of adopting more holistic approaches and positive aspirations. The case study of strawberry as a vehicle for increasing domestic vitamin C production is also analysed with respect to who, when, and how the case may be of benefit. Finally, the strengths and limitations of the approaches taken throughout the thesis are considered and used to suggest directions for future research.

Chapter 2 An Exploration of Seasonal and Regional Trends in UK Household Access to Vitamin C Through Purchases of Fresh Fruits and Vegetables

2.1 Introduction

To be food secure is to be able to, at all times, access sufficient, safe, and nutritious food that meets one's dietary needs and preferences to support a healthy life (FAO, 2024). In Chapter 1, attention was drawn to the fact that the nutritional components of food security have received relatively little attention when compared to the efforts that have been made to ensure food supply chains can meet calorific demands (Ingram, 2020; Section 1.1). Diets rich in calories but largely devoid of nutritional quality have become common throughout the global population and are driving increasing levels of malnutrition in low- and high-income countries alike (FAO, 2020; Willett et al., 2019; Chapter 1, Section 1.1). Such diets are particularly poor in delivering enough essential micronutrients (Derbyshire, 2018). Micronutrients are critical to human life and can only be obtained through an individual's diet (Shergill-Bonner, 2017), yet limited progress has been made toward understanding the capacity of modern food systems to support a state of micronutrient security.

Drawing on the key elements of its definition, food security is commonly considered in the context of four key facets or "pillars" - Availability, Accessibility, Utilisation, and Stability (FAO, 2008). Each of the four pillars must support one another to achieve a state of food security and, thus, it is critical to examine all the pillars concurrently when assessing food security in a population (Chapter 1, Section 1.1). The pillars of food security can equally be applied to assessments of micronutrient security to ensure that a food system can support the micronutrient requirements of the population that it serves (Chapter 1, Section 1.1).

Recent studies in the UK have used food supply data to estimate the availability of key micronutrients including iron, calcium, zinc, and vitamins A and C in the UK food system (Macdiarmid et al., 2018; Poppy et al., 2022; Chapter 1, Section 1.2.1). In 2016, a combination of domestic food production and international trade was shown to provide 149% of the UK's iron requirements, 201% for calcium, 148% for zinc, 167% for vitamin A, and 299% for vitamin C (Poppy et al., 2022). Based on these data, the UK food system can be said to have the capacity to provide surplus quantities of micronutrients that support the population's requirements. As discussed in Chapter 1, however, knowledge of the accessibility and stability of UK micronutrients is limited, and a surplus supply does not necessarily constitute a state of micronutrient security (Chapter 1, Section 1.2).

Of the micronutrients studied by Macdiarmid et al. (2018) and Poppy et al. (2022), vitamin C had the greatest availability relative to population requirements. Total supplies of vitamin C have increased by 33% per capita between 1961 and 2016, amounting to 299% of the UK recommended nutrient intake (RNI - 40 mg/capita/day) in 2016 (Poppy et al., 2022). Even considering the conceptual impacts of supply chain disruptions and that utilisation of vitamin C supplies will not be 100%, the UK's vitamin C supplies appear to be sufficient to be considered secure (Poppy et al., 2022). There are, however, several factors that could limit the extent to which the UK's vitamin C supplies can support the nutritional requirements of the UK population (Chapter 1, Sections 1.2.2 and 1.2.3).

In contrast to what is known about the availability of vitamin C in the UK, there is currently no direct indication of how accessible that vitamin C is (Chapter 1, Section 1.2.2). There are, however, reports of increasing difficulty accessing nutritious foods and decreasing fruit and vegetable purchases across the UK (Koltai et al., 2021; The Food Foundation, 2023). Given that 85% of the UK's available vitamin C came from fruits and vegetables in 2016 (Poppy et al., 2022), it is concerning that access to vitamin C may also be adversely affected by rising levels of food insecurity.

Whilst the increases in reported food insecurity are widespread, certain regions and sub-populations have been found to be disproportionately affected (The Food Foundation, 2023). Trends in UK food insecurity reflect existing regional inequalities between the wealthy South East and the rest of the country (Fai and Tomlinson, 2023; McCann, 2020). Regional disparities have driven the emergence of widespread health inequalities (Case and Kraftman, 2024; Corris et al., 2020) that include access to nutritious foods (Johnstone and Lonnie, 2023; Jones et al., 2014). Fruit and vegetable consumption in lower-income households, for example, has previously been reported to be as much as 128 g/day less than in the highest-income households (Maguire and Monsivais, 2015). Access to vitamin C is likely to be inequitable across different regions of the UK as a result, however, a direct assessment of regional access to vitamin C has yet to be undertaken.

The extent to which fruits and vegetables contribute to UK vitamin C supplies also creates concern regarding the seasonal stability of both vitamin C availability and access. Both the production and preference for different crops are known to vary seasonally (Macdiarmid, 2014; Revoredo-Giha et al., 2022; Toorang et al., 2013). For example, despite fresh strawberries being available year-round in the UK, British Summer Fruits observed that 85% of all strawberries were purchased between May and September 2015 (Pelham, 2017). It is unclear whether such seasonal trends impact the availability or access to vitamin C-rich fruits and vegetables at different times of the year. It will be important, however, to understand if seasonal patterns exist such that interventions can be developed to stabilise access to sufficient vitamin C.

2.1.1 Aims and Objectives

Seasonal instability and regional disparities therefore represent key components that must be considered with respect to the availability and accessibility of vitamin C supplies. As such, the study presented in Chapter 2 addressed the first aim outlined for this thesis; **To investigate access to vitamin C supplies at the household level and to explore seasonal and regional trends that could produce unstable or inequitable access to sufficient vitamin C.**

Key to addressing the aims of Chapter 2 was the establishment of a collaborative partnership with Kantar World Panel (Roberts, 2023). We thank Joe Shaw Roberts (Strategic Insight Director in Kantar's Produce Division) and his team for their generous sharing of valuable datasets that facilitated the work in Chapter 2. Having sourced suitable data, four key objectives were identified for Chapter 2.

1) To quantify the amount of vitamin C that reaches UK households through purchases of fruits and vegetables.

2) To identify the fruits and vegetables that contribute the most vitamin C to household provisions

3) To explore seasonal trends in household vitamin C provisions in relation to predictable instabilities that could threaten UK vitamin C security.

4) To explore regional differences in household vitamin C provisions in relation to disparate accessibility to vitamin C.

2.2 Materials and Methods

2.2.1 Data Sources and Wrangling

2.2.1.1 Kantar Consumer Purchase Data

Data describing UK consumer purchases of fruits and vegetables for at-home consumption were acquired from project collaborators at Kantar World Panel (Roberts, 2023). Whilst the data were leveraged by Kantar to provide companies with market insights, they also contained all the necessary information to address the aims outlined in Chapter 2. Initial conversations with Joe Shaw Roberts, Strategic Insight Director in Kantar's Produce Division, generated substantial enthusiasm and interest for this PhD project. Further discussions led to the development of a partnership with Joe and his team, in which Kantar would provide free access to the requested data in return for recognition of their contribution to the research that had been proposed. With its level of detail, contemporary time series, and coverage of foods that constitute most of the UK's vitamin C supplies, the data shared by Kantar enabled the aims of Chapter 2 to be addressed at a level of detail that otherwise would have been unachievable.

Kantar's UK consumer panel consists of 30,000 households that are selected to represent the UK's varied demography. Participants are required to scan shopping receipts with bar code scanners, provided by Kantar, which directly uploads the participant's purchase data to Kantar's storage servers. Both the volume and value of foods purchased for at-home consumption are gathered by the barcode scanners. The barcode scanners do not, however, capture foods that are purchased for consumption outside of the home. Kantar collects the purchase data automatically on a four-weekly cycle. Once gathered, Kantar applies an in-house survey weighting to the data to scale the values to the wider UK population. Kantar does not, however, provide specific details of how the weightings are applied to their survey design.

Two datasets were shared by Kantar as part of the collaborative partnership. The first dataset, received on 27th April 2022, described the total volume (thousand tonnes) and total value (thousand Great British Pounds (GBP)) of UK fresh fruit and vegetable purchases between April 2017 and March 2022. The dataset included 99 unique fresh fruit and vegetable commodities. Volumes and values for each commodity were provided at four-weekly intervals across the 5-year time series.

The data were categorised into purchase values from major UK regions. The regional categories included *London*, *South*, *Midlands*, *East*, *North*, *Scotland*, and *Wales*. The boundaries of the included regions were consistent with those used in official statistical and administrative documents. The data did not cover *Northern Ireland*. Some of the regions were a combination of several administrative regions; *South* (*South East* and S*outh West*), *North* (*North East*, *North West*, and *Yorkshire and the Humber*) and the *Midlands* (*East Midlands* and *West Midlands*).

The data were further categorised by retailer, which included market-leading retailers and an "other" category which captured purchases from other retailers and outlets. There was, however, one prominent retailer that was unexpectedly absent from the Kantar data without an indication as to the reason. It is possible that the retailer was present within the “other” retailers category but it was not possible to disaggregate the “other” category to confirm if this was the case. As such, the Kantar data may not provide full coverage of fruit and vegetable purchases. Direct analysis and comparisons between retailers were not conducted.

The second dataset, received on 9th May 2023, described the total volumes (thousand tonnes) and total values (thousand GBP) relating to purchases of foods from four fruit and vegetable categories between May 2018 and April 2023. The fruit and vegetable categories included fresh, frozen, juiced, and canned/tinned. There was, however, no breakdown of individual foods within the food categories. As with the first dataset, the data were also categorised by region of purchase.

The second dataset also provided breakdowns of retail purchases by National Readership Survey social grade (NRS; National Readership Survey, 2025). The NRS categorise households into grades that reflect the socioeconomic status of the household based on the occupation of the primary earner. The grades do not consider other earners that might live in a household, the size of the household, or the actual income being generated by all members of the household. In the context of the current study, the NRS grades were considered too imprecise to make any direct assessments of socioeconomic status in relation to retail purchases of vitamin C. The factors described above cover all information that were shared by Kantar for use in the current research.

The data received from Kantar were initially wrangled from multi-sheet ".xlsx" workbooks to a single data frame. Once collated into a single data frame, the data were methodically cleaned.

Instances of redundant or similar commodities were combined into a single entry. Sharon fruit and Persimmon Fruit, for example, are commonly grouped into a single category in food composition databases, meaning there isn’t separate nutritional information for each. As such, Sharon fruit and Persimmon Fruit were combined into a single "Persimmon Fruit" entry by summing the purchase volumes and values of both commodities.

Instances of broader groups of fruits and vegetables, for example, "Tropical fruit" or "Legumes" were also present in the initial dataset. The volumes and values for such groups were the direct summation of individual commodities that Kantar had assigned to those groups. As such, all grouped entries were filtered to retain only the individual commodities.

Overall purchase volumes and values were present in the data set for each commodity. The regional categorisation of the data, however, only included purchase values. To convert the regional purchase values to volumes, a relative GBP/Kg was calculated from the corresponding overall volumes and values. The GBP/Kg enabled the regional purchase values to be converted back to regional purchase volumes.

Each commodity was then manually assigned a code to enable subsequent integration of the Kantar and food composition data from the Composition of Foods Integrated Dataset (CoFID) (Public Health England, 2015; Roe et al., 2015). All the assigned codes were selected to match each Kantar commodity to the relevant raw food entry in the CoFID. For fruits and vegetables with non-edible parts, the nutrient reference values (NRV) for only the edible portion of the food were used. For herbs and spices, NRVs for dried commodities were used unless the Kantar commodity name explicitly stated that it was fresh. Uncommon fruits and vegetables, including Pineberries and Ugli fruit, which did not have an equivalent entry in the CoFID, were not assigned a code.

2.2.1.2 Composition of Foods Integrated Dataset

Food composition data were obtained through the publicly available Composition of Foods Integrated Dataset (Public Health England, 2015). The CoFID data were obtained as a multi-sheet ".xlsx" file. Sheets relevant to macronutrients, inorganic nutrients, and vitamins, were extracted and combined into a single data frame.

Similarly to the process of code assignment in the Kantar dataset, foods for which there were multiple entries in the CoFID were combined when the matching Kantar commodity only had one entry. The reference vitamin C value used for "Peppers", for example, is the mean vitamin C content described for red, yellow, and green peppers. Instances of NA and Tr (Tr = Trace: nutrient quantity lower than the limit of detection) values were replaced with zeros.

2.2.1.3 UK Dietary Recommendations

UK dietary recommendations were obtained as multiple PDF tables describing the Recommended Nutrient Intakes (RNI) for different age groups (Public Health England, 2016). The table of RNIs was extracted from the ".pdf" report to ".csv" and ".xlsx" formats using pdftables (https://pdftables.com/).

All tables in the dietary recommendations report (Energy, Macronutrients, Minerals, and vitamins) were standardised and combined into a single data frame.

2.2.1.4 Population Mid-Year Estimates

Population estimates for 2017 to 2021 were obtained through the Office for National Statistics (ONS) data portal (Office for National Statistics, 2023). The methodology and potential sources of inaccuracy are described in the dataset's supporting documentation (Office for National Statistics, 2023). Relevant mid-year estimate ".xlsx" sheets were again extracted and combined into a consolidated data frame.

The mid-year population estimates describe the population sizes of each geographic region in the UK. The regional categories in the ONS population estimates were combined in the same way as described for the Kantar data to enable the two datasets to be integrated (See Section 2.2.1.1). For example, the estimated population sizes for the *East Midlands* and *West Midlands* were summed to produce a population estimate for *Midlands*.

Population estimates were also categorised by age to match the age groups used in the dietary recommendations report (Public Health England, 2016). The final population estimates were categorised by geographic region, year, sex, and age group.

Food composition, Mid-year population estimates, and dietary recommendations were all accessed and utilised under the Open Government Licence 3.0.

2.2.2 Dataset Integration

Having standardised the variable names and formatting of each data source, the four data sources were combined into a final integrated dataset;

1) Kantar purchase data were combined with the CoFID reference values by matching the food commodity codes.

2) The mid-year population estimates were joined by matching the regional names to the regional names in the Kantar dataset.

3) The dietary recommendations were joined by matching the names of the nutrients to those in the CoFID data and the age groupings to those from the mid-year population estimates.

All data cleaning, wrangling, and processing were carried out in R (R Core Team, 2024), making extensive use of the {*tidyverse*} suite of packages (Wickham et al., 2019).

2.2.3 Data Analysis

Once combined, Household vitamin C provisions (HCP) were calculated in terms of the available mg/capita/person. Standardising the vitamin C supplies in this way was consistent with the analyses of Macdiarmid et al. (2018) and Poppy et al. (2022).

Calculating HCP first required the CoFID NRV to be converted from mg/100 g to mg/Kg. The converted NRV was then multiplied by the purchase volumes (Kg) from the Kantar data set. The calculation yielded the total amount of vitamin C purchased by consumers. The total amount of vitamin C was calculated for each food, four-week period, and region.

$$\textit{TotalvitaminC(mg)} = (\textit{CoFID}*10) * \textit{PurchaseVolume}$$

As an example, the CoFID reference value for apricot is 5 mg/100 g. In the East of England, the purchase volume of Apricots in the four weeks ending 23 April 2017 was 16132 Kg. Thus, the total vitamin C available from Apricots in the East of England in April 2017 was;

$$\textit{TotalvitaminC(mg)} = (5*10) * 16132 = 806604mg$$

Using the total vitamin C values, estimated HCPs were then calculated. The HCP was calculated for each year and four-week period to study annual and seasonal trends in HCP. The HCP was also calculated for each region to study geographic accessibility to vitamin C.

$$HCP = \text{vitamin C(mg)} / \text{population size} / 28$$

Returning to the apricot example, the population size in the East of England in 2017 was 6097161. Thus, the HCP provided by Apricots in the East of England during the four weeks ending 23 April 2017 was;

$$HCP = 806604 / 6097161 / 28 = 0.005 \text{ mg/capita/day}$$

Population-adjusted RNIs were also calculated based on the demographic compositions of each region in each year. The vitamin C RNI is 30 mg/day for children under 11 years, 35 mg/day for children aged 11-14 years, and 40 mg/day for all other age groups. As a result, the overall vitamin C requirement of a given UK population will be slightly less than 40 mg/day, depending on the number of young children present in that population.

The population-adjusted RNIs were applied to each geographic region based on the annual population estimates. The population-adjusted RNIs were used as a reference against which the contribution of HCP could be compared to population requirements.

2.3 Results

2.3.1 Household Vitamin C Provisions from Fresh Fruits and Vegetables

The volume (Kg) of fresh fruits and vegetables purchased between May 2018 and April 2023 accounted for 76.8% of all fruit and vegetable purchases. Purchases of other processed fruits and vegetables including juices, cans/tins, and frozen produce accounted for 13.5%, 5.5%, and 4.3% respectively. The relative contributions of each fruit and vegetable category to annual purchases remained consistent in the calendar years between 2019 and 2022 (Figure 2.1).

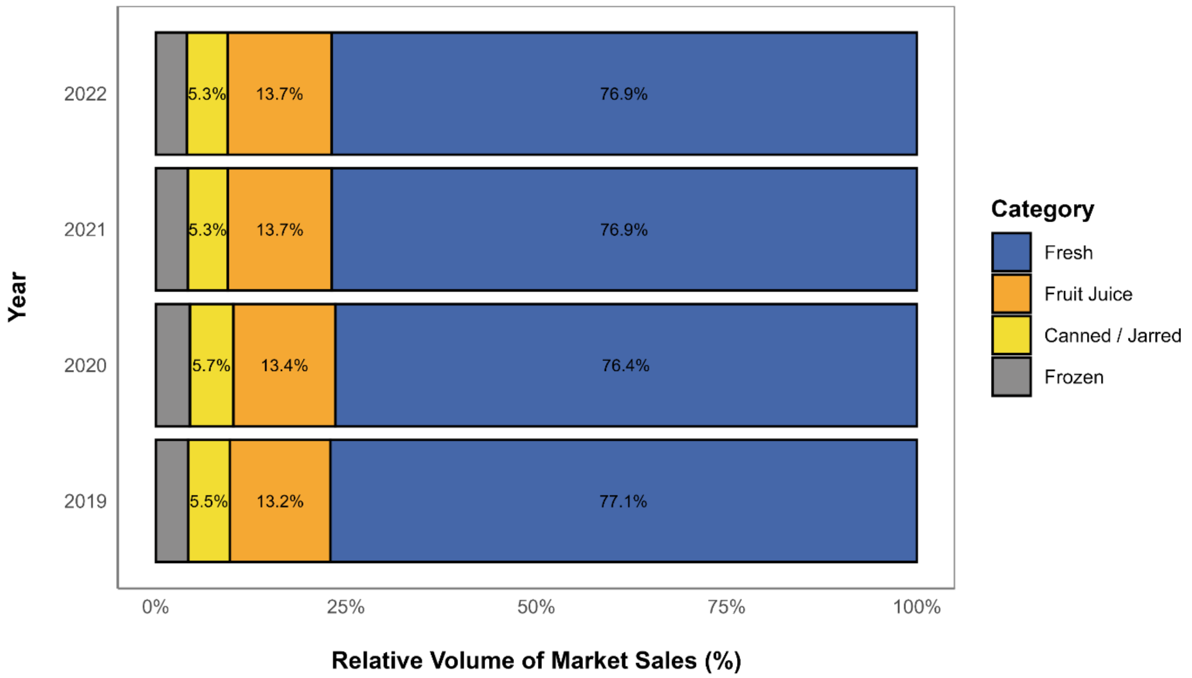


Figure 2.1 Stacked Bar-graph showing the relative contributions of fresh and processed fruits and vegetables to total UK sales volumes (Kg) between 2019 and 2022. Data from 2018 and 2023 were excluded because data were not available for all 12 calendar months.

Purchases of fresh fruits and vegetables between April 2017 and March 2022 were sufficient to provide UK households with a mean of 43.16 mg vitamin C/capita/day. The mean HCP was equivalent to 113.1% of the population-adjusted RNI. The mean annual HCPs were similar in 2018 (40.92 mg/capita/day; 106.2% of adjusted RNI) and 2019 (41.97 mg/capita/day; 108.9% of RNI). An increase in mean annual HCP of 4.4 mg/capita/day was observed between 2019 and

2020 (46.29 mg/capita/day; 120.1% of RNI). The increase in annual HCP in 2020 persisted into 2021 (45.47 mg/capita/day; 117.8% of RNI) (Figure 2.2).

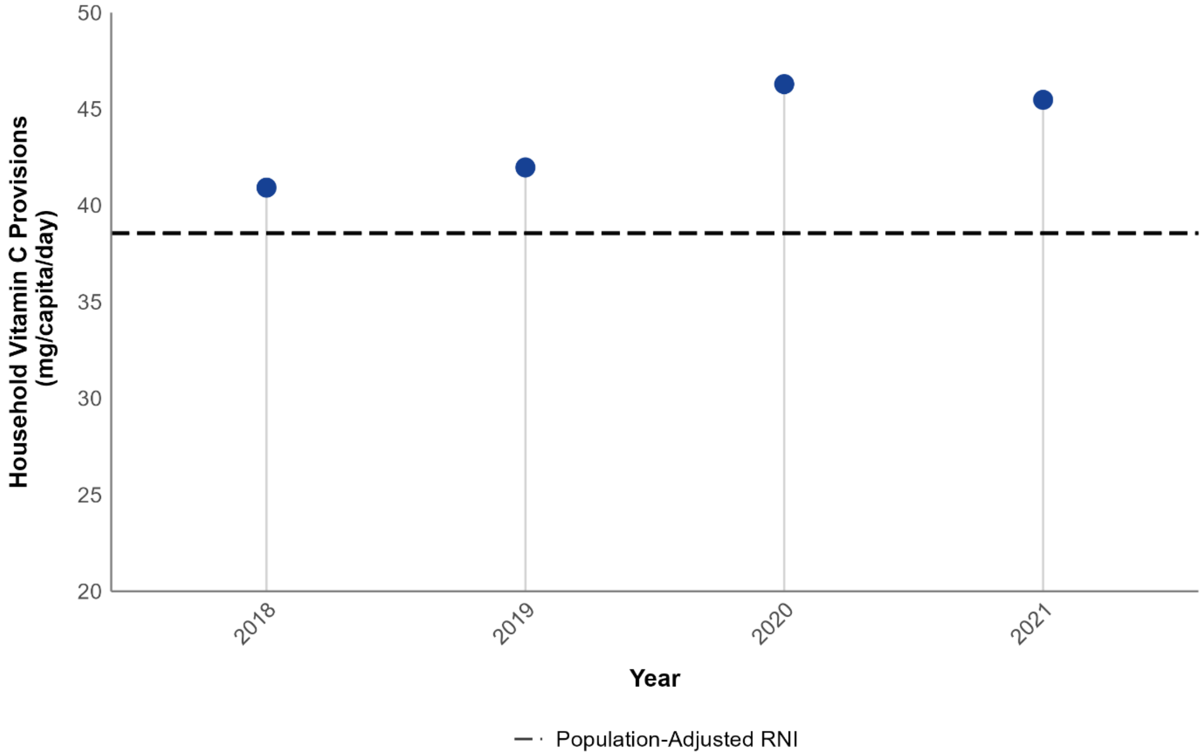


Figure 2.2 Lollipop-chart showing annual household vitamin C provisions from fresh fruits and vegetables in the United Kingdom between 2018 and 2021. Data from 2017 and 2022 were excluded because data were not available for all 12 calendar months.

2.3.2 The Contributions of Individual Fruits and Vegetables to Household Vitamin C Provisions

Between April 2017 and March 2022, 10 fruits and vegetables accounted for 69.0% of the total HCP (Table 2.1). The vitamin C provided by the top 10 fruits and vegetables was equivalent to a mean of 30.1 mg/capita/day (78.2% of adjusted RNI).

Sweet peppers were the greatest contributors to UK HCPs, amounting to 17.4% of total HCP between April 2017 and March 2022 (Table 2.1). The vitamin C provided by sweet peppers was equivalent to an annual mean of 7.6 mg/capita/day (19.7% of adjusted RNI). Broccoli, easy peeler oranges, strawberries, and cauliflower also contributed over 5% of the total HCP. The top 10 also included tomatoes, potatoes, oranges, cabbages, and Brussels sprouts (Table 2.1).

Table 2.1 Top 10 fresh fruits and vegetables contributing to UK household vitamin C provisions between April 2017 and March 2022.

Rank	Food Commodity	Total Volume of vitamin C (Kg)	Relative Supply (%)	Cumulative Supply (%)	Annual HCP (mg/capita/day)	Cumulative Annual HCP (mg/capita/day)
1	Sweet Pepper	56,206	17.38	17.38	7.59	7.59
2	Broccoli	28,519	8.82	26.20	3.85	11.44
3	Easy Peeler Orange[1]	25,845	7.99	34.19	3.49	14.93
4	Strawberry	24,764	7.66	41.85	3.34	18.27
5	Cauliflower	21,289	6.58	48.43	2.87	21.14
6	Tomato	16,558	5.12	53.55	2.24	23.38
7	Potato	16,444	5.08	58.63	2.22	25.60
8	Orange	11,877	3.67	62.30	1.60	27.20
9	Cabbage	11,144	3.45	65.75	1.50	28.70
10	Brussels Sprouts	10,667	3.30	69.05	1.44	30.14

[1]Includes clementines, mandarins, satsumas and tangerines.

The top 10 fruits and vegetables shown in table 2.1 remained consistent across each year between 2018 and 2021 (Figure 2.3). There were minor changes in the order of the top 10 between years, such as strawberries moving from fourth to third in 2021 and frequent switching of tomatoes and potatoes as the sixth and seventh highest contributors to HCP (Figure 2.3). Notably, the contribution of sweet peppers to HCP was 2.1% higher in 2021 than in 2018, whilst the relative contribution of other foods in the top 10 remained consistent between years (Figure 2.3).

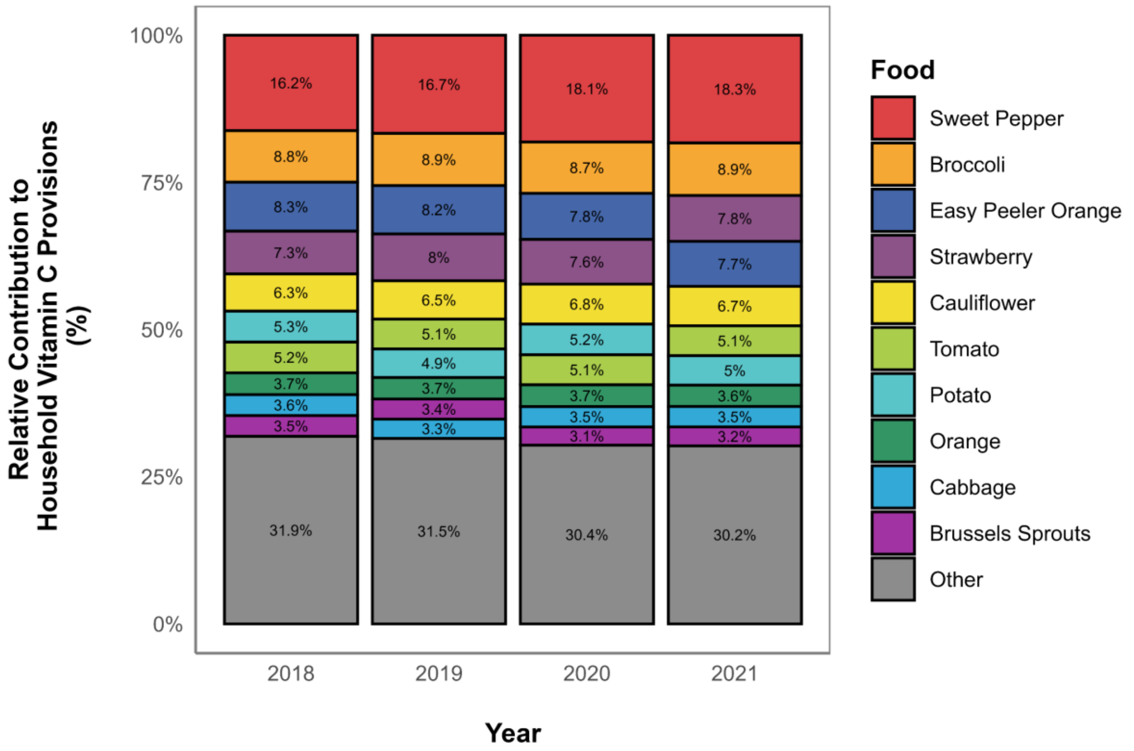


Figure 2.3 Stacked bar graph showing the top 10 fruits and vegetables and their relative contributions to annual UK household vitamin C provisions between 2018 and 2021. Fruits and vegetables outside of the top 10 in each year were combined into an "Other" category. Data from 2017 and 2022 were excluded because data were not available for all 12 calendar months.

2.3.3 Seasonal Instabilities in Household Access to Vitamin C Provisions

The lowest HCPs in each year were observed on 10 September 2017 (37.53 mg/capita/day), 06 October 2018 (37.33 mg/capita/day), 08 September 2019 (38.65 mg/capita/day), 06 September 2020 (42.27 mg/capita/day), and 28 November 2021 (39.97 mg/capita/day). The minimum HCPs observed for 2017 and 2018 were lower than the population-adjusted RNI (Figure 2.4). The highest HCPs were consistently observed at the end of December each year (Figure 2.4). The

HCPs in December of each year were between 16.6% and 23.8% higher than the minimums observed in the Autumn months.

In 2018 and 2019 HCPs remained relatively stable between January and August before decreasing in September, October and November (Figure 2.4). In 2020, the seasonal trends in HCPs deviated from previous years, characterised by a sharp increase between 23 February and 22 March (Figure 2.4). The monthly HCPs continued to increase until 20 June 2020 (50.55 mg/capita/day), at which point HCP was 17.6% higher than at the same time in 2019 (42.97 mg/capita/day). The same Autumnal reduction in HCP was still observed in 2020. Monthly HCPs reached their overall maximum on 27 December 2020 (52.32 mg/capita/day). A general decline in HCPs was observed during 2021 but HCPs remained higher than at the same time in 2019 (Figure 2.4).

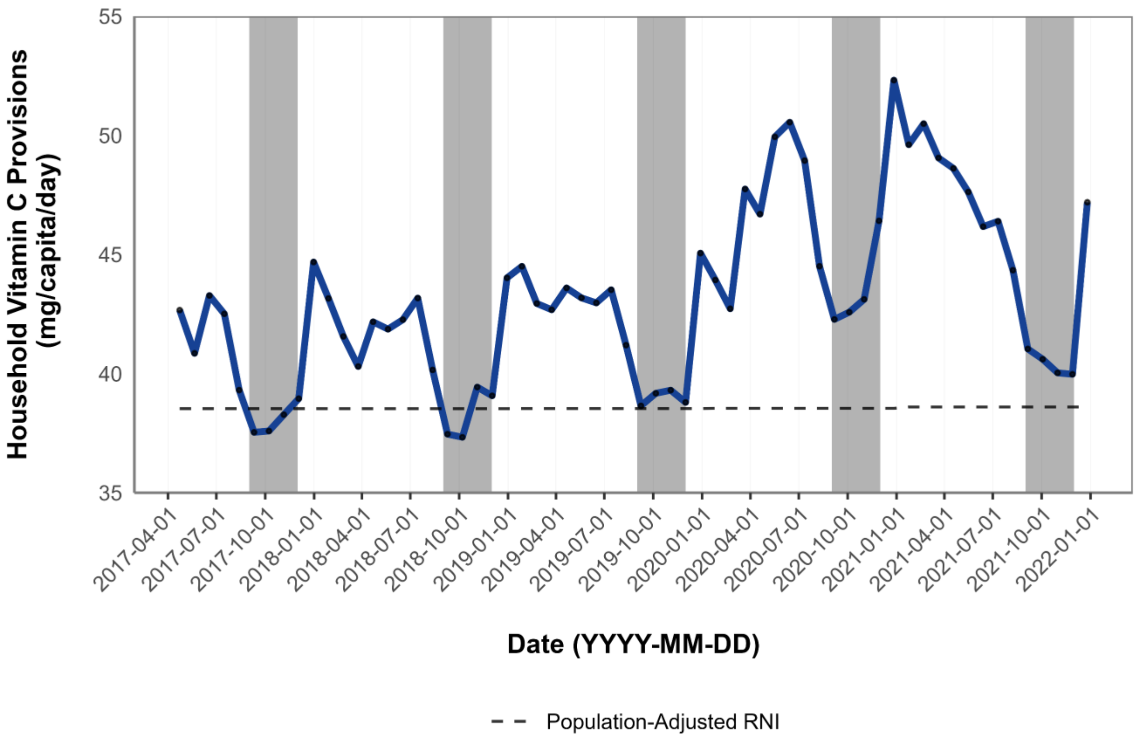


Figure 2.4 Time series showing monthly household vitamin C provisions from fresh fruits and vegetables in the UK between April 2017 and December 2021. The Autumn period (01 September – 30 November) in each year is highlighted in grey.

Overall purchases of fresh fruits and vegetables were found to follow a similar seasonal trend to that of HCPs (Figure 2.5). Seasonal purchases of fresh fruits and vegetables ranged between 446 – 496 (2017), 454 – 501 (2018), 453- 502 (2019), 488 – 574 (2020), and 470 – 571 (2021) thousand tonnes. The lowest annual purchase volumes occurred on 08 October 2017, 09 September 2018, 01 December 2019, 06 September 2020 and 28 November 2021.

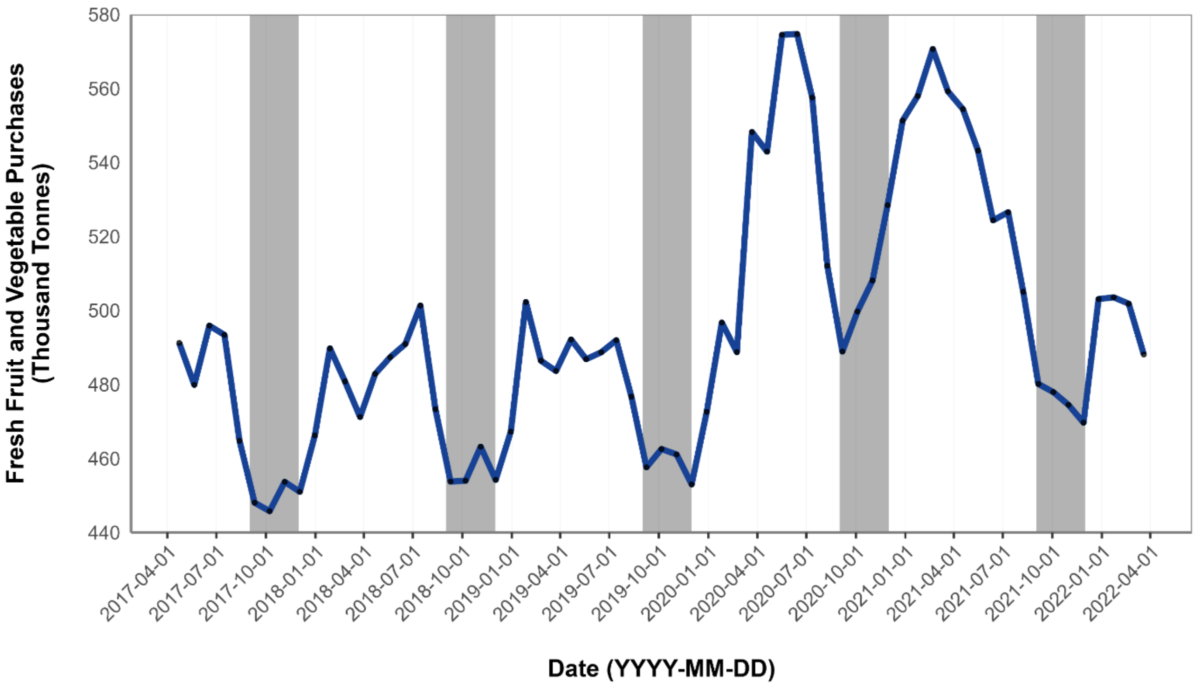


Figure 2.5 Time series showing monthly sales volumes of fresh fruits and vegetables in the UK between April 2017 and March 2022 The Autumn period (01 September – 30 November) in each year is highlighted in grey.

The fresh fruits and vegetables that were found to contribute most to HCP all exhibited unique but consistent seasonal trends (Figure 2.6). The contribution of broccoli to HCP ranged from 2.64 – 4.81 mg/capita/day with maximum HCP values occurring on 31 December 2017, 22 April 2018, 27 January 2019, 27 December 2020 and 21 February 2021. The contribution of broccoli to HCP remained relatively stable across the time series (Figure 2.6).

The seasonal HCP from sweet peppers ranged between 4.79 and 9.85 mg/capita/day. The seasonal maximums in 2017, 2018 and 2019 were consistent and occurred on 18 June (7.06 mg/capita/day), 15 July (7.84 mg/capita/day), and 19 May (7.63 mg/capita/day), respectively. The seasonal contribution of Sweet Pepper to HCP increased to 9.85 mg/capita/day in June 2020. Sweet peppers' contribution to HCP decreased between July and December each year, with seasonal minimums observed at the end of December (Figure 2.6). The seasonal contribution of strawberries to HCP was also greatest in June and July of each year. Annual maximum HCP values ranged between 6.11 and 6.81 mg/capita/day. Similarly to sweet peppers, the contribution of strawberries decreased between July and December, reaching annual minimums of between 1.00 – 1.38 mg/capita/day (Figure 2.6).

For Brussels sprouts, HCP values peaked between 6.44 and 7.17 mg/capita/day at the end of December in each year between 2017 and 2021 (Figure 2.6). Outside of the December peak, HCP from Brussels sprouts did not exceed 2.71 mg/capita/day. The contributions of both cauliflower and easy peeler oranges were also observed to peak at the end of December each year, ranging from between 3.69 – 4.60 mg/capita/day and 4.52 – 5.24 mg/capita/day, respectively. The contributions of cauliflower and easy peeler oranges to HCP decreased steadily to annual minimums in September of each year, before increasing again between September and December (Figure 2.6).

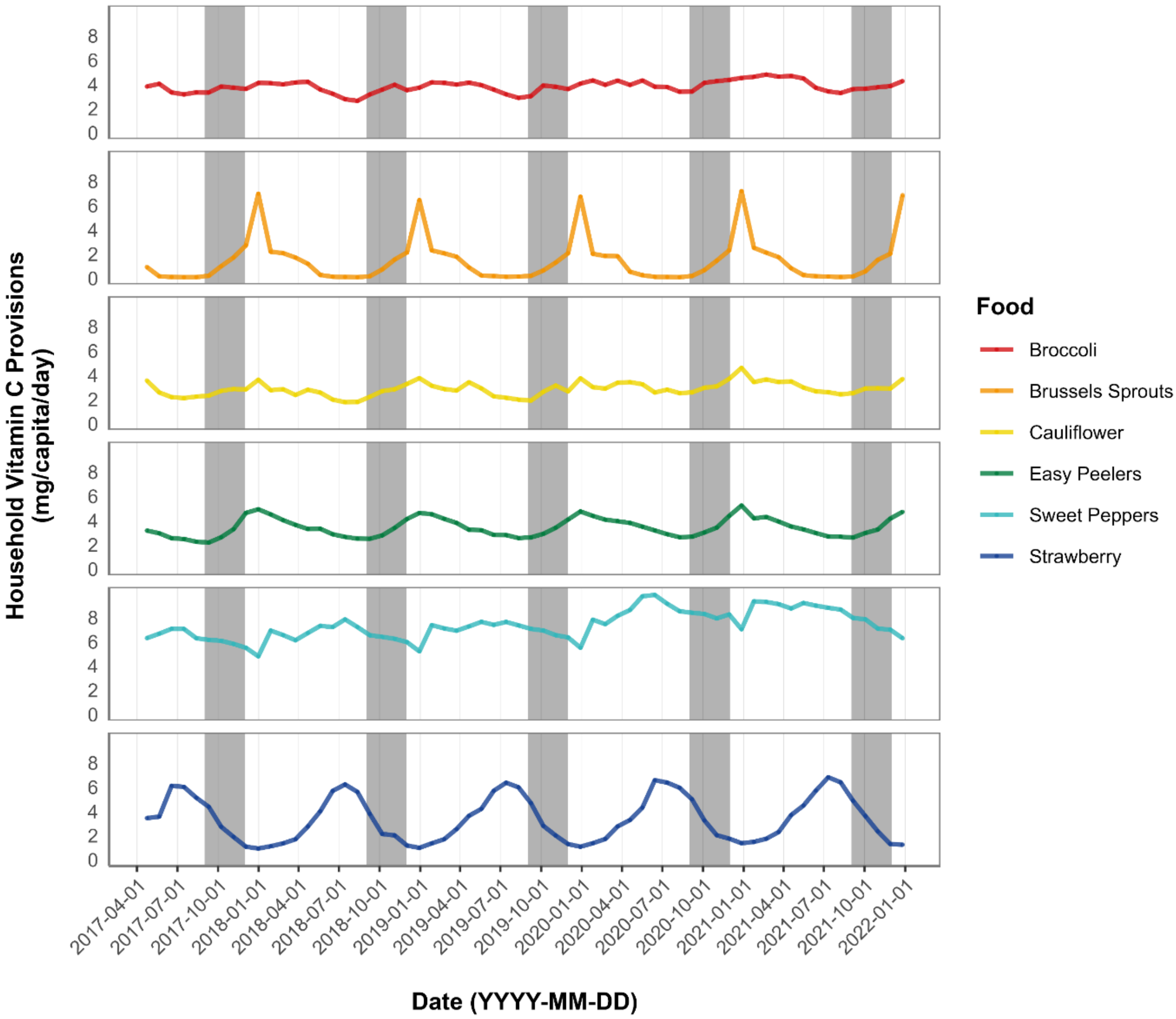


Figure 2.6 Time series showing the monthly contributions of selected fresh fruits and vegetables to household vitamin C provisions in the UK between April 2017 and December 2021 The Autumn period (01 September – 30 November) in each year is highlighted in grey.

2.3.4 Regional Disparities in Household Access to Vitamin C

Concerning regional differences in HCP, the greatest values were observed in the *South* (50.24 mg/capita/day) and *East* (48.06 mg/capita/day) of England (Figure 2.7; Table 2.2). The HCPs for the South and East were both greater than the national mean HCP of 43.6 mg/capita/day. The mean HCP for London (44.80 mg/capita/day) was also greater than the national average but lower than the HCPs observed in the *South* and *East* (Table 2.2).

Household provisions in the *Midlands* (41.83 mg/capita/day), *Scotland* (41.64 mg/capita/day), *Wales* (41.31 mg/capita/day), and the *North* of England (40.59 mg/capita/day) were comparably lower than in the South and East (Figure 2.7; Table 2.2). Regional HCPs were lowest in the *North* of England (40.59 mg/capita/day). The HCPs in these regions were all below the national mean of 43.16 mg/capita/day (Table 2.2).

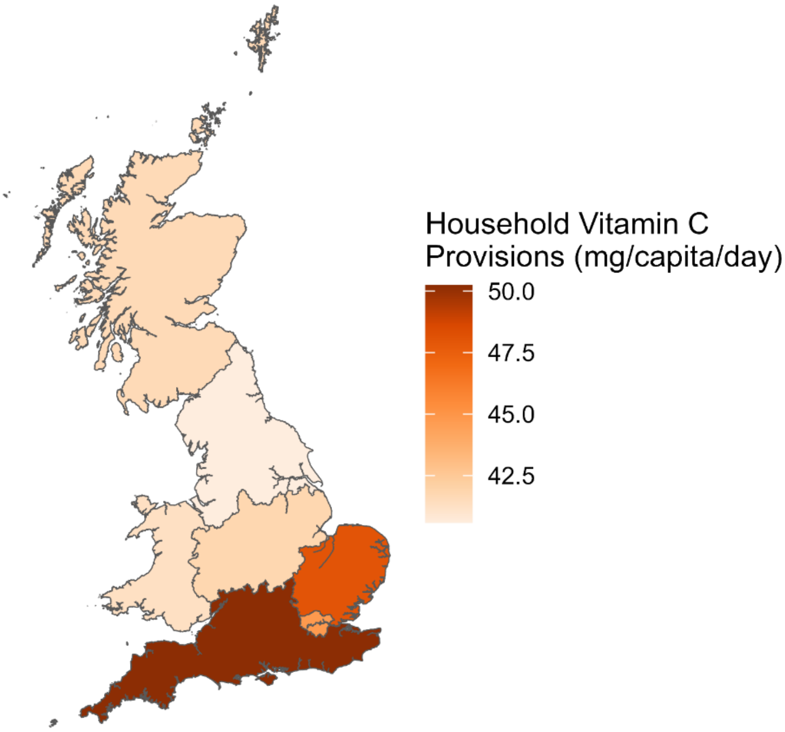


Figure 2.7 Choropleth map of the UK coloured by regional household vitamin C provisions between April 2017 and March 2022. Darker shades of orange indicate higher household vitamin C provisions.

Table 2.2 Regional mean household vitamin C provisions in the UK between April 2017 and March 2022.

Region	**Mean Household Vitamin C Provisions (mg/capita/day)**	**Regional Deviation from UK Mean (%)[1]**
South	50.24	+16.38
East	48.06	+11.34
London	44.80	+3.78
Midlands	41.83	-3.1
Scotland	41.64	-3.53
Wales	41.31	-4.3
North	40.59	-5.95

[1]Regional deviations are relative to the UK mean household vitamin C provisions of 43.16 mg/capita/day.

The seasonal trends in each region were consistent with the trends already described for national HCPs (Section 2.3.3). All regions exhibited the same seasonal trends in HCP, with the minimum values occurring between September and November and the maximum values occurring at the end of December in all years between 2017 and 2021 (Figure 2.8).

Seasonal HCPs in the *South* and *East* of England ranged between 43.85 - 61.22 mg/capita/day and 40.57 - 58.17 mg/capita/day, respectively. The HCPs in the *South* and *East* were always greater than the population-adjusted RNI (Figure 2.8).

Seasonal HCPs in the *Midlands*, *Scotland*, *Wales* and the *North* were similar over the same five-year period, ranging between 34.65 – 50.39 mg/capita/day. Lower overall HCPs in the *Midlands*, *Scotland*, *Wales* and the *North* translated into seasonal minimums that were lower than the population-adjusted RNI in 2017, 2018, 2019, and 2021 (Figure 2.8).

Between April 2017 and February 2020, seasonal HCPs in *London* closely resembled those of the *Midlands*, *Scotland*, *Wales* and the *North*, ranging between 36.15 - 45.35 mg/capita/day (Figure 2.8). After the sharp increase in HCPs between 23 February and 22 March 2020, HCPs in London became more similar to those observed in the *South* and *East* with values ranging between 44.11 – 55.74 mg/capita/day (Figure 2.8).

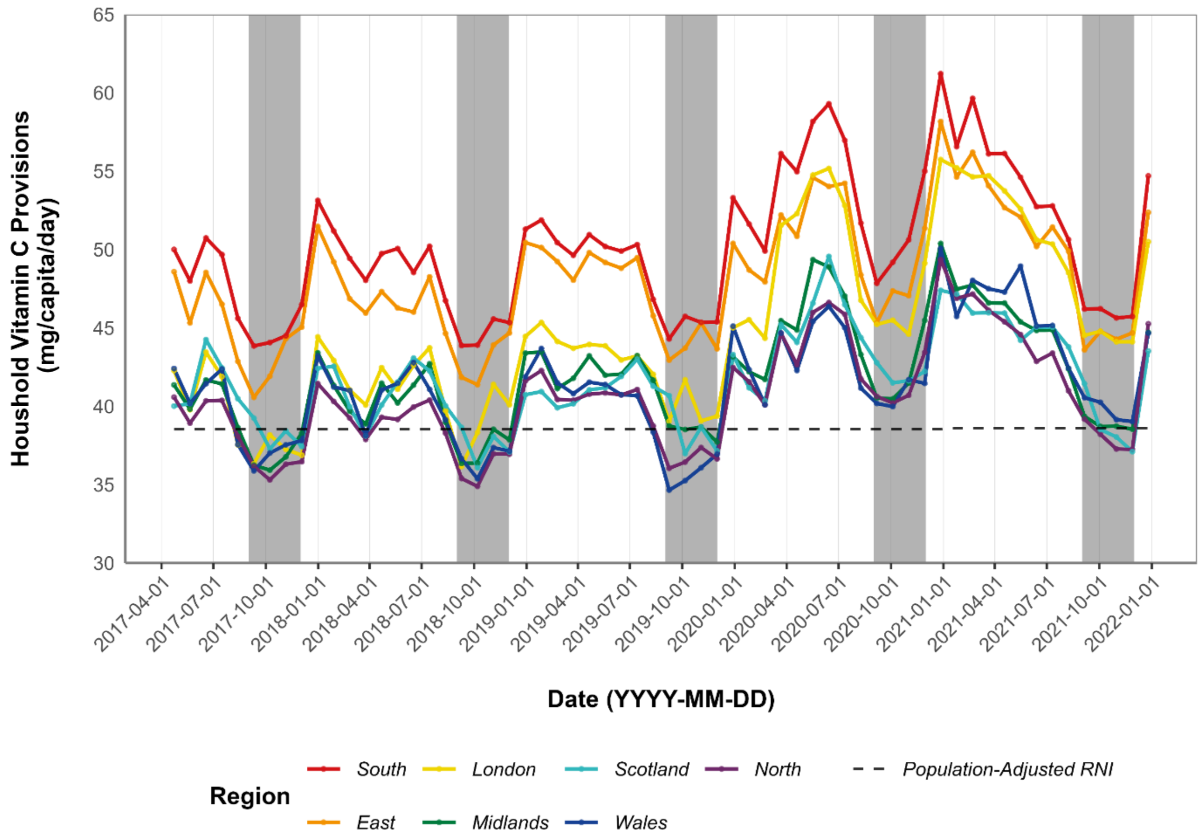


Figure 2.8 Time series showing monthly household vitamin C provisions from fresh fruits and vegetables in different regions of the UK between April 2017 and December 2021. The Autumn period (01 September – 30 November) in each year is highlighted in grey.

2.4 Discussion

2.4.1 The Contribution of Fresh Fruits and Vegetables to Household Vitamin C Provisions

Based on consumer purchase data from Kantar World Panel, HCPs from fresh fruits and vegetables were sufficient to support the UK's vitamin C requirements between April 2017 and March 2022. The estimated annual HCPs ranged between 106% and 120% of the population-adjusted RNI. Interestingly, an increase in annual HCP was observed between 2019 and 2020. Further analysis of seasonal trends in HCP revealed that this increase coincided with the start of the SARS-CoV-2 lockdowns in March 2020. Previous reports have indicated that the number of people cooking from scratch increased during the lockdown periods (Carroll et al., 2020). As the

Kantar data relate specifically to foods purchased for at-home consumption, the amount of fruits and vegetables captured by Kantar's consumer panels would also have been higher during the lockdowns (Revoredo-Giha et al., 2022; Scott and Ensaff, 2022). Consistent with these explanations, HCPs were observed to decrease back towards pre-pandemic levels in 2021 and early 2022. Despite concerns about reduced access to fruits and vegetables due to Brexit and SARS-CoV-2 (Poppy et al., 2022; The Food Foundation, 2017), fresh produce has continued to meet the UK's vitamin C requirements between 2017 and 2022. This is a positive indicator of the resilience of the UK food system in the face of global and regional crises (Moran et al., 2020).

Whilst HCP did exceed vitamin C requirements, the calculated values were far lower than previous estimates of the UK's total amount of available vitamin C (Macdiarmid et al., 2018; Poppy et al., 2022). Indeed, Poppy et al. (2022) found that overall food supplies contributed 299% of the UK's vitamin C RNI in 2016. There are, however, several factors that must be unpacked to give the HCP estimates context in relation to previous studies.

One immediate distinction is that the current study only considered fresh fruits and vegetables, rather than all food sources (Macdiarmid et al., 2018; Poppy et al., 2022). The choice to focus on fresh fruits and vegetables was driven by the fact that previous analyses had shown that fruits and vegetables accounted for between 85-90% of total vitamin C supplies (Poppy et al., 2022). The additional 10-15% of vitamin C supplied from other foods will not have been captured in the HCP estimates. Furthermore, the HCP estimates did not account for the contribution of frozen, juiced, or canned foods which made up 23.2% of household fruit and vegetable provisions (Figure 2.1). However, the contribution of processed fruits and vegetables to HCP is not directly comparable to that of fresh produce due to the variable effects of processing on vitamin C retention. Processes such as canning and blanching can result in vitamin C losses ranging from 10% to 80%, depending on the crop and processing method (Rickman et al., 2007). As such, fresh produce is still the largest constituent of food-derived vitamin C supplies in the UK. The HCP estimates therefore cover the majority of the UK's vitamin C and provide a strong foundation in the absence of similar data for a broader range of foods. Expanding the HCP analysis to include other food types would, however, be expected to result in higher estimated household provisions.

Beyond the apparent differences in coverage of food types, the HCP estimates also relate to a very specific point in the food supply chain. Whereas Macdiarmid et al. (2018) and Poppy et al. (2022) estimate the theoretical maximum amount of vitamin C available from UK food supplies, the current study was focused on the amount of vitamin C available from fresh fruits and vegetables that reach people's households. Between the points at which food enters the UK and subsequently reaches consumer households, there are multiple opportunities for foods and their constituent nutrients to be diverted away from households or lost entirely.

Food waste directly reduces the amount of food that reaches consumer households and, as such, would not be captured in HCP estimates. A recent POST-note examined the amount of food waste produced in the UK supply chain both upstream and downstream of consumer households (Bradshaw and Wentworth, 2024). Work by the Waste and Resources Action Programme (WRAP) estimated that food waste in processing, retail, and catering accounted for 2.7 million tonnes of waste in 2021 (25% of the total estimated waste) (WRAP, 2023). It has also been noted that waste values in these parts of the supply chain are likely underestimated due to the absence of mandatory waste reporting streams (Bradshaw and Wentworth, 2024). Thus, the amount of fruits and vegetables that reach consumers will be lower than overall supply estimates (Macdiarmid et al., 2018; Poppy et al., 2022). Rather than having to make assumptions about pre-household food waste, however, the HCP estimates inherently account for it.

It is also true that the Kantar consumer purchase data do not include foods that are consumed outside of the household, such as dining out or lunches purchased at a workplace, for example. However, the impact that non-household purchases would have on vitamin C provisions is likely to be negligible. Findings from the Living Costs and Food Survey showed that, for the three years from 2017 to 2021, household food consumption accounted for between 89.6% and 91.1% of estimated vitamin C intakes (DEFRA, 2022). These figures are suggestive that the consumption of vitamin C-rich fruits and vegetables is predominantly confined to the home. Even still, the Kantar data specifically describe retail purchases and do not consider other food outlets, such as local markets or food banks, that may also contribute to household fruit and vegetable provisions. Food banks are becoming an increasingly used method of accessing food (Safayet et al., 2024), with a recent report from the Trussel Trust indicating that 655,000 people used a food bank for the first time in 2023-2024, with a further 3.1 million food parcels delivered to support food insecure families (The Trussel Trust, 2024). This is another instance that highlights the importance of understanding the specificity of the Kantar data when making wider inferences about the UK food system.

Ultimately, it is reasonable that the HCP values derived from the Kantar data are lower than the overall supply estimates of Macdiarmid et al. (2018) and Poppy et al. (2022). Inconsistent coverage of foods between the studies does, however, make direct cross-examinations difficult. Moreover, the Kantar retail data do not incorporate alternative food outlets and will be an underestimation of the total vitamin C supplies that reach households as a result. However, even without 100% coverage of household food supplies and accounting for supply chain losses, the estimated HCPs are supportive of the conclusion that UK vitamin C supplies are sufficient to support population requirements at a macro scale (Poppy et al., 2022).

2.4.2 Popular Fruits and Vegetables in Vitamin C Provisions

Household vitamin C provisions were predominantly comprised of sweet peppers, broccoli, cauliflower, strawberries, easy peeler oranges, and tomatoes. Except for easy peeler oranges, the primary contributors to household vitamin C provisions are crops that the Food Foundation have previously described as "Brexit-Boosters" or "Channel Hoppers" (The Food Foundation, 2017). That is, the UK has the capacity to be more self-sufficient for the fruits and vegetables that contribute most to HCP (The Food Foundation, 2017). As such, increased self-sufficiency in these key crops could help to address concerns regarding future instabilities in vitamin C supplies due to shocks in international supply chains (Macdiarmid et al., 2018; Poppy et al., 2022; Scheelbeek et al., 2020).

The primary sources of HCP identified in the current study do, however, show several differences from the top 10 imported sources of vitamin C compiled by Poppy et al. (2022). In the current study, sweet peppers had the highest contribution to HCPs (17% of total vitamin C provisions). In contrast, sweet peppers did not feature at all in the top 10 presented by Poppy et al. (2022), whose analyses were based on HMRC trade data (HMRC, 2022). Similarly, Poppy et al. (2022) identified bananas as the number one contributor to imported vitamin C supplies in 2017 (24% of total supplies), yet bananas were ranked 11 with respect to HCP (3% of total provisions).

As pointed out by Poppy et al. (2022), bananas are not a vitamin C-dense fruit, containing 5 mg/100g of raw flesh compared to 120 mg/100g for sweet peppers (Public Health England, 2015). The supply of bananas would, therefore, need to be 24-fold greater than that of peppers to provide the same amount of vitamin C. In an analysis of both domestic and imported produce, Scheelbeek et al. (2020) showed that bananas made up 7.8% of total fruit and vegetable supplies compared to 1.3% for sweet peppers. The difference in overall supplies described by Scheelbeek et al. (2020) is not great enough for bananas to contribute more vitamin C than sweet peppers and thus supports the findings of the current study. Bananas are also subject to high levels of pre-household waste relative to other fruits and vegetables (Filimonau and Gherbin, 2017). As such, waste could further contribute to the reduced contribution of bananas to HCP relative to more vitamin C-dense crops such as sweet peppers, broccoli and tomatoes. It is important to note, however, that the Kantar retail data, and the HCPs that have been derived from them, do not extend to how much vitamin C is consumed from each fruit or vegetable. As such, further study of dietary survey data, such as in the NDNS

(Bates et al., 2019), would be useful to corroborate the top 10 suppliers of vitamin C with the top 10 most consumed sources of vitamin C. This distinction could be useful for identifying the sources of vitamin C that different population groups are most likely to consume and, thus, help to further refine targeted dietary interventions.

2.4.3 Predictable Seasonal Instabilities in Access to Vitamin C

One of the key strengths of the Kantar data was that HPC could be estimated at monthly intervals. A consistent trend that was observed in the Kantar data was that HCP from fresh fruits and vegetables were lower during the Autumn months than at any other point in the year. As a result, HCP consistently fell below the population-adjusted RNI between September and November. Concerningly, the reduction in HCP in the Autumn coincides with the start of seasonal increases in the incidence of Influenza and other respiratory infections (Nichols et al., 2021). Given the importance of vitamin C in mitigating the severity of such infections (Holford et al., 2020; McAuliffe et al., 2020), it will be important to understand the nature of the Autumnal reduction in HCP and whether that reduction translates into lower vitamin C intakes and utilisation of vitamin C.

In the present study, the Autumn was characterised by a consistent decrease in overall fresh fruit and vegetable purchases. Autumn also marked a transitional period in which the contributions of sweet peppers and strawberries to HCP declined following their peak in Summer, while the contributions of broccoli, cauliflower, and easy peeler oranges were beginning to increase toward their peak levels in Winter. Seasonal changes in consumer preferences for different fruits and vegetables have previously been reported in other studies (Toorang et al., 2013). Seasonal preferences can be driven by a range of factors including weather, locality of produce and seasonal holidays (He et al., 2021; Spence, 2021). Spence (2021) comments on the associations between warmer Summer weather and sales of fresh fruits like strawberries, whereas colder Winter weather drives a preference for hearty vegetables. This is consistent with the trends observed in the current study. Increasing preference for vegetables over fruits in the Autumn could lead to further vitamin C losses due to the effects of cooking on vitamin C retention. Boiling broccoli, for example, has been shown to reduce vitamin C content by 50% (Lee et al., 2018).

Given the dominant contribution of fruits and vegetables to vitamin C supplies, it is unlikely that seasonal preferences for other foods will make up for the Autumnal reduction in HCP. It will, however, be important to examine seasonal trends in dietary survey data to confirm if food purchase trends translate into lower vitamin C intakes and utilisation. A previous study in Finland identified seasonal trends in vitamin C intakes that were linked to seasonal consumption of fruits and vegetables (Paalanen et al., 2014). There have also been indications that the amount of vitamin C in people's blood is lower in the Autumn than in other seasons (Ness et al., 1999). There is, therefore, a growing body of congruent evidence to support the existence of a predictable Autumnal reduction in vitamin C accessibility that directly translates into lower utilisation of vitamin C. Seasonal instabilities in the UK are examined further in Chapter 3, using the nationally representative National Diet and Nutrition Survey (NDNS) to study seasonal changes in vitamin C intakes and blood biomarker levels (Section 3.4.1.1).

2.4.4 Regional Access to Vitamin C

Disparate trends were also observed in regional HCPs. The lowest HCPs were observed in the *North* of England, the *Midlands*, *Scotland* and *Wales*, compared to higher HCPs in the *South* and *East* of England. In combination with the consistent Autumnal reductions in HCP, regional HCPs regularly fell below the population-adjusted RNI in the *North* of England, the *Midlands*, *Scotland*, and *Wales*. Other direct analyses of regional HCP or fruit and vegetable supplies are currently lacking in the UK. Differences in vitamin C intakes have, however, been reported between geographic regions in Finland, Russia, and India (Paalanen et al., 2014; Ravindran et al., 2011). In the context of the UK, the well-documented socioeconomic disparities between the wealthy *South East* of England and the rest of the UK can provide context to the findings of the current study (Corris et al., 2020; McCann, 2020). These socioeconomic inequalities have subsequently been linked with inequalities in diet quality and health (Johnstone and Lonnie, 2023; Maguire and Monsivais, 2015; Roos et al., 2001). In relation to HCP, adults from the lowest income households in the UK have been found to consume at least one fewer portion of fruits and vegetables compared to the highest income households, with even greater disparities observed in specific age groups (Pinho-Gomes et al., 2021). Several studies have also found that individuals of lower socioeconomic status are at greater risk of insufficient vitamin C intake and vitamin C deficiency (Mosdøl et al., 2008; Schleicher et al., 2009). As a result, higher numbers of lower-income households in the *North*, *Midlands*, *Scotland* and *Wales* could be a primary contributing factor to the observed disparities in HCP.

Whilst adding more granular detail to the existing estimates of nationwide vitamin C supplies, the geographic regions used in the Kantar dataset are still an amalgamation of the major geographic regions of the UK (2.2.1.1). Indeed, high levels of food insecurity have been reported in local authorities in the *South West* of England, whilst levels of food insecurity were comparatively lower in local authorities in the *South East* (Moretti, Whitworth and Blake, 2021). Resultingly, our understanding of sub-regional disparities in access to vitamin C is still limited.

Of further concern in relation to regional health inequalities is the increased rates of obesity in low-income households (Case and Kraftman, 2024). Observational studies have estimated that for every 10 Kg increase in body weight, an individual requires an additional 10 mg/day of vitamin C to achieve similar blood biomarker levels (Carr et al., 2024, 2022). As a result, the population requirements of the *North*, *Midlands*, *Scotland* and *Wales* may be higher than the population-adjusted RNI used as a reference in the current study. Further analysis of regional vitamin C intakes and blood biomarkers in the UK are presented in Chapter 3 to corroborate the trends observed for HCP (Section 3.4.1.2).

2.5 Conclusions

At a macro-level, the analysis of HCPs presented in Chapter 2 showed that purchases of fresh fruits and vegetables in the UK were sufficient to meet the UK's vitamin C RNI. Sufficient HCPs occurred in every year between 2017 and 2022, demonstrating the resilience of the UK's food system to supply chain disruptions related to Brexit, SARS-CoV-2, and climate and geopolitical shocks.

When analysing the HCP data with greater granularity, however, it became evident that national and annual averages mask a more complex picture of UK vitamin C security. A consistent and predictable reduction in HCPs during the Autumn was associated with overall decreases in purchases of vitamin C-rich fruits and vegetables. The seasonal instabilities resulted in HCPs consistently falling below the UK RNI during the Autumn.

The impacts of the seasonal instabilities on HCPs were further confounded by regional disparities. Households in the *North*, *Midlands*, *Scotland* and *Wales* had lower HCPs than households from the *South* and *East* of England. The regional disparities enhanced the effects of the seasonal instabilities and resulted in HCPs well below the UK RNI in the *North* of England.

The accessibility and stability of UK vitamin C supplies could, therefore, be threatened by seasonal and regional trends that have not been observed in previous analyses of overall UK food supplies. Further validation of these trends using dietary survey data will be addressed in Chapter 3. The subsequent analysis presented in Chapter 3 will facilitate a greater understanding of how supply and HCP trends translate into vitamin C intakes and biological status in the UK, overcoming the limitations in supply chain coverage that have been discussed in relation to HCPs in Chapter 2.

Chapter 3 The Relationships Between Vitamin C Intakes and Status in a Large UK Cohort and Their Implications for Assessments of Vitamin C Security

3.1 Introduction

Developing a holistic understanding of micronutrient security requires the concerted examination of the availability, accessibility, utilisation and stability of a given micronutrient (FAO, 2008). Food supply and nutrient composition data underpin a relatively strong understanding of micronutrient availability in the UK. Multiple studies have found that UK food supplies are sufficient to meet the population's recommended nutrient intakes (RNI) for iron, calcium, zinc, and vitamins A, B9, B12, and C (Macdiarmid et al., 2018; Poppy et al., 2022). Household provisions of fresh fruits and vegetables alone have also been shown to be sufficient to support the UK RNI for vitamin C (Section 2.4.1). However, despite the positive and congruent indications micronutrient availability is sufficient to support population requirements, the public health burden of micronutrient deficiencies is of ever-increasing concern (Miller et al., 2016).

The disconnect between the macro, national-scale availability of micronutrients and public health concerns is an indicator of the need to better understand the accessibility and utilisation of UK micronutrient supplies. As discussed in Chapter 2, accessibility refers to the distribution of available micronutrients throughout a population (Section 2.1). Utilisation goes one step further, ultimately describing the amount of food or nutrients that are consumed and absorbed into people's blood (FAO, 2008). The extent to which studies can consider access and utilisation of micronutrient supplies is often limited by the availability of suitable data (Stevens et al., 2022). The UK is in an advantageous position in this regard, having access to datasets that monitor both micronutrient intakes and blood biomarkers on a national scale (Bates et al., 2020; Day et al., 1999).

The importance of more holistic examinations of micronutrient security is evident when comparing the outcomes of disconnected assessments of availability, access, and utilisation. National supplies of vitamin B9 (folate), for example, have been estimated to equate to ~200% of UK RNI (Macdiarmid et al., 2018). In contrast, analysis from the National Diet and Nutrition Survey (NDNS) has found that 52% of adults and 53% of children had levels of serum folate indicative of deficiency (Bates et al., 2020). Similarly, vitamin A supplies in 2016 were sufficient to support 167% of the UK's requirements (Poppy et al., 2022). In contrast, vitamin A intakes were observed to be below the lower reference nutrient intake (LRNI) in 10% of UK adults and 18% of older children (Bates et al., 2020). Sufficient micronutrient supplies do not, therefore, necessarily indicate a state of micronutrient security. In fact, specific sub-populations appear to experience variable levels of access and utilisation with respect to specific micronutrients. Further examination of both dietary intake and blood biomarker data will be crucial for identifying the factors that are limiting access and utilisation of specific micronutrients.

Concerning UK intakes and biological status, vitamin C is a micronutrient for which detailed analysis is lacking (Section 1.2.3). Findings from the NDNS have indicated that severe vitamin C deficiencies - indicated by plasma ascorbic acid (PAA) concentration < 11μmol/L - occur in less than 5% of the UK population. Analysis of the European Prospective Investigation of Cancer-Norfolk cohort (EPIC-Norfolk) (Day et al., 1999) has shown that median vitamin C intakes were 90% higher than the UK RNI of 40 mg/day (Carr et al., 2024). Carr et al. (2024) also found median PAA concentrations in the EPIC-Norfolk cohort to exceed 50 μmol/L, which is widely accepted as an "adequate" amount of circulating PAA (Ebenuwa et al., 2022; European Food Standards Agency, 2013; German Nutrition Society, 2015). Similarly to assessments of vitamin C availability, observational studies suggest that the overall outlook for UK vitamin C security is of lesser concern than for other key micronutrients such as folate, iron, and vitamin A (Bates et al., 2020).

However, Chapter 1 also detailed several UK-specific and international studies that have identified risk factors associated with inadequate access and utilisation of vitamin C (Section 1.2.3.2). The incidence of poor vitamin C status has been reported to be disproportionately high in certain sub-populations including South Asians, men, smokers, diabetics, and those of low socio-economic status (Mosdøl et al., 2008; Ness et al., 1999; Shohaimi et al., 2004). Body weight has also been directly associated with increased vitamin C requirements and reduced vitamin C intakes (Carr et al., 2024). The potential impacts of body weight on vitamin C security are of particular interest in the context of the increasing prevalence of overweight and obesity in the UK (Johnstone and Lonnie, 2023; Keaver et al., 2018). Finally, Chapter 2 identified seasonal instabilities and regional disparities in household vitamin C provisions (Sections 0 and 2.3.4). It will be important to corroborate these trends with intake and biomarker data to understand if insecurities further up the food supply chain translate into inadequate intakes and status.

3.1.1 Aims and Objectives

To facilitate a concerted assessment of UK vitamin C security, Chapter 3 will address the second key aim of this thesis: **To conduct a detailed analysis of vitamin C intakes and**

biological status in the UK population and to understand the prevalence and impact of known risk factors on an individual's vitamin C requirements. Specifically, Chapter 3 will interrogate the complete NDNS dataset, comprised of observations from 15,565 participants studied between 2008 and 2019. The analyses address three key objectives:

1) To describe vitamin C status in the UK population using conventional biomarker thresholds.

2) To understand whether the UK RNI is suitable to support adequate vitamin C status.

3) To identify social, health, and lifestyle factors that can influence an individual's ability to access sufficient vitamin C as well as their biological requirements to achieve adequate vitamin C status.

3.2 Materials and Methods

3.2.1 Data Sources

The NDNS is a rolling, cross-sectional programme funded by the UK government to survey dietary behaviours, nutrient intake, and nutritional status of the UK population living in private households aged over 1.5 years. The NDNS has been running since 2008 and to date has published data for 11 years, up to and including 2019. Each year, the survey aims to collect data from ~1000 individuals (500 adults (19+ years of age) and 500 children (1.5 - 18 years of age)). Households are randomly selected from postcode sectors (primary sampling units) across the UK to recruit a set of households that are representative of the varying demography of the UK. All individuals give informed consent, and the survey is conducted in accordance with ethical approval obtained from the Oxfordshire A Research Ethics Committee (Ref. No. 07/H0604/113) and the Cambridge South NRES Committee (Ref. No. 13/EE/0016) (Bates et al., 2020).

The survey comprised two main stages; Stage one involved the completion of an interviewer-administered four-day food diary (three weekdays and one weekend day). Food diaries were subsequently combined with information from the UK's Composition of Foods Integrated Dataset (CoFID) to estimate nutrient intakes. Physical measurements, lifestyle and physical activity information, and urine samples were also collected in stage one. Stage two occurred at a later date and comprised of a visit by a trained nurse who collected a blood sample (fasting or non-fasting depending on the ability or willingness of individuals to do so) from the participant, recorded additional physical measurements including waist and hip circumferences, demispan, and blood pressure, and recorded information about any prescribed medications. All data were subjected to curation and correction. The survey data were also weighted to correct for nonresponse bias and participant drop-outs. Full details on the household selection and random participant selection procedures, as well as full details regarding sample and data collection and processing, are available in the NDNS User Guide and supplementary methods documents (Bates et al., 2020; Page et al., 2019).

The NDNS data were accessed through the UK Data Service (https://ukdataservice.ac.uk/). The data were stored and used in accordance with the UK Data Service End User Licensing Agreement. Additional Ethical approval for the use of the data was granted by the University of Southampton under ERGO reference 89918.

3.2.2 Cohort Filtering

The cross-sectional analysis reported in Chapter 3 studied a subset of the 15,655 individuals surveyed between 2008 and 2019. Individuals were excluded if they were: Firstly, below 19 years of age; secondly, reported the use of dietary supplements; and thirdly, didn't provide a valid blood sample or had missing plasma ascorbic acid data. The final cohort size after these exclusions consisted of 3243 individuals, equivalent to 20.7% of the original cohort size (Figure 3.1).

Participants under the age of 19 years were excluded from the analysis because previous analyses of population status and the setting of RNI's have been derived from adult intake and status data (Carr and Lykkesfeldt, 2021; European Food Standards Agency, 2013; German Nutrition Society, 2015). The adult age group (19+ years of age) is consistent with the age groups used by the UK government in published dietary recommendations and previous analyses of the NDNS data (Bates et al., 2019; Public Health England, 2018)

Dietary supplement users were excluded for the same reason described for participants under the age of 19 years. Furthermore, as the current study was investigating vitamin C intakes derived from food, supplement users also had the potential to create a disproportionately large and skewed effect when considering the relationships between vitamin C intakes and status.

After sub-setting and combining data from multiple survey years, the survey weightings were adjusted according to NDNS documentation (Bates et al., 2019). For the current study, the relevant weightings were those for which a valid blood sample had been taken.

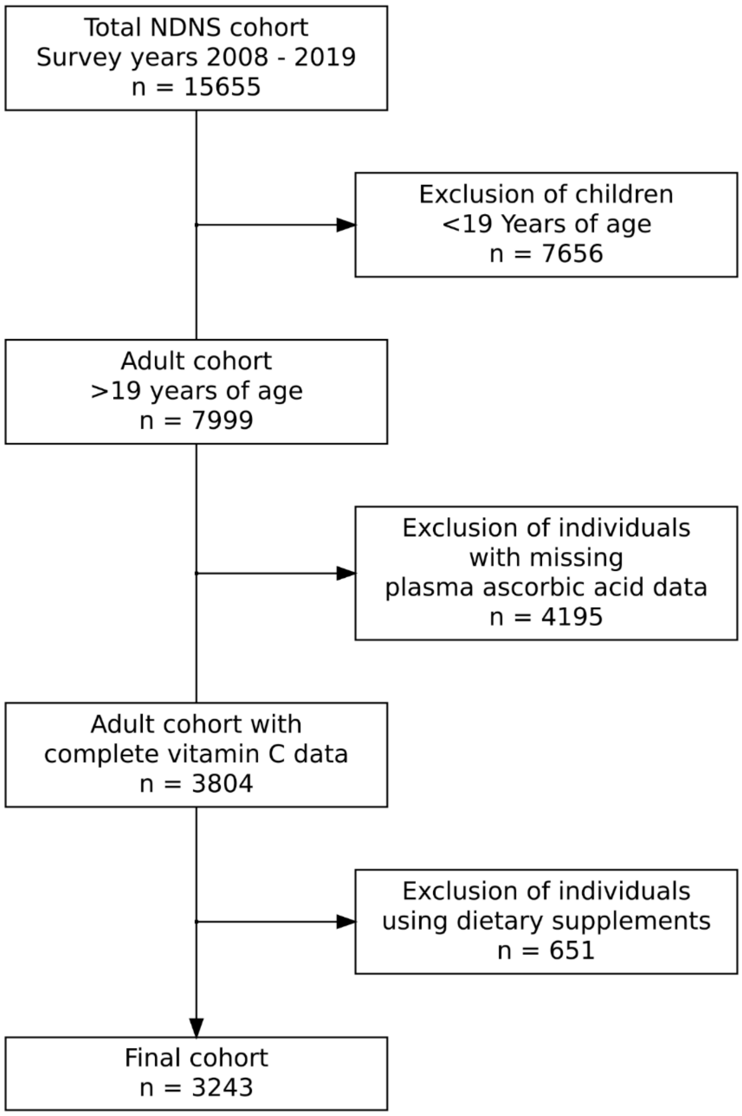


Figure 3.1 Exclusion criteria and filtering of the NDNS years 1-11 combined rolling cohort.

3.2.3 Defining Vitamin C Intakes, Biomarkers, and Status Thresholds

Vitamin C intakes were considered as the average daily amount of vitamin C consumed (mg/day) based on the complete food diary data collected by the NDNS. Vitamin C intakes were pre-determined in the NDNS dataset according to the conversion methodology described in section 3.2.1 and in the NDNS user guide (Bates et al., 2020; Page et al., 2019).

Vitamin C status biomarkers were presented as plasma ascorbic acid (PAA) concentration (μmol/L), which is widely accepted as an accurate indicator of vitamin C status in both clinical and observational settings (European Food Standards Agency, 2013). Information on the methods and quality control of PAA blood analyses can be found in Appendix Q of the latest NDNS report (Bates et al., 2020). Briefly, blood samples were immediately stabilised in 10%

(w/v) metaphosphoric acid, from which PAA quantification was carried out according to a fluorometric assay described by Vuilleumier and Keck (1989).

Vitamin C status thresholds were defined as follows: Deficient (PAA <11μmol/L); Marginal deficiency / Hypovitaminosis C (PAA 11-23 μmol/L); Inadequate (PAA 23-50 μmol/L); Adequate (PAA 50-70 μmol/L); and Saturating / Optimal (PAA <70 μmol/L). The selected thresholds were informed by those set out by Lykkesfeldt and Poulsen (Lykkesfeldt and Poulsen, 2010), which have been largely accepted in contemporary studies of vitamin C status and are based on the findings of randomised control trials and observational studies (Carr et al., 2024; Ebenuwa et al., 2022; European Food Standards Agency, 2013; German Nutrition Society, 2015). The threshold for deficiency also matches the one used in previous studies of the NDNS cohort (Bates et al., 2019).

3.2.4 Selection of Population Characteristics of Interest

A series of health, lifestyle, and social factors were selected as potential predictors of vitamin C intakes and PAA in the NDNS cohort. The factors selected for inclusion in logistic regression models were informed by the findings of previous studies concerning vitamin C status in various sub-populations (Table 3.1). Previous studies have identified age, ethnicity, low income, season, diabetes, smoking, body weight, and sex as factors that can impact an individual's vitamin C intake and/or PAA. The current study also included geographic sub-regions of the UK based on the findings presented in Chapter 2, concerning differences in regional household vitamin C provisions from fresh fruits and vegetables (Section 2.3.4). The selected factors, their definitions, and supporting literature are presented in Table 3.1.

Table 3.1 Selected health, lifestyle, and social factors known to impact vitamin C requirements.

Factor	Definition	Factor Levels	Supporting Literature
Season	Meteorological Season of NDNS house visit/blood sample.	Winter (December, January, February) Spring (March, April, May) Summer (June, July, August) Autumn (September, October, November)	(Ness et al., 1999; Ravindran et al., 2011; Toorang et al., 2013) (Section 0)
Geographic Region	Geographic regions of the UK in which individuals were residents. Regional geographies were consistent with those used in official statistical and administrative documents. To maintain continuity with the regions analysed in Chapter 2, the *South*, *North*, and *Midlands* regions were created by combining multiple specified regions (See Section 2.2.1.1).	*South* *London* *East of England* *Midlands* *North* *Scotland* *Wales*	(Mosdøl et al., 2008; Paalanen et al., 2014; Ravindran et al., 2011) (Section 2.3.4)
Sex	The biological sex of the individual as reported in the NDNS.	Female Male	(Jungert and Neuhäuser-Berthold, 2015; Ness et al., 1999; Wrieden et al., 2000)
Ethnicity	Ethnicity of individuals categorised according to ethnic groups reported in the NDNS and official UK Census data.	White Asian Black Mixed ethnicity Other ethnic identification	(Carr and Lykkesfeldt, 2023a; Donin et al., 2016; Ness et al., 1999)
Income	Equivalised household income split into tertiles.	Highest Middle Lowest	(Donin et al., 2016; Mosdøl et al., 2008; Pearson et al., 2017)

Factor	Definition	Factor Levels	Supporting Literature
Smoking Status	Smoking status of individuals based on response to NDNS.	Non-current smoker Current smoker	(Carr and Lykkesfeldt, 2023a; McCall et al., 2019; Schleicher et al., 2009)
Body Weight	Body weight groups based on established BMI categorisation (Ardern et al., 2004; Ross et al., 2020).	Healthy Weight (BMI 18.5-24.9) Overweight (BMI 25.0-29.9) Obese (BMI >29.9)	(Carr et al., 2022; Carr and Lykkesfeldt, 2023a; Lykkesfeldt and Carr, 2023)
Diabetes	Diabetes status of individuals based on response to NDNS. Diabetes status was based on clinical diagnoses of diabetes. Type-I and Type-II diabetics were not distinguished.	Not Diabetic Diabetic	(Carr et al., 2023; Ebenuwa et al., 2022; Wilson et al., 2017)
Age Group Tertile	Participants separated into tertiles based on their age in years.	Youngest (19-43 years) Middle (43-58 years) Oldest (58-91 years)	(Bates et al., 1999; Carr and Lykkesfeldt, 2023b; McCall et al., 2019; Ravindran et al., 2011)

3.2.5 Statistical Analysis

Continuous variables were described using the median and interquartile range. Categorical variables were described using counts and proportions relative to the total cohort size. Group comparisons of median values were conducted using a modified Kruskal-Wallis test that can account for complex survey designs (Lumley, 2024, 2004). Post-hoc tests were conducted using pair-wise Wilcoxon rank-sum tests. Statistically significant differences between groups were accepted at an alpha threshold of 0.05.

Binomial logistic regression models were fitted to identify which of the selected health, lifestyle, and social factors had a significant effect on vitamin C intakes and PAA in the NDNS cohort. The "Ethnicity" and "Diabetes Status" factors were excluded from regression analyses due to the group sizes being considered too unbalanced to make meaningful statistical inferences.

Models were fit using the {*survey*} (Lumley, 2024, 2004)and {*srvyr*} (Ellis and Schneider, 2024) packages in R (R Core Team, 2024), with estimates adjusted according to the individual weightings of the NDNS survey design. Model outcomes were presented as an odds ratio and upper and lower 95% confidence intervals. Within each factor, the odds ratios for each group were relative to a pre-selected reference group. Table 3.2summarises the selected reference groups for each factor and the reasoning behind their selection.

Table 3.2 Reference groups selected for logistic regression analysis.

Factor	**Reference Group**	**Reasoning**
Season	Winter	Winter was observed to be the meteorological season in which household vitamin C provisions were greatest (Section 0).
Geographic Region	*South*	The South was observed to be the region in which household vitamin C provisions were greatest (Section 2.3.4).
Sex	Female	Dichotomous factor. Females have been frequently observed to have better vitamin C status than males in other studies (Jungert and Neuhäuser-Berthold, 2015; Ness et al., 1999).
Income	Highest	Comparisons relative to the highest income tertile will enable inferences of any directional effects of income on the odds of vitamin C inadequacy (Maguire and Monsivais, 2015; Mosdøl et al., 2008).

Factor	Reference Group	Reasoning
Smoking Status	Non-current smoker	Dichotomous factor. Non-current smokers made up the majority of the cohort (84%) and current smokers have been shown to have attenuated dose-response relationships when compared to non-current smokers with similar vitamin C intakes in previous studies (Lykkesfeldt and Carr, 2023; Pearson et al., 2017).
Body Weight	Healthy Weight	Comparisons relative to the Healthy weight group will enable inferences of any directional effects of the weight group on the odds of vitamin C inadequacy (Carr et al., 2024, 2022).
Age Group Tertile	Youngest	Previous studies have reported attenuated responses in older individuals with similar vitamin C intakes to younger individuals. The youngest tertile will serve as the reference group as the relationship between vitamin C intake and status is expected to be normal (Carr and Zawari, 2023; McCall et al., 2019)

Concerning vitamin C intakes, the logistic regression modelled the effects of each selected factor, accounting for the co-varying effects of all other factors, on the odds of an individual having a vitamin C intake above or below the UK RNI of 40 mg/day. Concerning PAA, the model outcome related to the odds of an individual having a PAA above or below the Adequate threshold of 50 µmol/L.

Comparisons of vitamin C intakes and PAA between groups were conducted using the design-based Kruskal Wallis test for continuous variables and Pearson's chi-squared for categorical variables, as implemented in the {*gtsummary*} R package (Sjoberg et al., 2021). Statistically significant differences between groups were accepted at an alpha threshold of 0.05.

Log logistic four-parameter dose-response models (DRM) were fitted to investigate the relationship between vitamin C intakes and PAA in the full cohort and selected population sub-groups. Models were fit using the {*drc*} R package in accordance with package guidance (Ritz et al., 2015). The DRMs were used to predict the vitamin C intake required to achieve an Adequate PAA concentration of 50 µmol/L. The DRMs were also used to predict the PAA that was achieved by adhering to the UK RNI of 40 mg/day.

All tables were created using a combination of the {*flextable*} (Gohel and Skintzos, 2024) and {*gtsummary*} (Sjoberg et al., 2021) packages in R. All data visualisations were created using the {*tidyverse*} suite (Wickham et al., 2019) and {*ggplot2*} (Wickham, 2016) packages in R.

3.3 Results

3.3.1 Vitamin C Status in the NDNS Rolling Cohort

The final cohort of individuals included in the present study consisted of 3243 non-supplementing adults who had taken part in the NDNS rolling programme between 2008 and 2019 (Table 3.3). The median age of the cohort was 47 (Q1 - 34, Q3 - 61) years. Females and males made up 51% and 49% of the total cohort, respectively (Table 3.3). Individuals were predominantly of White ethnicity (89%), with smaller numbers of individuals from Asian (6%), Black (3%), and Mixed (1%) ethnic groups (Table 3.3). The proportion of individuals from the highest, middle, and lowest income tertiles was 39%, 29%, and 32%, respectively (Table 3.3).

Current smokers accounted for 17% of the filtered cohort, whilst overweight and obesity were prevalent in 65% of the cohort (Table 3.3). The filtered cohort had a median body weight of 76 (Q1 - 65, Q3 - 89) kg, a waist circumference of 92 (Q1 - 82, Q3 - 102) cm, and a BMI of 26.6 (Q1 - 23.6, Q3 - 30.1). Only 5.5% of the filtered cohort had been diagnosed with diabetes (Table 3.3).

Table 3.3 Characteristics of the filtered NDNS cohort.

Characteristic	**N = 3,243[1]**
Sex	
Female	1,657 (51%)
Male	1,586 (49%)
Ethnicity	
White	2,876 (89%)
Asian	188 (5.8%)
Black	81 (2.5%)
Mixed-Ethnicity	41 (1.3%)
other	56 (1.7%)
Income Tertile	
Highest	706 (39%)
Middle	528 (29%)
Lowest	570 (32%)

Characteristic	N = 3,243[1]
Smoking Status	
Non-current Smoker	2,411 (83%)
Current Smoker	480 (17%)
Weight Category	
Healthy Weight	1,067 (35%)
Overweight	1,150 (38%)
Obese	812 (27%)
Diabetes Status	
Diabetic	163 (5.5%)
Not Diabetic	2,770 (94%)
Age	47 (34, 62)
Body Weight (Kg)	76 (65, 89)
Waist Circumference (cm)	92 (82, 102)
Body Mass Index (Kg/m^2)	26.6 (23.6, 30.2)
Vitamin C Intake (mg/day)	70 (43, 107)
Plasma Ascorbic Acid (µmol/L)	50 (32, 65)

[1] Categorical variables show number of participants in each category - n (%); Continuous variables describe the Median (Q1, Q3) values for all participants in the filtered cohort.

Note: Categorical and continuous variables are separated by the horizontal line.

The median vitamin C intake was 70 (Q1 - 43, Q3 - 107) mg/day (Table 3.3). Vitamin C intakes were greater than the UK RNI of 40 mg/day for 78.4% of individuals (Figure 3.2A). Intakes between the UK and the upper EU RNI (110 mg/day for adult males) were reported in 54.8% of the cohort, whilst 23.6% of the cohort exceeded the upper EU RNI. Reported intakes of over 200 mg/day were observed for 2.9% of the filtered cohort (Figure 3.2A).

The median PAA was 50 (Q1 - 32, Q3 - 65) µmol/L, a value that was equal to the threshold associated with adequate vitamin C status (Section 3.2.3). Whilst 78.4% of individuals had intakes greater than the UK RNI (Figure 3.2A), the combined proportion of individuals with either adequate or saturating PAA was only 50.8% (Figure 3.2B). The remaining 49.2% of the filtered cohort all had inadequate PAA, with 33.8%, 10.6% and 4.8% of individuals categorised into the inadequate, hypovitaminotic, and deficient status groups (Figure 3.2B).

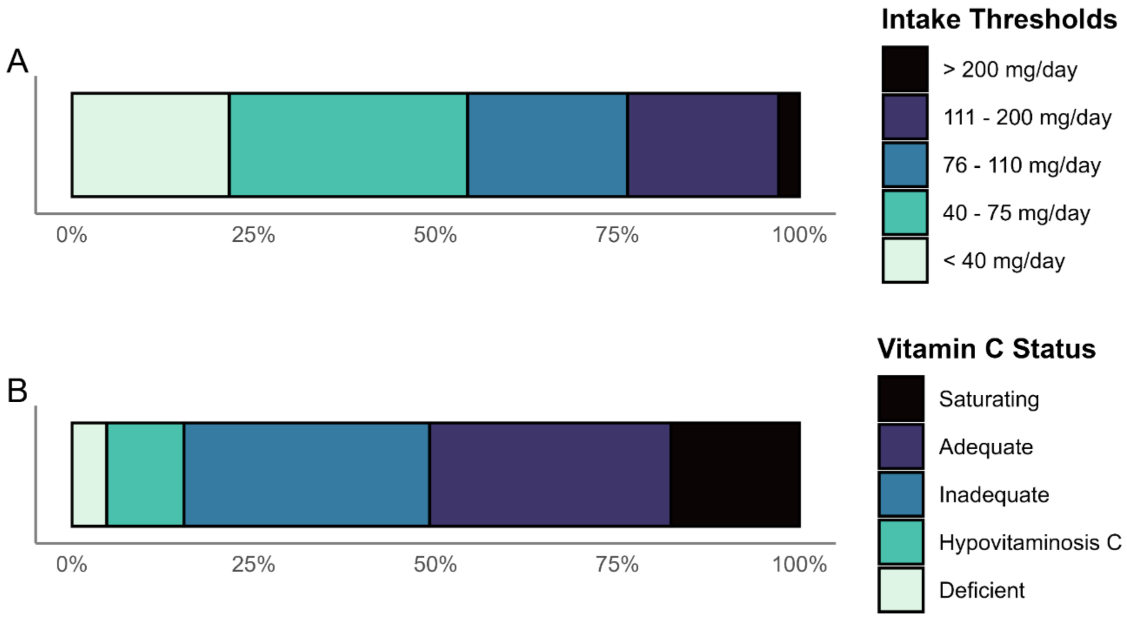


Figure 3.2 Stacked bar charts showing the relative proportion of UK adults meeting different vitamin C intake and plasma ascorbic acid thresholds. All subjects were non-supplementing adults from the National Diet and Nutrition Survey (n = 3963). The intake thresholds (A) are consistent with the following dietary recommendations: > 200 mg/day - Scientific consensus on intake required for saturating status (Frei, Birlouez-Aragon, and Lykkesfeldt, 2012), 111 - 200 mg/day - intakes above the EU RNI for adult males, 76 - 110 mg/day - intakes between the most commonly adopted RNI and the EU RNI for adult males, 40 - 75 mg/day - Intakes between the UK RNI and the most commonly adopted RNI, < 40 mg/day intakes below the UK RNI. B) Relative proportions of individuals with PAA concentrations indicative of different Vitamin C status groups: Saturating (>70 mol/L), Adequate (50 – 70 μmol/L), Inadequate (23 – 49 μmol/L), Hypovitaminosis C (11 – 22.9 μmol/L), and Deficient (< 11 μmol/L).

The dose-response relationship exhibited a sigmoidal shape with PAA increasing sharply at lower vitamin C intakes before slowing down as PAA reached saturating concentrations of ~70 μmol/L (Figure 3.3). The predicted PAA achieved with a vitamin C intake of 40 mg/day, in accordance with the UK RNI, was 40.28 (CI: 39.06, 41.50) μmol/L (Figure 3.3), which fell within the inadequate range of PAA concentrations. Inverse predictions indicated that the vitamin C intake required to reach an adequate PAA of 50 µmol/L was 73.23 (CI: 54.50, 91.97) mg/day (Figure 3.3).

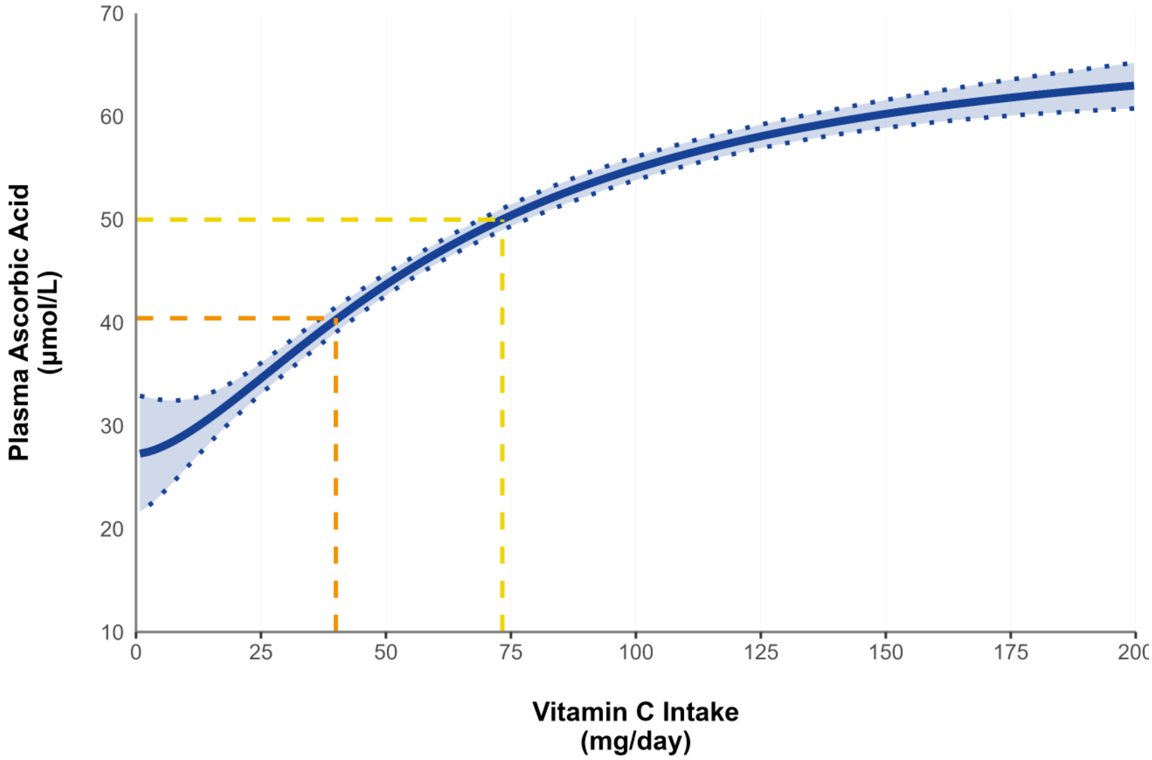


Figure 3.3 Dose-response relationship between vitamin C intakes and plasma ascorbic acid (PAA) concentrations in UK adults. All subjects were non-supplementing adults from the National Diet and Nutrition Survey (n = 3963). The dose-response relationship is illustrated by the four-parameter logistic curve (solid blue curve) and asymmetrical 95% confidence intervals (ribbon between the dotted blue lines). The fitted dose-response model was used to predict the PAA achieved by adhering to the UK recommended nutrient intake of 40 mg/day (orange dashed line) and to make an inverse prediction of the vitamin C intake required to achieve an "adequate" PAA concentration of 50 µmol/L (yellow dashed line).

3.3.2 Predictors of Inadequate Vitamin C Intakes and Status

Multiple logistic regression was applied to model the effects of selected health, lifestyle, and social factors on vitamin C intakes and PAA. The regression analyses quantified the odds of individuals in the filtered cohort having vitamin C intakes below the UK RNI (40 mg/day) and PAA concentrations below the adequate threshold (50 µmol/L).

Geographic region, household income, age, and smoking status were all found to have a statistically significant effect on the odds of an individual achieving the UK vitamin C RNI (Table 3.4). Concerning PAA, there were significant differences in the odds of an individual achieving an adequate PAA depending on the season, household income, sex, weight category, and smoking status (Table 3.4).

The survey-weighted median vitamin C intake was greater in the Winter (74 (Q1 - 45, Q3 – 112) mg/day) compared to the Autumn (65 (Q1 - 40, Q3 – 97) mg/day; p = 0.007; Figure 3.4; Appendix A.1).The median PAA values ranged between 49 (Q1 – 32, Q3 - 64) µmol/L in the Spring and 51 (Q1 – 30, Q3 - 66) µmol/L in the Winter (Figure 3.4). There were no significant differences in the median PAA values observed in each season (p = 0.11; Appendix A.1)

The odds of an individual having inadequate PAA were greater in the Autumn when compared to the Winter (OR = 1.55 [CI: 1.03, 2.33], p = 0.037; Table 3.4). The differences between the odds of an individual having a vitamin C intake below the RNI were not, however, statistically significant (OR = 1.59 [CI: 0.99, 2.55], p = 0.057; Table 3.4).

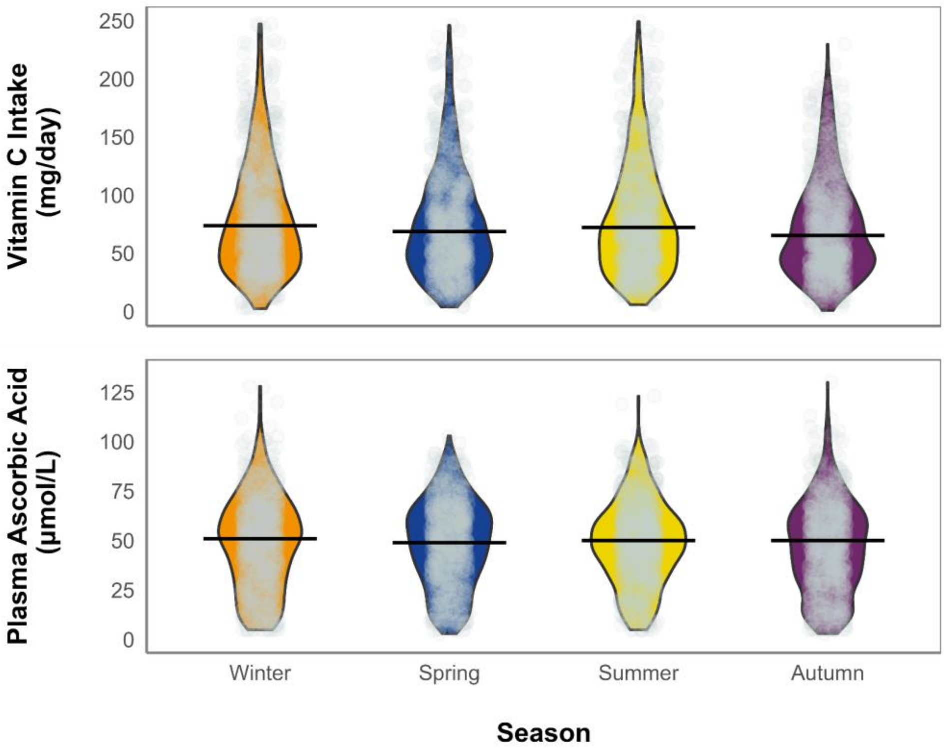


Figure 3.4 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in each season for non-supplementing adults in the NDNS cohort. Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each season are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

The survey-weighted median vitamin C intake was greatest in the East of England (79 (Q1 - 52, Q3 - 115) mg/day) and the South (75 (Q1 - 47, Q3 - 109) mg/day; Figure 3.5). Significantly lower vitamin C intakes were observed in the North (66 (Q1 - 39, Q3 - 99) mg/day), Scotland (63 (Q1 - 38, Q3 - 91) mg/day), and Wales (66 (Q1 - 44, Q3 - 105) mg/day; $p < 0.001$; Appendix A.3). Median PAA values were also greatest in the East (53 (Q1 - 36, Q3 - 66) µmol/L), South (54 (Q1 - 34, Q3 - 68) µmol/L) and London (52 (Q1 - 35, Q3 - 68); Figure 3.5), whilst median values in the North, Midlands, Scotland, and Wales were significantly lower and all below the adequate threshold of 50 µmol/L (48 – 49 µmol/L; Figure 3.5; $p = 0.007$; Appendix A.3).

Individuals from the East of England had significantly lower odds of having a vitamin C intake below the UK RNI when compared to individuals from the South of England (OR = 0.47 [CI: 0.24, 0.93], p = 0.031; Table 3.4). Despite affecting the odds of inadequate vitamin C intake, geographic region was not found to affect the odds of achieving adequate PAA (Table 3.4).

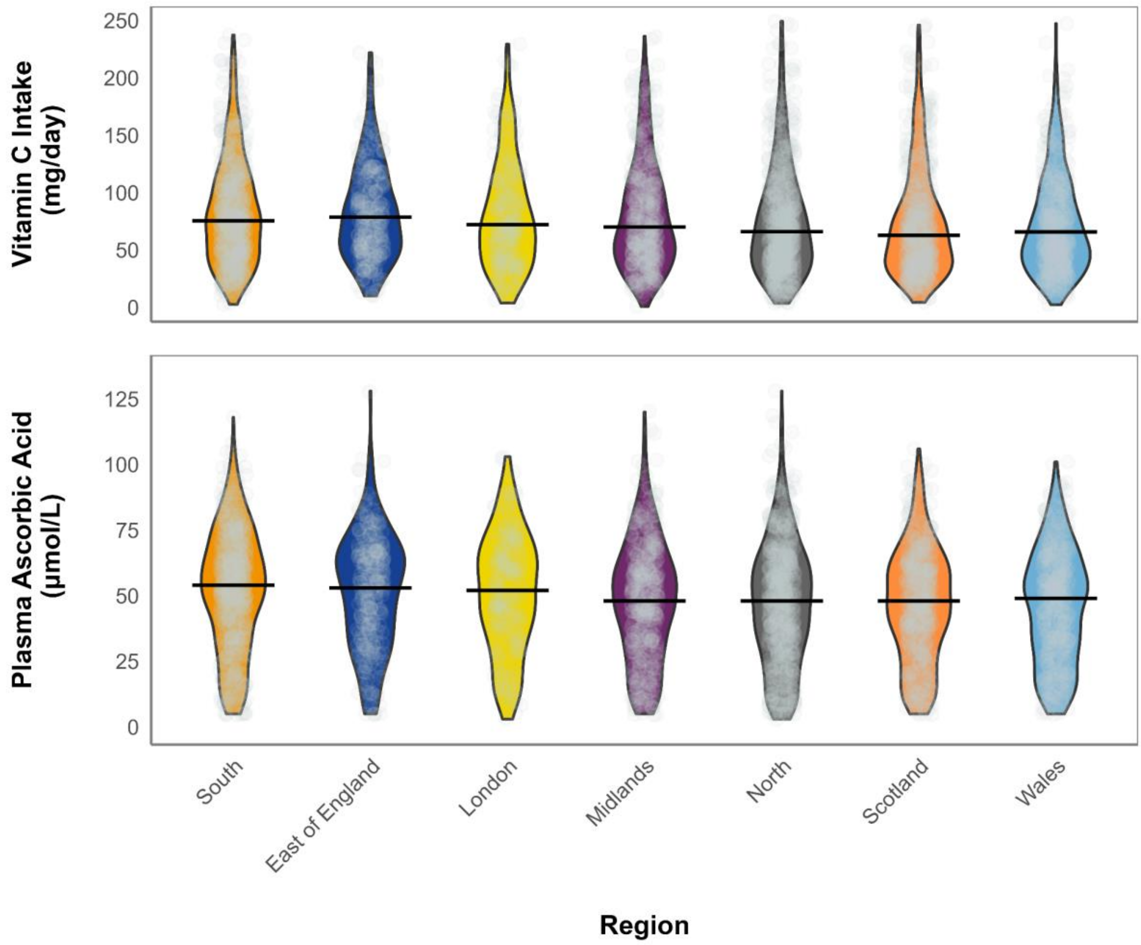


Figure 3.5 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in different geographic regions of the UK for non-supplementing adults in the NDNS cohort. Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each region are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

Median vitamin C intake was greatest in the highest income tertile (83 (Q1 - 55, Q3 - 119) mg/day; Figure 3.6). Significant reductions in vitamin C intake were observed in the middle (67 (Q1 - 42, Q3 - 97) mg/day) and lowest income tertiles (53 (Q1 - 35, Q3 - 91) mg/day; Figure 3.6; $p < 0.001$; Appendix A.4). Median PAA values followed the same trend, with incrementally decreasing values from the highest (57 (Q1 - 42, Q3 - 68) µmol/L) to the lowest income tertile (42 (Q1 - 23, Q3 - 60) µmol/L; Figure 3.6; $p < 0.001$; Appendix A.4). Median PAA in the lowest income tertile was notably lower than the adequate threshold of 50 µmol/L.

Individuals from the lowest and middle-income tertiles had significantly greater odds of having vitamin C intakes below the UK RNI, relative to the highest income tertile (lowest tertile: OR = 2.27 [1.45, 3.54], p = <0.001; middle tertile: OR = 1.68 [1.04, 2.70], p = 0.032; Table 3.4). Similarly to vitamin C intakes, individuals from the middle and lowest income tertiles had significantly greater odds of having inadequate PAA, relative to those from the highest income tertile (lowest tertile: OR = 2.12 [1.49, 3.01], p = <0.001; middle tertile: OR = 1.52 [1.09, 2.12], p = 0.015; Table 3.4).

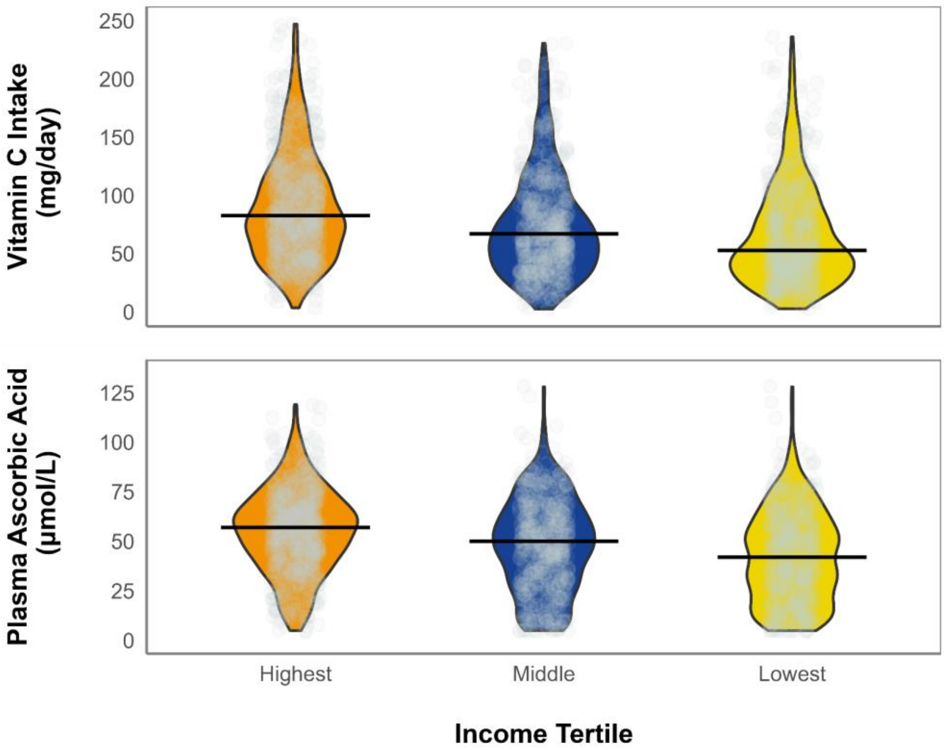


Figure 3.6 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in each household income tertile for non-supplementing adults in the NDNS cohort. Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each income tertile are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

Table 3.4 Adjusted odds ratios for inadequate vitamin C intakes (<40 mg/day) and plasma ascorbic acid concentrations (<50 µmol/L) by selected known risk factors.

		Vitamin C Intake Below UK RNI (40 mg/day)			**Inadequate Plasma Ascorbic Acid (50 µmol/L)**		
Characteristic	**N = 3,243[1]**	**OR[2]**	**95% CI[2]**	**p-value**	**OR[2]**	**95% CI[2]**	**p-value**
Season							
Winter	828 (26)	—	—		—	—	
Autumn	799 (25)	1.59	0.99, 2.55	0.057	1.55	1.03, 2.33	**0.037**
Spring	779 (24)	1.20	0.72, 2.00	0.5	1.40	0.91, 2.15	0.12
Summer	824 (26)	1.21	0.69, 2.12	0.5	1.48	0.97, 2.26	0.067
Region							
South	708 (22)	—	—		—	—	
East of England	319 (10)	0.47	0.24, 0.93	**0.031**	0.94	0.56, 1.59	0.8
London	393 (12)	1.11	0.51, 2.41	0.8	1.78	0.98, 3.22	0.059
Midlands	522 (16)	0.81	0.45, 1.46	0.5	1.24	0.77, 2.00	0.4
North	789 (25)	1.33	0.84, 2.13	0.2	1.38	0.88, 2.16	0.2
Scotland	296 (9.3)	1.47	0.75, 2.89	0.3	1.50	0.77, 2.93	0.2
Wales	164 (5.1)	1.20	0.68, 2.12	0.5	1.34	0.82, 2.17	0.2

		Vitamin C Intake Below UK RNI (40 mg/day)			Inadequate Plasma Ascorbic Acid (50 µmol/L)		
Characteristic	**N = 3,243**[1]	**OR**[2]	**95% CI**[2]	**p-value**	**OR**[2]	**95% CI**[2]	**p-value**
Income Tertile							
Highest	706 (39)	—	—		—	—	
Middle	528 (29)	1.68	1.04, 2.70	**0.032**	1.52	1.09, 2.12	**0.015**
Lowest	570 (32)	2.27	1.45, 3.54	**<0.001**	2.12	1.49, 3.01	**<0.001**
Age Group Tertile							
Youngest	1,320 (41)	—	—		—	—	
Middle	949 (29)	0.62	0.41, 0.95	**0.027**	0.73	0.51, 1.03	0.072
Oldest	974 (30)	0.53	0.35, 0.80	**0.003**	0.95	0.67, 1.34	0.8
Sex							
Female	1,657 (51)	—	—		—	—	
Male	1,586 (49)	1.15	0.80, 1.64	0.5	1.57	1.18, 2.09	**0.002**
Weight Category							
Healthy Weight	1,067 (35)	—	—		—	—	
Overweight	1,150 (38)	1.20	0.79, 1.84	0.4	1.82	1.28, 2.60	**0.001**
Obese	812 (27)	1.33	0.82, 2.14	0.2	3.18	2.24, 4.52	**<0.001**

		Vitamin C Intake Below UK RNI (40 mg/day)			Inadequate Plasma Ascorbic Acid (50 µmol/L)		
Characteristic	**N = 3,243**[1]	**OR**[2]	**95% CI**[2]	**p-value**	**OR**[2]	**95% CI**[2]	**p-value**
Smoking Status							
Non-current Smoker	2,411 (83)	—	—		—	—	
Current Smoker	480 (17)	3.31	2.10, 5.21	**<0.001**	2.57	1.68, 3.92	**<0.001**

[1]n (%)

[2]OR = Odds Ratio, CI = Confidence Interval

3.3.3 Relationships Between Vitamin C Intakes and Plasma Ascorbic Acid in Selected Population Sub-groups

As potential biological predictors of vitamin C status, further investigation was conducted into the impact of smoking, body weight, sex and age on the dose-response relationships between vitamin C intake and PAA. Ethnicity was not included in dose-response modelling due to the small sample sizes in the filtered cohort.

3.3.3.1 Smoking

Vitamin C intake amongst current smokers (48 (Q1 - 29, Q3 - 74) mg/day) was significantly lower than in non-current smokers (76 (Q1 - 48, Q3 - 111) mg/day; Figure 3.7; $p < 0.001$; Appendix A.7). Correspondingly, current smokers had a median PAA of just 34 (Q1 - 16, Q3 - 51) µmol/L, compared to 52 (Q1 - 16, Q3 - 51) µmol/L in non-current smokers (Figure 3.7; $p < 0.001$; Appendix A.7).

Current smokers were found to have significantly greater odds of having a vitamin C intake below the UK RNI than non-current smokers (OR = 3.31 [CI: 2.10, 5.21], p = 0.000; Table 3.4). Current smokers also had significantly higher odds of having inadequate PAA than non-current smokers (OR = 2.57 [CI: 1.68, 3.92], p = 0.002; Table 3.4).

Non-current smokers required a vitamin C intake of 69.40 (CI: -16.85, 155.65) mg/day to achieve a PAA of 50 µmol/L (Figure 3.8). Current smokers required 141.68 (CI: 5.21, 278.15) mg/day to achieve a PAA of 50 µmol/L (Figure 3.8). A vitamin C intake of 40 mg/day was associated with inadequate PAA concentrations in both current smokers (33.06 [CI: 30.82, 35.31] µmol/L) and non-current smokers (42.95 [CI: 41.18, 44.71] µmol/L).

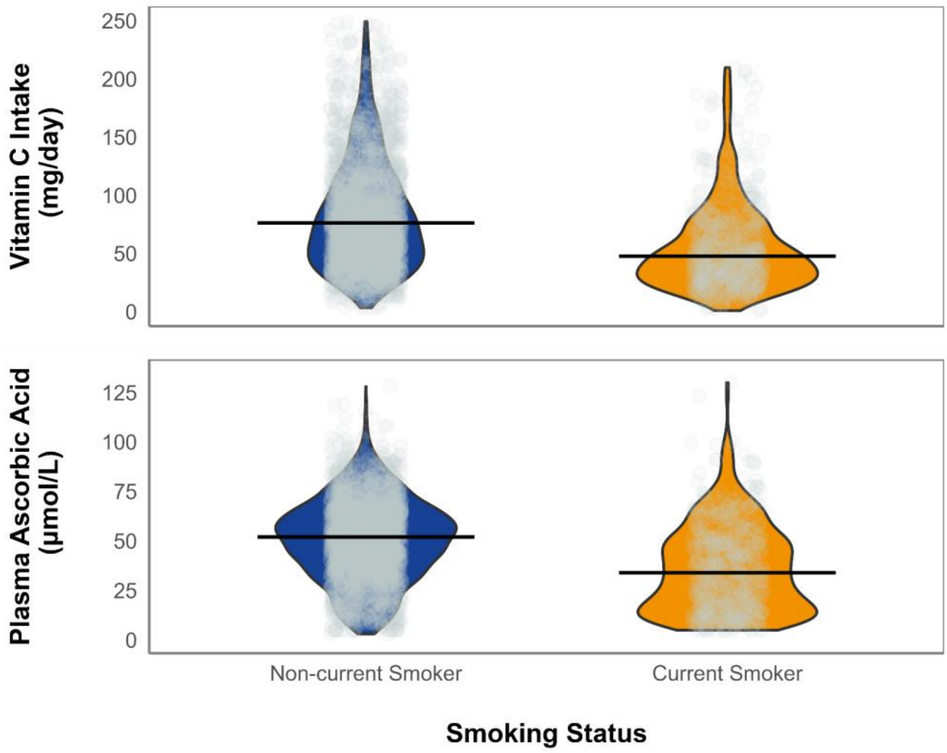


Figure 3.7 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in current and non-current smokers within non-supplementing adults in the NDNS cohort. Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each current- and non-current smokers are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

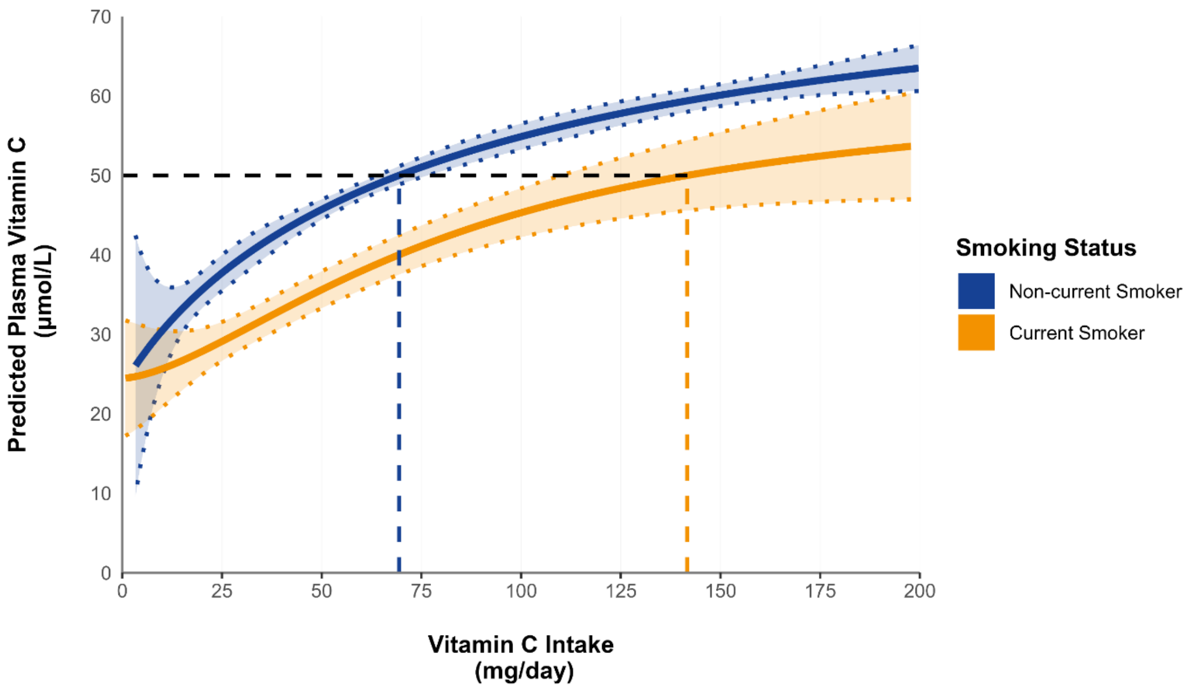


Figure 3.8 Dose-response relationship between vitamin C intakes and plasma ascorbic acid concentrations, relative to smoking status, in non-supplementing adults in the NDNS cohort. The dose-response relationship is illustrated by four-parameter logistic curves for non-current smokers (solid blue curve, *n* = 2411) and current smokers (solid orange curve, *n* = 480), and their associated asymmetrical 95% confidence intervals (ribbon between the dotted lines). The dose-response model was also used to make inverse predictions of the vitamin C intake required to achieve an "adequate" plasma ascorbic acid concentration of 50 µmol/L (dashed intersecting lines).

3.3.3.2 Body Weight

Vitamin C intake was above the UK RNI in all weight categories; however intake was greatest in healthy weight individuals (75 (Q1 - 47, Q3 - 111) mg/day) when compared to overweight (68 (Q1 - 45, Q3 - 104) mg/day) and obese individuals (66 (Q1 - 43, Q3 - 103) mg/day; Figure 3.9; p = 0.019; Appendix A.6). There were greater differences between the median PAA for each weight category, with healthy weight individuals observed to have a median PAA of 59 (Q1 - 40, Q3 - 71) µmol/L, whilst obese individuals were observed to have a median PAA of 43 (Q1 - 26, Q3 - 59) µmol/L (Figure 3.9; $p < 0.001$; Appendix A.6). Despite having comparable intakes to overweight individuals, obese individuals were the only weight category to have a median PAA below the adequate threshold of 50 µmol/L (Appendix A.6).

There were no statistically significant differences in the odds of having a vitamin C intake below the UK RNI in healthy weight, overweight, or obese individuals (Table 3.4). Conversely, significant differences were observed between weight groups with respect to the odds of achieving adequate PAA. Overweight individuals had greater odds of inadequate PAA compared to healthy-weight individuals (OR = 1.82 [CI: 1.28, 2.60], p = 0.001; Table 3.4). Obese individuals had even greater odds of inadequate PAA when compared to healthy-weight individuals (OR = 3.18 [CI: 2.24, 4.52], p = 0.000; Table 3.4).

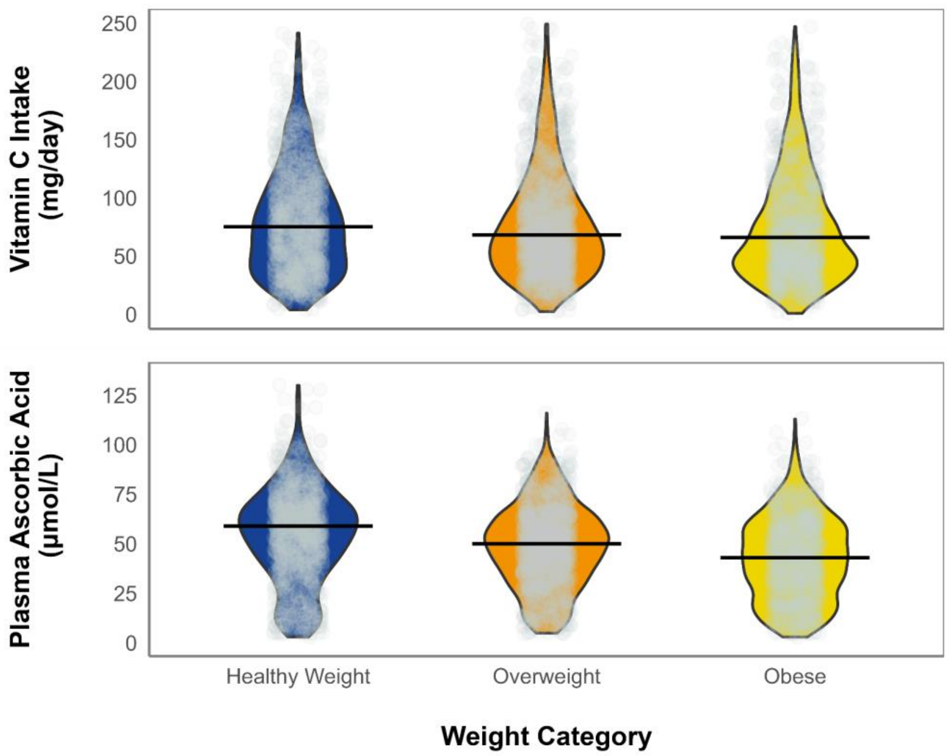


Figure 3.9 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in different body weight categories for non-supplementing adults in the NDNS cohort. Weight categories were derived from Body Mass Index (BMI): Healthy weight (BMI 18.5 – 24.9), Overweight (BMI 25.0 – 29.9) and Obese (BMI > 29.9). Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each weight category are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

Healthy-weight individuals required a vitamin C intake of 52.94 (CI: 30.21, 75.65) mg/day to reach adequate PAA concentrations (Figure 3.10). The amount of vitamin C required by overweight and obese individuals was 71.62 (CI: 38.78, 104.47) mg/day and 108.89 (CI: 0.90, 216.88) mg/day respectively (Figure 3.10). A vitamin C intake of 40 mg/day was, again, associated with inadequate PAA in all weight groups, (Healthy weight - 45.06 [CI: 42.80, 47.31] µmol/L; Overweight - 41.46 [CI: 39.50, 43.42] µmol/L; Obese - 36.31 [CI: 34.19, 38.43] µmol/L).

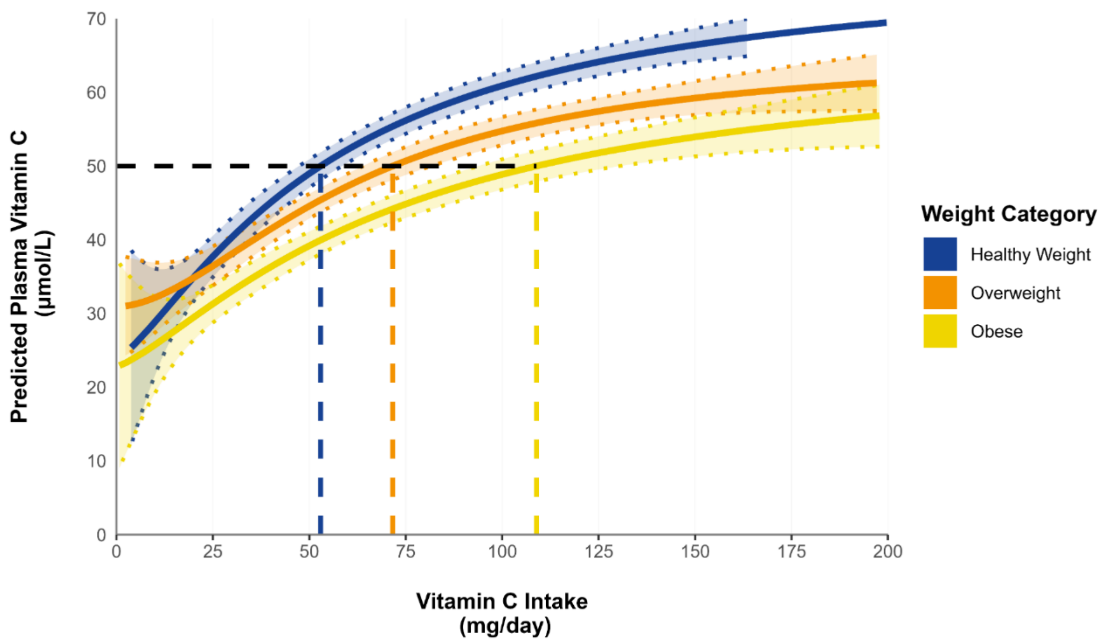


Figure 3.10 Dose-response relationship between vitamin C intakes and plasma ascorbic acid concentrations, relative to weight category, in non-supplementing adults in the NDNS cohort. The four-parameter logistic curves for healthy-weight (solid blue curve, *n* = 1067), overweight (solid orange curve, *n* = 1150) and obese (solid yellow curve, *n* = 812) individuals and asymmetrical 95% confidence intervals (ribbon between the dotted lines) illustrate the dose-response relationship. The dose-response model was also used to predict the vitamin C intake required to achieve an "adequate" plasma ascorbic acid concentration of 50 µmol/L (dashed intersecting lines).

3.3.3.3 Sex

Vitamin C intakes were comparable between females and males; both sexes having median intakes of 70 mg/day (Figure 3.11; $p = 0.7$, Appendix A.5). Median PAA was, however, significantly greater in females (55 (Q1 - 36, Q2 - 68) µmol/L) when compared to males (46 (Q1 - 30, Q2 - 61) µmol/L; Figure 3.11; $p < 0.001$; Appendix A.5). The median PAA for males was below the adequate threshold of 50 µmol/L (Appendix A.5)

There were no statistically significant differences between males and females with respect to the odds of having a vitamin C intake below the UK RNI. Males did, however, have significantly greater odds of inadequate PAA than females (OR = 1.57 [CI: 1.18, 2.09], p = 0.002; Table 3.4).

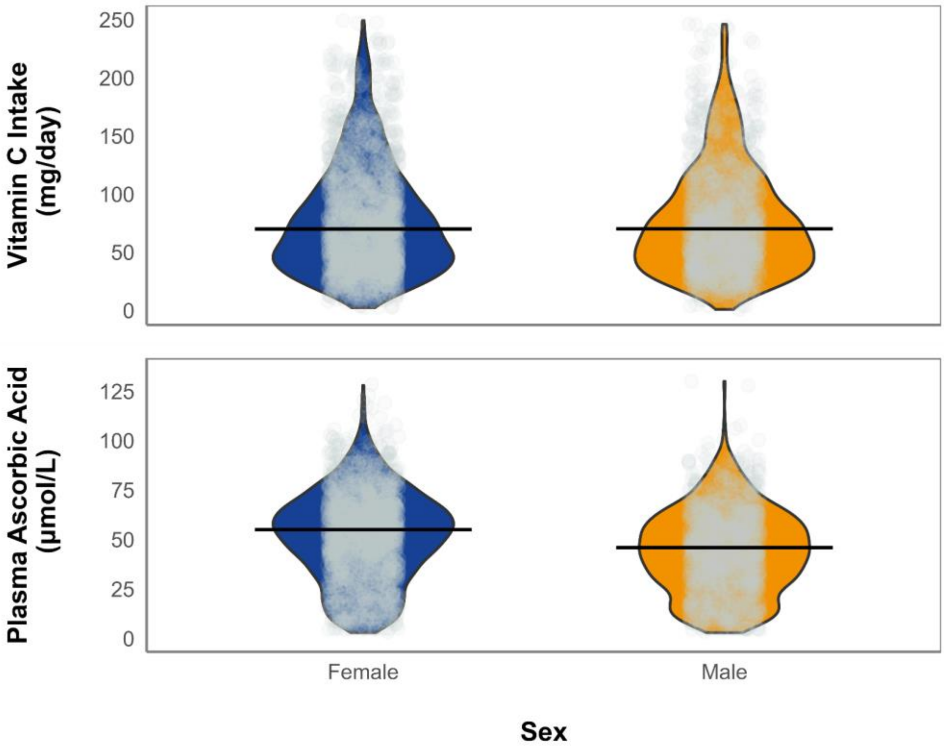


Figure 3.11 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in females and males from non-supplementing adults in the NDNS cohort. Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each sex are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

Concerning the dose-response relations, females were predicted to achieve an adequate PAA of 50 µmol/L at a vitamin C intake of 60.25 (CI: 40.95, 79.56) mg/day (Figure 3.12). Males, on the other hand, required a vitamin C intake of 94.64 (CI: 48.46, 140.81) mg/day to achieve the same PAA of 50 µmol/L (Figure 3.12). Adherence to the UK RNI resulted in a predicted PAA of 43.73 (CI: 42.19, 45.28) µmol/L for females and 35.29 (CI: 33.85, 37.59) µmol/L for males, both of which were in the inadequate PAA range.

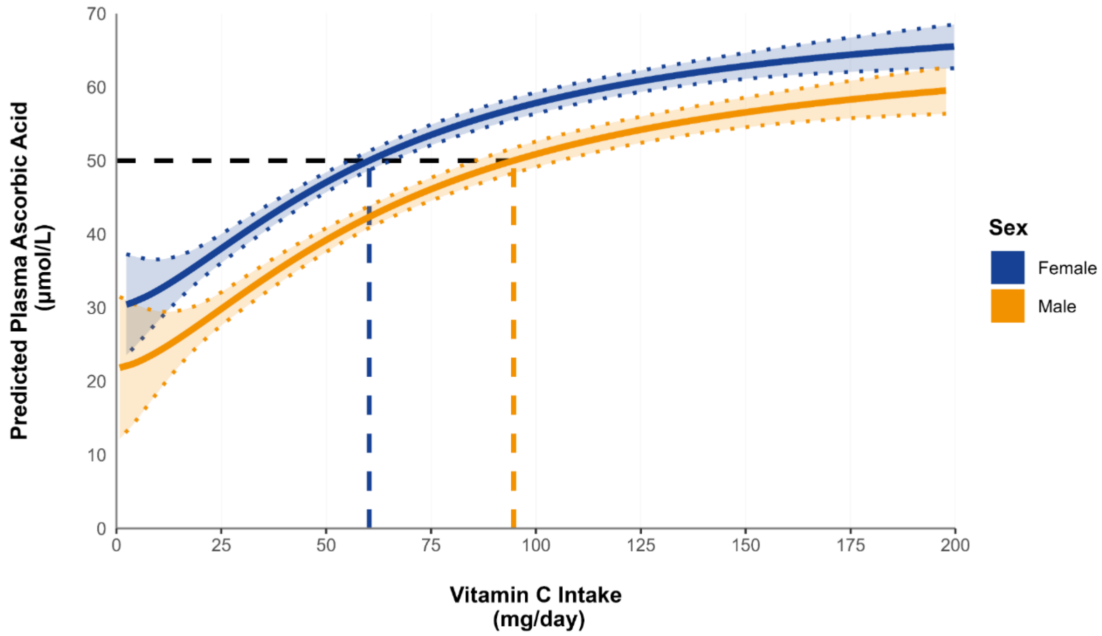


Figure 3.12 Dose-response relationship between vitamin C intakes and plasma ascorbic acid concentrations in non-supplementing males and females in the NDNS cohort. The four-parameter logistic curves for males (solid orange curve, *n* = 1586) and females (solid red curve, *n* = 1657) and asymmetrical 95% confidence intervals (ribbon between the dotted lines) illustrate the dose-response relationship. The dose-response model was also used to predict the vitamin C intake required to achieve an "adequate" plasma ascorbic acid concentration of 50 µmol/L (dashed intersecting lines).

3.3.3.4 Age

The oldest and middle age tertiles were observed to have similar vitamin C intakes of 77 and 74 mg/day, respectively (Figure 3.13). The youngest age tertile had a significantly lower vitamin C intake of 63 mg/day (Figure 3.13; $p < 0.001$; Appendix A.2). There were, however, no significant differences in the median PAA for each age tertile, with values of 49, 50, and 52 for the oldest, youngest, and middle tertiles, respectively (Figure 3.13; $p = 0.2$; Appendix A.2).

Individuals from the middle and oldest age group tertiles had significantly lower odds of having a vitamin C intake below the UK RNI, relative to the youngest tertile (oldest tertile: OR = 0.53 [CI: 0.35, 0.80], p = 0.003; middle tertile: OR = 0.62 [CI: 0.41, 0.95], p = 0.027; Table 3.4). There were, however, no statistically significant differences between the age group tertiles with respect to the odds of achieving adequate PAA (Table 3.4).

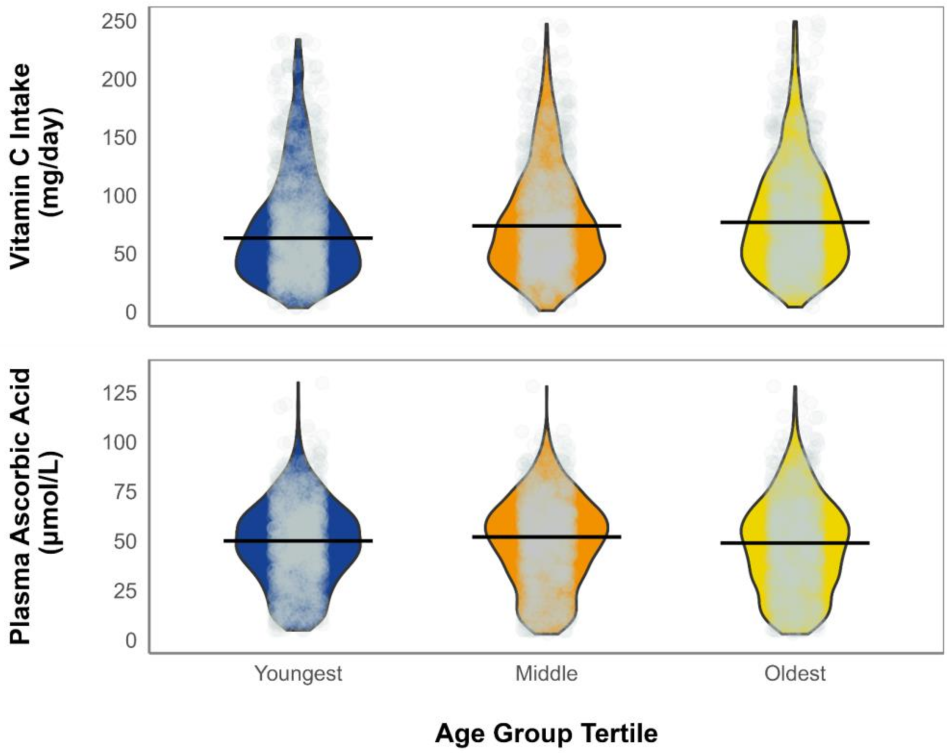


Figure 3.13 Violin plots illustrating the distribution of vitamin C intakes and plasma ascorbic acid concentrations in each age group tertile from non-supplementing adults in the NDNS cohort. The youngest tertile contained individuals aged between 19 - 43 years, the middle tertile 43-58 years, and the oldest tertile 58-91 years. Vitamin C intake and plasma ascorbic acid values are illustrated in the top and bottom panels, respectively. The survey-weighted median is signified by the horizontal black bar overlayed on each violin. All individual data points for each age tertile are also overlayed on the violins (grey dots). Individuals with a reported vitamin C intake > 250 mg/day were excluded from the plots for visual clarity but were included in the calculation of the survey-weighted median.

The youngest tertile was predicted to achieve an adequate PAA of 50 µmol/L at a vitamin C intake of 74.64 (CI: 41.37, 107.90) mg/day (Figure 3.14). Comparatively, the amount of vitamin C required by the middle and oldest tertiles was predicted to be 66.18 (CI: 44.77, 87.58) mg/day and 79.50 (CI: 20.59, 138.41) mg/day respectively (Figure 3.14). Adherence to the UK RNI was predictive of inadequate PAA in all age groups but was worst in the oldest tertile (Youngest - 42.26 [CI: 40.37, 44.16] µmol/L; Middle - 41.13 [CI: 38.90, 43.36] µmol/L; Oldest - 37.10 [CI: 34.91, 39.29] µmol/L).

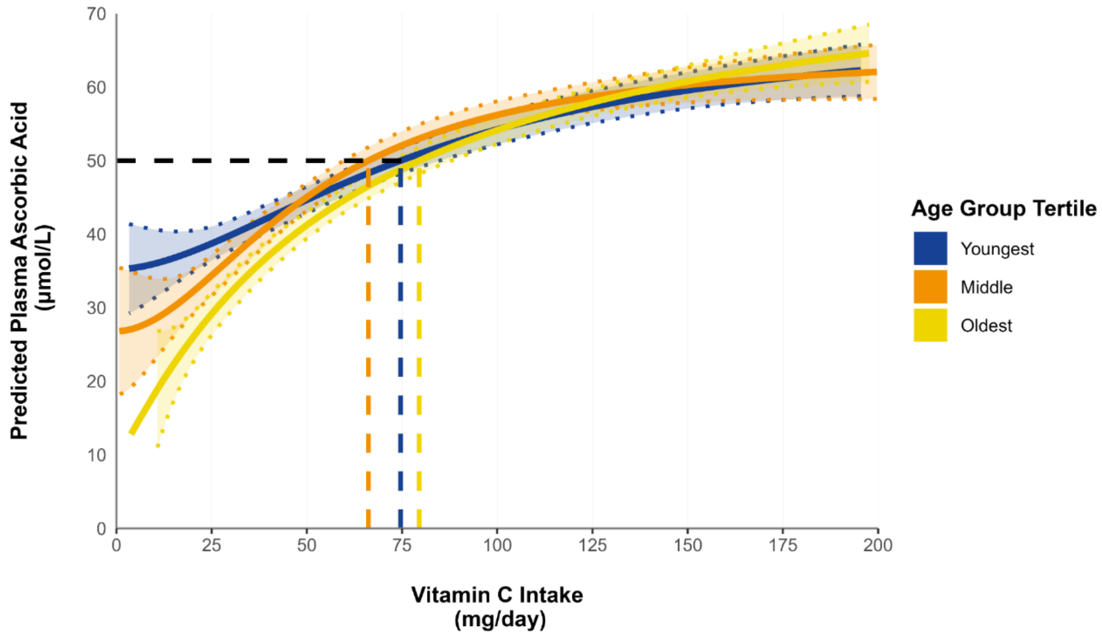


Figure 3.14 Dose-response relationship between vitamin C intakes and plasma ascorbic acid concentrations relative to age group tertile in the NDNS cohort. The youngest tertile contained individuals aged between 19 - 43 years, the middle tertile 43-58 years, and the oldest tertile 58-91 years. The four-parameter logistic curves for the youngest (solid blue curve, *n* = 1320), middle (solid orange curve, *n* = 949) and oldest tertiles (solid yellow curve, *n* = 974) and asymmetrical 95% confidence intervals (ribbon between the dotted lines) illustrate the dose-response relationship. The dose-response model was also used to predict the vitamin C intake required to achieve an "adequate" plasma ascorbic acid concentration of 50 µmol/L (dashed intersecting lines).

3.4 Discussion

Considering the reported vitamin C intakes and PAA in the NDNS cohort, the overall accessibility and utilisation of vitamin C in the UK appears to be in a positive position. More than half of the NDNS cohort had intakes that exceeded the RNIs of other authorities that have been implemented with the intent of maximising the health benefits of vitamin C (Carr and Lykkesfeldt, 2021; Nordic Council of Ministers, 2014). The PAA concentrations of the NDNS cohort were similarly indicative that most of the UK population can meet the biological thresholds associated with positive health outcomes (Carr and Lykkesfeldt, 2021).

Similar values for vitamin C intakes and PAA have been reported in the EPIC-Norfolk cohort (Carr et al., 2024). A key strength of the NDNS, relative to EPIC-Norfolk, is the fact that the survey was designed and weighted to be as representative of the national population as possible. As an example, the weighted proportions of individuals in the high-level ethnic groups were all within 3% of the census data from 2011 (Office for National Statistics, 2022). To have corroborated similar findings in both the NDNS and EPIC-Norfolk datasets adds confidence to the conclusions made from both studies and is a positive indicator that the UK food system is currently supporting a state of vitamin C security.

Although the overall outlook for both accessibility and utilisation of vitamin C in the NDNS cohort was positive, there were still a substantial number of individuals with inadequate vitamin C intakes and PAAs. As previously highlighted, the UK RNI is based on refuted science regarding the intakes required to prevent vitamin C deficiencies (Carr and Lykkesfeldt, 2021; Kallner et al., 1979; Department of Health, 1991). As a result, it will be important for future research efforts to understand who these individuals are and what factors are preventing them from accessing sufficient vitamin C.

Notably, there were also a greater number of individuals with inadequate PAA relative to the number of individuals with inadequate vitamin C intakes. In comparison to the 23% of individuals with inadequate intakes, 50% of individuals had PAA concentrations below the threshold associated with inadequate vitamin C status (Figure 3.2). To understand the discrepancy between inadequate intakes and biological status, further analysis of the relationship between vitamin C intakes and PAA was conducted in the NDNS population.

Judging the UK RNI against its intended purpose, an intake of 40 mg/day was sufficient to mitigate the prevalence of vitamin C deficiency (PAA < 23 μmol/L). However, adequate vitamin C status was only achieved with vitamin C intakes of 73.23 mg/day, 1.8-fold more than the UK RNI. Resultingly, figure 3.2 highlights the ineffectiveness of using the UK RNI as an indicator of vitamin C adequacy.

If the UK were to adopt a more contemporary position with respect to vitamin C recommendations – i.e. one that seeks to exploit the health benefits of adequate vitamin C status (European Food Standards Agency, 2013; German Nutrition Society, 2015; Nordic Council of Ministers, 2014) – a general recommendation of 75 mg/day would seem appropriate. This would bring the UK's RNI in line with that of the Nordic countries (Nordic Council of Ministers, 2014).

When interpreting the dose-response modelling presented in Chapter 3, it is important to consider a further limitation of the NDNS data. The NDNS was implemented in such a way that the food diaries and blood sampling were not necessarily conducted at the same time. It is possible, therefore, that an individual's diet or lifestyle could have changed between completing the food diary and providing a blood sample. As a result, the individual's PAA may not be reflective of their vitamin C intake and utilisation at the time of the blood sample, which could introduce uncertainty when modelling the relationships between intakes and PAA. That said, the overall relationship between vitamin C intake and PAA in the NDNS cohort followed the expected four-parameter sigmoidal curve that has been reported in previous pharmacological and observational studies (Carr and Lykkesfeldt, 2023a; Levine et al., 1996). As such, it is unlikely that potential mismatches between intake and blood data have had adverse effects on the findings of the current study.

3.4.1 Factors Affecting Access to Sufficient Vitamin C

3.4.1.1 Seasonal Instabilities

In Chapter 2, analysis of consumer purchase data indicated that household vitamin C provisions were lower during the Autumn months than at any other time of year (Section 2.4.3). The analysis of dietary intakes and PAA data from the NDNS appear to corroborate the existence of these autumnal instabilities.

Autumnal reductions in PAA of 15% were previously reported by Ness et al. (1999) in a smaller UK study but they did not discuss the reasons behind this observation. Seasonal variation in vitamin C status has also been reported in India and Finland, but the seasonal reductions in these other countries do not necessarily align with that observed in the UK (Paalanen et al., 2014; Ravindran et al., 2011). In Northern India, individuals were 30% more likely to experience vitamin C deficiencies between September and November (Ravindran et al., 2011). In contrast, individuals in Southern India were more likely to experience deficiencies between December and February (Ravindran et al., 2011). In Finland, PAAs have been observed to be greatest in the Autumn and lowest during the Spring (Paalanen et al., 2014). That is to say that whilst seasonal instabilities in vitamin C accessibility are seemingly a common occurrence, the timing and scale appear to be region-specific.

As discussed in Chapter 2 (Sections 2.4.2 and 2.4.3), seasonality in access to vitamin C could be driven by the seasonal availability and preference for different fruits and vegetables (Paalanen et al., 2014; Toorang et al., 2013). The current study did not find significant differences in the number of fruit and vegetable portions consumed per day between the seasons (Appendix A.1). Statistically significant differences were, however, observed for vitamin C intakes between seasons (Appendix A.1). These contrasting findings suggest that the UK Autumn is perhaps characterised by a reduction in vitamin C utilisation, as well as an overall reduction in fruit and vegetable consumption. It is important to note, however, that the analysis of the NDNS includes intake and PAA data from 2008 – 2019, meaning that 9 years of data precedes the period covered by the Kantar data that started in 2017 (2.2.1.1). Direct comparisons between the two datasets may be unreliable as a result. However, the fact that similar trends were observed in both datasets in isolation of one another is a strong indicator that seasonal instabilities in accessing vitamin C is a real issue for UK vitamin C security. As such, it would be prudent to investigate seasonal trends in more detail when the next release of NDNS data, expected to cover the period between 2019 and 2023, is released.

3.4.1.2 Regional and Socioeconomic Disparities

Similarly to seasonal instabilities, a consistent trend for reduced access to vitamin C in specific regions of the UK has now been reported across multiple data sources in relation to household provisions, dietary intakes, and biological status. Consistent with the trends observed in Chapter 2 (Section 2.3.4), individuals from the South and East of England had higher vitamin C intakes and PAA than individuals from the North, Scotland, and Wales (Appendix A.3).

The UK's North-South divide is most commonly attributed to regional disparities in socioeconomic status, with the South East – and London – being the UK's wealthiest regions (Corris et al., 2020; Fai and Tomlinson, 2023; McCann, 2020). In the current study, lower socioeconomic status was found to significantly increase an individual's odds of having inadequate vitamin C intakes and PAA. Previous analysis of the Low-Income Diet and Nutrition Survey has also shown that lower socioeconomic status is a key predictor of poor vitamin C deficiency and status (Mosdøl et al., 2008). Similar findings have also been reported in the EPIC-Norfolk (McCall et al., 2019) and US National Health and Nutrition Examination Survey (NHANES) cohorts (Schleicher et al., 2009). It is, perhaps, socioeconomic status, rather than region, that plays a greater role in driving the observed disparities in vitamin C access and status.

The differences between individuals with high and low incomes can again be related to the consumption of fruits and vegetables. Maguire and Monsivais (2015) reported a difference of 128 g/day of fruits and vegetables between high- and low-income households - equivalent to 1.6 portions – which is consistent with the observations in the current study (Appendix A.4). Low-income has also been more generally linked with inaccessibility to high quality and nutritious foods, which in turn can result in higher rates of malnutrition (Darmon and Drewnowski, 2015; Jones et al., 2018, 2014).

Reduced access to fruits and vegetables, and by extension vitamin C, in low-income households is of considerable concern in the context of UK vitamin C security. As discussed in Chapter 1 (Section 1.2.2), the number of food-insecure households in the UK has risen sharply in the years between 2019 and 2023 (The Food Foundation, 2023). Rising food insecurity has also been linked to reductions in the purchases of fruits and vegetables (The Food Foundation, 2023). As a result, financial barriers that limit access to vitamin C are likely to affect a greater proportion of the population now as compared to the NDNS population, which covers the period from 2008 to 2019.

3.4.2 Factors Affecting an Individual's Vitamin C Requirements

Chapter 3 also examined the relationship between vitamin C intake and PAA in the context of selected health and lifestyle factors. In the logistic regression analyses presented in Table 3.4, smoking status, body weight, sex, and age were identified as factors that could underpin differences in the odds of an individual achieving adequate vitamin C status.

3.4.2.1 Smoking

Within the NDNS cohort, smokers were more likely to have vitamin C intakes below the UK RNI compared to non-smokers, in conjunction with having increased vitamin C requirements. Independent of intake, current smokers were predicted to require an additional 100 mg/day over the current UK RNI to achieve adequate PAA. In other studies, smokers have been predicted to be between 1.5-fold and 7-fold more likely to experience vitamin C deficiencies than non-smokers, depending on the population that was studied (McCall et al., 2019; Mosdøl et al., 2008; Wrieden et al., 2000). The increased risk of vitamin C deficiency occurs independently of vitamin C intake due to an increased turnover in response to the oxidative stress caused by tobacco smoke (Kallner et al., 1981; Schectman et al., 1989). Even mild exposure to secondary tobacco smoke has been found to result in lower PAA in children (Preston et al., 2003). Emerging evidence suggests that the use of vapes and e-cigarettes may also lead to increased cellular and oxidative stress (Daiber et al., 2023; Münzel et al., 2024), similarly driving increased vitamin C turnover (Rezk-Hanna et al., 2021; Tolba et al., 2023. Since disposable e-cigarettes gained widespread popularity in 2021 (Jackson et al., 2024), the use of vapes and e-cigarettes has surged in teenagers and young adults (Tattan-Birch et al., 2024), making the potentially negative effects of vapes on vitamin C utilisation of particular concern for future nutrition security in the UK. As an emerging societal trend, more research into the long-term health impacts and their effects on vitamin C status will be needed to ensure e-cigarette users receive adequate dietary guidance.

Lower vitamin C intakes in smokers have also been observed in the NHANES cohort (Carr and Lykkesfeldt, 2023a). This could be linked to the fact that the proportion of current smokers was highest in the lowest income tertile which, as already discussed, was associated with lower intakes due to reduced financial accessibility (Appendix A.7). The combination of both increased requirements and reduced intakes makes smokers a sub-population at particularly high risk of vitamin C insecurity.

Several global authorities, including the UK, have published additional guidance for smokers with respect to vitamin C intakes. The UK guidance, published in 1991, suggests that smokers should consume an additional 80 mg/day of vitamin C compared to non-smokers (Department of Health, 1991). Thus, the current dietary guidance for UK smokers is to consume 120 mg/day (UK RNI = 40 mg/day + 80mg/day). In relation to the findings of the current study, this recommendation falls short of the 140 mg/day that was predicted to facilitate adequate PAA in current smokers. Other observational studies have suggested even higher requirements for smokers, ranging between 167 and 233 mg/day (Carr and Lykkesfeldt, 2023a; Schectman et al., 1989). It is also concerning to note that despite having had additional guidance for smokers since 1991, vitamin C intakes and PAA in smokers are still inadequate in the UK. This is a clear demonstration that adjusting RNIs must occur in conjunction with interventions that make those RNIs achievable.

3.4.2.2 Body Weight

Healthy-weight individuals were found to require substantially less vitamin C to achieve adequate vitamin C status than overweight and obese individuals. Body weight and obesity have been associated with worse vitamin C status in two key contexts. First is that of a dilution effect, whereby the same vitamin C intake will be diluted in a larger body when compared to a smaller one. Experimental data have previously been used to suggest that every 10 Kg increase in body weight equates to an increased vitamin C requirement of 10 mg/day (Block et al., 1999; Carr et al., 2022). In the current study, the difference between the median weights of the healthy weight and obese categories was 30 Kg (Appendix A.6), which should equate to an increased requirement of 30 mg/day for obese individuals. However, the actual predicted increase required to achieve adequate PAA in obese individuals was 56 mg. Similarly to the observations of Carr et al.(2024), the experimentally derived predictions appear to underestimate the increasing vitamin C requirements of obese individuals in observational cohort studies. These findings suggest that obesity may constitute multiple risk factors beyond just an increase in body weight.

In addition to dilution, overweight and obesity are risk factors for morbidities that can increase rates of vitamin C turnover. Obesity is known to cause an increase in oxidative stress and inflammation (Fernández-Sánchez et al., 2011; Martínez-Martínez and Cachofeiro, 2022), both of which serve to deplete the body’s stores of vitamin C (Carr et al., 2022). Obesity is also a risk factor for type-2 diabetes which is known to cause increased renal leakage of vitamin C and thus further contributes to lower PAA and body pool (Ebenuwa et al., 2022; Lunt et al., 2023; Wilson et al., 2017).

The combination of vitamin C intakes effectively decreasing through dilution and vitamin C requirements increasing due to physiochemical stress presents a concerning outlook for overweight and obese individuals in the UK. The prevalence of obesity has been projected to increase to as high as 48% of men and 43% of women in the UK by 2030 (Wang et al., 2011), and more severe cases of morbid obesity have been projected to be as high as 20% by 2035 in some parts of the UK (Keaver et al., 2018). Increases in overweight and obesity are especially prevalent in lower-income households (Burgoine et al., 2017; Cronin et al., 2022), for whom access to sufficient vitamin C is already limited by the high cost of healthy foods.

Despite overweight and obesity presenting such clear threats to vitamin C security, guidance related to increased vitamin C requirements for heavier individuals is lacking. What is certain, however, is that the current UK RNI is insufficient to support the vitamin C requirements of overweight and obese individuals.

3.4.2.3 Sex

Within the NDNS cohort, males were found to require 1.6-fold more vitamin C than females to achieve adequate PAA. These findings are consistent with previous observations of PAA in males and females, where PAA in females has been reported to be ~1.2-fold greater than in males (Jungert and Neuhäuser-Berthold, 2015; Ness et al., 1999). The amount of vitamin C required to achieve adequate PAA has similarly been estimated at ~1.2-fold greater for males compared to females (Carr and Lykkesfeldt, 2023a). This is lower than the increased requirement of males in the NDNS dataset. It is important to note that Carr and Lykkesfeldt (2023a) conservatively based their relative fold changes on the difference between the upper 95% CI for females and the lower 95% CI for males. In the current study, the 95% CIs were overlapping for all sub-group comparisons. For example, the upper 95% CI for females in the NDNS cohort was 79.56 mg/day, whilst the lower 95% CI for males was 48.46 mg/day. The width of the CIs highlights that the effective sample size of males at the adequate PAA threshold was relatively small. Thus, the power and precision of the inverse predictions at the adequate PAA threshold were limited. The differences in mean predicted requirements between the current study and that of Carr and Lykkesfeldt (2023a) are, however, similar at 1.58 and 1.76-fold, respectively.

The differences between male and female PAA have previously been attributed to differences in fat-free mass (Jungert and Neuhäuser-Berthold, 2015). A dilution effect, similar to that already discussed in relation to obesity, is therefore reasoned to affect males to a greater extent than females due to males having greater fat-free mass (Jungert and Neuhäuser-Berthold, 2015). Whilst fat-free mass was not measured in the NDNS, males were observed to have greater body weight compared to females, which supports the dilution effect hypothesis (Appendix A.5).

The differences in vitamin C requirements between males and females have been acknowledged in the RNI's for most EU countries (European Food Standards Agency, 2013; German Nutrition Society, 2015). The overall EU RNI, for example, recommended 110 mg/day for males and 95 mg/day for females, a 1.16-fold difference. In the current study, adherence to the UK RNI facilitated near-adequate PAA in females but males were closer to the threshold for hypovitaminosis C than they were to the threshold for adequate status. It may be prudent for the UK to consider separate guidance for males and females if dietary recommendations are updated.

3.4.2.4 Age

Younger individuals were found to have worse access to vitamin C than older individuals in the NDNS cohort. Individuals in the youngest age group tertile (19-43 years) were found to be 47% more likely to have vitamin C intakes below the UK RNI than individuals in the oldest tertile (58-91 years). Moreover, younger individuals consumed 1.02 fewer portions of fruit and vegetables and had median vitamin C intakes 22% lower than older individuals (Appendix A.2).

The same observation was made by Derbyshire (2018) concerning vitamin C intakes in a smaller subset of the NDNS. Derbyshire (2018) also showed that lower intakes in younger individuals applied to other micronutrients including vitamin A, folate, calcium, iron, and zinc. Lower vitamin C intakes in younger individuals have also been observed in the NHANES cohort, with Carr and Lykkesfeldt (2023b) finding that vitamin C intakes were 10% lower in the youngest age group tertile (18-36 years) when compared to the middle (37-58 years) and oldest (59-80 years) tertiles. This does not appear to be due to lower income in younger individuals as the median age of each income tertile was the same (47 years). Increased vitamin C intake in older individuals could, therefore, be the result of older individuals having more time to cook and prepare nutritious meals (Scientific Advisory Committee on Nutrition, 2008).

Despite the differences in vitamin C intakes between younger and older individuals, vitamin C status was observed to remain consistent across the age groups. Indeed, the dose-response modelling showed that vitamin C utilisation was less efficient in older adults. At intakes of 40 mg/day. There were, however, no observable differences in the predicted intakes required to achieve adequate PAA between the age group tertiles. The vitamin C status of older individuals, therefore, appears to be more sensitive to lower vitamin C intakes. Carr and Lykkesfeldt (2023b) made a similar observation in the NHANES cohort. As discussed by Carr and Lykkesfeldt (2023b), however, vitamin C pharmacokinetics have been shown to remain consistent across age groups in studies on healthy adults (Blanchard et al., 1990a, 1990b). The increased sensitivity to low vitamin C intakes in older adults is more likely, therefore, the result of increased vitamin C demands. In the current study, the oldest age group tertile was characterised by significantly higher body weight and waist circumference than the youngest age group tertile (Appendix A.2). Both body weight and waist circumference indicate a higher prevalence of abdominal obesity in the oldest age group tertile, the impacts of which on vitamin C requirements have already been discussed. The prevalence of chronic health conditions is also higher in older populations which, in turn, has been associated with increased vitamin C turnover in older adults (Carr and Lykkesfeldt, 2023a). However, given that vitamin C intakes in older individuals appear to generally be high enough to overcome the potential negative effects of low intakes on vitamin C status, age does not appear to be as great of a concern with regards to UK vitamin C status.

One population group that could be of concern, however, is elderly citizens living in institutionalised care facilities. Bates et al. (1999) found that institutionalised individuals had significantly lower vitamin C intakes and PAA when compared to free-living individuals of the same age. Overcoming the age-related sensitivity to low vitamin C intakes may, therefore, require more attention in assisted care facilities.

3.5 Conclusions

Observational analysis of the UK's NDNS has revealed a series of risk factors that can limit an individual's ability to access sufficient vitamin C, as well as increasing an individual's vitamin C requirements.

With respect to accessibility, the analysis showed that the risk of vitamin C inadequacy was greater in the Autumn than at any other time in the year. These findings are consistent with the Autumnal instabilities in household vitamin C provisions observed in Chapter 2. Underpinning the seasonal instabilities appears to be a reduction in the consumption of fruits and vegetables that are high in vitamin C, as opposed to a general reduction in consumption. There is, therefore, a need to understand why this seasonal gap exists and if any vitamin C-dense crops can address shortfalls in access to sufficient vitamin C. These enquiries will be explored in Chapter 4, which presents an investigation of seasonality in the vitamin C content of UK strawberries.

Socioeconomic status was found to be the greatest limiting factor to vitamin C accessibility, with individuals in the lowest income households being more than twice as likely to have inadequate vitamin C intakes and status as individuals from the highest income households. Ensuring that interventions to address vitamin C insecurities are suitable for low-income households will be essential but presents a substantial challenge when considering the relatively high costs associated with fresh fruits and vegetables that are rich in vitamin C.

Younger adults were also found to have lower intakes than older adults, although this observation may be related to time-poverty more so than financial poverty.

Consistent with other studies, smoking status, body weight, sex, and age were all found to impact an individual's vitamin C requirement. Taken together, the impacts of body weight and obesity on vitamin C requirements are particularly concerning with respect to UK vitamin C security. Being obese more than tripled the odds of an individual having inadequate vitamin C status. As the prevalence and severity of obesity and associated co-morbidities continue to increase, it will be important to project how the UK's vitamin C requirements will be impacted. Crucially, it must also be understood whether the UK food system can support increasing nutrient demands.

The emerging situation is one in which vitamin C intakes could effectively decrease because of increasing poverty – both financial and time - and dilution of intakes in larger bodies. At the same time, vitamin C requirements are increasing in conjunction with the prevalence of poor health. As such, the UK should approach vitamin C security with concerted shifts towards more aspirational dietary recommendations that reflect the needs of today's population, as well as interventions to overcome barriers that limit access to vitamin C-rich foods.

Chapter 4 Seasonal Variation in the Vitamin C Content of UK-Marketed Strawberries

4.1 Introduction

Food Composition databases (FCD) provide valuable information relating to the average nutritional content of foods. The UK's Composition of Foods Integrated Dataset (CoFID) is a well-respected FCD and provides one of the most comprehensive nutrient panels of any large-scale FCD (Traka et al., 2020). The current edition of CoFID contains full nutritional profiles for 2887 foods and dishes that are commonly consumed in the UK (Roe et al., 2015; Traka et al., 2020). These include the fresh fruits and vegetables that are the primary contributors to vitamin C supplies and intakes (Macdiarmid et al., 2018; Poppy et al., 2022; Chapter 2, Section 2.3.1). Consequently, CoFID has become an integral part of studies that seek to investigate the availability, accessibility, and utilisation of vitamin C at various stages of the UK food supply chain (Bates et al., 2020; Macdiarmid et al., 2018; Poppy et al., 2022; Chapter 2, Section 2.2.1.2).

The nutrient reference values (NRV) in the CoFID are primarily derived from analytical surveys that sample foods from UK retailers (Roe et al., 2015). For foods that have not been included in CoFID surveys, the NRVs are supplemented with information from scientific literature and other FCDs (Roe et al., 2015). The resulting NRVs are presented as a mean value for each nutrient in each food. The mean NRVs are broadly applicable to macro-scale analyses of national or annual nutrient supplies (Macdiarmid et al., 2018; Poppy et al., 2022). However, a key limitation of the CoFID data in seasonal and sub-national analyses, such as those presented in Chapters 2 and 3, is that natural and seasonal variations in the nutrient composition of foods are rarely accounted for (Roe et al., 2015).

Natural variation in the vitamin C content of fruits and vegetables is determined by a combination of pre- and post-harvest factors. Pre-harvest factors can include the effects of genotype (Kim et al., 2015; Novotny et al., 2018; Valcarcel et al., 2015), environmental conditions during cultivation (Hanson et al., 2009; Oh et al., 2009), cultivation practices (Atkinson et al., 2006; Tilahun et al., 2018), and maturity at harvest (Aubert et al., 2021; Liang et al., 2017). Changes in vitamin C contents during post-harvest storage and transport are also commonly reported (Octavia and Choo, 2017; Olsson et al., 2004; Russell et al., 2009).

Accumulation of vitamin C in fruits and vegetables is driven by a combination of biosynthesis and recycling. In plants, the best-characterised biosynthetic pathway is the L-galactose pathway, which converts D-glucose to *L*-ascorbic acid via *D*-mannose and *L*-galactose intermediates (Bulley and Laing, 2016; Cruz-Rus et al., 2011; Muñoz et al., 2023). Because *L*-ascorbic acid synthesis draws directly on photosynthetically derived glucose and protects photosystems from oxidative damage, its flux is tightly controlled by light exposure (Bulley and Laing, 2016). The dominant biosynthetic route, however, varies by species and developmental stage. For example, strawberries shift from the *L*-galactose to the *D*-galacturonate pathway during ripening, driven by upregulation of *D*-galacturonate reductase (Cruz-Rus et al., 2011).

Once synthesised, cellular ascorbate levels are further modulated by the ascorbate–glutathione cycle, which recycles oxidised dehydroascorbic acid - produced during neutralisation of reactive oxygen species (ROS) - back into functional *L*-ascorbic acid. Under abiotic stress, ROS accumulate rapidly, and ascorbate recycling is upregulated to quench these radicals and prevent oxidative damage to key cellular structures (Gallie, 2012). Beyond controlled biological experiments, this integration of transcriptional control and dynamic recycling implies that the variable conditions encountered throughout fruit and vegetable supply chains could substantially affect the vitamin C content of produce reaching consumers.

In practice, Phillips et al. (2018) found that the vitamin C content of retail-marketed fresh fruits and vegetables differed significantly from the US Department for Agriculture's (USDA) published nutrient reference values depending on when and where they were purchased. The vitamin C content in Winter Spinach, for example, was 55% greater than the USDA mean NRV (Phillips et al., 2018). Moreover, Winter Spinach contained 2.4-fold more vitamin C than Summer or Autumn Spinach purchased from the same retailers (Phillips et al., 2018). Seasonal variation and deviations from the mean NRV were also observed in bananas, oranges, potatoes, and broccoli (Phillips et al., 2018).

In Chapter 2, several of the key contributors to household vitamin C provisions, including easy peeler oranges, cauliflower, strawberries, and Brussels sprouts, followed distinctive seasonal consumption patterns (Section 0). Strawberries, for example, were the fourth-highest contributor to overall HCPs between 2017 and 2022. Purchases of strawberries peaked during the Summer months, with a mean of 35.2% of annual sales occurring in the months between June and August (Chapter 2, Sections 2.3.2 and 0). With a relatively high proportion of strawberries being purchased during the UK Summer, it is important that the CoFID vitamin C reference value is reflective of strawberries purchased in the Summer.

4.1.1 Aims and Objectives

As such, Chapter 4 addresses the third aim outlined for this thesis: **To comprehensively describe the vitamin C content of UK-marketed fresh strawberries and explore potential seasonal instabilities at the crop level.** To address this aim, Chapter 4 comprises three key objectives.

1) To document the range of available fresh strawberry products available at major UK retailers in relation to season, variety, and country of origin.

2) To quantify the vitamin C content of fresh strawberry products throughout a calendar year.

3) To quantify seasonal variation in berry sweetness and retail prices as indicators of consumer preference.

4.2 Materials and Methods

4.2.1 Sampling Strategy

The sampling strategy was designed to balance the effective representation of UK-marketed strawberries with the practical constraints of collecting, processing, and analysing large numbers of samples. The sampling strategy targeted the retailers that occupied the largest share of UK strawberry sales. These retailers were identified using consumer purchase data acquired from Kantar World Panel (Chapter 2, Section 2.2.1.1).

Within the Kantar dataset, four retailers held a combined market share of 68.6% of strawberry sales between April 2017 and March 2022 (Figure 4.1). Extending to the five retailers increased the cumulative share to 76.5%. The 7.9% increase in total market share achieved by sampling from five retailers instead of four was deemed insufficient to justify the additional time and resources required. As such, the top four retailers were selected for inclusion in the study (Figure 4.1). The specific stores selected for sampling were the largest stores within the locality of the Research Station at Niab (East Malling, Kent, England).

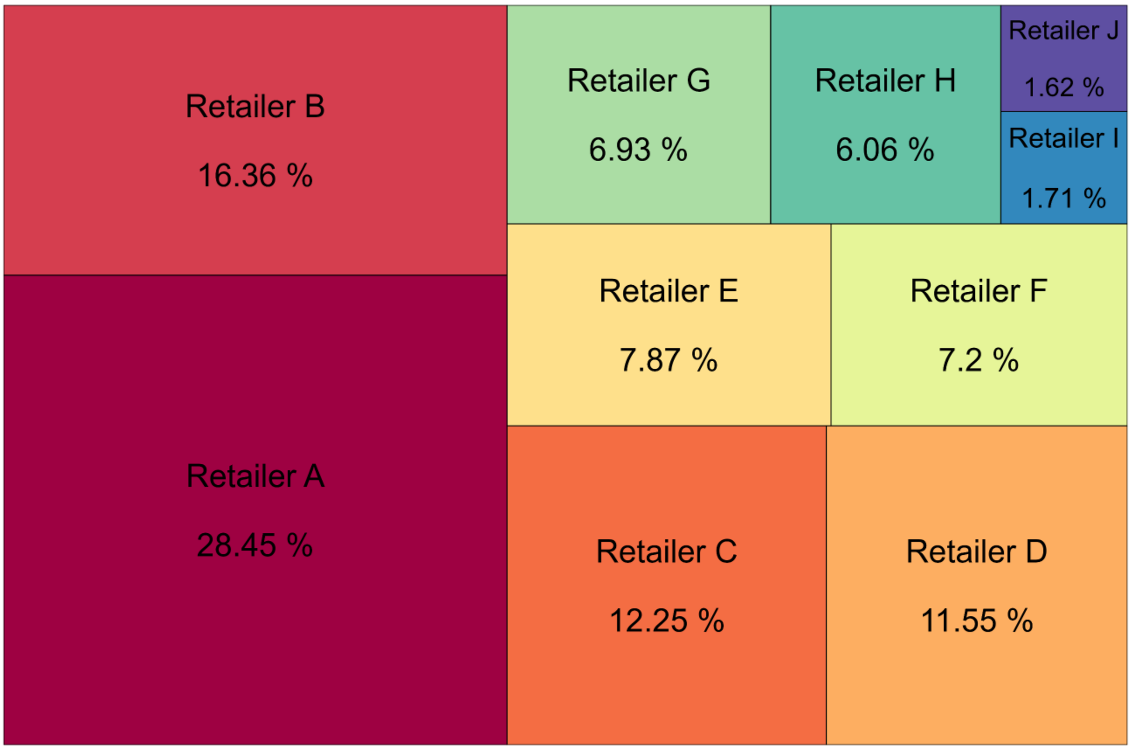


Figure 4.1 Tree Map showing the relative contributions of 10 UK grocery retailers to overall strawberry sales between April 2017 and March 2022. Each retailer was assigned a label from A-J to conserve anonymity. The size of each square is relative to the percentage of total strawberry sales made by each retailer. The sales figures were obtained from the Kantar Consumer panel data detailed in Chapter 2 (Section 2.2.1.1).

Sampling commenced on 24 March 2023 and concluded on 01 March 2024. Samples were purchased from the selected retailers on the first Monday and third Friday of each calendar month. Sampling on different days was incorporated to account for different consumer shopping habits and potential differences in the availability of fresh strawberries at different times of the week.

Each sampling event involved visiting each of the four selected retail stores. At each store, a minimum of 400 g of each distinct fresh strawberry product was purchased for sampling. A distinct strawberry product was identified based on the information available on the packaging. Key product identifiers included the variety, country of origin, and branding of the product (Figure 4.2). Product branding was categorised into "Premium", "Standard", "Organic", and "Budget" options.

Samples were excluded if they were not at least one day in advance of their best-before date. Samples were then selected based on their appearance, ripeness, and lack of visible damage.

The criteria for selecting samples were primarily designed to be representative of how a consumer would normally select a punnet of strawberries. Sampling every available unique product was also beneficial to capture the changing availability of fresh strawberries throughout the year. Fresh strawberries were available from all the selected retailers throughout the 12-month sampling period.

Figure 4.2 An example product label for a fresh strawberry product sampled from a major UK retailer. The label details the strawberry variety, where the strawberries were produced, who produced the fruit, and the best-before date of the product. Image taken on 12 July 2023.

The selected samples were returned to the laboratory within 2 hours after purchase. Each sample was photographed inside and outside of its packaging as a record of the general appearance of the strawberries. All information on sample packaging was recorded. Recorded information included purchase date, best before date, store, country of origin, region of origin, variety, product branding (“Premium”, “Standard”, “Organic” or “Budget”), and grower information.

Sub-samples were then selected at random consisting of 200 g of berries. The sub-sampled berries were washed in distilled water and hulled. Each sub-sample was then submerged in liquid nitrogen using a noodle strainer. Direct exposure to the liquid nitrogen was found to cause the berries to split and crack. The frozen berries were then shattered using a pestle and mortar. The fragments from the frozen berries were then mixed. A final sub-sample of 100 g of flash-frozen material was transferred to a plastic sample bag (Fisher Scientific, Nasco Whirl-Pak™ Standard Sample Bags, product code: 11781664). The flash freezing, shattering, and mixing was an effective way of ensuring that the final sub-samples contained a representative mix of material from all sampled berries.

The sub-samples were then lyophilised (SciQuip Ltd, Rotherham, UK. Christ Alpha 1-4 LSCplus, product code: 102141) for a minimum of 68 hours, or until a pressure increase test indicated a less than 5% change in drying chamber pressure. Once dried, the samples were then milled into a fine powder using a food processor. The milled samples were stored at -80 °C until analysis.

4.2.2 Chemical Analyses

4.2.2.1 Vitamin C, Malic, and Citric Acids

Vitamin C, measured as total ascorbic acid (AsA), and organic acids were extracted from the milled sample material simultaneously and analysed by HPLC. The extraction procedure followed the methods of Phillips et al. (2018) with some modifications. Briefly, 0.15 g of sample material was added to 5 ml 3% (w/v) meta-phosphoric Acid / 1mM EDTA (SigmaAldrich, meta-Phosphoric acid, CAS:37267-86-0; SigmaAldrich, Ethylenediaminetetraacetic acid, CAS:6381-92-6). The material was mixed by vortexing for 20 seconds, followed by 15 minutes in a dark room on a mechanical shaker operating at 500 rpm. The samples were then centrifuged under the following conditions: 20 minutes, 4 °C, 4700RPM, 4643RCF.

An aliquot of 200 µl was taken from the resulting supernatant and transferred to a 0.2 µm filter vial (Fisher Scientific Ltd, Loughborough, UK. Whatman™ Mini-UniPrep™ Syringeless Filters, product code: 11388734). The aliquot was then mixed with 200 µl 10 mM Tris(2-carboxyethyl)phosphine (TCEP; Sigma Aldrich, Tris(2-carboxyethyl)phosphine, CAS: 51805-45-9). The sample extracts were incubated at room temperature in the dark for 1 hour to allow the TCEP to reduce dehydroascorbic acid to *L*-ascorbic acid.

Chromatographic separation was performed using a Synergi 4 µm Hydro-RP 80 Ǎ LC 300 x 4.6 mm, Ea column (Phenomenex Ltd, Huddersfield, UK. part number: 00H-4375-E0), fitted with 3mm internal guard cartridge (Agilent Technologies LDS UK Ltd, Stockport, UK. Polaris C18-A, Chromsep Guard cartridge, 180 Å, 5 µm, 10 x 3.0 mm, part number: A2000GC3). Separation conditions were as follows: column temperature 21 °C, mobile phase – 10 mM potassium phosphate (Fisher Scientific, Potassium phosphate, tribasic, 97% pure, anhydrous, CAS: 7778-53-2) pH 2.7; flow rate 1 ml/min; injection volume 10 µl. The HPLC system incorporated a Waters 2960 separation module linked with a Waters 996 photodiode array detector. Detection of AsA was conducted at 243 nm and Malic and Citric acids were detected at 210 nm.

The amounts of AsA, malic, and citric acids were quantified using external calibration curves. The calibration series consisted of five standards containing Ascorbic acid (Sigma Aldrich, *L*-

Ascorbic acid, CAS: 50-81-7), Malic acid (Sigma Aldrich, D-Malic acid, CAS: 636-61-3) and Citric acid (Sigma Aldrich, Citric acid, CAS: 77-92-9) in quantities ranging from 0.25 - 2.00 mg/ml, 1.25 - 10 mg/ml, and 2.50 - 20.00 mg/ml, respectively. The calibration series was analysed each time a new mobile phase was prepared to account for any deviations in retention time.

Each set of sample extractions included a calibration standard, a BCR431 certified reference material extract (Sigma-Aldrich Co, Gillingham, UK. Brussels sprouts (vitamins), BCR®, certified reference material, UNSPSC Code: 85151701. note: Product is discontinued as of 01/10/2024), and an in-house strawberry reference extract. The standards were included to ensure the validity of the calibration and the precision and accuracy of the extractions. The measured malic and citric acid contents were presented as mg/g fresh weight. The measured AsA content was presented as mg/100g fresh weight.

4.2.2.2 Total Soluble Solids and Berry Sweetness

Total Soluble Solids (TSS) content was determined from the combined juices of five individual berries from each sample. The juice was extracted by crushing each of the berries by hand over a plastic cup. The juice from the five berries was mixed and the composite juice was analysed using a digital refractometer. Each juice composite was analysed three times. The measured TSS values were presented as °*Brix* (%).

Malic and Citric acid content, as quantified by HPLC, were used to calculate the theoretical titratable acidity (TTA) of each sample, as previously described by Manabe (1993) and Ikegaya (2024) $(TTA = (citric * 10^{-1} + malic * 10^{-1})/(6.4/6.71))$. The calculated TTA values were expressed as %Citric acid equivalents. The measured TSS and calculated TTA values were then combined to produce a TSS/TTA ratio. The TSS/TTA provided an index of overall berry sweetness which has been closely associated with expected consumer preference (Ikegaya et al., 2021).

Measurements of TSS were only started in July 2023, meaning that the time series for TSS and TSS/TTA was shorter than for the other analytes. The time series for TSS and TSS/TTA covers the period between 11 July 2023 to 01 March 2024.

4.2.3 HMRC Trade Data

Official HMRC trade data relating to UK strawberry imports between March 2023 and February 2024 were downloaded from the Food Standards Agency Trade Data Visualisation Portal on 15 August 2024 (https://foodstandards.shinyapps.io/TradeDataVis/; Food Standards Agency, 2024). These data were used as a reference to compare the relative contributions of different countries to the samples collected as part of the current study. The Trade data were visualised with the {*ggplot*} and {*rnaturalearth*} packages in R (Massicotte and South, 2023; R Core Team, 2024; Wickham, 2016).

4.2.4 Statistical Analysis

Comparisons between groups were analysed using Welch's F-test as implemented in the {*stats*} R package (R Core Team, 2024). Welch's F-test was selected due to its robustness to deviations from assumptions of normality and homoscedasticity (Delacre et al., 2019). The data residuals were checked for excessive violations of normality and homoscedasticity before analysis.

Post hoc comparisons of group means were conducted using pairwise student t-tests with Holm adjustments of *p*-values to correct for multiple comparisons. Where appropriate, results are presented as the mean ± standard error.

Correlation analyses were conducted using the {*correlation*} package in R (Makowski et al., 2020). Correlations were compared based on the computed Pearson's Correlation Coefficient between pairs of variables.

For all statistical tests, null hypotheses were rejected at an alpha threshold of 0.05. Visualisations of group-wise comparisons and correlation analyses utilised the {*ggplot}* and {*ggstatsplot*} packages in R (Patil, 2021; Wickham, 2016).

4.3 Results

4.3.1 Seasonal Availability of Fresh Strawberries

A total of 299 fresh strawberry samples were collected from local retail stores between 23 March 2023 and 01 March 2024. The availability of fresh strawberry products varied seasonally. A total of 79 samples (26%) were collected in the Spring (March-May 2023). The greatest number of samples were collected in the Summer, with 107 samples (36%) collected between June and August 2023 (Figure 4.3). The number samples decreased to 57 and 56 in the Autumn (September – November 2023) and Winter (December 2023 – February 2024), respectively.

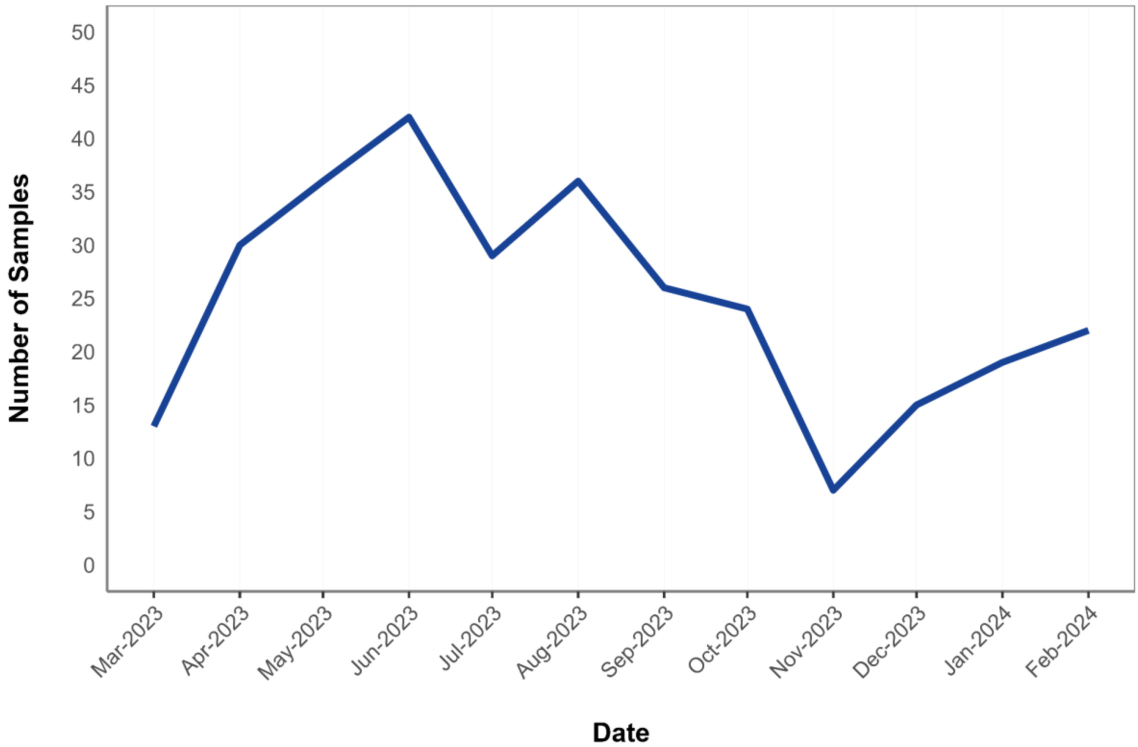


Figure 4.3 Time series depicting the number of fresh strawberry samples collected from UK retailers between 23 March 2023 and 01 March 2024.

The samples comprised 56 unique varieties, of which 29 were June-bearers (JB; 51.8%), 19 were Everbearers (EB; 33.9%), and 8 were not possible to classify (14.3%) due to a lack of information on the product label. A total of 117 JB and 76 EB samples were collected (Figure 4.4). *Favori* (EB, *n* = 33), *Malling Centenary* (JB, *n* = 27), *Inspire* (JB, *n* = 25) and *Fortuna* (JB, *n* = 19) were the most frequently sampled varieties. No variety information was available for 25 of the samples (Figure 4.4). The frequency with which each variety was sampled also varied seasonally across the study period (Figure 4.4). In the Spring, JB varieties accounted for 62.1% of all samples, with the most frequently sampled varieties being *Malling Centenary* (JB, 15.1%) and *Inspire* (JB, 12.7%). In the Summer, EB's became more prevalent, accounting for 68.2% of all samples with the most frequently sampled varieties being *Favori* (EB - 18.7%), *Driscolls Zara* (EB - 9.3%) and *Malling Centenary* (JB - 8.4%). In the Autumn, EB's (54.4%) remained more common than JB's (35.1%), with the most frequently sampled varieties being *Favori* (EB - 21.1%) and *Elsanta* (JB - 14.0%; Figure 4.4). In the Winter, JB's increased in frequency (57.1%), with the most frequently sampled varieties being *Fortuna* (JB - 23.2%), *Inspire* (JB - 16.1%), and *Sensation* (EB, 16.1%; Figure 4.4).

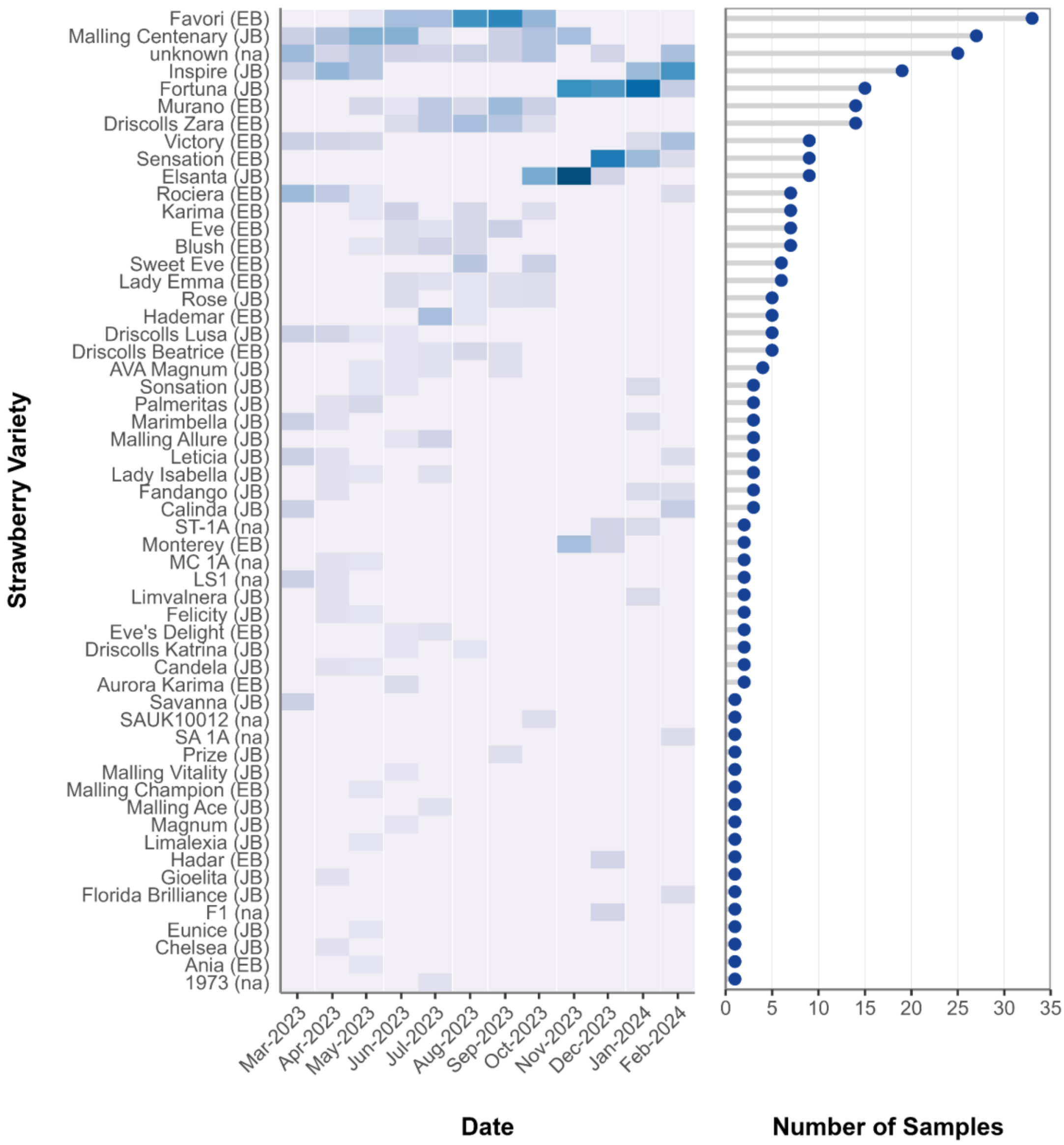


Figure 4.4 Relative and actual sampling frequencies of unique strawberry varieties from UK retailers Between 23 March 2023 and 01 March 2024. Left panel: Heat map illustrating the relative sampling frequency for each strawberry variety in each month, ranging from light purple (0%) to dark blue (100%). Varieties are marked as JB (June bearing), EB (Everbearing), or na (no variety information). Right panel: Lollipop chart illustrating the total number of samples collected for each variety between 23 March 2023 and 01 March 2024.

4.3.2 Geographic Origin of Fresh Strawberries

Fresh strawberry samples originated from a total of eight countries over the study period (Figure 4.5). In total, 181 samples (60.5%) were of UK origin. The earliest UK strawberries were sampled in March 2023 and the latest sampled in October 2023. Between June and August 2023, fresh strawberries were exclusively produced in the UK (Figure 4.5).

Strawberries of Spanish origin were the second most frequently sampled, accounting for 55 samples (18.4%). Spanish strawberries were only available during the Spring (March-May 2023) and Winter (December 2023-February 2024) months. North African (Morocco, Egypt, and Jordan) strawberries accounted for 46 samples (15.4%) and were only available in the Autumn and Winter. The remaining 17 samples were of Belgian or Dutch origin (5.7%) and were collected between September and December 2023.

Based on the HMRC import data between March 2023 and March 2024, UK strawberry imports were found to originate from a total of 27 countries. Imports from Spain (54.9%), North Africa (25.0%), Belgium and the Netherlands (13.1%) comprised 93% of all recorded fresh strawberry imports. Strawberry imports were reported in all seasons. This included the Summer months when only fresh strawberries of UK origin were available in the retailers sampled from in the present study (Figure 4.6).

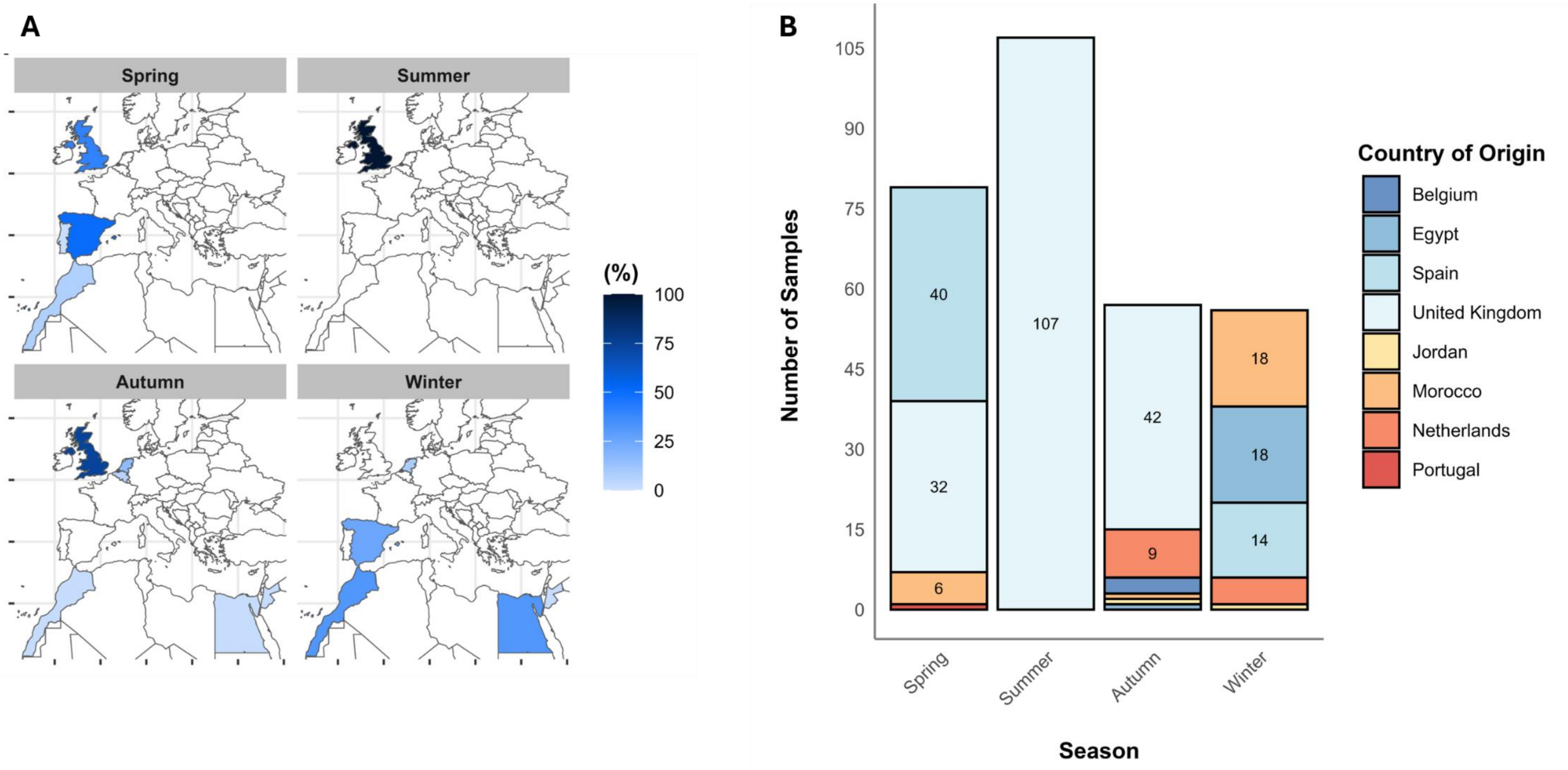


Figure 4.5 Seasonal origins of UK-marketed fresh strawberries sampled from UK retailers between 23 March 2023 and 01 March 2024. (A) A choropleth map illustrating the relative proportion of samples that originated from different countries in the Spring (March-May 2023), Summer (June-August 2023), Autumn (September-November 2023) and Winter (December 2023 – February 2024). (B) A stacked bar chart showing the number of samples that originated in different countries in each season.

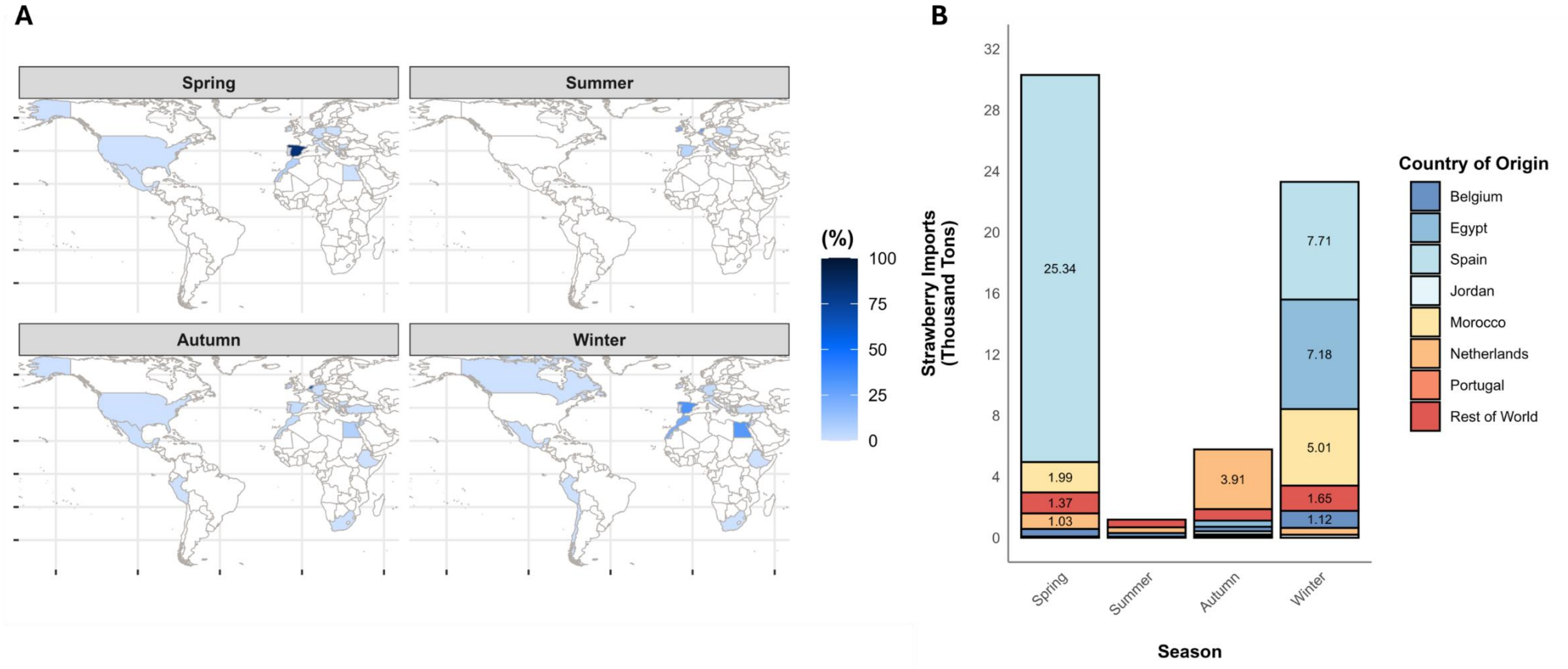


Figure 4.6 Seasonal origins of UK strawberry imports recorded by HMRC between 01 March 2023 and 28 February 2024. (A) A choropleth map illustrating the relative proportion of samples that originated from different countries in the Spring (March-May 2023), Summer (June-August 2023), Autumn (September-November 2023) and Winter (December 2023 – February 2024). (B) A stacked bar chart showing the number of samples that originated in different countries in each season. Imports from countries that were not observed in the samples gathered from UK retailers were grouped into a combined "Rest of World" category.

4.3.3 Seasonal Trends in the Vitamin C Content, Sweetness, and Cost of Fresh Strawberries

Vitamin C content, measured as AsA, ranged from 20.50 mg/100g to 119.40 mg/100g. The mean AsA was 60.44 ± 13.28 mg/100g. The data exhibited a high degree of centrality, as indicated by a median value of 59.31 mg/100g (Figure 4.7).

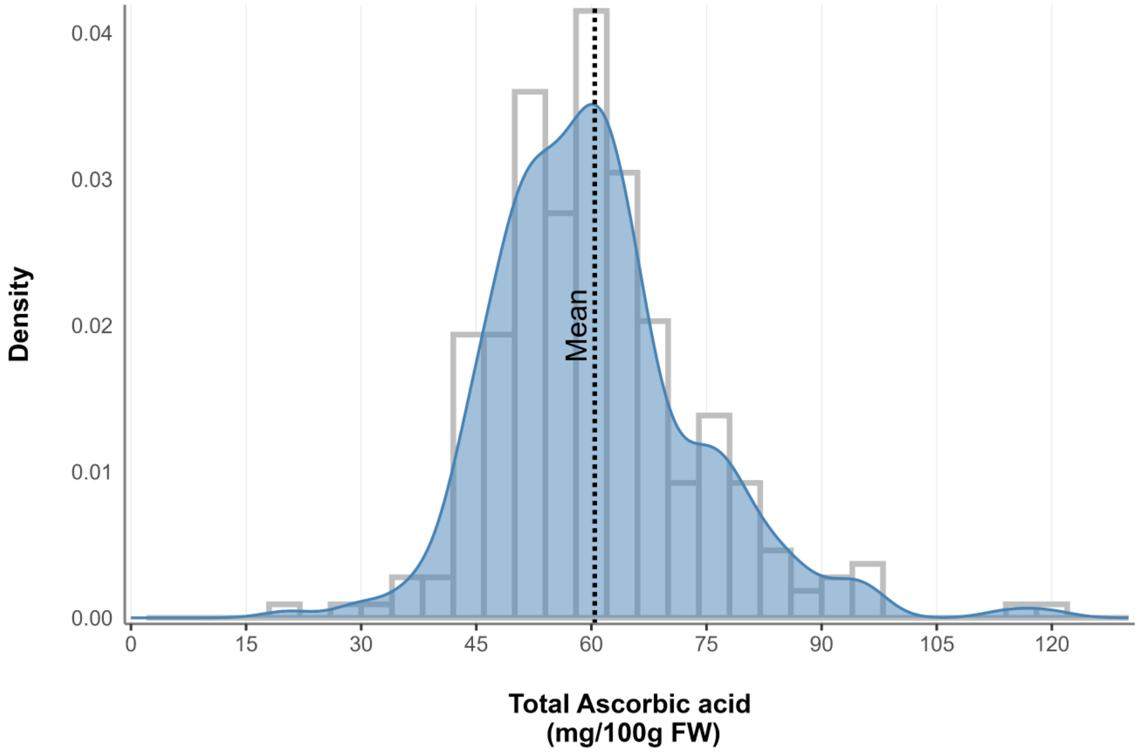


Figure 4.7 Distribution of measured vitamin C values in fresh strawberry samples purchased from UK retailers between 23 March 2023 and 01 March 2024. Vitamin C values are expressed as mg total ascorbic acid / 100g fresh weight.

Differences in the AsA of strawberries purchased in each calendar month were assessed with Welch's one-way ANOVA. Statistically significant differences in AsA were observed between calendar months ($F(11, 76.06) = [6.373]$, $p = 2.284e^{-07}$).

The greatest AsA values were observed in July (71.62 mg/100g FW), June (67.45 mg/100g FW) and August (65.23 mg/100g FW), respectively (Figure 4.8). The mean AsA observed in January (52.28 mg/100g FW) and February (52.01 mg/100g FW) 2024 were significantly lower than the values observed in June and July 2023 (Figure 4.8; $p < 0.03$).

The monthly AsA values were similar throughout the Spring of 2023 with values ranging between 54.66 and 59.57 mg/100g FW (Figure 4.8). Consistent AsA values were observed between September and December 2023, with values ranging between 55.17 and 60.80 mg/100g FW (Figure 4.8)

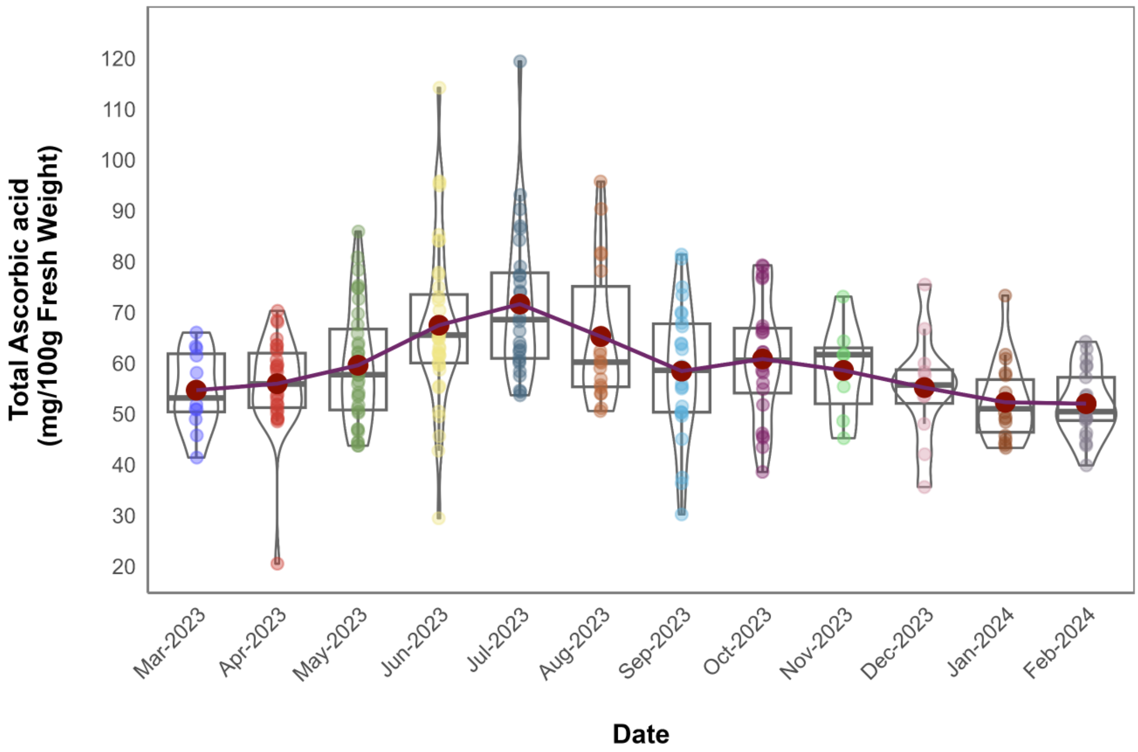


Figure 4.8 Box- and Violin-plots showing the mean total ascorbic acid content in fresh strawberry products sampled from UK retailers in each month between 23 March 2023 and 01 March 2024. The mean total ascorbic acid content for each month is represented as a red dot. The purple line intersecting the means indicates the directionality of the seasonal trends.

Differences in berry sweetness (TSS/TTA) between calendar months were assessed with Welch's one-way ANOVA. Statistically significant differences were observed between calendar months for TSS/TTA ($F(8, 45.63) = [5.183]$, $p = 1.245e^{-04}$).

The greatest TSS/TTA ratios were observed in January 2024 (9.15) and were significantly greater than in November 2023 (5.87; $p = 0.01$). The mean TSS/TTA values were comparable between July, August, September, October, and December 2023, ranging between 6.82 and 7.89 (Figure 4.9). Berry Sweetness data were not collected between March and June 2023.

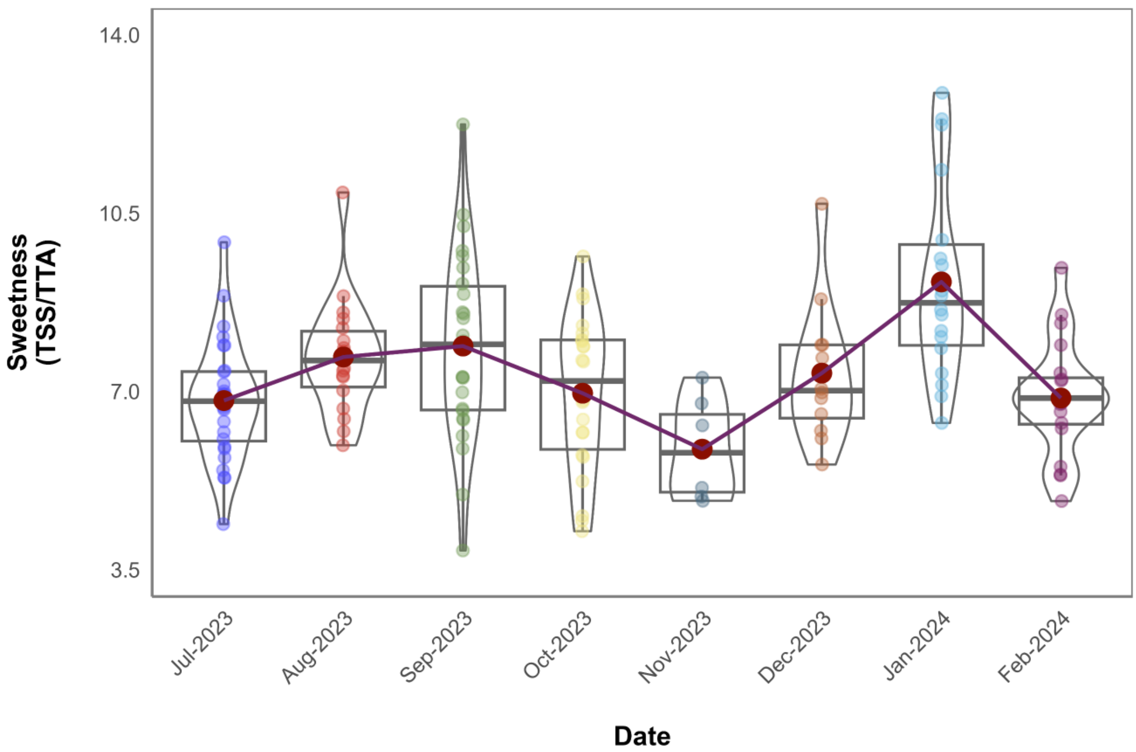


Figure 4.9 Box- and Violin-plots showing the overall berry sweetness in fresh strawberry products sampled from UK retailers in each month between 11 July 2023 and 01 March 2024. Berry sweetness is expressed as the ratio of Total Soluble Solids (TSS) to Theoretical Titratable Acidity (TTA). The mean TSS/TTA ratio for each month is represented as a red dot. The purple line intersecting the means indicates the directionality of the seasonal trends.

Differences in the price of strawberries sampled in each calendar month were assessed with Welch's one-way ANOVA. Statistically significant differences in the price of strawberries were observed between calendar months (F(11, 84.73) = [31.124], p = 2.200e^{-16}).

Strawberry prices followed an inverse trend to that described for vitamin C content (Figure 4.10). Strawberry prices were at their lowest between April 2023 and September 2023, ranging between £4.36/Kg and £5.04/Kg. The lowest mean price was observed in August 2023 (Figure 4.10). Prices increased between September (£4.63/Kg) and October 2023 (£5.96/Kg). Prices continued to increase and reached a maximum in December 2023 (£7.04/Kg). Prices remained above £6/Kg in January and February 2024 (Figure 4.10).

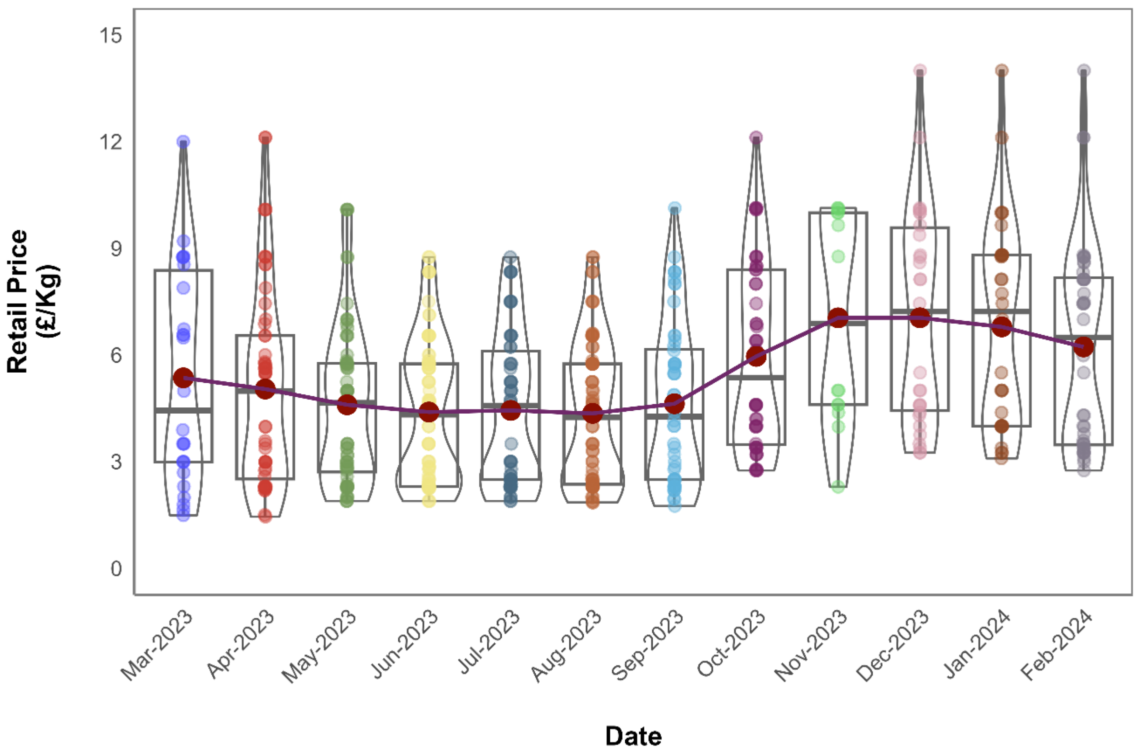


Figure 4.10 Box- and Violin-plots showing the retail prices of fresh strawberry products sampled from UK retailers in each month between 23 March 2023 and 01 March 2024. Retail price values are presented as Great British Pounds per Kg (£/Kg). The mean retail price for each month is represented as a red dot. The purple line intersecting the means indicates the directionality of the seasonal trends.

Further correlation analysis indicated that there were no detectable associations between berry sweetness and price ($r_{pearson}(148) = -0.06$, $p = 0.44$). Weak, negative correlations were observed between vitamin C and price ($r_{pearson}(148) = -0.18$, $p = 0.03$) and between berry sweetness and vitamin C ($r_{pearson}$ (148) = -0.26, $p = 0.001$; Figure 4.11).

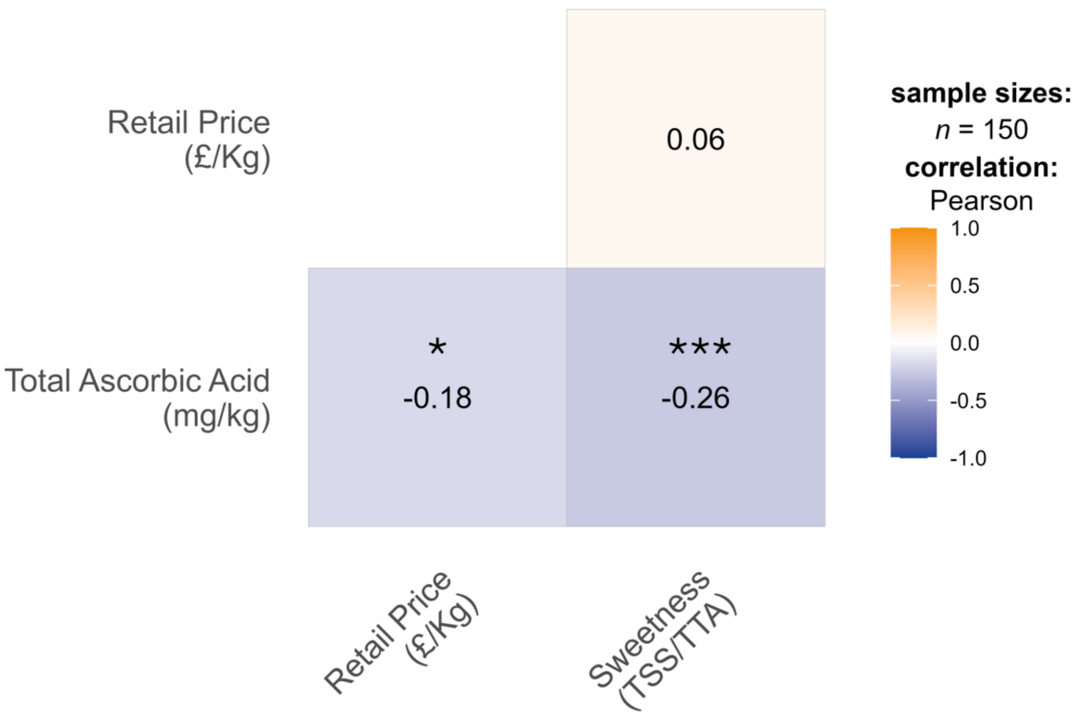


Figure 4.11 Correlation matrix illustrating the Pearson's correlation coefficient computed for pairs of observations relating to their retail price, sweetness, and vitamin C content. Retail price values are presented as Great British Pounds per Kg (£/Kg). Berry sweetness is expressed as the ratio of Total Soluble Solids (TSS) to Theoretical Titratable Acidity (TTA). Vitamin C content is presented as total ascorbic acid. Statistically significant correlations are marked with asterisks, with more asterisks signifying smaller *p* values ($p > 0.05$ *, $p > 0.01$ **, $p > 0.001$ ***).

4.3.4 Comparisons between Different Product Categories

The influence of product type on the vitamin C, sweetness, and price of strawberries was then considered. Each of the retailers offered a selection of strawberry products which were grouped into "Premium", "Standard", "Budget", and "Organic" options. Having accounted for the effects of the sampling month, statistically significant differences were found between the product categories for vitamin C ($F(3, 266) = [10.650]$, $p = 1.230e^{-06}$), sweetness ($F(3, 146) = [5.324]$, $p = 0.002$) and price ($F(3, 293) = [19.073]$, $p = 2.520e^{-11}$).

“Organic” strawberries had the greatest vitamin C contents (70.73 ± 15.21 mg/100g FW), sweetness (9.84 ± 1.98), and price (8.81 ± 1.07 £/Kg) relative to the other product categories (Table 4.1). The vitamin C content and price of “Premium” strawberries were similar to that of Organic, but sweetness values were lower (7.53 ± 1.39) (Table 4.1). Budget and Standard strawberries had similar vitamin C, sweetness, and price values, all of which were significantly lower than the values in Organic strawberries (Table 4.1).

Table 4.1 Vitamin C content, sweetness, and price of different types of fresh strawberry products purchased between 23 March 2023 and 01 March 2024.

	Product Category			
Characteristic	**Budget** *n* **= 36**	**Organic** *n* **= 13**	**Premium** *n* **= 44**	**Standard** *n* **= 206**
Vitamin C (mg/100g FW)[1,2]	61.66 (14.14)	70.73 (15.21)	68.68 (16.81)	57.97 (11.19)
Berry Sweetness (TSS/TTA)[1,3,4]	6.78 (1.29)	9.84 (1.98)	7.53 (1.39)	7.40 (1.65)
Price (£/Kg)[1,5]	6.33 (1.13)	8.81 (1.07)	8.82 (2.27)	6.97 (1.80)

[1]Mean (SD)

[2]Measured as Total Ascorbic Acid

[3]Measured as the ratio of Total Soluble Solids to Theoretical Titratable Acidity (TSS/TTA).

[4]Berry sweetness values only cover the period between 11 July 2023 and 01 March 2024.

[5]Expressed as Great British Pounds per Kilogram (£/Kg)

4.4 Discussion

4.4.1 The Vitamin C Content of UK-Marketed Fresh Strawberries

Chapter 4 characterised the vitamin C content of 299 fresh strawberry samples collected from UK retailers between March 2023 and February 2024. The overall mean vitamin C content was found to be consistent with existing NRVs derived from other analytical surveys. Indeed, the observed mean of 60.44 ± 13.28 mg/100g fresh weight was within 2% of the official CoFID (Public Health England, 2015) and USDA (U.S. Department of Agriculture, 2024) NRVs.

Consistency in the mean vitamin C content observed in surveys from different regions and time periods supports the accuracy and reliability of macro-scale conversions of strawberry supply and intake data to equivalent vitamin C values (Bates et al., 2019; Macdiarmid et al., 2018; Poppy et al., 2022). However, inter-survey consistency in the case of strawberries and vitamin C is not necessarily representative of other crops and micronutrients. In a similar study of local fruits and vegetables, Phillips et al. (2018) found that the mean vitamin C content of apples, bananas, tomatoes, and potatoes all differed significantly from the USDA NRVs. Thus, broader conclusions about the accuracy and reliability of FCDs would require the approach of the current study to be expanded to more foods. Such studies would, however, be limited by the cost and resources required to source and analyse the necessary numbers of samples, which is already acknowledged as a reason for the often limited sample sizes in FCD analytical surveys (Roe et al., 2015).

4.4.2 Seasonal Variation in the Vitamin C Content of Fresh Strawberries

While the mean vitamin C content in strawberries was consistent with published NRVs, the current study also identified significant seasonal variations that have not been described in previous surveys (Public Health England, 2015; Roe et al., 2015). Seasonal trends in the vitamin C content of UK-marketed strawberries were characterised by higher values during the Summer months (June – August 2023). The vitamin C content observed in July 2023 was 37.7% higher than the vitamin C content observed in February 2024 and 18.5% higher than the survey mean. Similarly, Phillips et al. (2018) found that the vitamin C content of baby spinach leaves purchased in the Winter was 140% higher than baby spinach sampled in the Autumn. Phillips et al. (2018) also observed seasonal variation in the vitamin C contents of potatoes and oranges, but not in apples, bananas, or tomatoes. Crop- and nutrient-dependent seasonal variations in mineral contents have also been observed in a survey of 21 fruits and vegetables in Korea, which included strawberries (Kim et al., 2007). As such, the relative impact of strawberries on micronutrient availability and accessibility is likely to vary depending on the time of year and should be accounted for in seasonal analyses of micronutrient security.

In the case of strawberries, the seasonal peak in vitamin C content was observed to coincide with the seasonal peaks in the availability of UK-grown strawberries and the lowest retail prices. Similarly to the current study, Kim et al. (2007) also observed that nutrient values in fruits and vegetables tended to be higher during their respective domestic growing season. The combination of local produce (He et al., 2021) and lower prices (Bhat et al., 2015) between June and August 2023 may contribute to the peak in consumer preference for strawberries during the same period (Pelham, 2017; Chapter 2, Section 0). Interestingly, the alignment of seasonal preferences with peak vitamin C content in strawberries suggests that weighting the NRV to account for seasonal variation would result in greater estimated contributions to vitamin C intake. Having accurate NRVs for the times when consumption is highest could improve the accuracy of analyses of nutrient intake from foods.

4.4.3 Sources of Seasonal Variation in the Vitamin C Content of Fresh Strawberries

The additional metadata collected in relation to the genotype and country of origin of the strawberry samples offer key insights into some of the factors that may underpin the observed seasonal variation in vitamin C content.

A total of 56 unique strawberry genotypes were analysed in the current study, which greatly exceeds the number of genotypes documented in previous UK surveys (Roe et al., 2013). Indeed, the most recent CoFID survey analysed eight genotypes, of which only *Elsanta* and *Ava* overlapped with the current study (Roe et al., 2013). The impact of genotype on strawberry vitamin C content is widely documented, with numerous studies reporting up to 2-fold variations in vitamin C content under experimental conditions (Kim et al., 2015; Mezzetti et al., 2016; Skupień and Oszmiański, 2004). Consequently, the large number of genotypes captured in the current survey was likely a key factor in driving the observed variation.

During the UK Summer, the most frequently sampled varieties were *Malling Centenary* and *Favori*. Different varieties were more common in the Autumn (*Elsanta*), Winter (*Fortuna*), and Spring (*Inspire*). Currently, there have been no standardised studies that have compared the vitamin C content of the genotypes that were most frequently sampled in the current study. Future efforts to characterise the vitamin C contents of popular UK strawberry genotypes could provide useful context to the seasonal variation observed in the current study. Furthermore, standardised assessments could provide valuable information for breeders, growers, and retailers relating to genotypes that are of high nutritional value.

During the UK Summer, all collected samples were of UK origin whereas imports from Europe and North Africa became more abundant in the Autumn, Winter, and Spring. Cultivation practices are known to vary between regions and seasons and include the use of greenhouses, polytunnels or open fields in combination with either soil or soilless growth substrates (Calleja et al., 2012; Mordini et al., 2009; Van Delm et al., 2016). Experimental studies have found vitamin C content to be greater in open-field compared to greenhouse production systems (Rahim Doust et al., 2023). From a biochemical perspective, this may be because plants grown in an open field are exposed to a more variable environment. Abiotic stresses in open-field conditions will cause ROS to accumulate, subsequently driving an upregulation of ascorbate recycling (Gallie, 2012; Pandey et al., 2017). In practice, however, differences in the vitamin C content of retailed strawberries from different production systems are minimal (Pincemail et al., 2012).

A further consideration could be related to the shorter distance to market for UK strawberries as compared to imported strawberries. Domestically-produced strawberries likely spend less time in refrigerated storage and transit, which has been shown to cause vitamin C losses of 50% in as few as 5 days. (Octavia and Choo, 2017; Russell et al., 2009). This could be explained by reduced activity (*D*-galacturonate pathway) in the cold (Cruz-Rus et al., 2011), and likely limited activity of the light-dependent (*L*-galactose pathway; Maruta, 2022). Extended periods in the dark and cold during longer supply chains for imported strawberries during the Winter months could exacerbate these losses. It is also likely that local produce can be harvested closer to peak ripeness, which has been associated with higher vitamin C contents relative to harvests at earlier maturity stages in some studies (Muhammad Nisar and Serçe, 2022; Rahman et al., 2016). The need to pick before peak ripeness to ensure fruit do not rot in transit may inhibit the progressive upregulation of the *D*-galacturonate pathway in ripening strawberry fruit (Cruz-Rus et al., 2011), thus curtailing accumulation of vitamin C. Resultingly, retention and bioavailability may be greater in UK-grown strawberries and may contribute to higher vitamin C contents in the Summer months. Further metabolic studies using fruit from different stages in the supply chain would be useful to determine the exact mechanisms underpinning the observed seasonal variation.

Finally, the vitamin C content of Organic strawberries was found to be 22.0% greater than Standard strawberries. This aligns with a common belief that organically farmed fruits and vegetables are of higher nutritional quality due to greater exposure to biotic and abiotic stressors (Faoro et al., 2024; Orsini et al., 2016). As previously discussed, biotic and abiotic stress trigger responsive pathways in plants that often rely on ROS as a signalling intermediate (Baxter, Mittler, and Suzuki, 2014), subsequently driving increased production of vitamin C to quench the ROS (Pandey et al., 2017). Direct comparisons of farming systems have, however, shown that vitamin C content in strawberries was 17.4% greater in Standard produce when compared to Organic (Cardoso et al., 2011). Contrasting results have also been observed in raspberry, where Standard fruit contained 20% more vitamin C than Organic (Ponder and Hallman, 2020). Other studies have shown no difference between farming systems (Wunderlich et al., 2009; Kobi et al., 2018).

It is important to note, however, that the comparison of product types in the current study is a collective comparison of the different varieties, seasonal patterns, farming systems, and supply chains underpinning each product type. Organic samples were most frequently sampled between May and September in the current study (9/13 samples), and all but 2 of the Organic samples originated from the UK. These seasonal trends suggest shorter, more local supply chains are likely employed for Organic strawberries when compared to Standard strawberries. Other studies have also highlighted the use of non-standard post-harvest technologies in organic supply chains, such as UV light and Ozone treatments to extend shelf life in the absence of pesticides and fungicides (Phillips et al., 2019). Moreover, for the reasons already discussed, the higher mean vitamin C content may be a product of the time of year when Organic strawberries were available. As such, generalisations based on controlled horticultural experiments do not necessarily apply to the findings of the current study. Importantly, the current study presents a comparison of different product types from the perspective of a consumer. This means that, irrespective of the nature of the pre-consumer supply chain, inferences can be made as to the nutritional value of strawberries at the point of purchase. Caution must be taken, however, when inferring a difference between Organic and Standard strawberries in the current study due to the difference in sample sizes (13 Organic vs 206 Standard).

4.5 Conclusions

Chapter 4 described a comprehensive analytical survey of vitamin C contents in strawberries available to UK consumers at different times of the year. The survey found that peak vitamin C levels in UK-marketed strawberries occurred between June and August, which coincided with the peak of the UK production season, lowest retail prices, and peak consumer purchases of strawberries. This finding has key implications for seasonal analyses of strawberries in relation to UK vitamin C security as it indicates that strawberry-based interventions will likely have a greater impact during the Summer months.

Taken together, the observed variation in vitamin C contents is likely a product of *genotype x environment* interactions that were not controlled for in the current study. It is, therefore, difficult to identify the extent to which each factor may have impacted the seasonal differences in vitamin C content. From the perspectives of breeders and growers, further standardised studies of the effects of genotype and cultivation practices could provide useful information to facilitate the production of more vitamin C-dense strawberries. Similarly, from a retailer's perspective, a better understanding of how vitamin C contents are affected by post-harvest storage conditions could be useful to ensure that nutritional quality is preserved before reaching consumers. The effects of cultivation practices, genotype, and post-harvest storage are subsequently investigated in Chapters 5 and 6 of this thesis.

Chapter 5 The Effects of Foliar-applied Iron-Nanoparticles and Sodium Selenate on the Vitamin C Content of UK Strawberries Cultivated in Commercial Growing Systems

5.1 Introduction

Over the past 60 years, the UK has become increasingly reliant on imported foods to meet its population's nutritional needs. Analyses indicate that the composition of food supplies has shifted between 1960 and 2016, such that the UK is no longer self-sufficient in key micronutrients such as iron, zinc, and vitamins A and C (Macdiarmid et al., 2018; Poppy et al., 2022). While overall micronutrient supplies remain in surplus relative to requirements (Macdiarmid et al., 2018; Poppy et al., 2022), the trend toward declining self-sufficiency raises concerns about the stability of these supplies and the resilience of the food system to increasingly frequent supply chain shocks (Chapter 1, Section 1.2.1).

The observed declines in micronutrient self-sufficiency have coincided with a shift in consumer preferences toward fruits and vegetables that cannot be produced in the UK (Poppy et al., 2022; Scheelbeek et al., 2020). Imported fruits and vegetables — primarily from the EU (Poppy et al., 2022) and regions highly susceptible to climate shocks (Scheelbeek et al., 2020) — now account for 60% of total UK vitamin C supplies (Poppy et al., 2022). Without these imports, the UK's vitamin C supply would decrease from 300% of the recommended nutrient intake (RNI; 40 mg/capita/day) to just 120% of the RNI (Poppy et al., 2022). Furthermore, evidence from Chapter 3 suggests that the current RNI may underestimate the true vitamin C requirements of the UK population. Analyses showed that vitamin C requirements could be 1.8 times higher than the current RNI (Section 3.3.1), implying that disruptions to imported supplies could result in inadequate availability and accessibility to vitamin C.

The UK food system has demonstrated remarkable resilience to recent challenges, including Brexit, the SARS-CoV-2 pandemic, regional conflicts, and climate shocks (Moran et al., 2020). Nonetheless, exploring strategies to enhance domestic vitamin C production offers a proactive approach to stabilising and securing vitamin C supplies.

Chapter 1 introduced the concept of increasing UK vitamin C self-sufficiency through the biofortification of popular local crops (Section 1.3). Strawberries are particularly well-suited to this purpose, owing to their widespread popularity and the fact that domestic production comprises 65-70% of total strawberry supplies (Chapter 1, Section 1.4). Moreover, strawberries play a substantial role in UK vitamin C provisions. As shown in Chapter 2, strawberries were the fourth-highest contributor to household vitamin C provisions between 2018 and 2021 (Section 2.3.2). Consequently, strawberries represent an ideal target for vitamin C biofortification in the context of enhancing UK vitamin C self-sufficiency.

Compared to conventional breeding techniques, the manipulation of environmental conditions or cultivation practices offers a faster and potentially more cost-effective approach to increasing vitamin C content (Chapter 1, Section 1.4.2.2). Foliar spray treatments have shown positive results in their capacities to enhance crop nutritional quality (Chapter 1, Section 1.4.2.2). Moreover, given that foliar sprays for fungicides and pesticides are already widely used in UK agriculture — 98% of UK strawberries were treated with pesticides in 2006, for example (Calleja et al., 2012) — it is feasible that sprays aimed at boosting productivity and nutritional quality could be integrated into existing agricultural practices.

Selenium-based foliar (SEL) sprays are one such strategy that has shown positive effects regarding crop biofortification. Previous studies have shown that application of SEL treatments has led to an increase in the vitamin C content of strawberries by 25-42% (Lin et al., 2024; Lu et al., 2022) and tomatoes by 14-29% (Hu et al., 2023; Morais et al., 2024). In strawberries, Lu et al. (2022) showed that application of SEL led to increased activity of key enzymes in the ascorbate-glutathione cycle, including ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). The ascorbate-glutathione cycle is a key component of plant antioxidant defence pathways in which glutathione is required by DHAR to regenerate ascorbic acid from dehydroascorbic acid (Hasanuzzaman et al., 2019). A proposed mechanism by which SEL can positively impact vitamin C metabolism is through facilitating increased activity of Glutathione Peroxidase (GPX) – a reactive oxygen species (ROS) scavenger with a selenocysteine co-factor (Lu et al., 2022; Zhang et al., 2020b). The reduction of ROS by GPX utilises glutathione as a substrate, creating glutathione disulfide as a biproduct (Hasanuzzaman et al., 2019). Logically, increased GPX activity should lead to elevated levels of glutathione disulfide that must be reduced to glutathione again by GR. Increased activity of GR could then drive increased activity within the ascorbate-glutathione cycle, ultimately leading to increased accumulation of vitamin C (Lu et al., 2022).

SEL sprays have also been associated with increases in leaf chlorophyll content, which has been directly associated with enhanced photosynthetic efficiency and crop productivity (Hu et al., 2022; Silva et al., 2020; Yin et al., 2019). Additionally, SEL sprays have effectively increased the selenium content of edible crop tissues (Lin et al., 2024; Lu et al., 2022; Zhu et al., 2018). This is particularly relevant in the UK, where 50% of females and 26% of males have dietary selenium intakes below the lower recommended nutrient intake (Derbyshire, 2018). Thus, foliar selenium sprays could provide dual benefits to dietary vitamin C and selenium intakes in the UK population.

Iron nanoparticles (Fe-NPs) are another promising area of research for enhancing the productivity and nutritional quality of crops (Liu and Lal, 2015; Mgadi et al., 2024). The vitamin C content of strawberries infected with *Botrytis cinerea* was found to double in response to FE-NP treatments in a recent study (Mogazy et al., 2022). Similarly, the vitamin C content of Cherry tomatoes was shown to increase by 62% in response to FE-NPs under salt-stress conditions (Shahzad et al., 2024). Increases in vitamin C have been observed concurrently with increased activity in stress-response pathways that upregulate vitamin C production (Bidabadi et al., 2023; Shahzad et al., 2024). Under non-stressed conditions, Fe-NPs have yielded more modest increases, such as the 12% increase in the vitamin C content of oranges reported by El-Gioushy et al. (2021).

The mechanisms by which Fe-NP treatments drive increases in fruit vitamin C contents are not clearly understood. A commonly cited mechanism is related to a non-specific impact of metal nanoparticle application: in which the rapid influx of nanoparticles leads to the activation of stress response pathways, subsequently leading to an accumulation of ROS (Feng et al., 2022; Zoufan, Baroonian, and Zargar, 2020). The accumulated ROS must then be quenched by upregulation of antioxidant systems (Feng et al., 2022), including the ascorbate-glutathione cycle (Asthir, Kaur, and Kaur, 2020). This mode of action may also explain why nanoparticles have been documented to be particularly effective at reducing the impacts of environmental or biological stressors (Bidabadi et al., 2023; Shahzad et al., 2024). If the nanoparticle sprays cause further upregulation of defence pathways with minimal toxic effects, the treated plants may effectively be primed to tolerate the additional environmental or biological stressors to which they are exposed (Hossain et al., 2015; Rezayian, Niknam, and Arabloo, 2023). Under this proposed mode of action, it will be critical that the dosage of Fe-NP treatments is high enough to elicit a response in the plants antioxidant defence systems, but not so high as to create untenable oxidative stress.

Beyond their effects on vitamin C, Fe-NPs have been shown to enhance leaf chlorophyll content and photosynthetic efficiency (El-Desouky et al., 2021; Mozafari et al., 2018b; Shahzad et al., 2024), improve crop yields (Dola et al., 2022; Feng et al., 2022), and extend fruit shelf life (El-Gioushy et al., 2021). Fe-NPs have also been shown to increase the iron content of edible crop tissues (El-Gioushy et al., 2021; Mahmoud et al., 2022; Mogazy et al., 2022). Like selenium, iron deficiencies are also highly prevalent in the UK - particularly among UK adolescent and adult females (Derbyshire, 2018; Miller et al., 2016) - such that the adoption of Fe-NP foliar treatments could again offer additional nutritional benefits beyond enhanced vitamin C.

To the best of the authors' knowledge, the effects of SEL and Fe-NP treatments have not been studied in the context of UK commercial strawberry production. Given the promising agricultural and nutritional benefits reported in previous studies, investigating the application of SEL and Fe-NPs within UK strawberry production systems is of considerable scientific and practical interest.

5.1.1 Aims and Objectives

Chapter 5 addresses the Fourth aim of this thesis: **To evaluate the effects of previously tested foliar nutrient fertilisation strategies to enhance vitamin C accumulation in UK strawberries grown under standard commercial conditions.** Specifically, Chapter 5 addressed two key objectives:

1) To evaluate the effects of foliar Fe-NP and SEL treatments on the vitamin C content of two UK strawberry cultivars cultivated under standard commercial growing conditions.

2) To analyse additional nutritional (berry iron and selenium content), sensory (berry sweetness) and agronomic traits (chlorophyll fluorescence and yield) in response to the FE-NP and SEL treatments.

5.2 Methodology

5.2.1 Experimental Site

The experiment was conducted between June and August 2023 in a commercially standard tabletop polytunnel at Niab (United Kingdom – 51°17’09.6”N 0°27’10.8”E). The polytunnel contained six 18-meter rows that were oriented North to South and was fully covered with manually adjustable vents (Figure 5.1).

Figure 5.1 Tabletop polytunnel used for strawberry cultivation during foliar fertiliser experiments at Niab. Photograph taken on 16 July 2023.

5.2.2 Plant Material and Management

Grade A+ cold-stored bare-rooted strawberry plantlets of genotypes *Malling Centenary* and *Vibrant* were procured from R W Walpole Ltd (https://www.rwwalpole.co.uk/).

Genotype selection was informed by a preliminary genotype screen conducted between September 2020 and January 2021. The genotype screen analysed the vitamin C, total phenolic content, and total antioxidant capacity of historic strawberry samples collected as part of the study by Cockerton et al., 2021 (Figure 5.2). *Vibrant* was selected as the most nutrient-dense genotype in the preliminary screen. *Malling Centenary* was selected because of its popularity in the UK strawberry market (Chapter 4, Section 4.3.1). Both *Vibrant* and *Malling Centenary* are June bearing genotypes developed by the East Malling Strawberry Breeding Club. A key distinction between the two is that *Vibrant* was bred to crop very early whereas *Malling Centenary* was bred to produce a heavy, main season crop. *Vibrant* would be expected to crop around 1 week earlier than *Malling Centenary*, which must be considered when comparing the primary fruit of each variety.

The cold-stored plants were received on 24 April 2023 and held at -2 °C until being transplanted on 01 June 2023. Plants were cultivated in coir grow bags at a density of 6 plants/m2 (Cocogreen, Manchester, UK. Berryfusion Growbags, White, 6-hole).

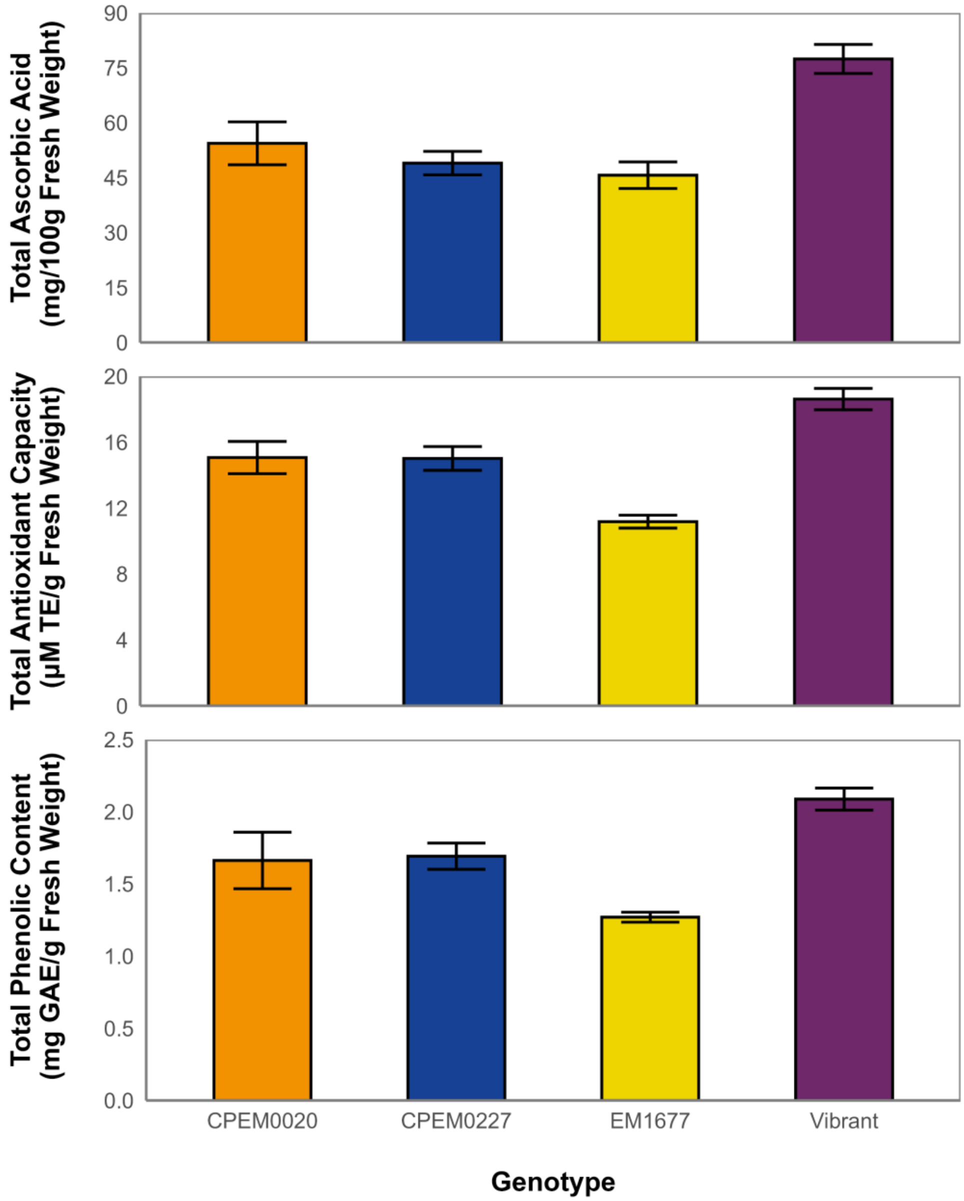


Figure 5.2 Bar graphs illustrating selected nutritional components of four strawberry genotypes from the East Malling Strawberry Breeding Club programme. Top panel - vitamin C content, measured as total ascorbic acid. Middle panel - total antioxidant capacity, measured as TROLOX Equivalent (TE) antioxidant capacity. Bottom panel – total phenolic content measured as Gallic Acid Equivalents (GAE). All values are presented as the means ± standard error.

Fertigation was supplied through an automated drip irrigation system. Each coir bag was supplied by seven drippers. The drip irrigation system was connected to three tanks of fertiliser containing macronutrients, micronutrients, and nitric acid (for pH adjustment). The full feed recipe is detailed in Table 5.1.

The applied dosage from each tank was controlled by separate Dosatrons (Dosatron, Tresses, FR. DG3L2). By default, the Dosatrons diluted the nutrient solutions to 1% with tap water. Further dosage adjustments were informed by twice-weekly measurements of coir electrical conductivity (EC; Delta-T, Cambridge, UK. WET KIT and WET-2 Sensor), and run-off pH and EC (MyronL Company, CA, USA. Ultrameter II). The dosages for tanks A and B were adjusted between 1% and 0.65%, as necessary, to maintain a coir and run-off EC of between 150-200 mS/m. The dosage for tank C was adjusted between 1% and 1.9%, as necessary, to achieve a run-off pH of 5.5-6.0.

Irrigation events occurred every two hours between 6 am and 8 pm. The duration of each irrigation event was adjusted throughout the experiment to maintain coir moisture content (CM) between 50-60%. The duration of irrigation events varied between 90 and 150 seconds depending on CM and weather conditions. The fertigation regime was provided by Niab's agronomist and was representative of a standard commercial fertigation regime used for growing *Malling Centenary* plants.

Plants were routinely sprayed once a week with fungicide to mitigate the onset and spread of powdery mildew. An additional insecticide spray treatment was applied to all plants on 14 June 2023 to control an aphid outbreak. Routinely applied bio-controls included predators for thrips (Koppert, Suffolk, UK. Thripex-V), spider mites (Koppert, Spical), and aphids (Koppert, Aphiscout). Predators were applied following the supplier's instructions (Koppert). Beehives (Koppert, Natupol Beehive) were placed in the tunnel to provide pollination services as the first flowers began to open. Hives were kept in the tunnel for the full duration of the cropping period between June and August 2023.

Table 5.1 Nutrient feed recipes applied during the strawberries' vegetative and fruiting growth stages.

Tank	Chemical	Vegetative Feed (g/25 L)	Fruiting Feed (g/25 L)
Tank A			
	Potassium Nitrate	662.75	916.50
	Calcium Nitrate	1716.00	1450.00
	Ammonium Nitrate	100.00	0.00
Tank B			
	Monopotassium Phosphate	423.50	423.50
	Magnesium Nitrate	728.25	541.75
	Magnesium Sulphate	292.75	216.75
	Manganese Sulphate	10.75	8.00
	Solubor	1.50	1.50
	Copper Sulphate	0.25	0.25
	Zinc Sulphate	5.00	3.00
	Sodium Molybdate	0.25	0.25
	Iron EDTA	48.75	35.75
Tank C			
	Nitric Acid	0.300	0.300

5.2.3 Preparation of Foliar Treatment Solutions

5.2.3.1 Iron Oxide Nanoparticles

The Fe-NP treatment comprised a spray solution containing 200 mg/L Fe_3O_4 nanopowder (Sigma-Aldrich Co, Gillingham, UK. Iron (II/III) oxide nanopowder, particle size 50-100nm, CAS: 1317-61-9). The concentration of the Fe-NP spray solution was selected based on the findings of Mogazy et al. (2022), who found that foliar Fe-NP treatments had a greater positive effect on strawberry vitamin C content at a concentration of 200 parts per million (ppm) compared to 100 ppm.

Several methods were tested to solubilise the Fe_3O_4 nanopowder without success. Moradbeygi et al. (2020) described the dissolution of Fe_2O_3 nanoparticles in deionised water by reducing the pH of the solution 1 mM Hydrochloric Acid (Sigma-Aldrich Co. Hydrochloric acid (HCL) 37% ACS reagent, CAS: 7647-01-0). In the current study, HCL was incrementally added to a solution of 200 ppm Fe-NP in de-ionised water. After each addition of HCL, the solution was sonicated at 40 KHz for 1 hour and mechanically shaken for 1 hour. No dissolution of the Fe_3O_4 nanoparticles was observed between pH 6.89 to 3.98. Subsequently, fresh Fe-NPs were added directly to undiluted HCL (pH 2.76), sonicated for 1 hour at 40 KHz and mechanically shaken for 1 hour. Again, no dissolution of the Fe_3O_4 nanoparticles was observed.

A further test followed the methods of Dola et al. (2022), in which Fe_3O_4 nanoparticles were stirred continuously at 1200 RPM on a hot plate at 60 °C for 16 hours. The solution was then sonicated at 40 KHz for 1 hour. Again, the Fe_3O_4 nanoparticles did not dissolve. That said, Dola et al. (2022) did not state that their nanoparticles were dissolved, but rather that they were mixed homogeneously in solution.

Even without heat application or pH adjustment, the combination of sonication and mechanical mixing was observed to be sufficient to homogeneously disperse the Fe-NPs in solution. The distinction between dispersion and dissolution of nanoparticles has been noted in the methods of other similar studies (Dola et al., 2022; Lian et al., 2022). As such, the stock Fe-NP spray solution used during the current study was prepared by adding 2000 mg Fe_3O_4 nanopowder to 1 L de-ionised water. The solution was then sonicated at 40 KHz for 1 hour and mixed by mechanical shaking for 1 hour at room temperature. The stock solution was made 10x more concentrated than the desired treatment after consulting the technical farm team regarding how spray treatments are normally prepared. Preparing 10x concentrated stocks therefore enabled consistency with normal farm protocols administered by experienced technical staff.

5.2.3.2 Sodium Selenate

The SEL treatment comprised a spray solution containing 30 mg/L Na_2SeO_4 (Sigma-Aldrich Co. Sodium selenate, CAS:13410-01). Similarly to the FE-NP treatment, the concentration of the SEL spray was selected based on the findings of earlier studies (Lu et al., 2022). Lu et al. (2022) found that the application of foliar Na_2SeO_3 had a greater positive effect on strawberry vitamin C content at a concentration of 30 mg/L, compared to 10 mg/L and 60 mg/L. The stock SEL solution was prepared by dissolving 300 mg Na_2SeO_4 in 1 L de-ionised water to create a 10x concentrated solution, followed by mechanical shaking for 1 hour at room temperature.

5.2.4 Experimental Design and Foliar Treatments

The experiment followed a completely randomised block design across the central two rows of the polytunnel (Figure 5.3). The experimental design consisted of five blocks with each block containing one experimental replicate. Experimental replicates were defined as the central four plants within a coir bag filled with 6 plants for each genotype x treatment combination. The two end plants in each coir bag acted as guard plants between treatments and were not sampled from for chemical analysis. The western and eastern edges of the experiment were both flanked with a row of guard plants (Figure 5.3). The guard plants were implemented into the experimental design to control for potential edge effects within each block and within the tunnel. The relative positions of each treatment and replicate were randomised within each block.

The experiment consisted of three treatment groups: FE-NP (200 mg/L Fe_3O_4 nanopowder), SEL (30 mg/L Na_2SeO_4), and Control (No foliar fertiliser treatment). The experimental design utilised a negative control group but did not include a positive control group – i.e. the application of an inert spray to verify that the physical application of the spray was not the cause of any treatment effects. As described in section 5.2.2, all plants were sprayed with fungicides on a weekly basis and this was deemed a suitable positive control measure. The treatments were applied once at the early fruiting stage on 19 June 2023. It was decided that one treatment application would be sufficient given the ~4-week cropping period of *Vibrant* and *Malling Centenary* and the relatively high concentrations of the Fe-NP and SEL solutions.

The stock Fe-NP and SEL solutions were prepared on the day of spray application and were diluted 1:10 with tap water before spraying. The Fe-NP solution was vigorously shaken before and after dilution with tap water to maintain homogeneous dispersion of the nanoparticles. Foliar-treated plants were sprayed with a hand-held electric sprayer until a visible film covered the leaves and excess spray began to drip off the canopy. Perspex shields were used to prevent over-spray onto adjacent plants. The Control treatment group did not receive any foliar spray treatment.

After the foliar treatments were applied to each experimental replicate, 150ml of the spray solution was sprayed into a plastic beaker. The Fe-NP and SEL spray samples were then sent to Yara Lancrop Laboratories (Lancrop Laboratories, York, UK) to analyse the iron and selenium content of the administered sprays. Analysing the iron and selenium content of the applied sprays is not common practice in similar studies (Lu et al., 2021; Mogazy et al., 2022) but was deemed important to verify that the applied dosages were consistent with the targeted dosages.

The mean selenium content of the applied SEL spray was 0.57 ± 0.01 mg/L. Selenium comprises 41% of the molecular weight of Na_2SeO_4, meaning that the mean concentration of Na_2SeO_4 in the analysed samples was approximately 1.39 mg/L. The approximate concentration of the spray was, therefore, 22-fold less concentrated than the targeted concentration of 30 mg/L.

The iron content of the applied Fe-NP spray was measured between 0.1 and 6.8 mg/L (1.66 ± 1.30 mg/L). Iron comprises 72% of the molecular weight of Fe_3O_4, meaning that the mean concentration of Fe_3O_4 in the analysed samples was approximately 2.31 mg/L. The spray concentration was, therefore, approximately 87-fold less concentrated than the targeted concentration of 200 mg/L.

The conversion of measured iron and selenium to Fe_3O_4 and Na_2SeO_4 using molecular weights only provides an estimate and likely does not represent the true concentrations in the water samples. While issues may have occurred during the dilution of the stock spray solutions with tap water, the sprays were prepared by professional technicians under supervision and is unlikely to account for the significant discrepancies between expected and measured concentrations. Another possibility for the Fe-NP sprays is that incomplete dissolution may have led to particles getting stuck in the sprayer nozzle, reducing the applied concentration. Additionally, as the water analysis was outsourced to a commercial lab, it is unclear whether the samples were mixed thoroughly prior to analysis (see Section 5.2.3.1 regarding the importance of homogenous mixing of the Fe_3O_4 nanoparticles). Thus, improper sample preparation could have contributed to the lower-than-expected iron concentrations.

The results of the water analysis were not seen until after the experiment had concluded. As such, no adjustments were made to the experimental design or additional treatment applications during the experiment. The spray concentrations have not been verified but should be considered when interpreting the effects of the applied treatments described in subsequent sections of Chapter 5. In future, it would be prudent to also analyse samples of the tap water as a positive control, such that comparisons can be made to the mineral contents of the treatment sprays.

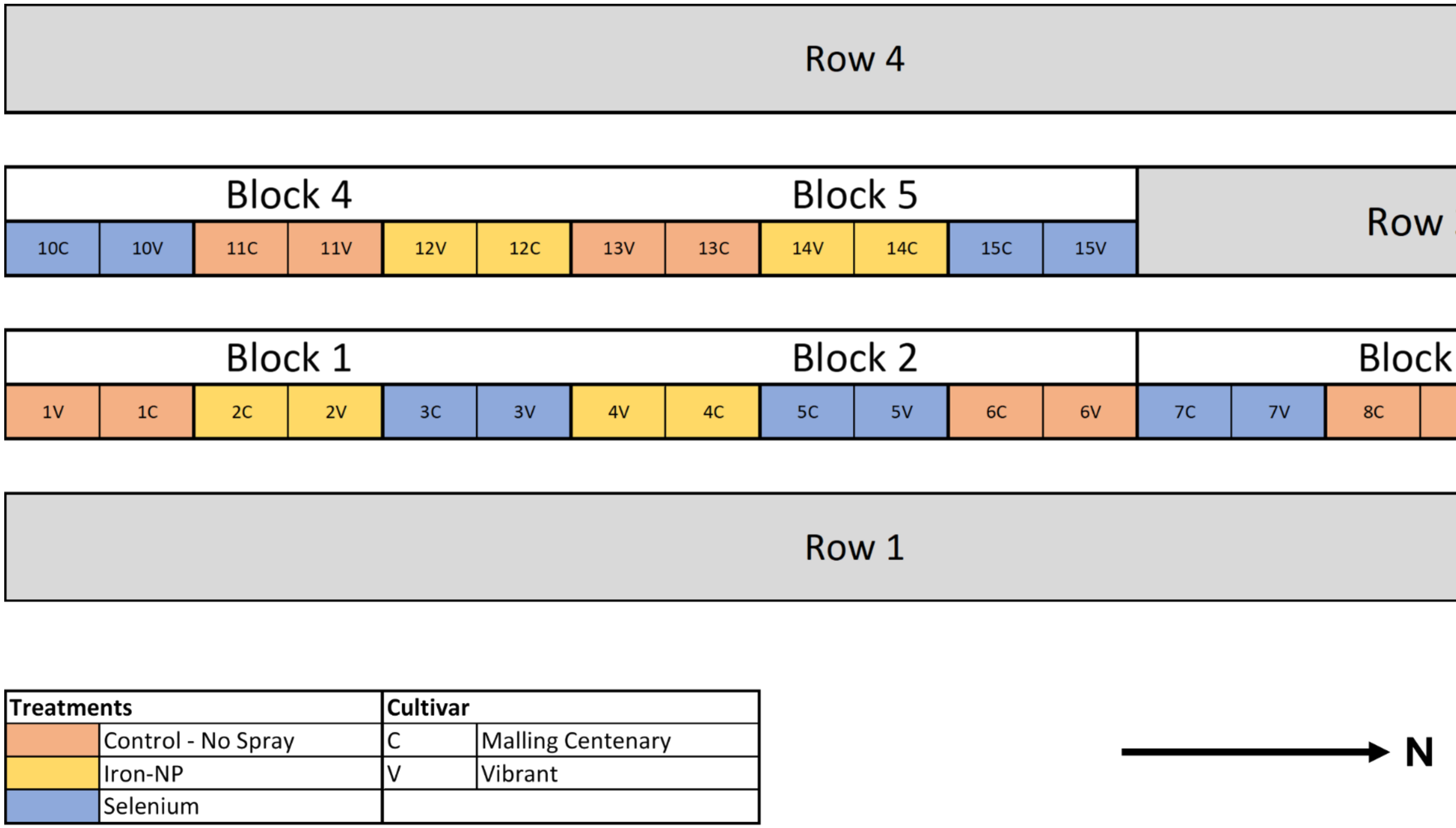


Figure 5.3 Diagrammatic representation of the experimental set-up and randomised position of two strawberry genotypes treated with foliar-applied iron nanoparticles (Fe-NP), sodium selenate (SEL), or no foliar treatment (Control). Grey portions of the rows indicate areas that were occupied by guard plants.

5.2.5 Sample Collection

Fully matured berries (uniform red colouration across >80% of the berry surface) were picked two times per week for the full cropping period. Cropping occurred from 03 July - 03 August 2023. Berries harvested from each experimental replicate were classified as Class I (>28mm diameter and uniform conical shape), Class II (22-28mm diameter and/or minor misshaping) or waste (>22mm diameter and/or misshapen or spoiled). The number of berries in each Class was counted and the combined weight from all three classes was recorded. Yield measurements include berries harvested from primary, secondary, and tertiary trusses that occurred sequentially throughout the cropping period.

Berries were sampled for chemical analyses on 03, 06, 10 and 13 July 2023 – 14 to 24 days after the spray treatments were applied. Sampling was conducted on these specific dates because they coincided with the peak yields for both *Malling Centenary* and *Vibrant* (Figure 5.13). The samples consisted of approximately 100g of Class I berries from the plant's primary trusses, pooled from all six plants in each experimental replicate. If the Class I yield was lower than 100g, samples consisted of all available Class I berries. The sampled berries were washed in de-ionised water to remove any spray residue and hulled before weighing. The mean sample weight over the four sampling dates was 82.59 ± 2.48 g, equating to a mean of 4.85 ± 0.14 berries per sample. Additional berries that were not sampled for chemical analysis were set aside for destructive analysis of total soluble solids (TSS; see section 5.2.6).

The samples for chemical analysis were then flash-frozen in liquid nitrogen, as previously described in Chapter 4 (Section 4.2.1). Sub-samples were taken from the flash-frozen material before further processing due to limited freeze-drier capacity at the time of sample collection. The flash-frozen sub-samples had a mean weight of 61.54 ± 2.42 g. The sub-samples were then freeze-dried and milled into a fine powder, as previously described in Chapter 4 (Section 4.2.1).

The processed samples from 03 and 06 July were combined to create a composite sample for the week commencing (w/c) 03 July 2023. Likewise, the processed samples of berries harvested on 10 and 13 July were combined to create a composite sample for the w/c 10 July 2023. The purpose of the composite samples was to ensure that there was sufficient and representative material from each experimental replicate to investigate differences between the genotypes and experimental treatments.

Ensuring that each replicate had sufficient sample material came at the expense of information regarding inter-day variability. Maintaining two composite samples per replicate did, however, retain the ability to analyse inter-week variability. Analysing samples from multiple weeks was considered important to account for variable weather conditions during the experiment (Figure 5.4). Furthermore, sampling across multiple weeks would capture any potential diminishing effects of the foliar treatments over time, which was an important consideration given that the treatments were only applied once.

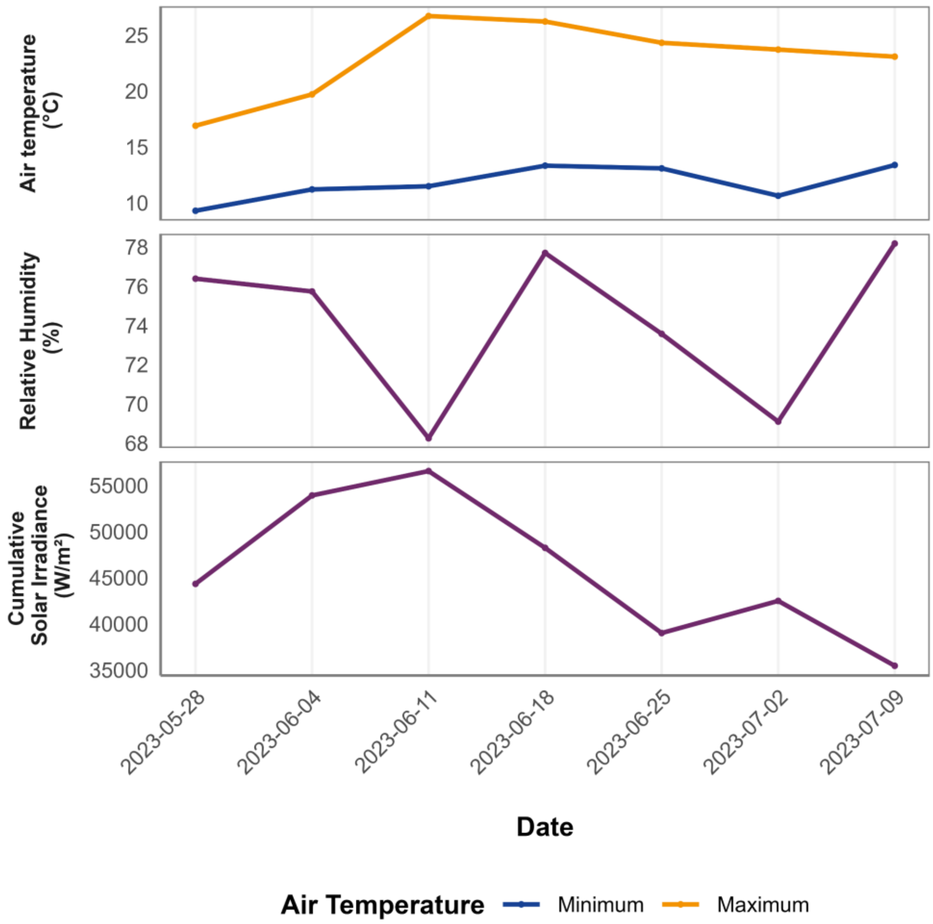


Figure 5.4 Line graphs illustrating weather conditions during the experimental period between 28 May and 15 July 2023. Values for air temperature (top panel) and relative humidity (middle panel) are the mean values recorded during the week commencing on each plotted date. Values for cumulative solar irradiance (bottom panel) are the summation of all measured solar irradiance in the week commencing on each plotted date.

5.2.6 Chemical Analyses

The vitamin C content of the berries was measured as AsA. The extraction and quantitation of AsA, Malic acid, and Citric acid, followed the same procedures described in Chapter 4 (Section 4.2.2.1). Values for AsA were presented as mg/100g fresh weight (FW). Malic and Citric acid values were presented as mg/g FW. As described in Chapter 4 (Section 4.2.2.2), Malic and Citric acid values were also used to calculate the theoretical titratable acidities (TTA) for each sample. TTA calculations followed the formula derived from the work of Manabe (1993) and Ikegaya (2024) ($TTA = (citric * 10^{-1} + malic * 10^{-1})/(6.4/6.71)$).

The remaining composite sample material from the w/c 03 July 2023 was sent to Yara Lancrop Laboratories (Lancrop Laboratories, York, UK) for iron and selenium analysis. Analysis of the sample composites from the w/c 03 July was prioritised as these samples were collected closer to the date of treatment application than the composite samples from the w/c 10 July. As such, it was reasoned that any treatment effects would be more easily detected in the composite samples from the w/c 03 July. Iron and selenium were quantified by Inductively Coupled Plasma Mass Spectrometry and the measured values were presented as ppm.

TSS was measured using fresh berries on the day of harvest by the same methods described in Chapter 4 (Section 4.2.2.2). Briefly, five berries of mixed Class were randomly sampled from each replicate and squeezed by hand to extract the juices into a single plastic cup. The TSS of the composite juice was measured in triplicate using an ATAGO digital refractometer (ATAGO Co. Ltd, Tokyo, Japan). Values for berry TSS were expressed as °Brix (%).

The ratio of TSS/TTA was then calculated as a measure of overall berry sweetness. The balance of sugars to acids in strawberries is a key component of the sensory experience and has been previously shown to be a strong determinant of consumer preference (Ikegaya et al., 2021). The calculation of the TSS/TTA ratio was consistent with the analyses conducted in Chapter 4 (Section 4.2.2.2).

5.2.7 Non-destructive Leaf Analysis

Leaf chlorophyll fluorescence (CHL) and epidermal flavanols (FLAV) were measured using a Dualex leaf-clip meter (Dualex Scientific, Force A, France). The ratio of CHL to FLAV, referred to as the Nitrogen Balance Index (NBI), was calculated from the collected data. Whilst CHL was the primary parameter of interest in the current study, NBI data were also presented as they provide an index of general plant health, growth and nitrogen status (Fan et al., 2022).

Dualex measurements were taken on four occasions between 28 June and 13 July 2023. Measurements were taken during this period so that the data were aligned with the peak cropping periods and collection of berry samples (Figure 5.13; Section 5.2.5). Each recorded observation was the combination of two measurements taken from a single mature leaf: one from the adaxial surface and one from the abaxial surface. At each time point, measurements were taken from four mature leaves from each experimental replicate. The CHL and NBI at each time point are presented as the mean ± standard error (SE).

5.2.8 Statistical Analyses

The individual and interacting effects of genotype, foliar treatment, and harvest date on measured variables were analysed by Analysis of Variance (ANOVA). Data residuals were visually inspected using histograms and quantile-quantile plots to assess conformity to the ANOVA assumptions of normality and homogeneity. The normality and homogeneity assumptions were further confirmed by Shapiro-Wilk and Levene's tests, respectively. Measured variables with non-normal distributions were presented as means ± SE only. Post-hoc pair-wise comparisons of group means were conducted using Tukey's Honestly Significant Differences (HSD) test. The resulting p-values were adjusted for multiple testing and statistically significant differences were accepted at an alpha threshold of 0.05.

All statistical analyses and visualisations were conducted in R (R Core Team, 2024). Statistical analyses were conducted using the {stats} package. Visualisations were produced using the {*ggplot2*} (Wickham, 2016), and {*ggsci*} (Xiao, 2024) R packages.

5.3 Results

5.3.1 Vitamin C, Malic, and Citric Acid Contents

Plants of strawberry genotypes *Malling Centenary* and *Vibrant* were treated with either foliar Fe-NP or SEL to investigate their effects on berry vitamin C – measured as AsA – and organic acid contents.

Having accounted for potential blocking effects, there were no statistically significant two- or three-way interactions between genotype, foliar treatment, and sampling date with respect to berry AsA. There were, however, differences in the measured AsA values between genotypes (F(1,44) = [61.037], p = 7.510e-10) and sampling dates (F(1,44) = [36.224], p = 3.170e-07). The mean AsA content of *Vibrant* berries was 83.03 ± 1.65 mg/100g FW, which was greater than the mean AsA content of *Malling Centenary* at 67.14 ± 2.03 mg/100g FW (Figure 5.5). *Vibrant* and *Malling Centenary* berries had higher AsA content in the w/c 10 July (81.21 ± 2.36) when compared to berries sampled in w/c 03 July (68.96 ± 1.74 mg/100g FW). AsA remained consistent in both genotypes across each of the foliar treatment groups at both sampling dates (F(1,44) = [36.224], p = 0.308).

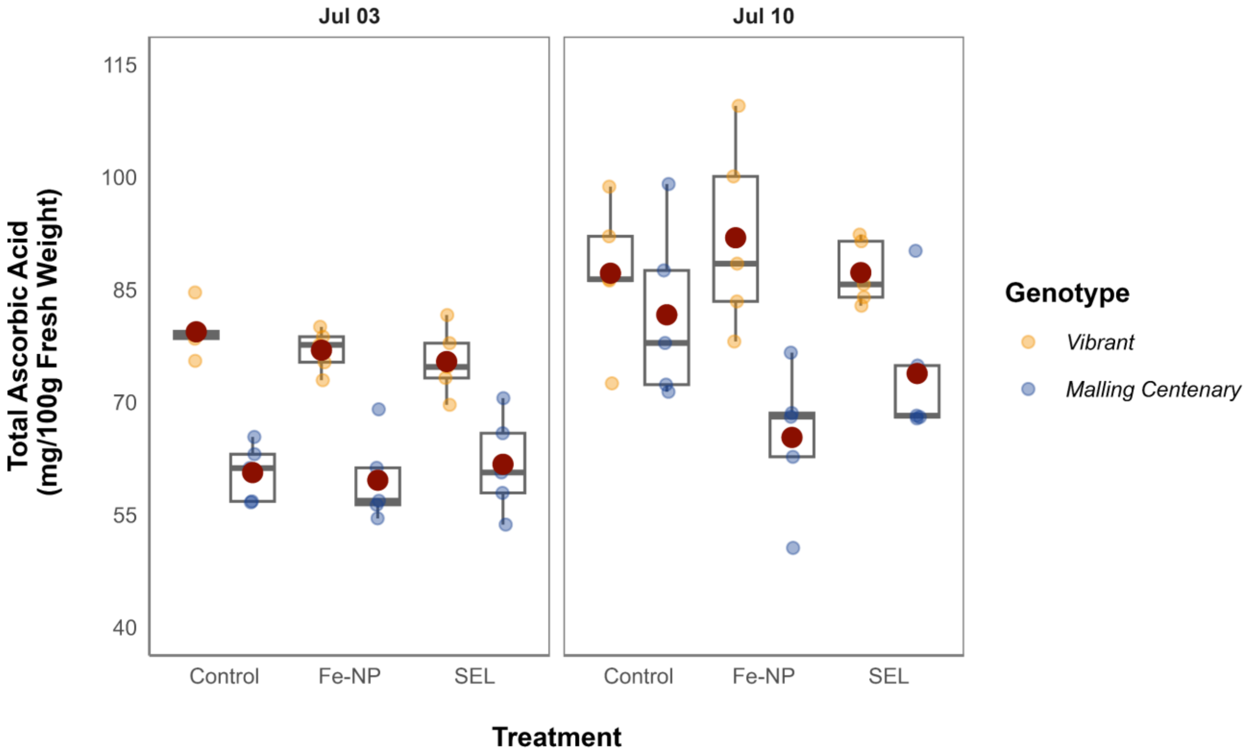


Figure 5.5 Box-plots illustrating the vitamin C content of strawberries from two genotypes treated with foliar iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). Berries were analysed from two sampling dates in the weeks commencing 03 July 2023 (left panel) and 10 July 2023 (right panel). All plants were cultivated in a commercially standard polytunnel between 01 June and 03 August 2023. Vitamin C content is expressed as total ascorbic acid. The mean ascorbic acid content for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for total ascorbic acid. The distance between the first and third quartiles illustrates the interquartile range within the data.

Malic acid contents were also higher in *Vibrant* berries (2.61 ± 0.07 mg/g FW) when compared to *Malling Centenary* (2.00 ± 0.07 mg/g FW; [F(1,44) = [72.421], p = 7.560e-11]). Conversely to AsA, however, malic acid contents were higher in the berries sampled in the w/c 03 July (2.52 ± 0.08 mg/g FW) when compared to berries sampled in the w/c 10 July (2.08 ± 0.07 mg/g FW; [F(1,44) = [36.224], p = 1.820e-07]; Figure 5.6). There were, again, no observable differences between the foliar treatment groups (F(1,44) = [36.224], p = 0.104) and no observable interactions between any combination of the three experimental variables.

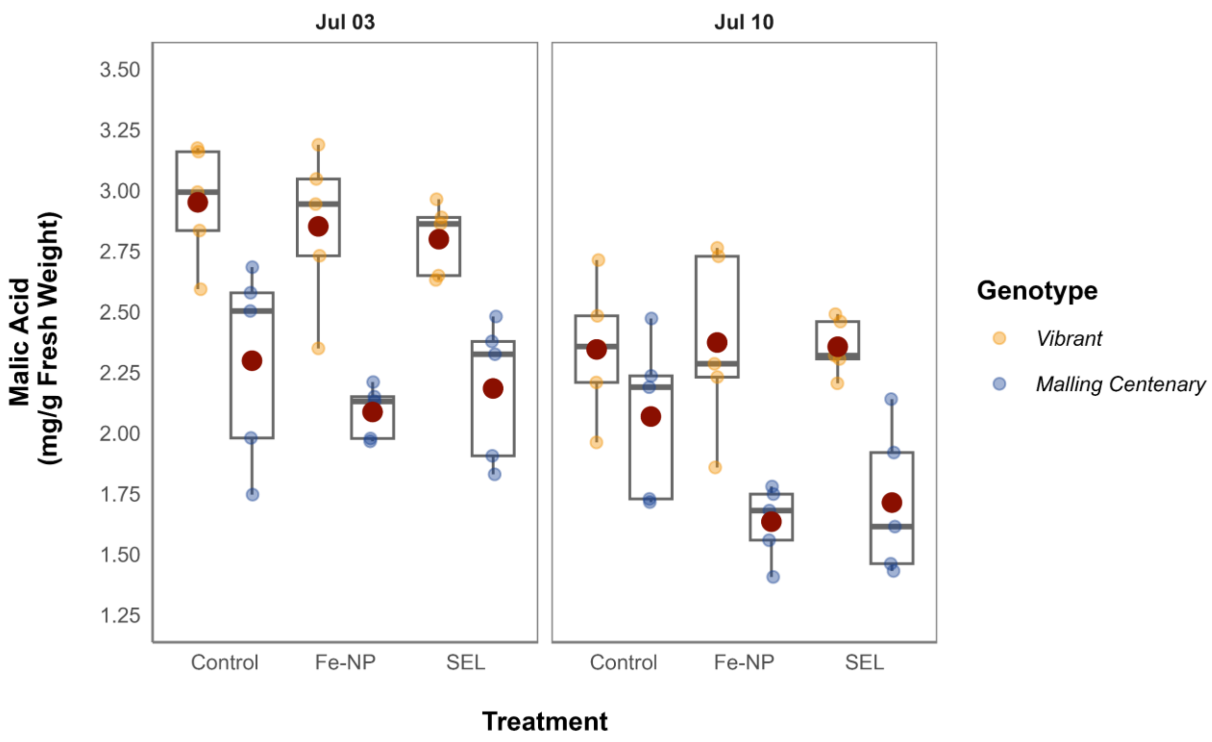


Figure 5.6 Box-plots illustrating the malic acid content of strawberries from two genotypes treated with foliar iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). Berries were analysed from two sampling dates in the weeks commencing 03 July 2023 (left panel) and 10 July 2023 (right panel). All plants were cultivated in a commercially standard polytunnel between 01 June and 03 August 2023. The mean malic acid content for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for malic acid content. The distance between the first and third quartiles illustrates the interquartile range within the data.

Citric acid proved to be an exception in that the foliar treatments had a statistically significant effect on berry citric acid content (Figure 5.7). A two-way interaction between genotype and foliar treatment was observed with respect to berry citric acid content ($F(2,44) = [4.836]$, p = 0.013).The citric acid content in *Malling Centenary* berries was found to be lower in the Fe-NP treatment group (7.78 ± 0.24 mg/g FW) relative to the Control (9.08 ± 0.47 mg/g FW) and SEL treatment groups (8.80 ± 0.39 mg/g FW). The citric acid content in *Vibrant* berries, on the other hand, was observed to remain consistent across all three treatment groups (Control: 10.04 ± 0.23 mg/g FW, SEL: 9.79 ± 0.23 mg/g FW, Fe-NP: 10.38 ± 0.44 mg/g FW).

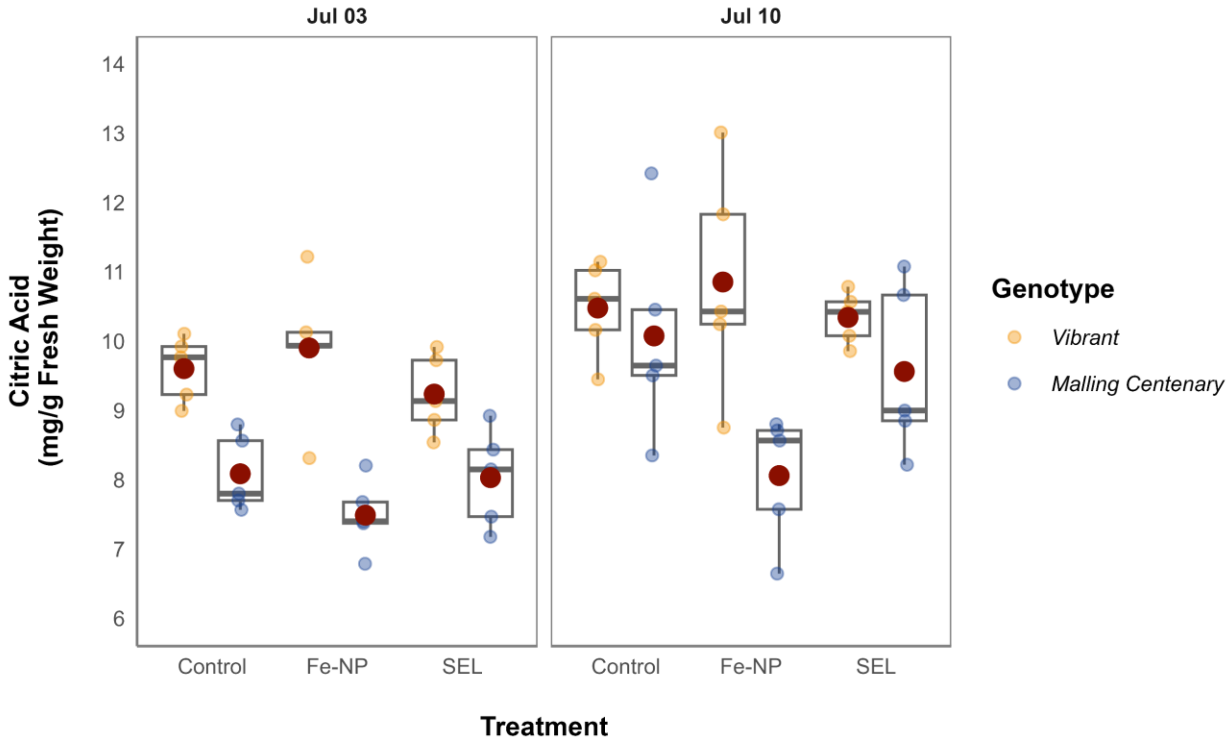


Figure 5.7 Box-plots illustrating the citric acid content of strawberries from two genotypes treated with foliar iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). Berries were analysed from two sampling dates in the weeks commencing 03 July 2023 (left panel) and 10 July 2023 (right panel). All plants were cultivated in a commercially standard polytunnel between 01 June and 03 August 2023. The mean citric acid content for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for citric acid content. The distance between the first and third quartiles illustrates the interquartile range within the data.

5.3.2 Berry Iron and Selenium Contents

Further investigations were conducted concerning the iron and selenium content of the berries sampled in w/c 03 July 2023. Iron and selenium were analysed under the hypothesis that foliar application of an FE-NP or SEL fertiliser would have a direct effect on the iron or selenium content of the berries.

Two-way ANOVA indicated that there was no statistically significant interaction between genotype and foliar treatment with respect to iron content ($F(2,20) = [2.208]$, $p = 0.136$). Foliar treatment was found to exert an independent main effect on iron content ($F(2,20) = [3.675]$, $p = 0.044$).

Iron content was found to be greatest in the Control treatment group (28.60 ± 1.78 ppm) when compared to the Fe-NP (24.4 ± 1.09 ppm) and SEL (24.1 ± 0.81 ppm) treatment groups. The greatest mean iron content was observed in *Malling Centenary* berries from the Control treatment group (31.20 ± 2.73 ppm) and the lowest in the *Malling Centenary* berries from the FE-NP treatment group (23.40 ± 2.08 ppm). All other iron values ranged between 23.60 ± 0.87 ppm and 26.00 ± 1.84 ppm (Figure 5.8).

Genotype was not found to have a statistically significant independent effect on berry iron content ($F(1,20) = [0.234]$, $p = 0.634$).

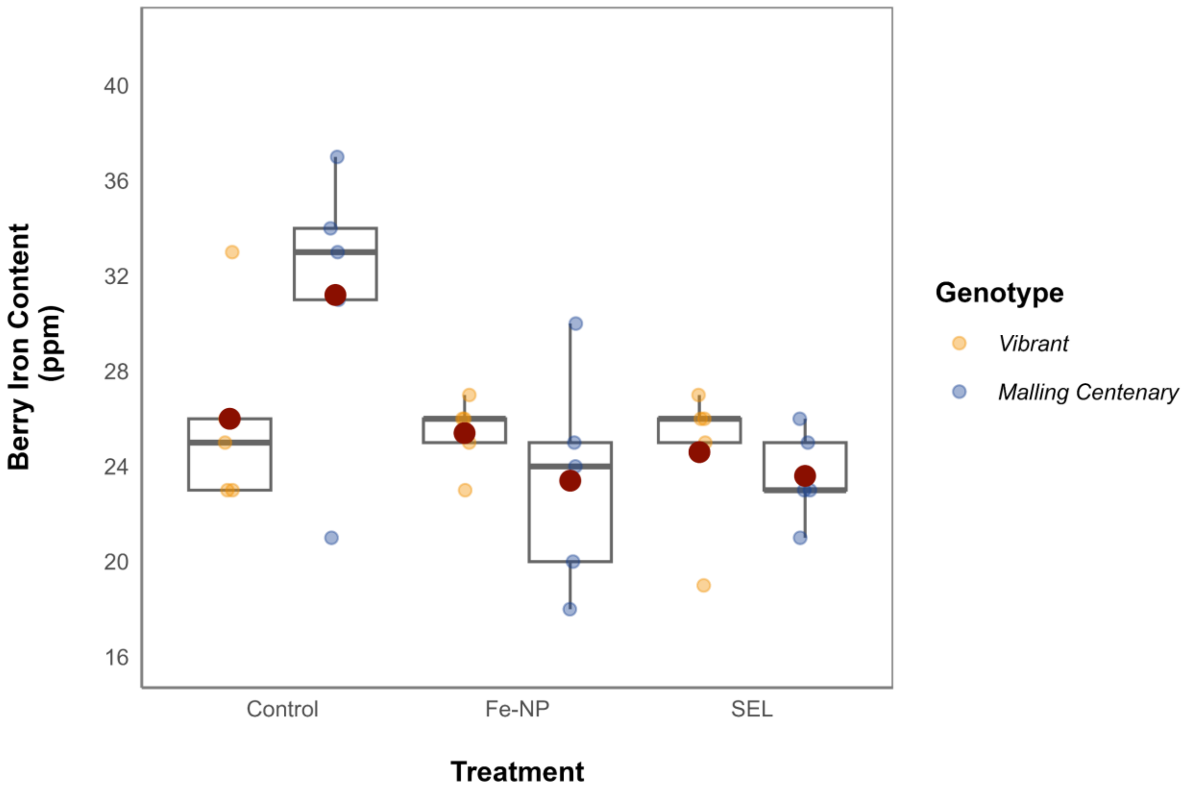


Figure 5.8 Box-plots illustrating the iron content of strawberries from two genotypes treated with foliar iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). All plants were cultivated in a commercially standard polytunnel between 01 June and 03 August 2023. The mean iron content for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for iron content. The distance between the first and third quartiles illustrates the interquartile range within the data.

The selenium data were found to not meet the assumptions of the ANOVA procedures. The highest selenium contents were observed in the SEL foliar treatment group (0.08 ± 0.01 ppm) when compared to the Control (0.04 ± 0.00 ppm) and Fe-NP (0.04 ± 0.01 ppm) treatment groups (Figure 5.8). The increase in selenium content under the SEL treatment was consistent for both *Vibrant* and *Malling Centenary* (Figure 5.9).

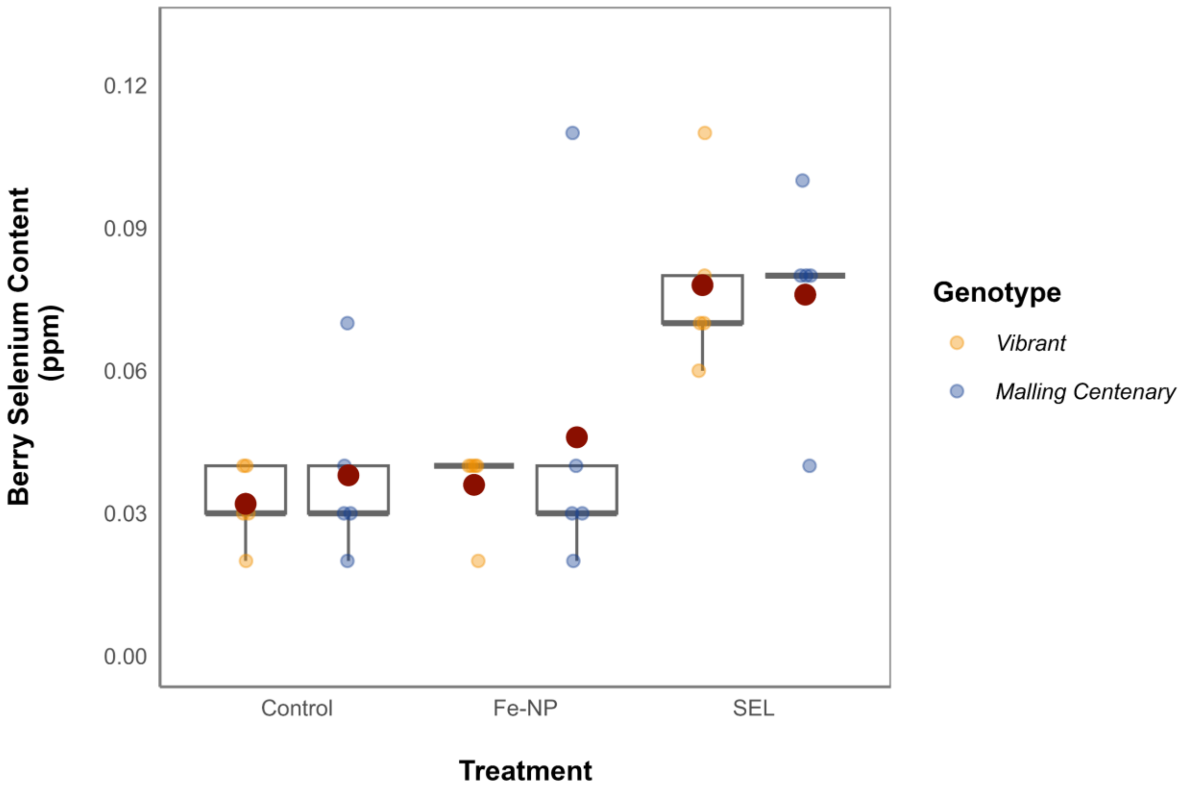


Figure 5.9 Box-plots illustrating the selenium content of strawberries from two genotypes treated with foliar-applied iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). All plants were cultivated in a commercially standard polytunnel between 01 June and 03 August 2023. The mean selenium content for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for selenium content. The distance between the first and third quartiles illustrates the interquartile range within the data.

5.3.3 Overall Berry Sweetness

The effects of genotype and foliar treatment on implied strawberry sensory qualities were considered in the context of berry TSS and the ratio between TSS/TTA. Analysis revealed a statistically significant two-way interaction between genotype and sampling date with respect to TSS (F(2,40) = [16.279], p = 2.390e-04). Independent main effects of genotype (F(1,40) = [6.032], p = 0.018) and sampling date (F(1,40) = [58.622], p = 2.320e-09) were also observed, having accounted for the effects of the other modelled factors and their interactions.

TSS was greater in *Vibrant* berries sampled in w/c 10 July (8.41 ± 0.16 °Brix) compared to *Vibrant* berries sampled in w/c 03 July (6.35 ± 0.17 °Brix; p = 0.000). In contrast, TSS in *Malling Centenary* was consistent across both sampling dates (03 July - 7.48 ± 0.22 °Brix; 10 July - 8.07 ± 0.16 °Brix; *p* = 0.138; Figure 5.10).

TSS was greater in *Malling Centenary* (7.83 ± 0.15 °Brix) than in *Vibrant* (7.39 ± 0.22 °Brix), and greater in w/c 10 July (8.24 ± 0.12 °Brix) compared to w/c 03 July (6.83 ± 0.17 °Brix; Figure 5.10).

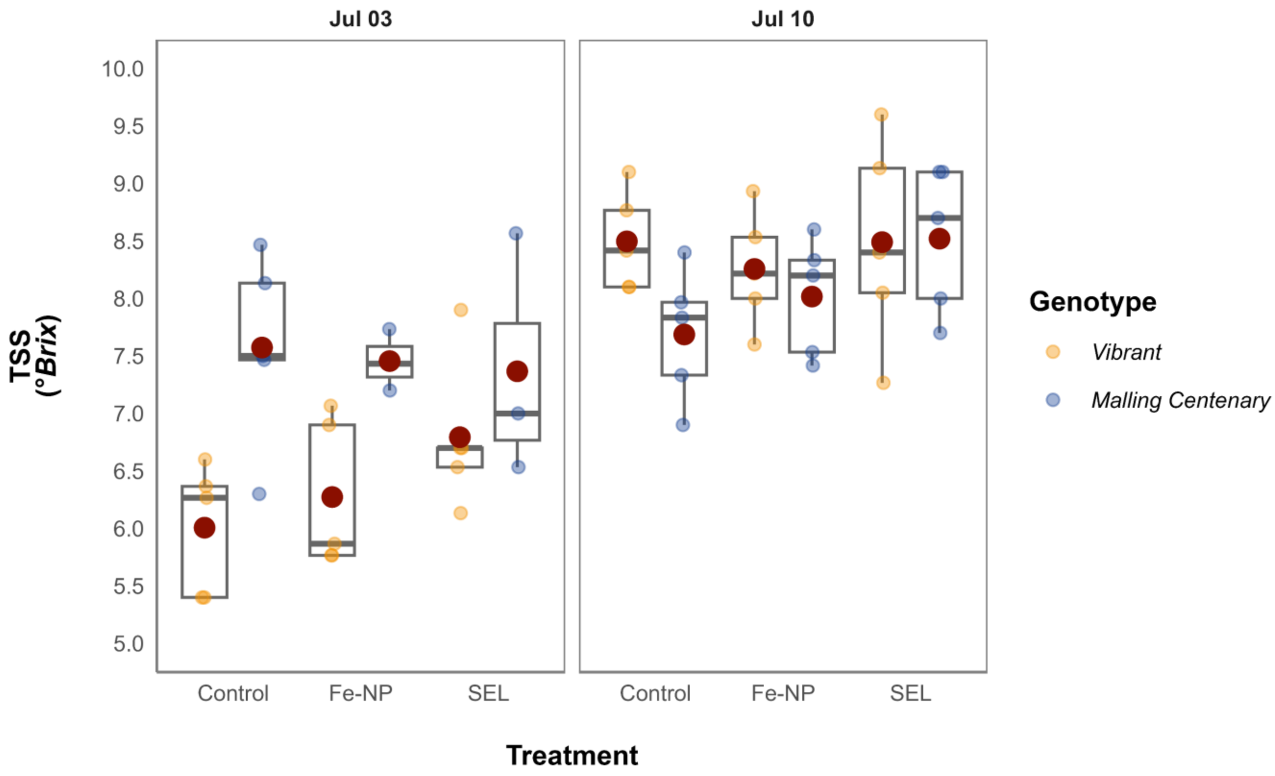


Figure 5.10 Box-plots illustrating differences in the total soluble solids (TSS) content of strawberries from two genotypes treated with foliar-applied iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). Berries were analysed from two sampling dates in the weeks commencing 03 July 2023 (left panel) and 10 July 2023 (right panel). All plants were cultivated in a commercially standard polytunnel between 01 June and 03 August 2023. The mean TSS content for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for TSS. The distance between the first and third quartiles illustrates the interquartile range within the data.

Overall berry sweetness was found to be affected by statistically significant interactions between genotype and treatment ($F(2,40) = [3.295]$, $p = 0.047$) and between genotype and sampling date ($F(2,40) = [11.397]$, $p = 0.002$).

The observed two-way interactions were underpinned by genotype-dependent responses to either foliar treatment or sampling date. There were no observed differences in the overall sweetness of *Vibrant* berries between the different foliar treatments ($p > 0.75$; Figure 5.11). *Malling Centenary* berries, however, had greater sweetness values in the Fe-NP treatment group compared to the Control group (FE-NP - 7.83 ± 0.22; Control - 6.57 ± 0.35; $p = 0.035$).

Concerning sampling date, *Vibrant* berries were observed to be sweeter in w/c 10 July compared to w/c 03 July (03 July - 4.90 ± 0.17; 10 July - 6.27 ± 0.20; p = 0.000), whereas *Malling Centenary* berries were of comparable sweetness at both sampling dates (03 July - 7.13 ± 0.26; 10 July - 7.12 ± 0.30; $p = 0.995$; Figure 5.11).

Independent main effects were observed for genotype ($F(1,40) = [51.754]$, $p = 1.010e-08$), and sampling date ($F(1,40) = [10.963]$, $p = 0.002$) having accounted for the effects of experimental blocking and foliar treatments. Berry sweetness was greater in *Malling Centenary* than in *Vibrant* (*Malling Centenary* - 7.12 ± 0.20; *Vibrant* - 5.58 ± 0.18), and greater in w/c 10 July compared to w/c 03 July (03 July - 5.84 ± 0.26; 10 July - 6.69 ± 0.19; Figure 5.11).

Having accounted for the other modelled factors and their interactions, foliar treatment was not found to have statistically significant independent main effects on overall berry sweetness ($F(2,40) = [2.754]$, $p = 0.076$).

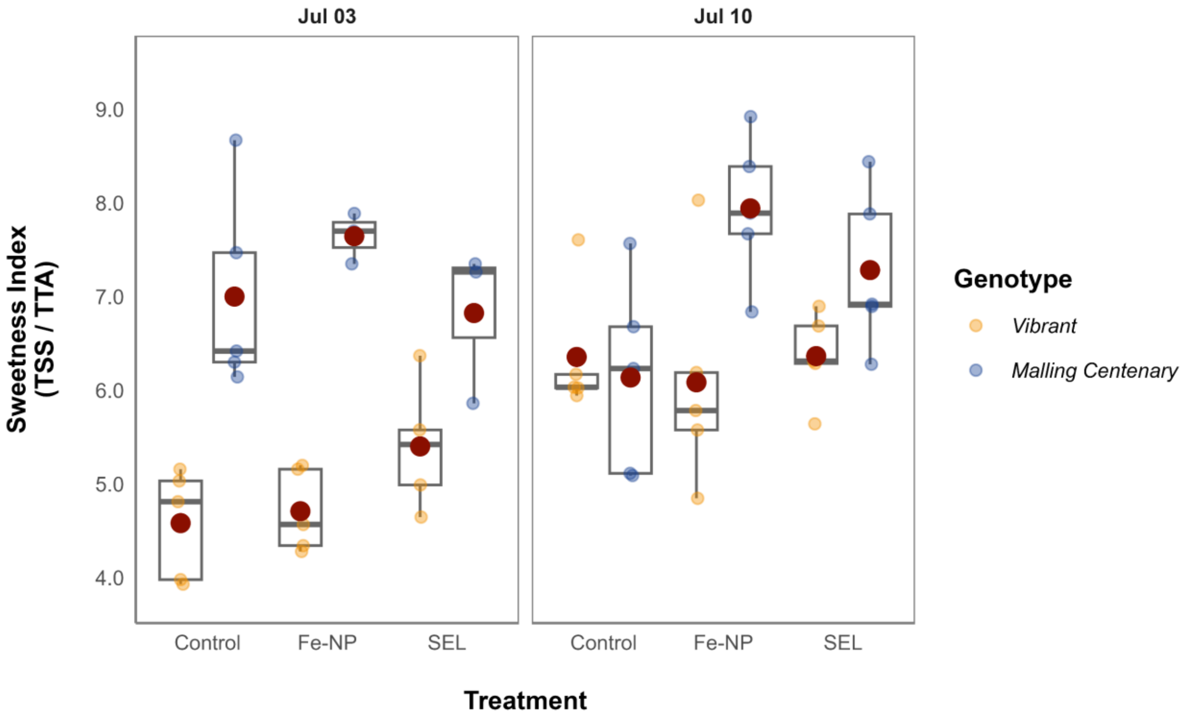


Figure 5.11 Box-plots illustrating differences in the overall sweetness of strawberries from two genotypes treated with foliar-applied iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Malling Centenary* (Blue) and *Vibrant* (Orange). Berries were analysed from two sampling dates in the weeks commencing 03 July 2023 (left panel) and 10 July 2023 (right panel). All plants were grown in a commercially standard polytunnel between 01 June and 03 August 2023. Berry sweetness is presented as the ratio between total soluble solids (TSS) and theoretical titratable acidity (TTA). The mean TSS/TTA ratio for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum values for TSS/TTA. The distance between the first and third quartiles illustrates the interquartile range within the data.

5.3.4 Leaf Chlorophyll, Flavonoids, and Nitrogen Balance Index

During the experimental period, measurements of leaf CHL and NBI were taken on four occasions. Measurements of CHL and NBI were observed to remain stable over the experimental period (Figure 5.11). Two-way ANOVA showed that there was no statistically significant interaction between genotype and foliar treatment with respect to CHL (F(2,107) = [0.334], p = 0.717) or NBI (F(2,107) = [0.560], p = 0.573).

Genotype was found to have had a statistically significant independent main effect on CHL (F(1,107) = [28.842], p = 4.610e-07) and NBI (F(1,107) = [49.200], p = 2.180e-10). Leaf CHL values were higher in *Vibrant* leaves (33.13 ± 0.29 DX units) across the study period when compared to *Malling Centenary* (31.02 ± 0.31 DX units; Figure 5.10A). The mean NBI value was also higher for *Vibrant* (18.84 ± 0.16) when compared to *Malling Centenary* (17.20 ± 0.18; Figure 5.10B).

Having accounted for the effects of genotype, date, and experimental blocking, there was no statistically significant main effect of foliar treatment on CHL (F(2,107) = [1.811], p = 0.168) or NBI (F(2,107) = [0.243], p = 0.785).

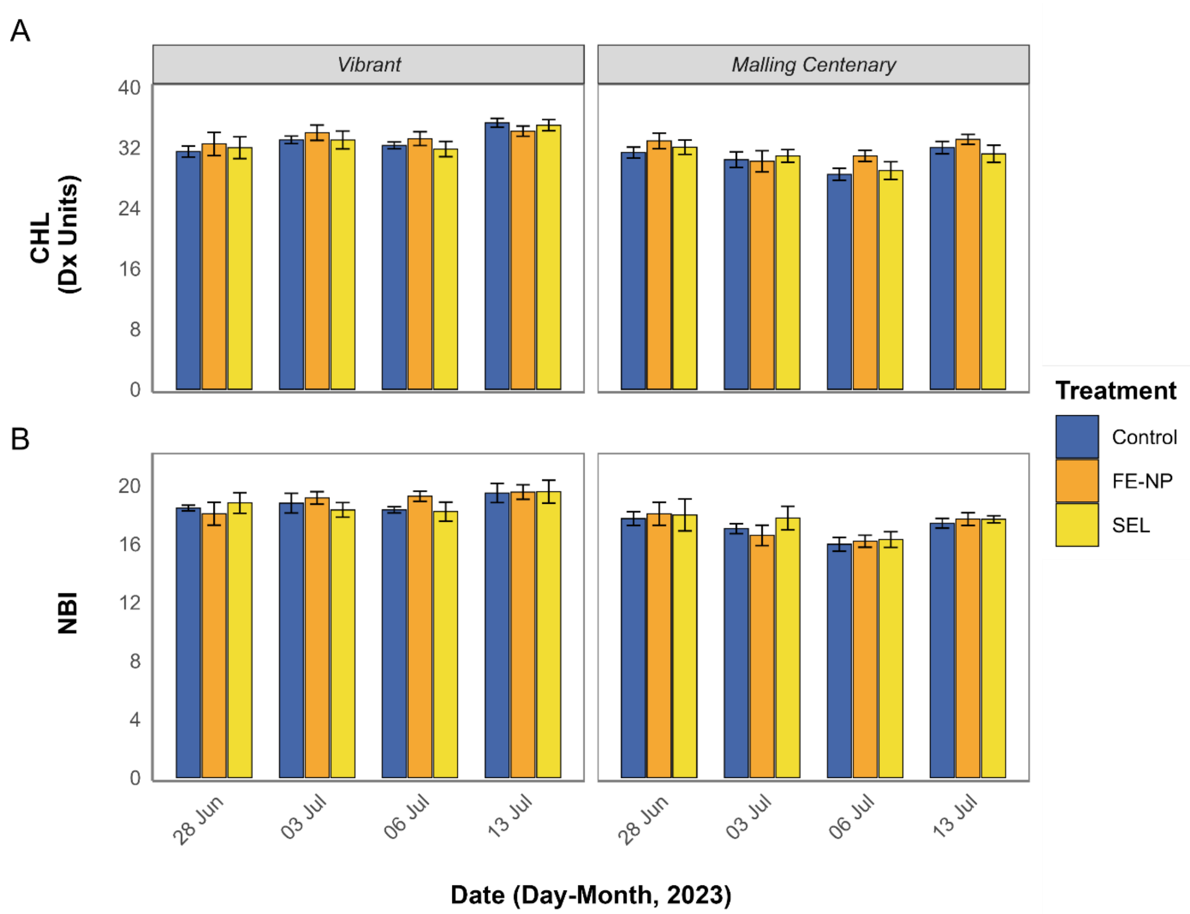


Figure 5.12 Bar-plots illustrating (A) chlorophyll fluorescence (CHL) and (B) Nitrogen Balance Index (NBI) in strawberry leaves treated with foliar-applied iron nanoparticles (FE-NP - orange), sodium selenate (SEL - yellow), or with no treatment (Control- blue). Chlorophyll fluorescence values are presented in proprietary Dualex units (Dx). Leaf CHL and NBI were analysed in two strawberry genotypes: *Vibrant* (left panels) and *Malling Centenary* (right panels). All plants were grown in a commercially standard polytunnel between 01 June and 03 August 2023.

5.3.5 Crop Yields

The final trait assessed in the current experiment was crop yield. The peak yields for *Malling Centenary* and *Vibrant* both occurred between 03 and 10 July 2023 (Figure 5.13).

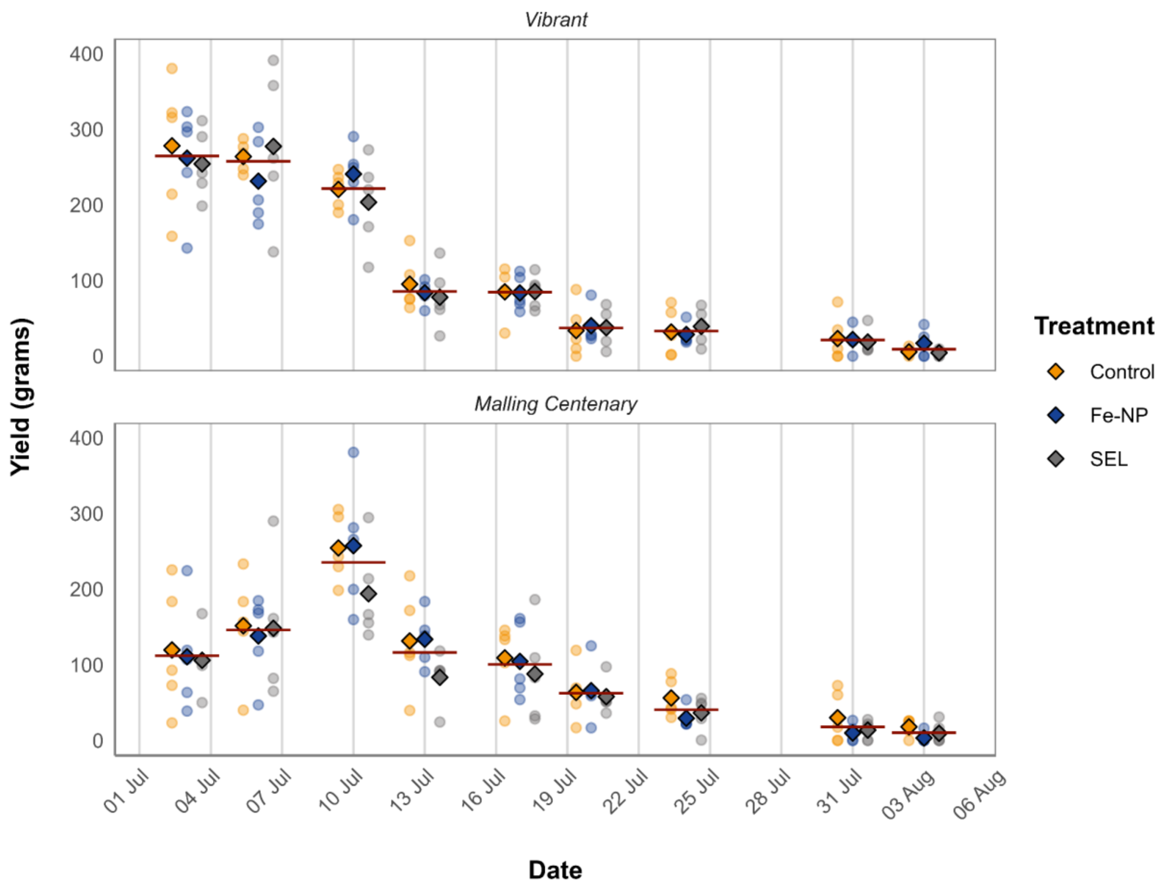


Figure 5.13 Dot plots showing strawberry yield profiles for two genotypes treated with foliar-applied iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were *Vibrant* (Top panel) and *Malling Centenary* (Bottom panel). Coloured points represent the yields recorded from each biological replicate within each treatment group. The coloured diamonds represent the mean yield recorded for each treatment group. The red bars represent the mean yield from all three treatment groups at each harvest.

Analysis showed that there was no statistically significant interaction effect between genotype and foliar treatment on total yield per plant ($F(2,20) = [0.603]$, $p = 0.557$), number of Class I berries ($F(2,20) = [0.723]$, $p = 0.498$), or mean berry size ($F(2,20) = [0.606]$, $p = 0.555$).

An independent main effect of genotype was observed on total yield ($F(1,20) = [8.437]$, $p = 0.009$) and the number of Class I berries ($F(1,20) = [16.860]$, $p = 0.001$), having accounted for potential variance between experimental blocks. Genotype was not found to have a statistically significant effect on mean berry size ($F(1,20) = [1.198]$, $p = 0.287$) or the number of Class II /Waste berries ($F(1,20) = [0.107]$, $p = 0.747$). Foliar treatment had no statistically significant effect on any of the measured yield parameters ($p > 0.200$).

Vibrant total yields ranged between 249.58 ± 15.99 g/plant in the SEL treatment group and 259.34 ± 18.55 g/plant in the Control treatment group (Figure 5.14). The number of Class I berries from *Vibrant* ranged between 14.05 ± 1.08 berries/plant in the SEL treatment group and 14.25 ± 1.13 berries/plant in the Fe-NP treatment group (Figure 5.14).

Malling Centenary total yields ranged between 184.21 ± 17.78 g/plant in the SEL treatment group and 233.50 ± 27.63 g/plant in the Control treatment group (Figure 5.14). The number of Class I berries from *Malling Centenary* ranged between 9.20 ± 1.06 berries/plant in the SEL treatment group and 11.35 ± 1.48 berries/plant in the Control treatment group (Figure 5.14).

The mean berry sizes were comparable for both *Vibrant* (13.61 ± 0.17 g) and *Malling Centenary* (13.96 ± 0.27 g). The number of Class II and Waste berries was also consistent for both genotypes (Figure 5.14)

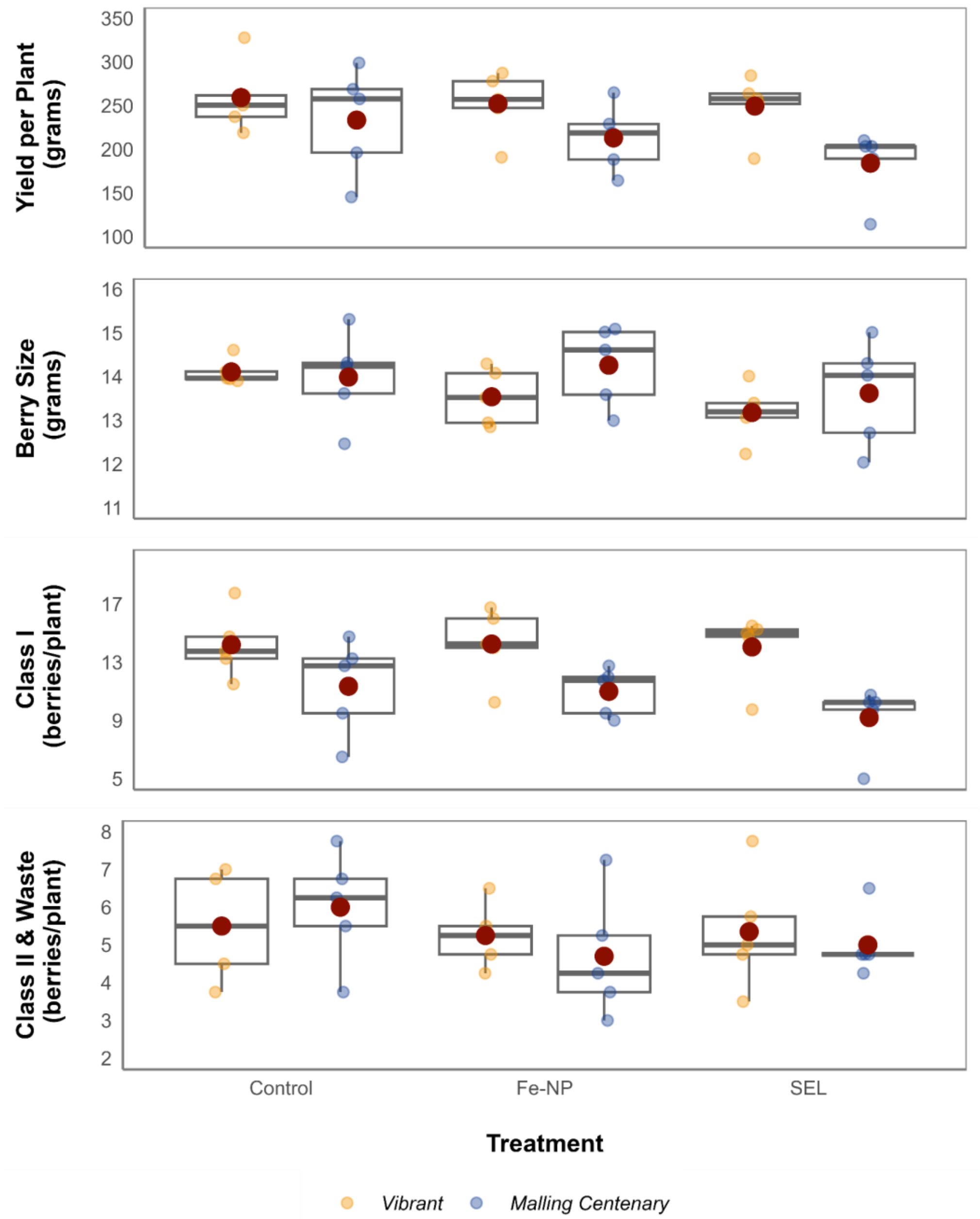


Figure 5.14 Box-plots illustrating differences in selected yield parameters from two strawberry genotypes treated with foliar-applied iron nanoparticles (Fe-NP), sodium selenate (SEL), or with no treatment (Control). The genotypes used were Malling Centenary (Blue) and Vibrant (Orange). From top to bottom, yield parameters include total yield per plant (1st panel), berry size (second panel), number of class I berries (3rd panel) and number of class II and waste berries (4th Panel). The mean yield value for each genotype/treatment is represented as a red dot. The boxplots summarise the minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum yield values for each parameter. The distance between the first and third quartiles illustrates the interquartile range within the data.

5.4 Discussion

5.4.1 Vitamin C Content

The findings of the present study reaffirmed the important role of genotype in determining berry AsA. In the Control treatment group, AsA was 1.17-fold greater in *Vibrant* berries when compared with *Malling Centenary* berries. The measured AsA values for *Vibrant* (83.30 ± 2.52 mg/100g FW) and *Malling Centenary* (71.15 ± 4.35 mg/100g FW) in the Control treatment group were comparable to those reported in Chapter 6 (Section 6.3.1.1). *Malling Centenary* AsA was also consistent with the findings of Woznicki et al. (2023), who found that *Malling Centenary* plants grown in 100% coir produced berries with a mean AsA content of ~66 mg/100g FW. Having a well-characterised baseline for UK-relevant genotypes, studied in commercially applicable growing systems, will be of great utility when investigating strategies that could enhance the AsA content of those genotypes.

In Chapter 5, Fe-NP and SEL foliar treatments were found to have had no statistically significant effect on AsA in the two studied genotypes. *Vibrant* AsA values across the three treatment groups were all within 3.08 mg/100g FW of each other. Greater variation between treatments was observed in *Malling Centenary*, where AsA content was highest in the Control group (71.15 ± 4.35 mg/100g FW) and lowest in the Fe-NP group (62.47 ± 2.55 mg/100g FW). The lack of a difference between AsA in the different treatment groups contrasts with other published studies (El-Gioushy et al., 2021; Lin et al., 2024; Lu et al., 2022).

Concerning SEL treatments, Lu et al. (2022) applied treatments of 30 mg/L sodium selenite (Na_2SeO_3) every seven days to strawberries grown in garden soil and observed an increase of ~50% in AsA content in fully ripe berries. Furthermore, the observed increases were associated with variable activities of ascorbate peroxidase (APX), monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase (GR), L-galactono-1,4-lactone dehydrogenase, and ascorbate oxidase (Lu et al., 2022). These results indicated that the SEL treatment had driven changes in the metabolic regulation of AsA and led to an increased accumulation in the berries (Lu et al., 2022). Lin et al. (2024) also reported increases in AsA in response to the application of sodium selenite sprays ranging from 10 mg/L to 70 mg/L every 5 days. It is important to note the distinction between the use of selenate (Na_2SeO_4) in the current study and selenite (Na_2SeO_3) in the studies by Lu et al. (2022) and Lin et al. (2024). With regards to the activity of APX and GR in plants, however, Na_2SeO_4 has been reported to exert a greater effect than Na_2SeO_3 (Silva et al., 2020). Consequently, an increase in AsA in the current study would still have been expected in response to the SEL treatments.

A further key distinction between the current study and those already discussed is the frequency of treatment application. In the current study, a single spray treatment was applied, whereas Lu et al. (2022) utilised three applications, and Lin et al. (2024) implemented six. Both Lu et al. (2022) and Lin et al. (2024) also used June-bearing cultivars (*Sweet Charlie* and *Benihoppe*, respectively), which have cropping periods comparable to those of *Vibrant* and *Malling Centenary*. Consequently, the cumulative dose applied by Lu et al. (2022) and Lin et al. (2024) would have been higher than in the current study. The dosage applied in the current study could have been further limited by under-concentrated spray solutions (Section 5.2.4), although further investigation will be required to confirm the accuracy of the reported spray concentrations. Notably, however, even the lowest SEL dose applied by Lin et al. (2024) (10 mg/L) resulted in increased berry AsA levels. It is plausible, therefore, that less frequent application of a more concentrated spray, as used in the current study (30 mg/L), could yield similar effects if cumulative dosage is a critical determinant of treatment efficacy. Further research is needed to clarify the interplay between dosage and application frequency in enhancing berry AsA content. However, such investigations were beyond the scope of the current study.

Published data on the effects of Fe-NP treatments on AsA content are less inconclusive. Supporting the findings of the current study, an experiment conducted at Niab East Malling during 2023/2024 also reported that Fe-NP treatments inconsistently reduced AsA levels in tomato fruit (Stavridou et al., data unpublished). Conversely, other studies have documented increases in AsA content following Fe-NP treatments, but these effects were observed exclusively in plants subjected to drought or salinity stresses (Bidabadi et al., 2023; Moradbeygi et al., 2020; Shahzad et al., 2024). No significant response was noted under non-stressed conditions in these studies (Bidabadi et al., 2023; Moradbeygi et al., 2020; Shahzad et al., 2024). Mogazy et al. (2022) also reported increases in strawberry AsA content in response to two 200 mg/L FE-NP sprays applied over a 7-day interval. However, these plants had been deliberately infected with *Botrytis cinerea* prior to treatment, as the study's objective was to mitigate pathogenic effects. Under non-stressed conditions, El-Gioushy et al. (2021) noted only marginal increases in orange AsA content following Fe-NP treatments. Thus, the lack of a significant effect of Fe-NP treatments in the current study may, in part, be attributed to the absence of prolonged environmental or biotic stress on the strawberry plants.

Although berries sampled in w/c 03 July were chronologically closer to the date of foliar treatments, their AsA content was lower than that of berries sampled in w/c 10 July. This inter-week variation was statistically significant, with AsA levels 15% and 21% higher in w/c 10 July compared to w/c 03 July for *Vibrant* and *Malling Centenary*, respectively. Similarly, Tavarini et al. (2008) reported significant differences in the AsA content of fully ripe kiwi fruit harvested just one week apart, although their study did not investigate or comment on potential reasons for these temporal variations. In the current study, one key difference between the two sampling dates was climatic conditions (Table 5.3). Specifically, cumulative solar irradiance was lower in w/c 25 June (38,993 W/m^2/day), which preceded the first sampling date, compared to w/c 03 July (42,482 W/m^2/day), which preceded the second sampling date. Light exposure is a well-documented factor influencing AsA accumulation in various crops, including strawberries (Pineli et al., 2012), tomatoes (Gautier et al., 2008), and brassica microgreens (Liu et al., 2022). Therefore, it is plausible that berries harvested in w/c 10 July had higher AsA levels due to increased sunlight exposure in the preceding week.

5.4.2 Organic Acid Content

Organic acids are important secondary metabolites involved in plant stress responses. They are also a key determinant of strawberry palatability, with berries containing lower amounts of organic acids reported to be favoured by consumers (Ikegaya, 2024; Ikegaya et al., 2019).

Genotype was again found to be a key factor in determining berry malic and citric acid contents. Malic and Citric acid contents were 1.31- and 1.18-fold greater in *Vibrant* when compared to *Malling Centenary*, respectively. These genotypic differences in strawberry organic acid content are concurrent with the wider literature (Chiomento et al., 2021; Crespo et al., 2010; Kapur et al., 2018; Kim et al., 2013).

The foliar treatments were not found to have a direct independent effect on malic or citric acid content in the current study. There was, however, a statistically significant interaction between genotype and foliar treatment, whereby *Malling Centenary* citric acid content was reduced by 14% under the Fe-NP treatment relative to the Control treatment.

Previously reported effects of SEL treatments on organic acid content are inconsistent between studies. Similarly to the current study, Lin et al. (2024) reported a decrease in strawberry citric acid content in response to Na_2SeO_3 treatments of 10, 40, or 70 mg/L, compared to their control treatment. For malic acid, Lin et al. (2024) also observed no differences between non-treated plants and plants treated with 10 or 40 mg/L Na_2SeO_3, which were similar doses to that used in the current study (30 mg/L). Moderate increases in malic acid were observed at concentrations of 70 and 100 mg/L. Hu et al. (2022) found that both citric and malic acid increased in cucumbers treated with two 0.025 mM (1.15 mg/L) Na_2SeO_4 sprays. Repeating their experiment in tomato, Hu et al. (2023) found fruit citric acid content to increase in response to foliar SEL treatments, but that malic acid and total organic acid did not vary relative to their control treatment. Zhu et al. (2018) also reported no change in tomato malic or citric acid content in response to foliar treatment with 1 mg/L Na_2SeO_4. Given the available evidence, organic acid responses to selenium treatments appear to be dependent on a combination of species, genotype, form of selenium, and concentration of selenium spray. As such, it is difficult to determine a specific mode of action by which selenium sprays can impact crop organic acids.

The effects of Fe-NP sprays on crop organic acid contents appear, again, to be contextual regarding pre-existing oxidative stress. Sun et al. (2016) have recently shown that organic acid content in *Spinacia oleracea* roots decreased after the application of Fe-NP treatments under arsenic stress. Reductions in root citric acid content were also observed in the absence of arsenic stress. Sun et al. (2016) also showed that the Fe-NP treatments reduced the levels of oxidative stress biomarkers. Consequently, Sun et al. (2016) suggest that by reducing the chemical signals associated with oxidative stress, the plant’s production of stress-responsive organic acids decreases. At the time of this writing, however, additional evidence regarding the effects of Fe-NP treatments on organic acid contents in other crops or plant tissues is lacking. The reductions in *Malling Centenary* citric acid content observed in the current study could be explained by the mode of action suggested by Sun et al. (2016), but further investigation into changes in oxidative stress markers would be required to confirm if this was the case. The reduction in strawberry citric acid content was also genotype-dependent, which presents an additional layer of complexity for which a conclusive explanation is currently lacking. This is, however, a finding of scientific interest and practical application, as reducing berry organic acid content could be relevant to production of sweeter and more palatable berries (Ikegaya, 2024).

5.4.3 Total Soluble Solids and Berry Sweetness

Organic acid content and TSS are commonly combined as a ratio as the balance of sugars to acids is a better reflection of the sweetness of a berry than TSS content alone (Ikegaya, 2024). Combining the findings related to organic acid and TSS contents in the current study, genotype-dependent effects on berry sweetness were observed with respect to both foliar treatment and sampling date.

Malling Centenary berries were sweeter under the Fe-NP foliar treatments, predominantly caused by the reduction in organic acid content relative to the Control and SEL treatment groups. *Vibrant* berries were sweeter in w/c 10 July, caused by the lower TSS values observed in w/c 03 July in conjunction with the consistent organic acid contents across both sampling dates. Given that berry sweetness is such an important quality parameter in strawberries, the potential for Fe-NP sprays to reduce organic acid contents without reducing sugar content could be of commercial interest.

There were, again, no direct effects of foliar treatment on overall sweetness. Sweetness values (TSS/TTA) ranged between 6.02 ± 0.27 and 6.48 ± 0.36 in the Control and Fe-NP treatment groups, respectively. Whilst not found to be statistically significant, there were marginal increases in overall sweetness in the SEL and Fe-NP treatment groups, relative to the control group. These observations could, again, be of interest for future optimisation of treatment dosage or application strategy to maximise the size of the observed effects.

Lin et al. (2024) found the TSS content of strawberries to increase in strawberries treated with 70 mg/L Na_2SeO_3. The increases in TSS reported by Lin et al. (2024) were, however, only ~ 1 °Brix between the control and 70 mg/L treatments, a similar effect size to the 0.4 °Brix observed in the current study. Application of less concentrated SEL treatments (1 - 1.5 mg/L), has also been shown to cause elevated TSS content in tomatoes (Hu et al., 2023), but not in peaches (Pezzarossa et al., 2012). Findings from other studies therefore support the idea that sufficient doses of SEL can drive increases in TSS, but that the increases are likely to be marginal and that optimal dosages are crop specific.

Reports on the effects of Fe-NP treatments on strawberry TSS and sweetness are lacking. El-Gioushy et al. (2021) showed in oranges that Fe-NP treatments of either caused an increase in fruit TSS and sweetness relative to their control treatment group. These increases were observed in conjunction with a decrease in fruit acid content, similar to the current study. Dose-dependent increases in tomato soluble sugars were observed by (Shahzad et al., 2024), with the greatest increase in sugar content achieved in fruits treated with 100 mg/L Fe-NP. Similarly to SEL, it appears that Fe-NP treatments have the potential to improve strawberry sweetness through a combined reduction of organic acid content and elevation of TSS. As observed in the current study, however, it appears that the achievable gains are relatively small and are unlikely to be of commercial significance to growers at this stage. It is also important to highlight that TSS/TTA is just one chemical component related to strawberry sensory quality. Whilst TSS/TTA has been found to associate with improved sensory quality in sensory panel studies (Ikegaya et al., 2021), further analysis of other flavour compounds, such as amino acid content and volatile compounds (Fan et al., 2021), in addition to direct sensory panels, will be necessary to support stronger conclusions related to the potentially beneficial effects of Fe-NP and SEL.

5.4.4 Iron and Selenium Content

Selenium content was higher in the strawberries from the SEL treatment group relative to the Control and Fe-NP treatments, irrespective of genotype. The selenium content in the SEL treatment group was measured at 0.08 ± 0.01 ppm, which represented a 100.0% increase in selenium contents compared to the Control and Fe-NP treatment groups (both 0.04 ± 0.01 ppm).

The observed increase of 100.0% between the Control and SEL treatments is similar to that observed by Lu et al. (2022). In fully ripe strawberries treated with 10 mg/L Na_2SeO_3, Lu et al. (2022) observed a 97.4% increase in berry selenium. The increase was reported to be even greater at a Na_2SeO_3 concentration of 30 mg/L (242.1%). Other studies that have applied foliar selenium treatments to a range of crops have consistently reported increased selenium content in edible tissues (Hu et al., 2023; Lin et al., 2024; Zhu et al., 2018). More broadly, the foliar application of nutrients has been widely documented as an effective way of increasing the nutrient content in edible fruit tissues (Niu et al., 2021). The increase in berry selenium content observed in the current study is, therefore, a good indication that the SEL treatment was sufficient to increase selenium availability for the plants.

In contrast, the iron content of *Malling Centenary* berries was found to have decreased in response to both the Fe-NP and SEL treatments. The iron content of *Malling Centenary* from the Control treatment was 31.20 ± 2.73 ppm, whilst all other iron values ranged between 23.40 ± 0.87 ppm and 26.00 ± 1.84 ppm. The iron content in *Vibrant* berries was not affected by either treatment.

The difference in iron content between the *Malling Centenary* Control samples and all other groups was unexpected given the intuitive trends observed with respect to selenium content. Other studies that have applied Fe-NP treatments have reported increases in fruit iron content (El-Gioushy et al., 2021; Mahmoud et al., 2022). In strawberries, Mogazy et al. (2022) found that the application of 20 and 40 mg/L Fe-NP treatments caused a dose-dependent increase in berry iron content. Mozafari et al. (2018b), however, did not observe any significant increase in berry iron content at an Fe-NP concentration of 0.8 mg/L. The fact that iron content did not increase in the Fe-NP treatment group in the current study may, therefore, indicate that the Fe-NP dose was not sufficient to increase iron availability to the levels required to observe increased iron accumulation. This does not, however, explain why the iron contents of Fe-NP-treated berries would decrease relative to untreated berries.

Concerning the effects of SEL treatments on iron content, Zhu et al. (2018) found no statistically significant differences between the iron content of tomato fruit treated with 1 mg/L Na_2SeO_4, relative to their control treatment. Further information regarding the effects of SEL treatments on iron content is lacking.

One possible explanation could be that the lack of an applied spray treatment somehow facilitated greater nutrient uptake in the *Malling Centenary* Control group. That said, all plants in the experiment were subject to weekly fungicide sprays throughout the experimental period. This means that any impacts of the physical application of a spray should have been negated. As a precaution, future studies could incorporate a treatment group that is sprayed with an inert solution (positive control) at the same time as the SEL and Fe-NP treatments are applied. The use of a positive control would confirm that the application of the spray itself was not the cause of the observed differences.

Another explanation could be that the Control and Fe-NP treatments were applied to the wrong plants by mistake. This is also highly unlikely, however, as the Fe-NP sprays were noted to leave visible dark spots on the treated leaves. Having frequently recorded CHL measurements from the plants, these dark spots were certainly only present on plants assigned to the Fe-NP treatment.

Identifying the potential causes of the unexpected decrease in iron content is further confounded by the fact that no differences were observed in the iron content of the *Vibrant* samples across the three treatment groups. Given the incongruent nature of the findings, further investigations will be required to determine whether these observations are repeatable and, if so, to determine the nature of the observed effects.

5.4.5 Chlorophyll Fluorescence and Crop Productivity

Crop productivity was assessed both by indirect measures of CHL and direct measures of berry production. Genotype was found to be the only factor that had a direct effect on CHL, NBI, or crop yield in the current study, whereas the foliar treatments were not found to have any statistically significant effect on productivity.

Considering CHL and NBI, values were greater in *Vibrant* leaves than in *Malling Centenary*. The mean CHL values for *Vibrant* and *Malling Centenary* were 33.13 ± 0.29 and 31.02 ± 0.31, respectively. The corresponding NBI values for *Vibrant* and *Malling Centenary* were 18.84 ± 0.16 and 17.20 ± 0.18, respectively. Total yields and numbers of Class I berries were also higher in *Vibrant*. *Vibrant* total yield was 253.71 ± 9.25 g/plant, compared to 210 ± 12.67 g/plant for *Malling Centenary*. Similarly, *Vibrant* plants produced a mean of 3.5 extra Class I berries compared to *Malling Centenary*. The effect of genotype on the yields of *Vibrant* and *Malling Centenary* was consistent with the findings reported in Chapter 6 (section 6.3.1.4). Previous studies have also reported genotypic variation with respect to both CHL and yield parameters in strawberries (Ferreira et al., 2019; Ullah et al., 2024). Choi et al. (2016) also showed that CHL was positively correlated with strawberry yields, which could explain why *Vibrant* yields were higher than *Malling Centenary* in the current study.

Woznicki et al. (2023) cultivated *Malling Centenary* plants under similar conditions to the current study and observed CHL and NBI values of ~50 and ~30, respectively. These values are nearly double those observed in the current experiment. They also recorded total yields of ~320 g/plant, and ~18 Class I berries/plant, which are approximately 1.5-1.6 time greater than those recorded in the current study. Whilst Woznicki et al. (2023) did not present the environmental conditions during their experiment, both light and temperature (Choi et al., 2016; Tang et al., 2020) are known to be key determinants of both CHL and yield that could explain the observed differences between the studies. The factors associated with the lower-than-expected yields in the current study do not, however, appear to have affected berry AsA, organic acids, and TSS, all of which were within expected ranges.

No differences in CHL, NBI, or yield were observed between the foliar treatment groups. Whilst the absolute differences were marginal, recorded yield parameters tended to be slightly worse under the SEL and Fe-NP treatments for both genotypes. Increases in CHL have been commonly reported in studies that have applied either SEL (Hu et al., 2022; Lu et al., 2022; Zahedi et al., 2019) or Fe-NP treatments (El-Desouky et al., 2021; El-Gioushy et al., 2021; Rahman et al., 2023). Similarly, the association between increases in CHL, photosynthetic efficiency, and crop yields have been reported (Hu et al., 2022; Yin et al., 2019). As has been already discussed with respect to the other nutritional and sensory parameters, the lack of an effect of the foliar treatments in the current experiment could be related to an inadequate dosage or application frequency. These factors appear to be the key differences between the current study and the wider literature and warrant further investigation to understand how the treatments could be adapted in future research.

5.5 Conclusions

Chapter 5 reported an investigation into the effects of Fe-NP and SEL treatments on selected nutritional, sensory, and agronomic parameters in UK-relevant strawberry cultivars. The primary aim of the study was to assess whether these treatments could be applied to increase AsA accumulation, and thus vitamin C content, within the edible berries.

The SEL and Fe-NP treatments were not found to affect berry AsA, but they did have positive effects on other nutritional parameters and specific sensory traits. In response to the application of the 30 mg/L SEL treatment, berry selenium content was found to increase by 100%. Additionally, the Fe-NP treatment was observed to have a genotype-dependent effect on *Malling Centenary* Citric acid content, effectively reducing the acidity and increasing the overall sweetness of the berries. With further sensory panels and nutritional analysis, the increased selenium content and increased sweetness may both be of interest and value to commercial growers, demonstrating the potential to produce fruit of increased nutritional and sensory quality.

The SEL and Fe-NP treatments were not found to have any significant negative effects on any of the measured parameters, but the lack of an effect on AsA, CHL, and yield was in contrast to expectations based on other published studies. Unlike previous reports, however, the current study was conducted under commercial growing conditions in the absence of severe environmental or biotic stresses. It may be the case that SEL and Fe-NP are simply not effective in driving increases in strawberry vitamin C content under UK commercial strawberry growing conditions.

Whilst beyond the scope of this thesis, addressing the limitations of the current study is warranted given the beneficial reports of similar treatments elsewhere in the literature. Further investigation will, therefore, be required to optimise the dosage and frequency of treatment applications to reach a conclusion as to the potential of SEL and Fe-NP treatments in commercial strawberry production systems.

Strawberry AsA content was also observed to be directly affected by the strawberry genotype. The strong influence of genotype reaffirms the potential for selective breeding to be applied to UK-relevant genotypes cultivated in commercial production systems. Genetic potential in UK strawberries will be further explored in Chapter 6, examining a broader range of genotypes grown over multiple seasons.

Chapter 6 Characterisation of the Vitamin C Profiles of British Strawberry Genotypes Grown Under Commercial Cultivation Systems

6.1 Introduction

Strawberry is a crop that has been extensively crossed in modern breeding programs seeking to improve commercial and sensory qualities such as yield, disease resistance, and flavour (Mezzetti et al., 2018). Despite its relatively low importance in current breeding programmes, the nutritional content of strawberries has also been shown to vary significantly between genotypes (Capocasa et al., 2016, 2008; Dragišić Maksimović et al., 2015; Pineli et al., 2012; Scalzo et al., 2005). As discussed in Chapter 1, vitamin C alone has been found to vary between commercial varieties, breeding selections, and back-crosses by as much as 3-fold (Mezzetti et al., 2016) (Section 1.4.2.1). Further to the exploration of nutrition-smart cultivation practices reported in Chapter 5, selective breeding could present an alternative route to biofortifying UK strawberries which, in turn, could contribute to increased domestic vitamin C production (Section 5.1). There has, however, been relatively limited work conducted on the nutritional quality of UK-relevant strawberry genotypes. As such, the potential to biofortify UK strawberries through selective breeding remains unclear.

In Chapter 4, the vitamin C content of UK-marketed strawberries varied by 5.6-fold (Section 4.3.3). Genotype was highlighted as a key factor that could underpin the observed variation, with 56 unique strawberry varieties sampled between March 2023 and March 2024 (Section 4.3.1). As an observational study, however, it was impossible to determine the extent to which genotype influenced the variation in vitamin C content. This was due to the lack of control over other variables including harvest times, cultivation practices, and countries of origin. Indeed, the interacting effects of genotype, environmental conditions, and cultivation practices on vitamin C contents are well documented (Crespo et al., 2010; Pincemail et al., 2012; Pineli et al., 2012). For example, Crespo et al. (2010) found that the vitamin C content of four strawberry genotypes was differentially affected when grown in different regions of Switzerland. Significant differences in strawberry vitamin C content and antioxidant capacity have also been reported in the same genotype when grown under open field and glasshouse production systems (Rahim Doust et al., 2023).

As such, determination of the extent to which genotype contributes to variation in the nutritional quality of UK strawberries will require studies to be conducted under standardised conditions. In Chapter 5, a preliminary screen of selected breeding lines from the East Malling Strawberry Breeding Club found up to 1.69-fold differences between genotypes (Section 5.2.2). Subsequently, comparisons of the vitamin C contents of two UK commercial genotypes, *Malling Centenary* and *Vibrant*, were found to vary by 1.17-fold under standardised cultivation conditions (Section 5.3.1). Building on these initial investigations, it is of interest to expand the scope of the aforementioned analyses to include a greater number of UK strawberry genotypes. To account for seasonal variation, it will also be beneficial to conduct these studies over multiple seasons such that the stability of any genotypic effects can be assessed (Capocasa et al., 2008; Cervantes et al., 2019; Pineli et al., 2012).

A further, often overlooked, consideration in characterising the vitamin C content of UK strawberry genotypes is the effects of post-harvest handling, transit, and storage (Chapter 4, Section 4.4.2). Before reaching consumers, strawberries undergo a period of post-harvest chilling, storage, transport, and display on retailers’ shelves (Russell et al., 2009). Most studies of genotypic variation only analyse samples at the point of harvest (Kim et al., 2015; Mezzetti et al., 2016). This could mean that the findings of such studies are not representative of the vitamin C content of the strawberries at the point of consumption.

As discussed in Chapter 4, there are conflicting reports on the effects of different storage conditions on vitamin C contents in strawberries (Chapter 4, Section 4.4.2). Generally, storage periods of between 2 and 6 days at refrigerated and ambient temperatures have shown no significant negative effects on strawberry vitamin C content (Cordenunsi et al., 2005, 2003; Shin et al., 2007). However, the experiments in these studies do not necessarily reflect the conditions and timings that strawberries are exposed to in the strawberry supply chain (Russell et al., 2009). To the best of the author's knowledge, no studies have considered changes in vitamin C content through the UK strawberry supply chain. If biofortified crops are to be successful, any increases in vitamin C must be carried through to the point of consumption.

6.1.1 Aims and Objectives

Chapter 6 addresses the fourth aim of this thesis: **To assess the vitamin C content of a range of popular UK strawberry genotypes grown under standard commercial conditions.** Specifically, the study presented in Chapter 6 had four key objectives.

1) To characterise the vitamin C content of five commercialised UK strawberry varieties and two heritage varieties when grown under typical UK cultivation conditions.
2) To investigate associations between vitamin C, antioxidant capacity, and phenolic content.
3) To investigate associations between vitamin C, sensory qualities, and productivity.
4) To measure changes in vitamin C content during typical post-harvest supply chain scenarios

6.2 Materials and Methods

6.2.1 Experimental Site and Plant Material

The experiments were conducted in the same polytunnel described in Chapter 5 (Section 5.2.1). Cultivation of the experimental plants took over two consecutive seasons in 2023 and 2024. All plant material was procured from R W Walpole Ltd (https://www.rwwalpole.co.uk/). The experimental plants were purchased as cold-stored bare-rooted plants that were lifted from the soil when fully dormant and held at -2°C until planting. All plants were cultivated in coir grow bags at a density of 6 plants/m^2 (Cocogreen, Manchester, UK. Berryfusion Growbags, White, 6-hole).

6.2.2 Experimental Design

6.2.2.1 Characterisation of Genotypes

The experiments in both years followed the same core designs concerning cultivation practices and the analysis of vitamin C content (Table 6.1). A series of modifications were made to the experiment in 2024 based on the learnings and findings from 2023. These modifications are described in the subsequent sections and summarised in Table 6.1.

In both 2023 and 2024, the selected genotypes were set up in a completely randomised block design. Each experimental replicate was defined as a coir bag filled with 6 plants from a single genotype.

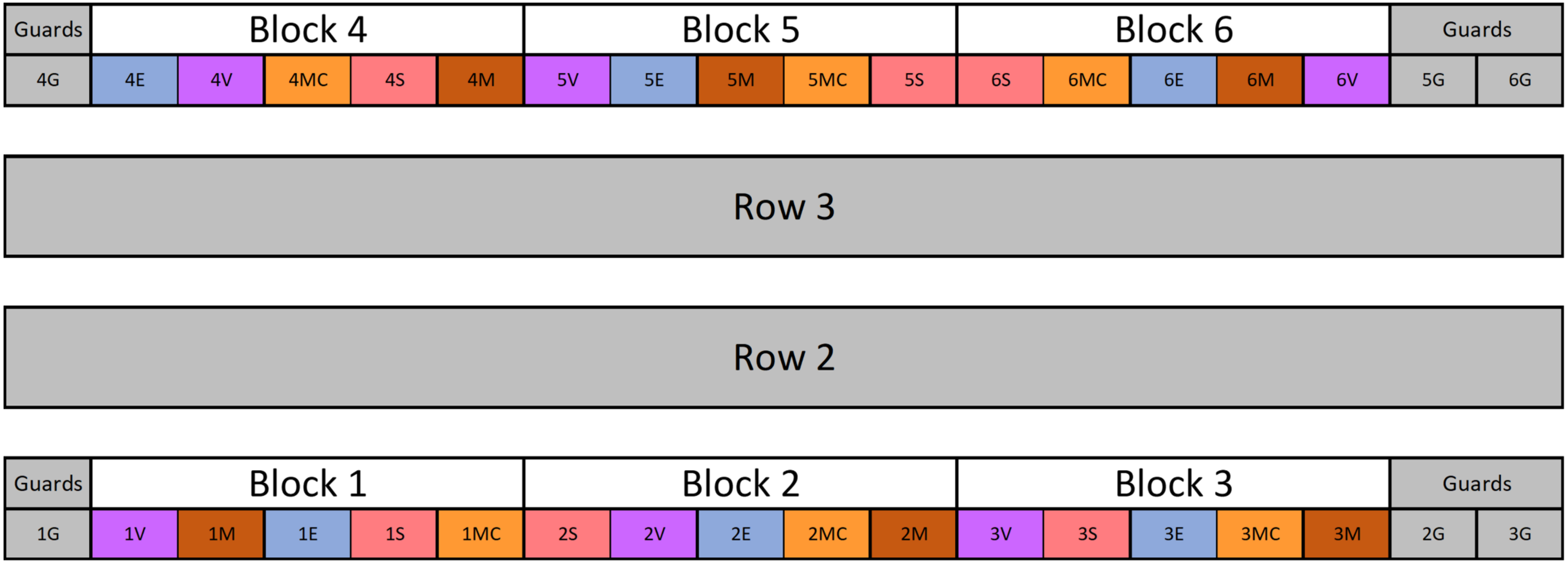
In 2023, the experimental set-up consisted of six blocks across 2 rows (Figure 6.1). Each block contained one experimental replicate. The relative positions of each replicate were randomised within each block (Figure 6.1). Each row of plants was flanked by a full bag of guard plants to control for edge effects.

The genotypes studied in 2023 were *Elsanta*, *Malling Centenary*, *Malwina*, *Sonata*, and *Vibrant*. Based on the findings of Chapters 4 and 5, these genotypes were selected for their diverse vitamin C phenotypes and varying popularity in the modern UK strawberry market.

Plants were ordered from A+ quality stock: R W Walpole's grading system is based on the crown diameter of the plantlets. A+ graded plants are the largest 25-30% of the total crop and are, therefore, the easiest to establish. The Plants arrived at Niab on 24 April 2023 and were stored at -2 °C until planting on 14 June 2023. The fertigation and pest management practices applied in 2023 were the same as those described in Chapter 5 (Section 5.2.2).

Table 6.1 Summary of the modifications made to the experimental designs implemented in 2023 and 2024.

Design Element	2023	2024	Reason for Modification
Number of Biological Replicates	Six	Eight	Increased statistical power.
Number of Genotypes	Five	Seven	Increased genetic diversity.
Stock Plant Quality	A+ Grade	A Grade	Shortage of A+ plant material in 2024.
Planting Date	14 June	09 April	To better align the experimental period with commercial planting times for June-bearing genotypes.
Fertigation Control	Manually adjusted timer	Automated by environmental data loggers.	To make it easier to ensure that all plants were receiving sufficient fertigation.
Yield Assessments	Total weight and number Class I number Class II number Waste number	Total weight and number Class I weight and number Class II weight and number Waste weight and number	To align the 2024 yield assessments with commonly reported measures, namely Class I g/plant, and percentage Class I.
Sensory Quality Assessments	None	Berry colour, firmness, and sweetness.	To quantify subjective assessments of sensory qualities made in 2023.
Composite Sampling	Yes	No	Sufficient sample material to conduct all chemical analyses was collected from a single pick in 2024.
Post-harvest Handling Scenarios	No	Yes	To investigate a hypothesis that was developed based on the findings of the 2023 experiment.



Varieities	Label
Elsanta	E
Malwina	M
Malling Centenary	MC
Sonata	S
Vibrant	V

Figure 6.1 Diagrammatic representation of the experimental set-up and randomised position of selected strawberry genotypes in a standard commercial polytunnel between June and August 2023.

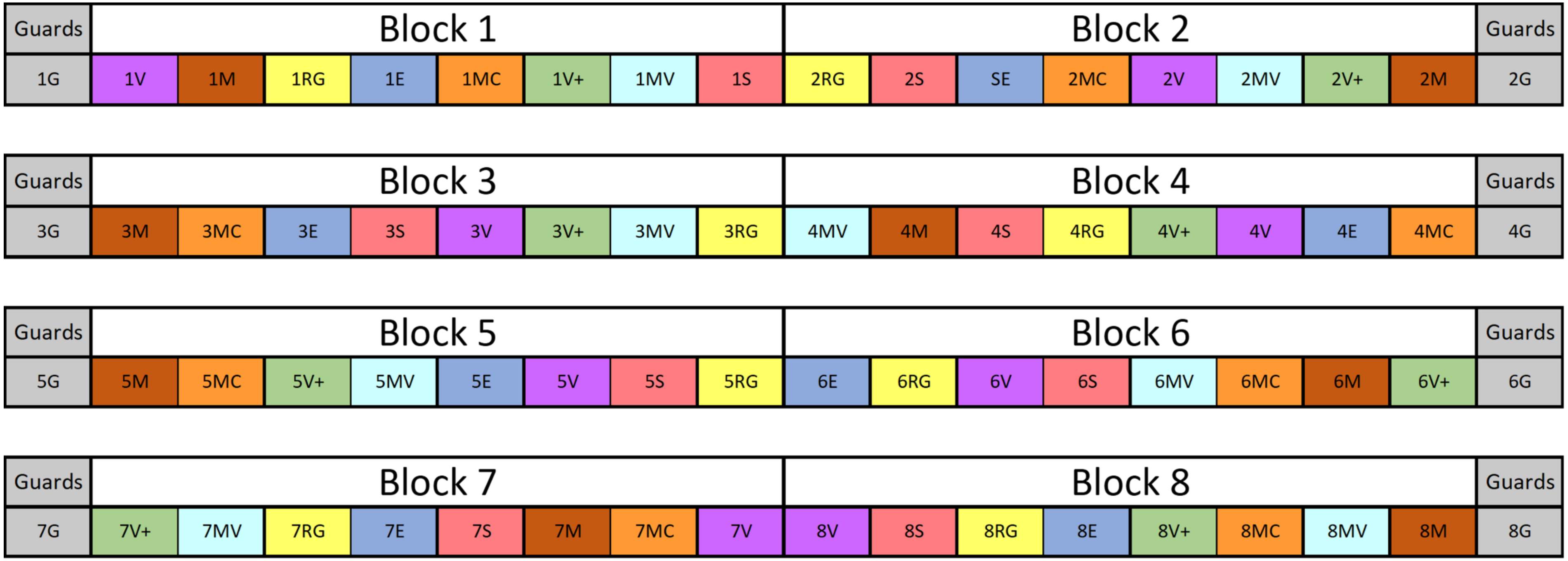
In 2024, eight replicate blocks were distributed over four rows. Extra available space facilitated the increased number of replicates which, in turn, increased the statistical power of between-group comparisons in 2024 (Table 6.1). The positioning of experimental replicates was randomised in the same way as in 2023 (Figure 6.2).

The additional space available in 2024 also allowed for additional genotypes to be studied (Table 6.1). In addition to the five genotypes studied in 2023, plants from *Malling Vitality* and *Red Gauntlet* were added to the 2024 experiment. The addition of more genotypes was deemed a suitable modification for increasing the potential variation that could be captured.

Another change in 2024 concerned the quality of the plant stock. The plants obtained in 2024 were from A-grade quality stock due to a shortage of A+ quality stock with R W Walpole (Table 6.1). A-grade plants represent the next 50% of the total crop after A+ for crown diameter (25%-75%). This means that the potential variability in the size and quality of A-grade plants is greater relative to A+ grade plants. To account for variability due to plant quality, additional A+ graded plants were obtained for *Vibrant*, hereby referred to as *Vibrant+*.

The experimental plants arrived at Niab on 27 March 2024 and were stored at -2 °C until planting on 09 April 2024. The planting in 2024 was, therefore, two months in advance of the plantings in 2023 (Table 6.1). Planting in early April is more consistent with the commercial planting of June bearing strawberries. As one of the core elements of the current study was to characterise strawberry vitamin C content under standard commercial conditions, the change to planting time in 2024 was considered appropriate.

A further modification made in 2024 was the use of GP2 data loggers (Delta-T, Cambridge, UK. GP2 Data Logger and Controller) to automate plant fertigation (Table 6.1). Moisture and temperature sensors were used to monitor temperature-corrected moisture content (TCM) of eight coir bags in the Eastern half of the tunnel and eight coir bags in the Western half of the tunnel. Irrigation to each half of the tunnel was controlled separately. Irrigation was triggered every 30 minutes if the mean TCM was below 65%. The pest management practices applied in 2024 were the same as those applied in 2023.



Varieities	Label
Elsanta	E
Malwina	M
Malling Centenary	MC
Malling Vitality	MV
Red Gauntlet	RG
Sonata	S
Vibrant	V
Vibrant+	V+

Figure 6.2 Diagrammatic representation of the experimental set-up and randomised position of selected strawberry genotypes in a standard commercial polytunnel between April and July 2024.

Fully matured fruit (uniform red colouration across the entire berry surface) were picked two times a week for the full cropping period. Cropping occurred from 03 July – 10 August in 2023 and from 28 May – 01 July in 2024. Table 6.2 summarises the local weather conditions during the experimental periods in 2023 and 2024. Weather data were obtained from a local weather station located at the experimental site.

Table 6.2 Summary of weather conditions during the experimental periods between 14 June and 03 August 2023, and 09 April – 01 July 2024.

		Maximum Air temperature (°C)	Minimum Air temperature (°C)	Relative Humidity (%)	Daily Solar Irradiance (W/m^2)	Cumulative Solar Irradiance (W/m^2)
2023	June	23.57	11.98	73.90	260.62	49,552.66
	July	22.75	12.35	76.55	185.64	35,294.72
	August	22.75	12.15	80.25	168.21	31,981.49
2024	April	14.30	6.28	79.18	150.30	28,576.89
	May	19.03	8.84	85.19	196.05	37,274.95
	June	20.68	8.46	77.73	242.04	46,018.23
	July	22.72	11.90	81.71	204.94	38,965.08

Immediately after each pick, the berries were classified as Class I (>28mm diameter and uniform conical shape), Class II (22-28mm diameter and/or minor misshaping) or waste (>22mm diameter and/or misshapen, presence of powdery mildew or grey mould). In 2023, the number of berries in each Class was counted but only the combined weight from all three classes (grams) was recorded. In 2024, the weights of each Class were also recorded separately (Table 6.1).

Berries were sampled for chemical analysis on selected dates in each season that coincided with the peak yields (Figure 6.12and Figure 6.13). All berries selected for chemical analysis were from the plant's primary trusses to account for potential differences in resource allocation between primary, secondary, and tertiary berries. All berries were washed and hulled before sampling. Sampling consisted of randomly selecting 100 g of Class I fruit. If less than 100 g of Class I fruit had been picked, all Class I fruit were sampled. The mean sample size across both years was 87.47 ± 23.76 g. The samples were then flash-frozen, freeze-dried, and milled into a fine powder, as previously described in Chapter 4 (Section 4.2.2).

In 2023, sampling took place on 10, 13, 17, and 20 July. The samples were reduced to ~50g before freeze-drying due to limited freeze-drier capacity. Once milled, sample composites were created by combining all sample material collected in the same week, as previously described in Chapter 5 (Section 5.2.5). Composite samples were prepared for the week commencing (w/c) 10 July and w/c 17 July 2023. The purpose of the composite samples was to ensure that there was sufficient material from each experimental replicate for analysis, whilst also accounting for inter-day and inter-week variability.

In 2024, sampling took place on 06 and 20 June 2024. The samples collected in 2024 were dried at their original sample weight and were not composited with samples from any other picks (Table 6.1). Composite samples were not deemed necessary in 2024 as sufficient sample material was collected from a single pick.

Samples from *Elsanta*, *Malling Centenary*, *Malling Vitality*, *Red Gauntlet*, *Sonata*, *Vibrant*, and *Vibrant*+ were collected on 06 June 2024. The samples from *Malwina* were collected on 20 June 2024 due to the genotype's later cropping cycle (Figure 6.13). The delay in *Malwina* cropping was such that the other genotypes had finished cropping by the time *Malwina* had started (Figure 6.13) As such, there was no suitable date to collect samples from all genotypes simultaneously. Consistency was maintained, however, in that the samples of all genotypes were collected close to the peak of their respective cropping periods.

6.2.2.2 Post-harvest Handling Scenarios

An additional experiment was designed for 2024 to investigate post-harvest changes in strawberry vitamin C content (Table 6.1). The experiment was designed to simulate the conditions that strawberries are exposed to from the point of harvest through to retailers and consumer households. Scenario development was based on personal communications with Dr Richard Colgan (Senior Fellow in Post-harvest Physiology, University of Greenwich) regarding ongoing commercial projects studying post-harvest handling of soft fruit in the UK.

The typical post-harvest handling of strawberries can be summarised in four key stages:

1) After being harvested, the berries remain at ambient temperature in the field for 2 - 4 hours.

2) The berries are rapidly chilled at 2 °C for 0.5 - 2.5 hours.

3) The berries are transported to retail distribution hubs on refrigerated lorries.

4) The berries remain in refrigerated conditions at the distribution hubs for between 24 and 72 hours before being dispatched to retail stores.

The first stage of all post-harvest scenarios was designed to mimic the typical timings and conditions that strawberries experience before being displayed on retailer shelves. All the post-harvest scenarios started with the same three steps:

1) Harvested berries would spend 4 hours at ambient temperature (T=4).
2) The berries were transferred to commercial chilling rooms for 2 hours at 2 °C (T=6).
3) The berries were then stored at 4 °C for 24 hours (T=30). The total time spent in pre-retail was 30 hours (Figure 6.3).

After the pre-retail stage, eight scenarios were developed that varied based on the timings and simulated storage conditions during retail display and within a consumer's household (Figure 6.3). The berries were kept in the retail display stage for either 24 (T=54) or 48 (T=78) hours after the pre-retail stage. Half of the berries were stored at ambient room temperature and half were stored at 4 °C to simulate conditions at retailers with standard and chilled display cabinets. After the retail display stage, all samples were kept at ambient room temperature for 1 hour to simulate the journey of the berries from retailer to consumer household (Figure 6.3). The berries then spent a further 48 hours within the consumer household stage (Figure 6.3).

The nomenclature for each scenario was abbreviated according to the stage (R = Retail, H = Household), conditions (A = Ambient, RF = Refrigerated), and time since harvest. For example, scenario RA54-HRF describes strawberries that had been displayed at a retailer (R) at ambient room temperature (A) for 24 hours (RA54) beyond the pre-retail stage. The berries were then transferred to a consumer household and stored in refrigerated (HRF) conditions thereafter. Combining the retail and household elements of the scenario derives the nomenclature RA54-HRF. Details of all eight scenarios are summarised in Figure 6.3.

The experiment used Class I berries from *Vibrant*+ plants to investigate the effects of each scenario on the vitamin C content of the berries. The material was sampled from *Vibrant*+ plants that were cultivated under the same conditions described in section 6.2.2.1. Each scenario started with ~1 kg of strawberries that were randomly selected from the *Vibrant*+ berries harvested on 06 June 2024.

Sub-sampling consisted of randomly selecting three berries from the starting pool of berries (~50 g). Sub-samples were taken from each scenario at T=0 and T=30. Further sub-samples for the scenarios in which berries spent 24 hours in the retail stage (RA54, RRF54) were taken at T=55, and T=103 (Figure 6.3). For the scenarios in which berries spent 48 hours in the retail stage (RA78, RRF78), sub-samples were taken at T=79, and T=127 (Figure 6.3). The sub-samples were processed for chemical analysis in the same way as described in section 6.2.2.1.

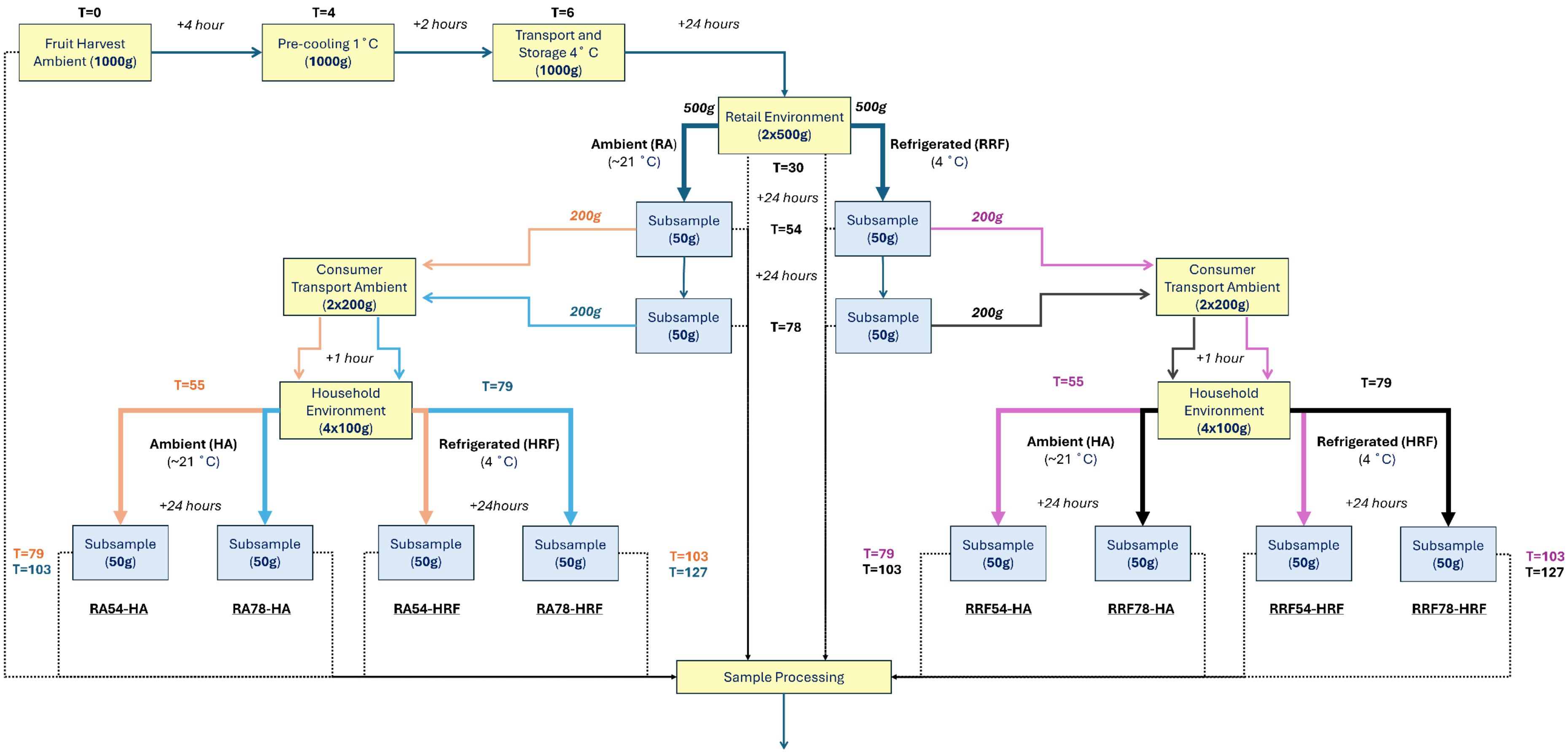


Figure 6.3 Diagrammatic representation of eight post-harvest scenarios designed to simulate typical conditions in the UK strawberry supply chain. Key milestones in each scenario are highlighted in the cream-coloured boxes. The solid Navy arrows connecting each milestone indicate the movement of the berries between milestones. The number of hours the berries spend at each milestone is indicated along the solid arrows. The subsequent timing notations at each milestone describe the cumulative hours since harvest. After sub-samples are taken at each milestone, the amount of remaining material is indicated within the cream-coloured boxes. Sub-sampling events are highlighted in the light blue boxes.

6.2.3 Chemical Analyses

6.2.3.1 Total Ascorbic and Organic Acids

Vitamin C content was measured as total ascorbic acid (AsA). The AsA, Malic acid, and Citric acid contents of the samples were extracted and quantified following the same procedures described in Chapter 4 (Section 4.2). Briefly, the organic acids were extracted from 0.15g of sample in 5ml 3% MPA / 1mM EDTA. An aliquot of 400µl from the resulting supernatant was then analysed by HPLC. The HPLC components and conditions were identical to those described previously (Chapter 4, Section 4.2.2.1).

Each sample was extracted from once. Samples of certified reference material BCR431 (Sigma-Aldrich Co, Gillingham, UK. Brussels sprouts (vitamins), BCR®, certified reference material, UNSPSC Code: 85151701. note: Product is discontinued as of 01/10/2024), an in-house strawberry reference material, two L-ascorbic acid standards, and two Malic/Citric acid standards were analysed concurrently with every batch of experimental samples. The references and standards enabled monitoring of the inter-extraction accuracy and precision, whilst also verifying that the calibration curve used for quantitation remained valid in each set of extractions.

Total ascorbic acid values were presented as mg/100g fresh weight. Malic and Citric acids were presented as mg/g fresh weight. Malic and Citric acid values were subsequently used to calculate the theoretical titratable acidities (TTA) for each sample using the same formula as described in Chapter 4 ($TTA = (citric * 10^{-1} + malic * 10^{-1})/(6.4/6.71)$).

6.2.3.2 Total Antioxidant Capacity and Total Phenolic Content

Sample extracts for analysis of total antioxidant capacity (AC) and total phenolic content (TPC) were prepared from 100 mg of sample in 10 ml 80% (v/v) methanol. The subsequent mixing and centrifugation steps were identical to those described for the AsA/organic acid extractions (Chapter 4, Section 4.2). Samples collected in 2023 were extracted in triplicate on three independent days. Samples from 2024 were extracted from once in conjunction with five samples of in-house reference material to monitor technical accuracy and precision.

Determination of AC was measured relative to the capacity of Trolox (Sigma Aldrich, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, CAS: 53188-07-1) in stabilising the radical cation ABTS.+ (Sigma Aldrich, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate), CAS: 30931-67-0), as described by Re et al. (1999).

Trolox equivalent antioxidant capacity (TEAC) assays were performed on the same day as the sample extraction. An ABTS.+ working solution was prepared by diluting ABTS.+ stock with analytical grade ethanol to an absorbance of ~0.900 at 734 nm and kept at 40 °C for the duration of the assay. For the TEAC assay, 3 ml of ABTS.+ working solution was added to 30 µl sample extract and incubated for 15 minutes in a 40˚C water bath. Absorbance at 734 nm was then measured and used to calculate the percentage inhibition relative to a blank ethanol standard.

A calibration series of five Trolox standards, ranging from 0.1 - 2 µM, was analysed before each set of sample extracts. Blank and Trolox standards were analysed with each set of sample extracts to enable the calculation of percentage inhibition and to verify that the calibration remained valid. Trolox standard concentrations were used to convert percentage inhibition to the proportional antioxidant concentration, expressed as µM Trolox equivalent per gram fresh weight (µM TE/g). Once analysed, the sample extracts were stored overnight in the dark at 4˚C in preparation for analysis of TPC.

Quantification of TPC was carried out using the Folin-Ciocalteu (FC) assay (Singleton and Rossi, 1965; Slinkard and Singleton, 1977). The sample extracts were diluted to a ratio of 100 µl sample extract to 200 µl MilliQ water. The diluted extracts were then mixed with 600 µl 10% (v/v) FC reagent (Sigma Aldrich, Folin & Ciocalteu's phenol reagent, Product number: F9252). After two minutes, 2500 µl 700 mM sodium carbonate was added (Fisher Scientific Ltd, Loughborough, UK., Sodium carbonate, 99.5%, extra pure, anhydrous, CAS: 497-19-8). The sample extracts were then incubated at room temperature for one hour in complete darkness before absorbance was measured at 765 nm. Gallic acid (GA) standards (0 - 80 µg/ml) were used for calibration before each set of sample extracts. The TPC of the sample extracts was expressed as mg Gallic acid equivalent (GAE) per gram fresh weight (mg GAE/g).

6.2.3.3 Sensory Traits

Additional assessments of berry colour, firmness, and sweetness were conducted in 2024 (Table 6.1). Having subjectively observed differences in colour and flavour during the 2023 experiment, it was deemed interesting to quantify these differences to investigate potential associations with AsA content.

Samples were taken for colour, firmness, and sweetness assessments on 10 June 2024 for all genotypes excluding *Malwina*. *Malwina* samples were analysed on 27 June 2024, again due to the late cropping of *Malwina* (Figure 6.13). Sampling consisted of a random selection of five Class I berries from each experimental replicate. The five selected berries were used to sequentially analyse berry colour, firmness, and total soluble solids content (TSS).

Berry colour was assessed using a Konica Minolta Chroma Meters CR-400 colourimeter (Konica Minolta Sensing Americas Inc, NJ, USA). The colour meter produced L*a*b* colour values for each berry separately. The five individual colour values were then used to calculate a mean colour for each sample. Mean L*a*b* colour values were subsequently used to calculate Hue angle ($Hue = arctan(b^*a^*)$) and Chroma $\left(Chroma = \sqrt{a^{*2} + b^{*2}}\right)$ values.

Berry firmness was then assessed using the same five berries previously analysed for colour. Firmness was measured using a Texture Analyser TA.XTPlus (Stable Micro Systems Ltd., Godalming, UK) with an 8mm probe diameter. Firmness was measured as the maximum recorded force (N) required to reach a depth of 8mm.

All five berries were then squeezed by hand to extract fruit juice into a single plastic cup. The TSS content of the composite juice was measured using an ATAGO digital refractometer (ATAGO Co. Ltd, Tokyo, Japan). Each juice composite was analysed three times to produce a mean TSS content for each sample. The TSS values were recorded as °*Brix* (%). The TSS values were then combined with the corresponding TTA values to calculate a TSS/TTA ratio. The TSS/TTA ratio provided an indexed measure of berry sweetness that has been previously shown to be a determinant of consumer preference (Ikegaya et al., 2021).

6.2.4 Statistical Analyses

Differences between genotypes on individual measures were analysed by one-way Analysis of Variance (ANOVA) as implemented in the {*car*} R package (Fox and Weisberg, 2019). Data residuals were visually inspected using histograms and quantile-quantile plots to assess conformity to the ANOVA assumptions of normality and homogeneity.

Sample sizes for each genotype varied due to some of the genotypes being assessed in both 2023 and 2024 (*Elsanta*, *Sonata*, *Vibrant*, *Malling Centenary*, *Malwina*: n = 20) and the others only being assessed in 2024 (*Red Gauntlet*, *Malling Vitality*, *Vibrant*+: n = 8). Model errors were adjusted using the Huber-White standard error corrections for heteroscedasticity, as implemented in the {*car*} R package, as a precaution against the potential effects of unequal group sizes. The fitted models also incorporated potential errors arising from the effects of the experimental blocks (variable ~ genotype + block). For those variables assessed in both 2023 and 2024 (AsA, AC, TPC), the fitted models further incorporated potential errors arising from the year of measurement (variable ~ genotype + year + block). Statistically significant differences between genotypes were accepted at an alpha threshold of 0.05.

Post-hoc comparisons were made using the Games-Howell test implemented in the {*rstatix*} R package (Kassambara, 2023). Games-Howell was chosen as the most suitable post hoc test due to its robustness to heteroscedasticity, again as a precaution to the potential effects of comparing groups of different sizes. The resulting *p*-values were adjusted for multiple testing and statistically significant differences were accepted at an alpha threshold of 0.05.

Post-harvest scenario data were analysed by two-way ANOVA as implemented in the {*stats*} R package. Two-way ANOVA was deemed a suitable model for the post-harvest scenarios due to the potential interactions between time since harvest and the unique set of conditions in each scenario.

Visualisations of between-group comparisons were inspired by the {*ggstatsplot*} R package (Patil, 2021) and colour palettes available in the {*ggsci*} R package (Xiao, 2024). All data points are presented for each set of measures. For AsA, AC, and TPC, data from each year are presented separately.

6.3 Results

6.3.1 Genotypic Variation

6.3.1.1 Vitamin C Content

A one-way ANOVA was carried out to study the effect of genotype on berry vitamin C content, measured as AsA. Having accounted for experimental blocking and year of harvest, the analysis showed that there was a statistically significant difference in AsA between at least two of the studied genotypes ($F(7,107) = [72.586]$, $p = 9.869e^{-38}$).

In both years, the highest AsA values were observed in *Elsanta*, *Vibrant*, *Vibrant+*, and *Malwina* (Figure 6.4). The measure AsA values ranged between 87.67 ± 1.85 (*Malwina*) and 94.35 ± 2.46 mg/100g FW (*Elsanta*). The post hoc analysis found no statistically significant differences between the AsA of *Elsanta*, *Vibrant*, *Vibrant+*, and *Malwina* ($p > 0.05$; Figure 6.4).

The AsA contents of *Malling Centenary*, *Malling Vitality*, *Red Gauntlet*, and *Sonata* ranged between 58.36 ± 1.85 (*Sonata*) and 69.97 ± 2.46 mg/100g FW (*Malling Centenary*) and were significantly lower than the AsA contents of *Elsanta*, *Vibrant*, *Vibrant+*, and *Malwina* ($p < 0.05$; Figure 6.4).

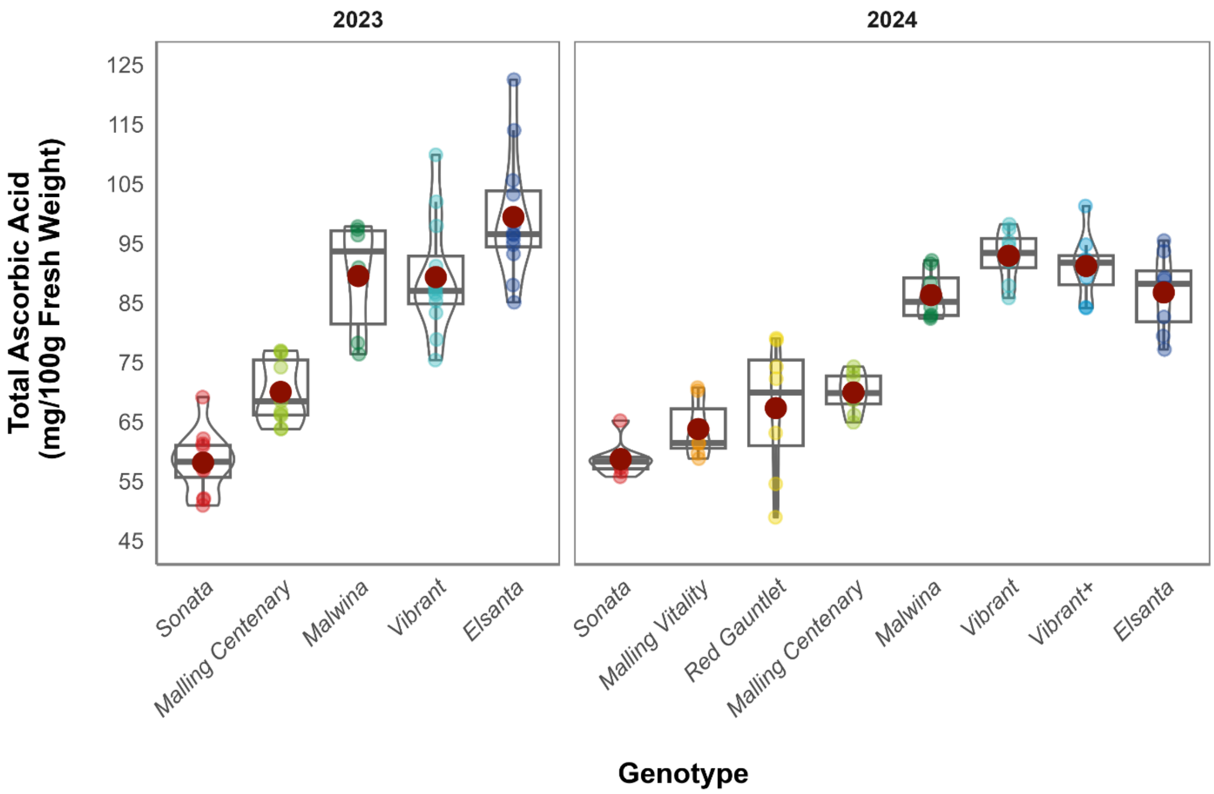


Figure 6.4 Box- and Violin-plots illustrating the differences in vitamin C content between seven UK strawberry genotypes. All genotypes were cultivated in a standard commercial polytunnel. The data are faceted to separate the values collected in 2023 (left panel) and 2024 (right panel). Vitamin C content is expressed as total ascorbic acid. The mean ascorbic acid content for each genotype is represented as a red dot.

6.3.1.2 Total Phenolics and Antioxidant Capacity

The effect of genotype on secondary nutritional components, namely AC and TPC, was subsequently investigated to understand if variability in vitamin C was an isolated trait or one that was associated with wider nutritional quality. Analysis by one-way ANOVA found a statically significant effect of genotype on both AC ($F(7,106) = [25.689]$, $p = 3.169e^{-20}$; Figure 6.5) and TPC ($F(7,107) = [75.846]$, $p = 1.431e^{-38}$; Figure 6.6).

Differences between the genotypes followed an almost identical pattern for AC and TPC (Figure 6.5 and Figure 6.6). *Malwina* had the highest mean AC (18.01 ± 0.45 μM TE/g FW) and TPC (2.07 ± 0.04 mg GAE/g FW). The AC and TPC of *Malwina* were significantly higher than the AC and TPC of *Sonata*, *Malling Vitality*, *Malling Centenary*, *Vibrant*, and *Vibrant+* ($p < 0.05$). The AC and TPC of *Malwina* were not significantly different from those of *Elsanta* (16.89 ± 0.27 μM TE/g, 1.94 ± 0.03 mg GAE/g, $p > 0.05$). *Sonata* had the lowest AC (12.89 ± 0.29 μM TE/g FW) and TPC (1.41 ± 0.02 mg GAE/g FW).

No statistically significant differences were observed between the AC measurements made in 2023 and 2024 ($F(1,106) = [1.672]$, $p = 0.199$). However, the year of harvest was found to have had a statistically significant random effect on TPC ($F(1,107) = [29.410]$, $p = 3.662e^{-07}$).

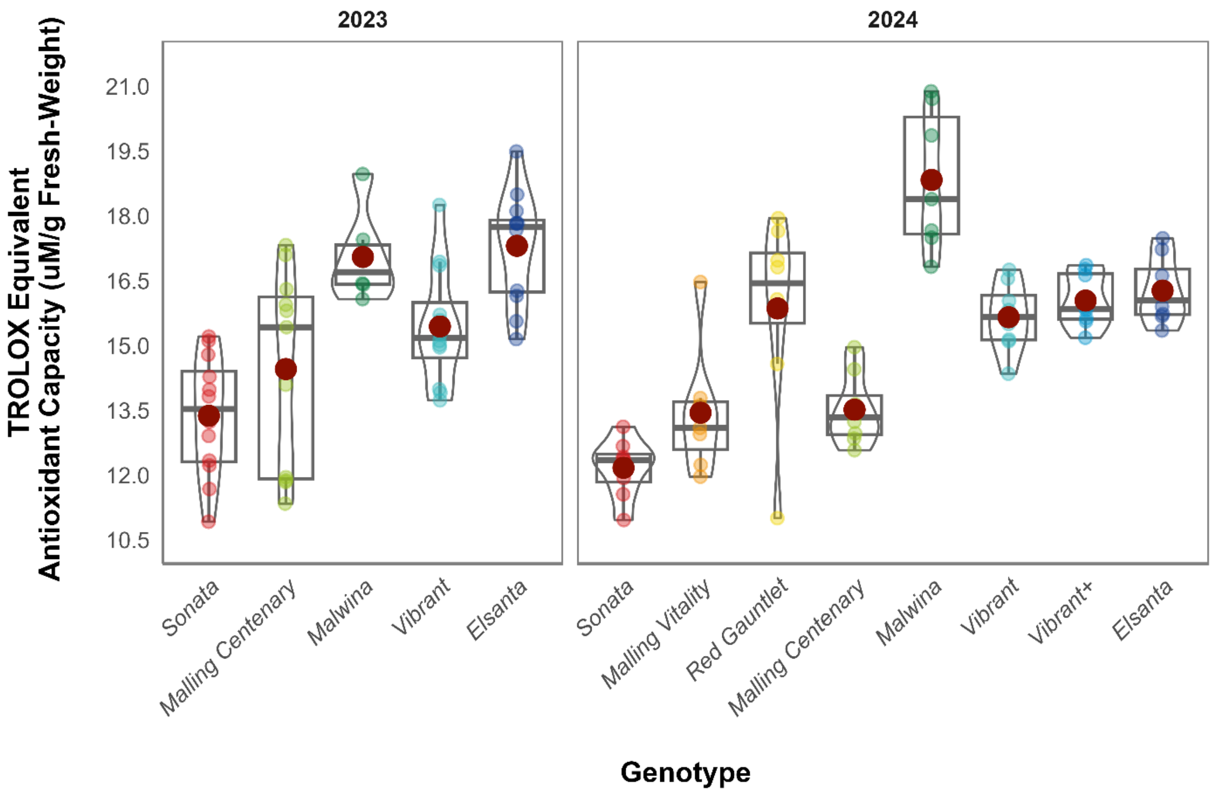


Figure 6.5 Box- and Violin-plots illustrating the differences in total antioxidant capacity (AC) between seven UK strawberry genotypes. All genotypes were cultivated in a standard commercial polytunnel. The data are faceted to separate the values collected in 2023 (left panel) and 2024 (right panel). Berry AC is expressed as TROLOX equivalent antioxidant capacity. The mean AC for each genotype is represented as a red dot.

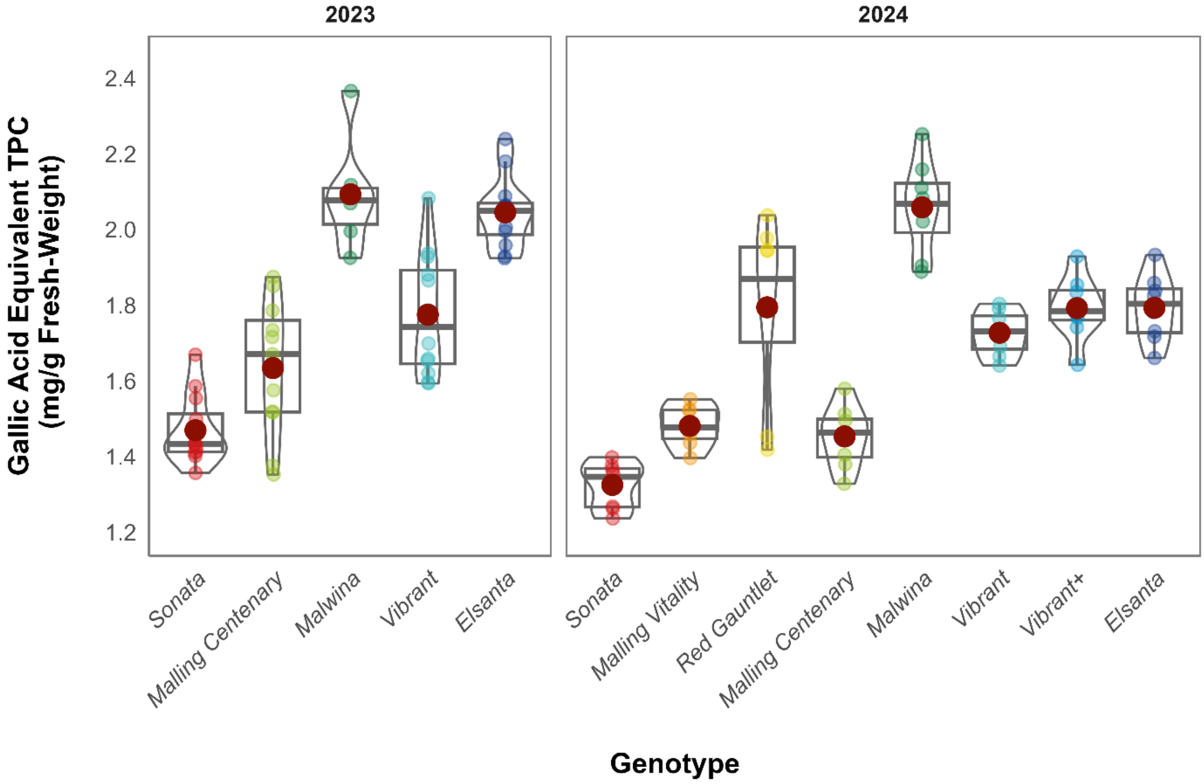


Figure 6.6 Box- and Violin-plots illustrating the differences in total phenolic content (TPC) between seven UK strawberry genotypes. All genotypes were cultivated in a standard commercial polytunnel. The data are faceted to separate the values collected in 2023 (left panel) and 2024 (right panel). The mean TPC for each genotype is represented as a red dot.

Correlation analyses were conducted to further investigate possible associations between AsA, AC, and TPC. Pearson's correlation coefficient analysis found significant correlations between AC and TPC ($t_{students}$(113) = 18.251, p = 1.749e^{-35}, $r_{pearson}$ = 0.86), AC and AsA ($t_{students}$ (113) = 9.811, p = 8.279e^{-17}, $r_{pearson}$ = 0.68), and AsA and TPC ($t_{students}$ (113) = 12.035, p = 5.678e^{-22}, $r_{pearson}$ = 0.75). The correlations between each pair of variables were all positive. The strength of the correlation was greatest between AC and TPC (r = 0.86) when compared to TPC and AsA (r = 0.75) and AC and AsA (r = 0.68) (Figure 6.7).

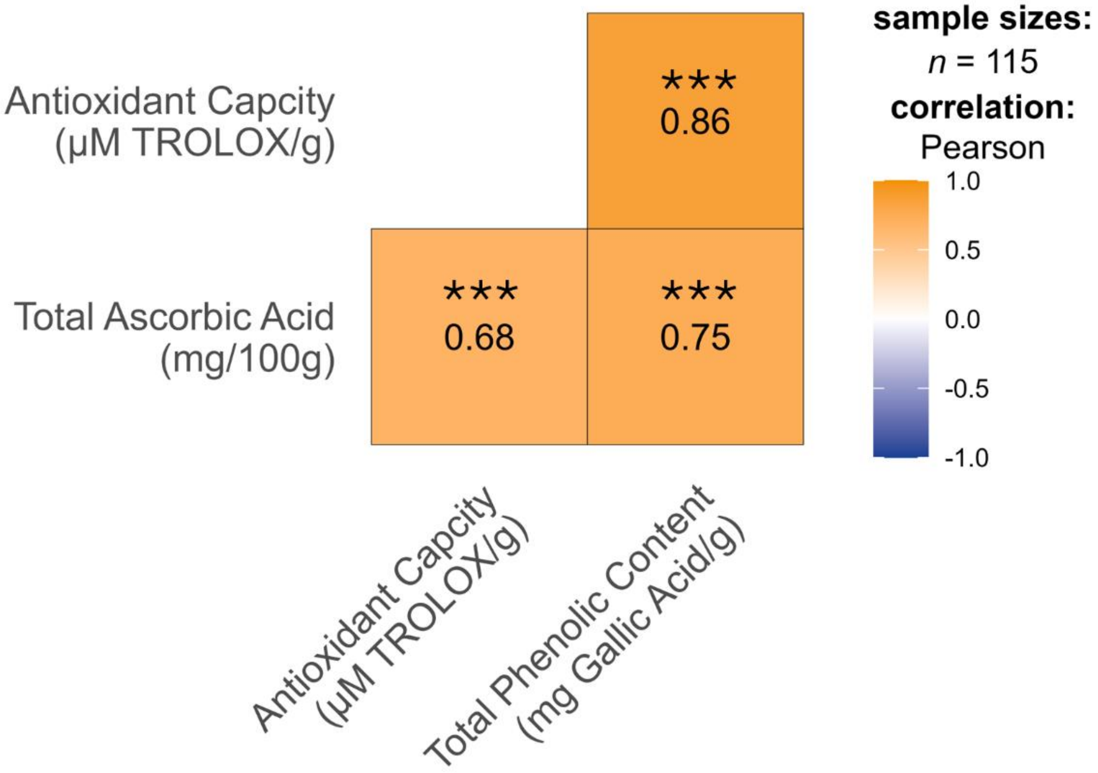


Figure 6.7 Correlation matrix illustrating the associations between berry ascorbic acid (AsA), antioxidant capacity (AC), and total phenolic content (TPC). The correlations are based on data collected from seven UK strawberry genotypes grown in standard polytunnel conditions in 2023 and 2024. The Pearson's correlation coefficient computed for each pair of observations is displayed on the matrix. The colour of each matrix square is determined by the strength of the correlation between the two variables, scaling from strongly negative (blue), neutral (white), and strongly positive (orange). Statistically significant correlations are marked with asterisks, with more asterisks signifying smaller *p* values ($p > 0.05$ *, $p > 0.01$ **, $p > 0.001$ ***).

6.3.1.3 Berry Colour, Firmness, and Sweetness

The epidermal colour was presented as a *Chroma* value. For a strawberry's predominantly red epidermis, higher *Chroma* values indicate a more vibrant red colour, whereas lower *Chroma* values indicate a darker red/purple colour. Statistically significant differences in *Chroma* were found between the genotypes ($F(7,47) = [196.942]$, $p = 1.331e^{-32}$; Figure 6.8).

The highest *Chroma* values were observed for *Malling Vitality* (55.05 ± 0.63), *Red Gauntlet* (53.76 ± 0.53), and *Sonata* (52.62 ± 0.38). The lowest *Chroma* values were observed in *Vibrant* (40.90 ± 0.62), *Vibrant*+ (39.57 ± 0.22), and *Malwina* (33.84 ± 0.60). Both *Elsanta* (49.70 ± 0.53) and

Malling Centenary (47.19 ± 0.76) were observed to have intermediate *Chroma* values relative to the other genotypes (Figure 6.8).

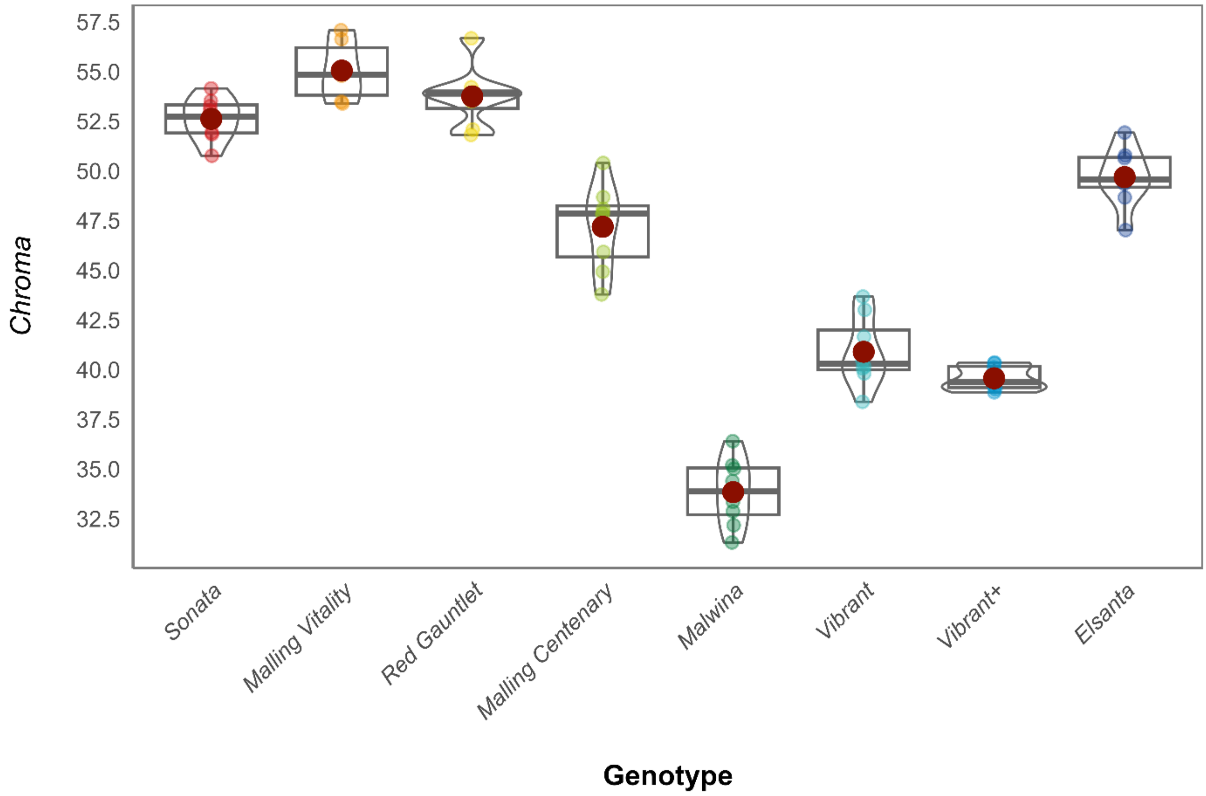


Figure 6.8 Box- and Violin-plots illustrating the differences in berry epidermal colour (Chroma) between seven UK strawberry genotypes. All genotypes were cultivated in a standard commercial polytunnel in 2024. The mean *Chroma* value for each genotype is represented as a red dot.

Visually, *Malwina* and *Vibrant* berries had a distinctly darker colouration relative to the other genotypes that otherwise shared a visually similar red colouration (Figure 6.9A). Side-by-side visual comparisons of *Malwina* and *Malling Centenary* berries further illustrate the contrast in epidermal colours (Figure 6.9B).

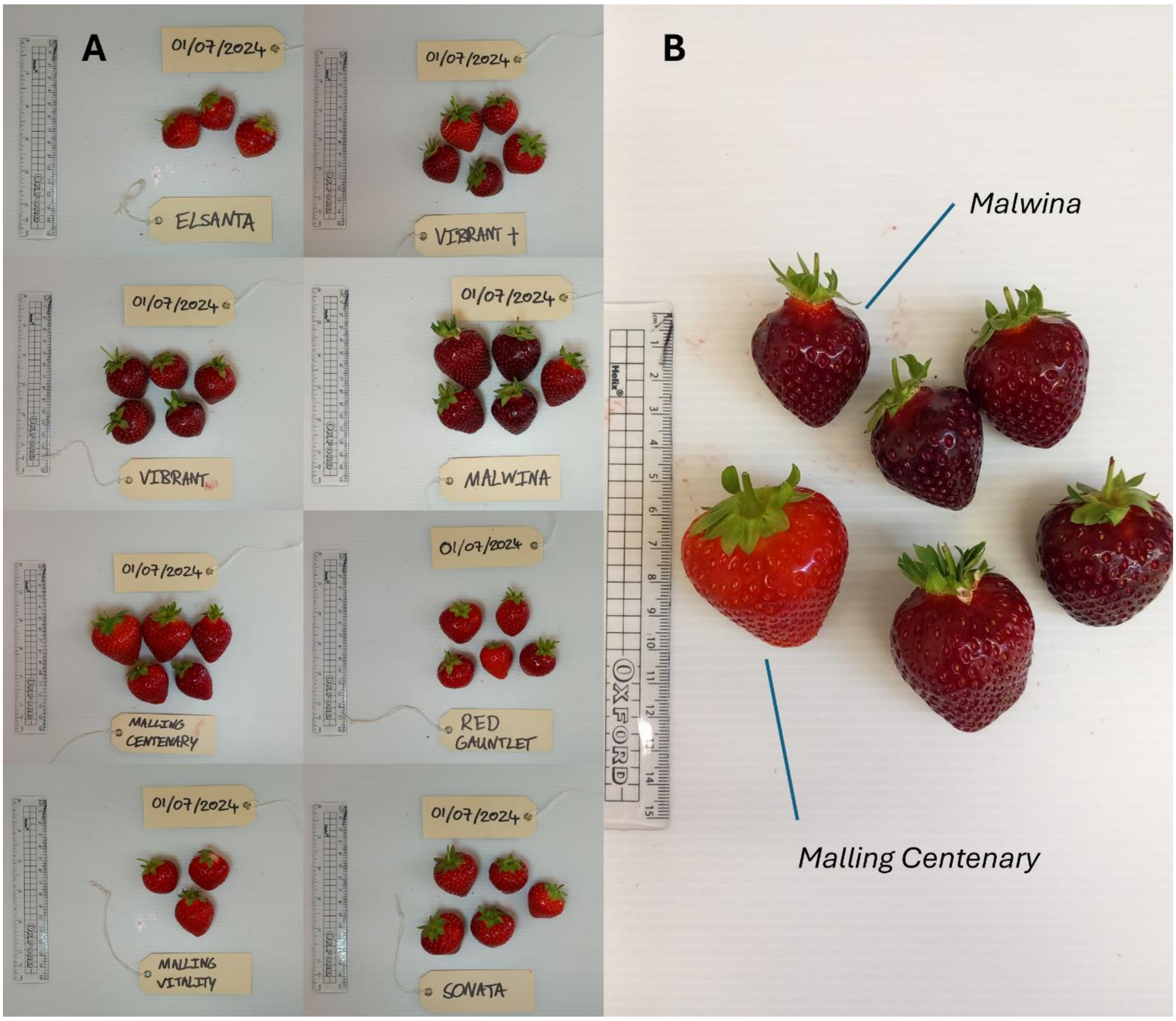


Figure 6.9 Images of berries produced from seven UK strawberry genotypes, illustrating the visual differences in epidermal colours. A) Images of berries harvested on 01 July 2024 for each genotype. B) Side-by-side images of berries from genotypes Malwina (five berries on the right of the image) and Malling Centenary (Single berry, furthest left on the image).

One-way ANOVA also identified statistically significant differences in berry firmness between genotypes (F(7,47) = [11.445], p = 2.247e-^{08}). The firmest berries came from *Malling Vitality* (6.11 ± 0.55 N). *Malling Centenary* berries had comparable firmness values (5.35 ± 0.23 N; $p > 0.05$; Figure 6.10). All other genotypes were significantly less firm than *Malling Vitality* and *Malling Centenary* ($p < 0.05$). *Elsanta*, *Vibrant*, *Vibrant+*, *Red Gauntlet*, and *Sonata* all exhibited similar firmness values, ranging between 2.95 ± 0.17 N (*Sonata*) and 3.67 ± 0.26 N (*Vibrant*; $p > 0.05$; Figure 6.10).

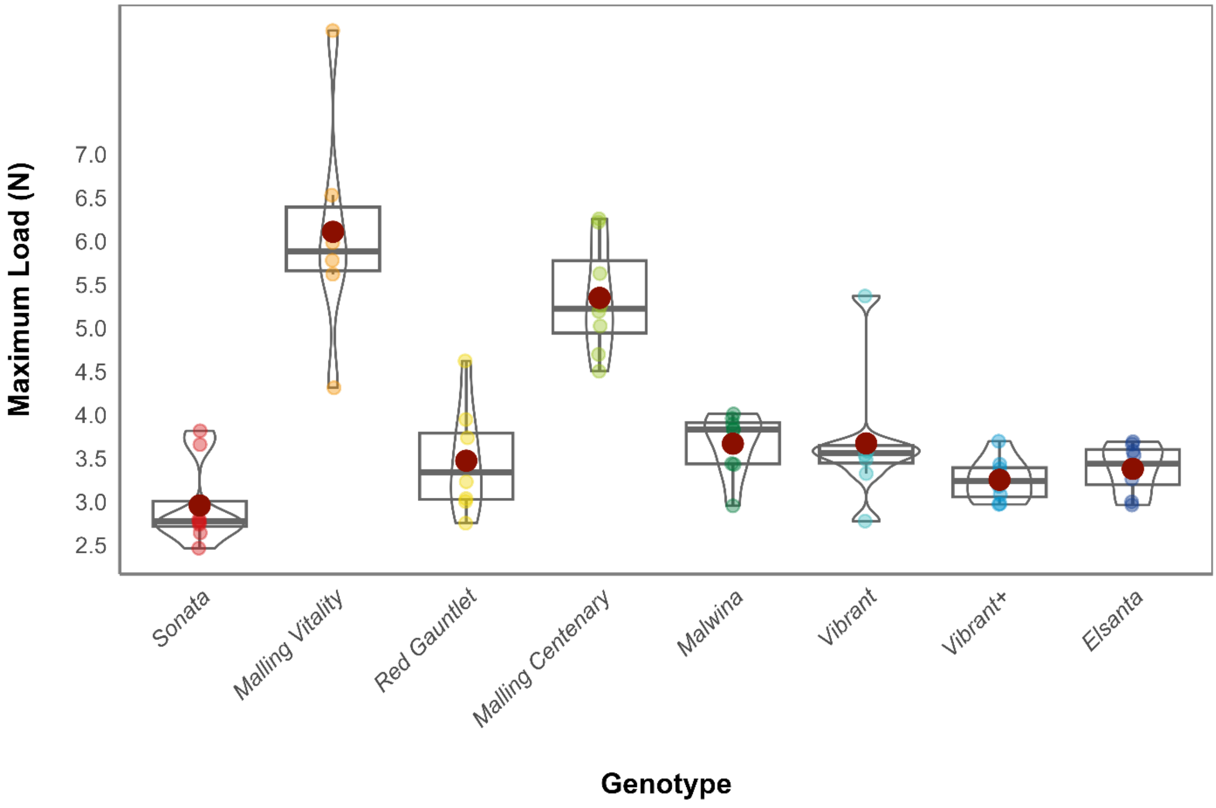


Figure 6.10 Box- and Violin-plots illustrating the differences in berry firmness (maximum load N) between seven UK strawberry genotypes. All genotypes were cultivated in a standard commercial polytunnel in 2024. The maximum load recorded for each genotype is represented as a red dot.

The ratio of TSS/TTA reflected the overall sweetness of the berries and was the final parameter of sensory quality assessed in the current study. One-way ANOVA found statistically significant differences in sweetness between the genotypes ($F(7,46) = [39.430]$, $p = 2.316e^{-17}$).

The highest mean sweetness value was observed for *Malling Centenary* (10.35 ± 0.27) and was significantly greater than the sweetness values for all other genotypes (Figure 6.11). No statistically significant differences were observed between the other genotypes ($p < 0.05$). Sweetness values ranged between 6.95 ± 0.15 (*Malwina*) and 7.76 ± 0.38 (*Elsanta*).

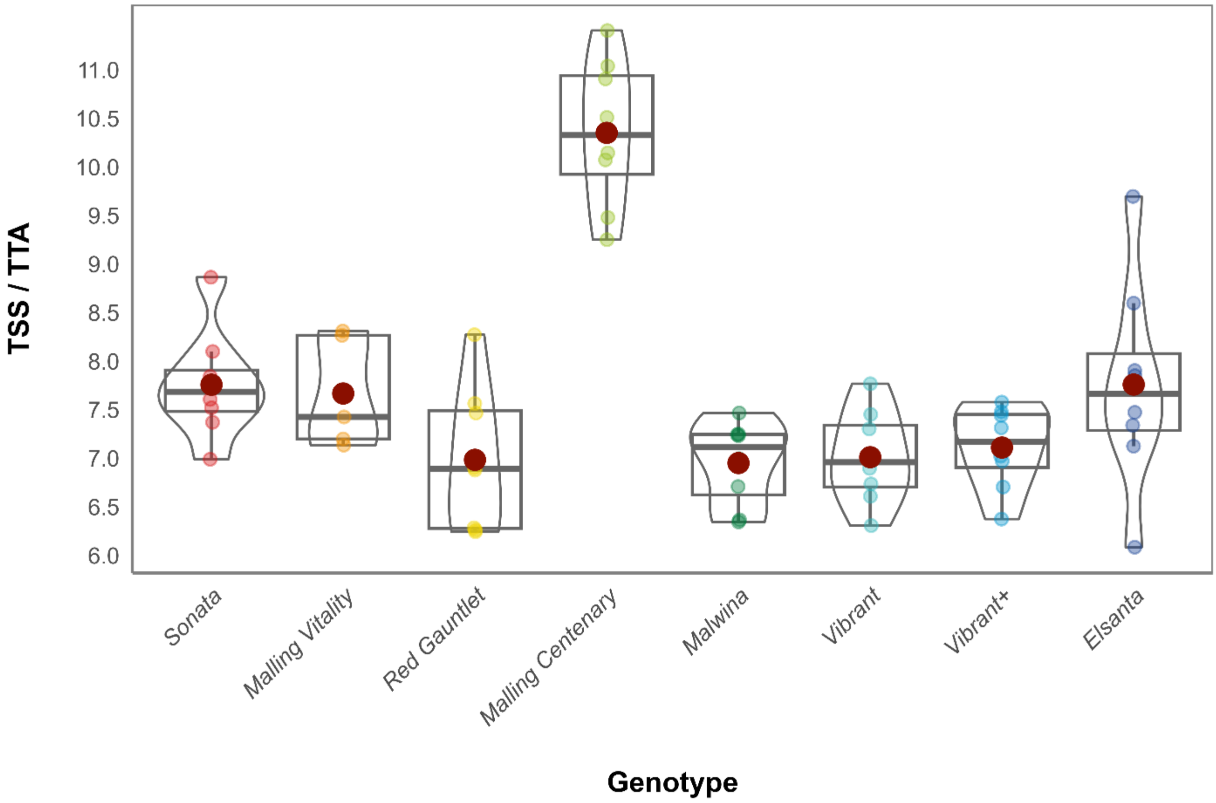


Figure 6.11 Box- and Violin-plots illustrating the differences in berry sweetness between seven UK strawberry genotypes. Values for overall sweetness are a relative index derived from the ratio of total soluble solids (TSS) and theoretical titratable acidity (TTA). All genotypes were cultivated in a standard commercial polytunnel in 2024. The mean TSS/TTA ratio for each genotype is represented as a red dot.

6.3.1.4 Yield

In 2023, the peak yields for *Sonata*, *Malling Centenary*, *Vibrant*, and *Elsanta* occurred in the w/c 10 July 2023. The peak yields for *Malwina* occurred 10 days later than the other genotypes, between 20 and 24 July 2023 (Figure 6.12).

The productivity of each genotype was subsequently compared based on the total mass of berries produced per plant (Table 6.3). One-way ANOVA found statistically significant yield differences between genotypes ($F(4,25) = [24.275]$, $p = 2.687e^{-08}$). The greatest yields were recorded for *Sonata*, which produced 254 ± 8 g/plant. Intermediate yields were observed for *Elsanta* (207 ± 6 g/plant) and *Vibrant* (202 ± 6 g/plant), whilst the lowest yields occurred in *Malwina* (134 ± 12 g/plant) and *Malling Centenary* (122 ± 18 g/plant).

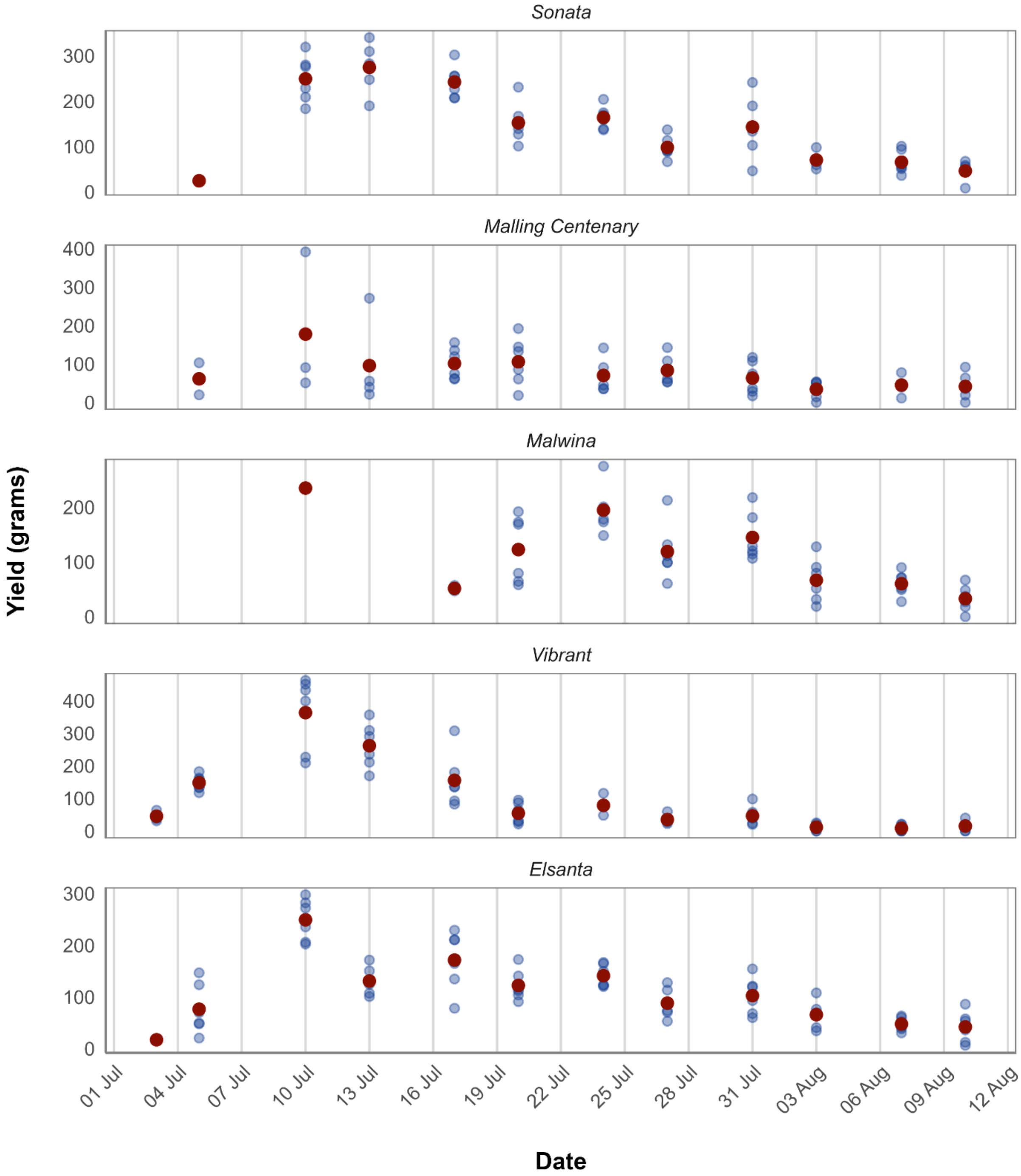


Figure 6.12 Dot plots showing strawberry yield profiles for five genotypes cultivated under standard commercial conditions between 14 June and 10 August 2023. Blue points indicate the yields recorded from each biological replicate. The mean yield recorded at each pick is represented as a red dot.

Table 6.3 Strawberry yields recorded for five genotypes cultivated between 14 June and 10 August 2023.

	Genotype						
Characteristic	***Sonata*** ***n* = 6**	***Malling Centenary*** ***n* = 6**	***Malwina*** ***n* = 6**	***Vibrant*** ***n* = 6**	***Elsanta*** ***n* = 6**	**F-value[1]**	**p-value[1]**
Total Yield (g/plant)						20	<0.001
Mean (SE)	254 (8)	122 (18)	134 (12)	202 (6)	207 (6)		

[1]One-way analysis of means (not assuming equal variances)

Concerning the relative timings of peak yields for each genotype, the trends observed in 2024 were similar to those described for 2023 (Figure 6.13). The peak yields for *Malling Vitality*, *Malling Centenary*, *Elsanta*, *Vibrant*, *Vibrant*+, *Red Gauntlet*, and *Sonata* all occurred between 06 and 10 June 2024 (Figure 6.13). The delay in Malwina cropping was greater in 2024 than in 2023. Peak yields for Malwina occurred on 23 June 2024, 17 days later than the other genotypes.

In 2024, the collection of additional data meant that the productivity of each genotype could be compared based on the total mass of berries produced per plant, the total mass of Class I berries produced per plant, the percentage of Class I/II/waste yields, and the average mass of Class 1 berries (Table 6.4). One-way ANOVA showed that yield differences between genotypes were statistically significant for all considered yield parameters ($p < 0.001$).

Similarly to 2023, *Sonata* was the highest-yielding genotype in the 2024 study. Class I yields for Sonata amounted to 250 ± 12 g/plant, which accounted for 93% of *Sonata*'s total yield. *Malling Centenary* (96%), *Malwina* (97%), and *Vibrant* (94%) showed similarly high percentage Class I yields. However, the total masses of Class I fruit were between 69 and 111 grams/plant less than that of *Sonata*. *Elsanta* produced a similar mass of Class I berries to *Malling Centenary* (155 ± 11 g/plant). *Elsanta*'s Class I yields only accounted for 71% of total yields, which was significantly lower than the genotypes already described. *Red Gauntlet* and *Malling Vitality* were the only genotypes to yield less than 100 g/plant of Class I fruit. *Malling Vitality* also had the lowest Class I percentage of all the studied genotypes at 63% (Table 6.4).

Concerning the size of Class I berries, *Malwina* and *Malling Centenary* produced larger berries than all other genotypes and were the only genotypes with a mean Class I berry size greater than 20 grams (21.42 ± 0.22 and 21.17 ± 0.54 g, respectively).

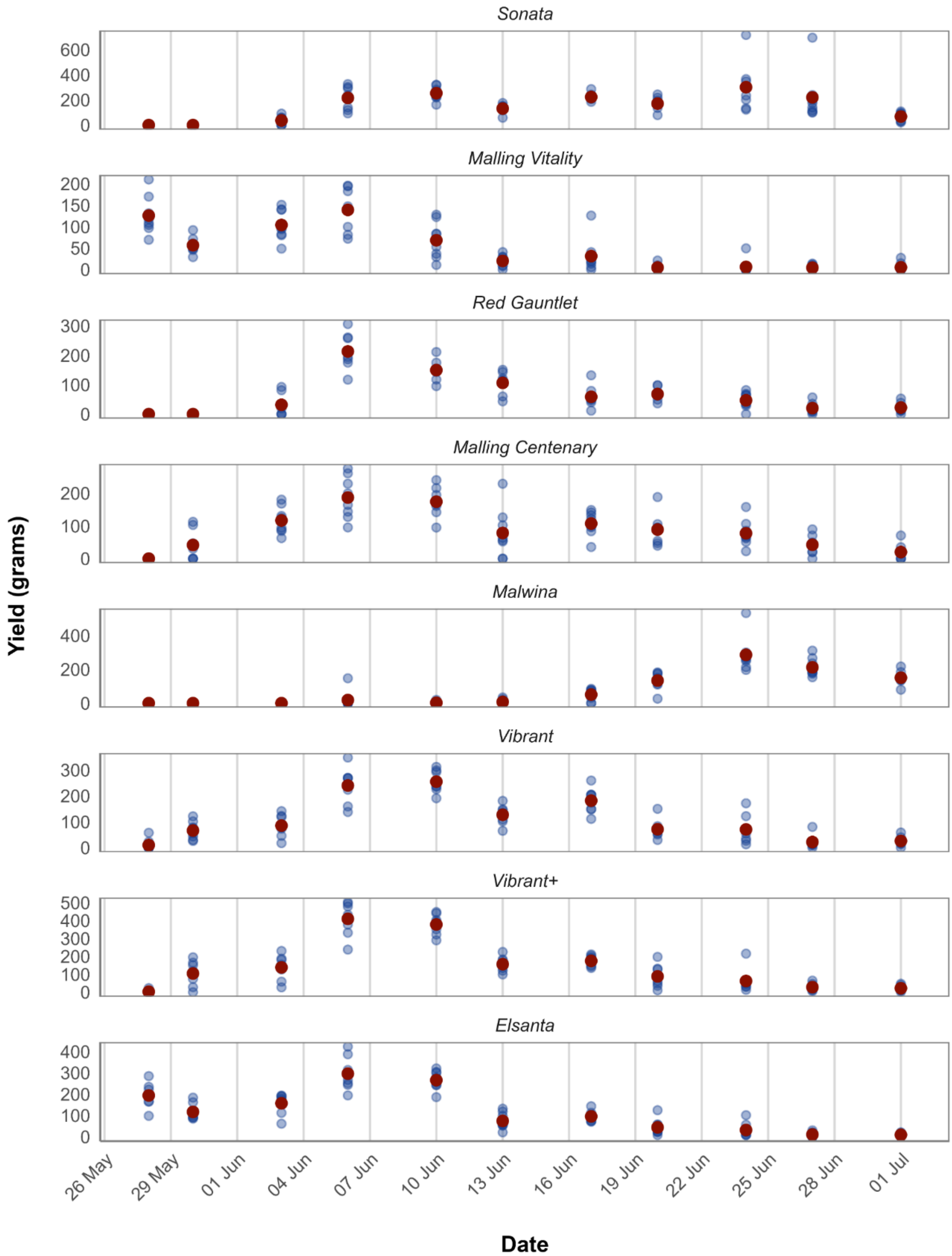


Figure 6.13 Dot plots showing strawberry yield profiles for seven genotypes cultivated under standard commercial conditions between 09 April and 01 July 2024. Blue points indicate the yields recorded from each biological replicate. The mean yield recorded at each pick is represented as a red dot.

Table 6.4 Strawberry yields recorded for seven genotypes cultivated between 09 April and 01 July 2024.

	Genotype									
Characteristic	***Sonata* *n* = 8**	***Malling Vitality* *n* = 8**	***Red Gauntlet* *n* = 8**	***Malling Centenary* *n* = 8**	***Malwina* *n* = 8**	***Vibrant* *n* = 8**	***Vibrant+* *n* = 8**	***Elsanta* *n* = 8**	**F-value[1]**	**p-value[1]**
Total Yield (g/plant)									36	**<0.001**
Mean (SE)	270 (14)	94 (7)	120 (7)	156 (16)	144 (7)	193 (5)	253 (12)	216 (12)		
Class 1 Yield (g/plant)									62	**<0.001**
Mean (SE)	250 (12)	60 (4)	99 (5)	150 (16)	139 (7)	181 (6)	222 (11)	155 (11)		
Percentage Class 1 (%)										
Mean (SE)	93 (1)	63 (1)	83 (2)	96 (1)	97 (1)	94 (1)	87 (1)	71 (2)		
Average Class 1 Berry Size (g)									48	**<0.001**
Mean (SE)	18.60 (1.19)	17.54 (0.58)	15.36 (0.49)	21.17 (0.54)	21.42 (0.22)	17.53 (0.36)	16.57 (0.16)	16.86 (0.39)		

[1]One-way analysis of means (not assuming equal variances)

6.3.2 Post-Harvest Handling and Storage Scenarios

A final experiment was carried out to study changes in vitamin C content over time in eight different post-harvest scenarios. Two-way ANOVA showed that there was no interaction between scenario and time-since-harvest ($F(20,98) = 0.605$, $p = 0.901$). Simple main effects analysis showed that scenario also had no significant effect on AsA ($p = 0.661$) whereas the time since harvest did have a statistically significant effect on AsA ($p = 4.051e^{-09}$).

The mean AsA at T=0 was 90.04 ± 1.58 mg/100g fresh weight. For all scenarios, AsA was found to remain stable between T=0, T=30, and T=55 (Figure 6.14; $p > 0.05$).

For the scenarios that incorporated 24 hours at the retail stage, statistically significant differences were found between the AsA at T=0 and T=103 in scenarios RA54-HA (105.81 ± 1.61 mg/100g), RRF54-HA (100.76 ± 3.90 mg/100g), and RRF54-HRF (97.86 ± 1.82 mg/100g; $p < 0.05$). In contrast, AsA in berries from scenario RA54-HRF were found to remain stable between T=0 and T=103 (90.84 ± 1.55 mg/100g; $p > 0.05$ Figure 6.14).

Similar trends were observed in the scenarios that incorporated 48 hours at the retail stage (Figure 6.14). Statistically significant differences were found between the AsA of berries at T=0 and T=127 in all scenarios (RA78-HA - 111.23 ± 1.97 mg/100g, RA78-HRF - 99.46 ± 1.70 mg/100g, RRF78-HA - 103.41 ± 6.03 mg/100g, RRF78-HRF - 101.96 ± 4.41 mg/100g; $p < 0.05$).

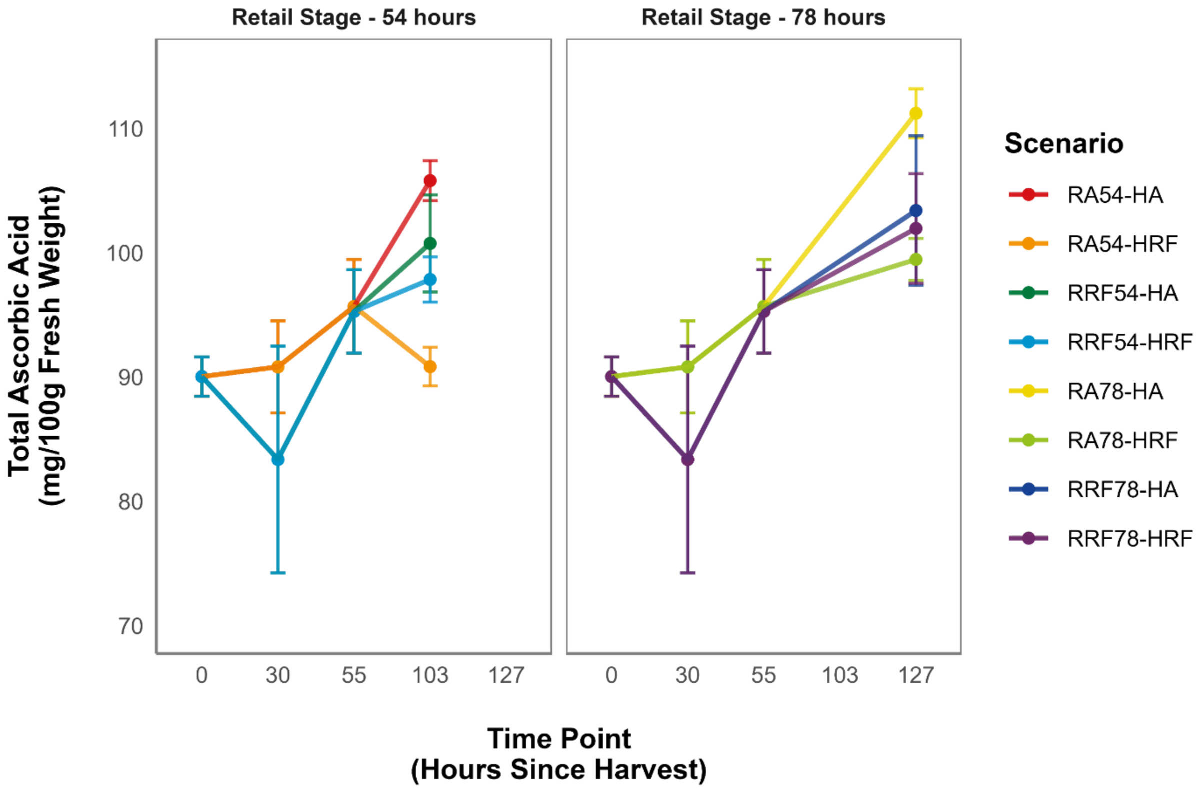


Figure 6.14 Line graph illustrating changes in strawberry total ascorbic acid content in eight post-harvest handling scenarios. The scenarios were designed to simulate similar conditions to those in commercial strawberry supply chains. All scenarios used strawberries produced from genotype *Vibrant* in 2024. Berry ascorbic acid content was measured at four time points in each scenario. The scenario names refer to the time and conditions at the pre- and post-retail stages of the supply chain, e.g. RA54-HA = Retail Ambient (54 hours) – Household Ambient; RRF78-HRF = Retail Refrigerated (78 hours) – Household Refrigerated. The plot is split into the scenarios that spent 24 hours at the retail stage (left panel) and the scenarios that spent 48 hours at the retail stage (right panel).The time points represent the ascorbic acid content at key milestones in the simulated strawberry supply chain; Harvest (T=0), arrival at retail markets (T=30), arrival at consumer households (T=55), and after 48 hours in consumer households.

6.4 Discussion

6.4.1 Genotypic Variation in the Vitamin C Contents of UK Strawberries

In Chapter 6, the vitamin C contents of seven UK strawberry genotypes were characterised under standard commercial cultivation conditions. The studied genotypes were found to segregate into two groups of either "high" (>85 mg AsA/100g FW) or "low" (58-70 mg AsA/100g FW) vitamin C content, with the highest values occurring in *Elsanta*, *Vibrant*, and *Malwina*.

The vitamin C contents observed in the current study were consistent with previous studies conducted under commercial cultivation conditions. Previous characterisations of *Elsanta* (Skupień and Oszmiański, 2004) and *Malling Centenary* (Woznicki et al., 2023) have reported values within 10% of those reported in the current study. The reported values for *Malling Centenary* and *Vibrant* are also consistent with the data presented in Chapter 5 (Section 5.3.1).

In the context of UK strawberry genotypes, the findings of the current study therefore indicate that *Elsanta*, *Vibrant*, and *Malwina* could be useful resources in efforts to breed for increased vitamin C content in new genotypes.

The relative differences in vitamin C content of the seven genotypes amounted to 1.6-fold. This variation was consistent over two consecutive seasons in 2023 and 2024. A greater number of genotypes were characterised in Chapter 6 when compared to Chapter 5 and this has exposed greater variation than was initially observed between *Vibrant* and *Malling Centenary* (Section 5.3.1). Other studies have reported similar variations in strawberry vitamin C content under commercial cultivation conditions. For example, Skupień and Oszmiański (2004) observed 1.6-fold variation between six European strawberry genotypes, whilst Kim et al. (2015) observed 1.9-fold variation between 14 Japanese and Korean strawberry genotypes. The variation in UK-relevant genotypes is, therefore, consistent with the relative variation previously observed in non-domestic strawberry genotypes.

However, the true extent of variation in UK strawberries may be greater than that observed in the current study. The Marche Polytechnic strawberry breeding programme in Italy has reported 3.1-fold variation in vitamin C content between 15 genotypes (Capocasa et al., 2016; Mezzetti et al., 2016). Mezzetti et al. (2016) suggest that the greater variation in their study can be attributed to the inclusion of non-commercialised breeding lines and commercially available genotypes. The authors also found that the greatest vitamin C contents occurred in the breeding lines and not in the commercial genotypes (Mezzetti et al., 2016). Genome-wide association Studies (GWAS) have also reported greater than 2.6-fold variation in vitamin C content using diverse strawberry mapping populations (Muñoz et al., 2023). Similar observations have also been made in breeding populations for Pepper (Geleta and Labuschagne, 2006), Spinach (Rueda et al., 2022), and tomatoes (Bhandari et al., 2016). As a result, further analysis of larger and more diverse UK strawberry populations may be required to effectively evaluate the potential to selectively breed for increased vitamin C content.

Populations consisting of over 400 UK strawberry genotypes have previously been used to study variations in other quality traits (Cockerton et al., 2021; Li et al., 2020). However, the vitamin C content in these mapping populations has yet to be characterised. The preliminary genotype screen described in Chapter 5 found 1.7-fold variation between one commercially available cultivar and three UK breeding lines (Section 5.2.2). Although the variation in the preliminary screen was consistent with the current study, the number of breeding lines that remain uncharacterised for vitamin C content could harbour greater variation. Considering that the necessary genetic material already exists, there is certainly potential to increase the scope of vitamin C characterisation in UK strawberries.

Expanding the characterisation of vitamin C contents to a greater number of genotypes could also facilitate a better understanding of the genetic determinants of vitamin C-dense phenotypes. Variable vitamin C content in food crops has been associated with mutations in several specific genes, primarily related to the D-Mannose/L-Galactose Pathway (Lin et al., 2021; Paciolla et al., 2019). *In situ*, however, the allo-octoploid nature of the cultivated strawberry genome has made it difficult for GWAS to identify robust markers that explain variations in vitamin C content (Muñoz et al., 2023; Vallarino et al., 2019). The genomes of ancestral diploid strawberry species, such as *Fragaria vesca*, have a high degree of synteny with the sub-genomes of cultivated strawberry and are commonly relied upon to validate potential genetic markers (Vallarino et al., 2019). There is still, however, a need to functionally characterise potential genetic markers in cultivated strawberry to account for potential interactions between gene homologues on each of the sub-genomes. The study of the genetics underpinning the variation in vitamin C content was beyond the scope of the current study. Continued efforts to identify robust genetic loci associated with vitamin C will, however, enhance our understanding of the observed variation. This enhanced understanding would, in turn, enable more targeted efforts to improve vitamin C content in strawberries.

6.4.2 Associations Between Vitamin C Content and Secondary Metabolites

The genotypes with higher vitamin C content tended to have higher amounts of phenolic and antioxidant compounds. This apparent co-variation between vitamin C, antioxidant capacity, and total phenolic content can be understood by considering the composition and antioxidant activity of individual phenolic compounds. Vitamin C has been shown to contribute more than 30% to the total antioxidant capacity of strawberries (Tulipani et al., 2008). It therefore follows that genotypes that are higher in vitamin C also exhibit greater antioxidant capacities (Capocasa et al., 2008), as was observed in the current study.

Furthermore, the primary phenolic constituents of strawberries include anthocyanins, tannins and phenolic acids (Aaby et al., 2012; Nowicka et al., 2019). Anthocyanins and ellagitannins have been shown to make significant contributions to overall AC in strawberries, which could explain the correlation between TPC and AC (Tulipani et al., 2008). That said, not all studies have observed correlations between TPC and AC (Dzhanfezova et al., 2020). The presence or absence of a correlation in different studies could be due to the genotypes used in those studies. The specific composition of phenolic compounds has been found to strongly vary between genotypes (Nowicka et al., 2019). This means that some genotypes could have a high phenol content, but relatively low antioxidant capacity if those phenols are comprised of compounds with lower antioxidant activity (Proteggente et al., 2002). The fact that a positive correlation was observed in the current study is suggestive that the UK genotypes comprised relatively high amounts of tannins and anthocyanins. Further analysis would, however, be required to quantify the contents of individual phenolic compounds in the UK genotypes.

Concerning human health, anthocyanins, ellagitannins, flavonols, and other potent antioxidants in strawberries have been linked to cardioprotective, neuroprotective, anti-inflammatory, anti-diabetic, anti-carcinogenic and obesity suppressive benefits (Afrin et al., 2016; Giampieri et al., 2015). As a result, breeding for increased vitamin C content in UK strawberries could also yield higher levels of health-promoting secondary metabolites, providing a double benefit to both vitamin C security and wider dietary quality.

6.4.3 Associations Between Vitamin C and Sensory Traits

In the current study, *Elsanta*, *Malwina* and *Vibrant* had consistently high vitamin C, AC and TPC. Both *Malwina* and *Vibrant* were also observed to be among the darkest, softest, and most acidic of the studied genotypes. Several studies of consumer preference have highlighted the importance of high sweetness and moderate firmness values in strawberries (Bhat et al., 2015; Fan et al., 2021; Ikegaya et al., 2021). As such, it is important to consider whether selective breeding for higher vitamin C content in UK strawberry genotypes could be associated with lower consumer preference.

Of the genotypes included in the current study, *Malling Centenary* could be considered the most popular in the current UK strawberry market due to it being the second most frequently sampled genotype in Chapter 4 (Section 4.3.1). *Malling Centenary* had significantly lower vitamin C, AC and TPC when compared to the more nutrient-dense genotypes but was also the firmest and sweetest. *Malling Centenary* was also significantly lighter in colour than *Vibrant* and *Malwina*. Similar trends were observed for *Malling Vitality*, which was the most recently released commercial genotype included in the current study.

The relative popularity of the studied genotypes suggests that high vitamin C contents and desirable sensory qualities have become decoupled in UK strawberries. However, higher vitamin C content is not exclusively related to less desirable sensory qualities. Other studies have found that vitamin C content was positively correlated with sweetness and firmness in some genotypes (Aubert et al., 2021; Temocico et al., 2019). Furthermore, recently released genotypes for the European strawberry market emphasise the possibility of breeding for both good sensory and nutritional quality (Capocasa et al., 2016).

In the context of the current study, *Elsanta* provides an example of a genotype that had both high nutritional quality and sufficient sensory and commercial quality to still occupy a share of modern UK strawberry markets (Chapter 4, Section 4.3.1). Balancing improvements in nutritional and sensory quality is certainly possible. Ultimately, the findings of the current study reaffirm the need to expand future studies to larger and more diverse populations.

6.4.4 Associations Between Vitamin C and Productivity

Significant variation was also observed in the productivity of each genotype. This was to be expected given that increased productivity is a primary goal of modern breeding programmes (Mezzetti et al., 2018). As such, the importance of genotype in determining crop productivity is commonly reported in the literature (Ariza et al., 2021; Zannat et al., 2023).

There was, however, no clear association between productivity and vitamin C content in the current study. In fact, the genotypes with the lowest vitamin C content, *Sonata* and *Malling Vitality*, were the most and least productive genotypes, respectively. The Class I yield recorded for *Malling Vitality* in the 2024 experiment was, however, much lower than expected. Unpublished data from other trials at Niab East Malling have recorded Class I yields between 395-457 grams/plant for Malling Vitality. These yields are 6-fold greater than the yields recorded in the current study. As was discussed in Chapter 5, there are several possible explanations for the lower-than-expected yields (Section 5.4.5). However, the inferences that can be made from the yield data in the current study are limited due to the yields being inconsistent with expectations for all genotypes.

In addition to total yield, berry size is another primary objective in contemporary strawberry breeding programs (Simkova et al., 2023). In the present study, *Malwina* exhibited the largest mean berry weight at 21 g/berry during the 2024 season. *Malwina* was also among the genotypes with the highest levels of vitamin C, TPC, and AC. Previous research by Simkova et al. (2023) reported a similar positive association between berry size and both sugar and vitamin C concentrations, whereas smaller berries tended to contain greater concentrations of anthocyanins. Tin relation to vitamin C, these findings suggest that nutrient accumulation may scale with berry size, rather than being diluted in larger fruit. As such, secondary and tertiary berries, which are typically smaller than primary berries, may contain lower concentrations of vitamin C. Further investigation will be required to elucidate the mechanisms of nutrient allocation within strawberry fruit, how these scale with berry size, and whether there is a reduction in vitamin C content in secondary and tertiary fruit. Such investigations would be particularly interesting in day-neutral/everbearing genotypes that are harvested over much longer periods than the short-day/June bearing varieties studies in Chapter 6. Nonetheless, the present findings suggest a promising alignment between vitamin C biofortification and breeding for increased berry size, indicating that there is potential to develop of cultivars that are both high-yielding and nutritionally enhanced.

Future studies must analyse vitamin C contents when commercially acceptable yields are achieved. This will ensure that there are no negative associations between selecting for increased vitamin C content and maximizing crop productivity. Synergistically, future research could also investigate whether there are any benefits associated with increased vitamin C content. Many plant phytochemicals, including vitamin C, are involved in environmental stress responses to heat or drought (Macknight et al., 2017; Zhang et al., 2020a). Similar positive associations have also been made between vitamin C content and decreased susceptibility to fungal infection (Davey et al., 2007). Such traits are all relevant to crop productivity and commercial value. Identifying direct links between vitamin C and productivity could create an incentive for breeders to target high vitamin C genotypes in future breeding efforts.

6.4.5 Changes in Vitamin C Content During Post-harvest Handling

The final element of the current study considered the effects of post-harvest handling on strawberry vitamin C content. In *Vibrant* berries, vitamin C content was found to increase over time under all the tested scenarios. This is in contrast to a similar study that analysed the vitamin C contents of Canadian strawberries at harvest, wholesale, and retail stages of the supply chain (Russell et al., 2009). The storage conditions and overall timings between harvest and retail were similar to those imposed in the current study. This is a positive reflection that the scenarios designed for the current study were a good reflection of real-world strawberry supply chains. However, Russel et al. (2009) observed an opposing trend to the current study, finding vitamin C content to decrease between harvest and wholesale/retail markets by ~10% over 48-54 hours.

Other studies of post-harvest changes in strawberry vitamin C have reported mixed findings. Shin et al. (2007) found that the vitamin C content of *Jewel* strawberries remained stable for 96 hours at 10 °C, but decreased by ~10% after 48 hours at 0.5 °C and 20 °C. In *Dover*, *Campinero*, and *Oso Grande* strawberries, Cordenunsi et al. (2005) observed vitamin C contents to remain stable for 72 hours when stored at room temperature. Moreover, Cordenunsi et al. (2005) observed statistically significant increases in vitamin C content for berries stored at 16 °C for 6 days. Similar trends have also been reported for post-harvest increases in antioxidant capacity (Ayala-Zavala et al., 2004; Jin et al., 2011). This could be due to vitamin C synthesis and recycling continuing in strawberries post-harvest, although there are currently no studies that have confirmed if this is the case.

6.5 Conclusions

The study presented in Chapter 6 characterised the vitamin C content of seven UK-relevant strawberry genotypes when cultivated under standard commercial conditions. The findings have shown that variability in the studied genotypes is consistent with studies of non-domestic genotypes, although it is unclear whether that variation is sufficient to practically support selective breeding. The data do, however, provide a reliable reference for the vitamin C content of the studied genotypes that can be directly applied to the most widely used cultivation system in UK strawberry production.

Within the studied genotypes, nutritional and sensory qualities tended to be negatively correlated. The flavour, colour, and texture of a strawberry are critical in determining the commercial viability of new and existing genotypes. If these negative associations persist beyond the seven genotypes studied here, interest in vitamin C as a selective breeding target could be limited.

Positively, it appears that changes in the vitamin C content between harvest and retail are likely to be negligible. This is an important finding as it indicates that assessments of strawberry vitamin C content at harvest are a good reflection of vitamin C contents at the point of consumption in the UK.

Considered together, larger and more genetically diverse panels of genotypes must be assessed to properly evaluate the potential to selectively breed for increased vitamin C content in UK strawberries. In this effort, the true extent of vitamin C variability could be revealed and the potential outlooks for improving vitamin C content will become clearer. It will also be crucial to better understand the co-heritability of nutritional, sensory, and agronomic qualities, such that nutritional quality can be effectively integrated with the existing goals of selective breeding programmes. The findings of the current study make a positive contribution towards this ultimate evaluation and will provide a useful foundation for future studies of genotypic variation in UK strawberry production systems.

Chapter 7 General Discussion

7.1 Introduction

This thesis has presented an investigation into the current state of UK vitamin C security and the potential to biofortify UK strawberries to enhance vitamin C self-sufficiency. The primary purpose of Chapter 7 is to bring together the collective findings and to discuss the conceptual progress that has been made toward understanding vitamin C security in the UK.

Chapter 7 is organised into three main sections that highlight advances in the way in which the pillars of availability, accessibility, utilisation, and stability are perceived in relation to vitamin C. Specifically, the sections will address: 1) How assessments of vitamin C availability align with vitamin C requirements, in comparison to alignment with the UK RNI; 2) How health, social, and lifestyle factors, combined with seasonal instabilities in fruit and vegetable consumption, can further change perceptions of vitamin C availability, accessibility, and utilisation; 3) How, for whom, and when biofortified strawberries could contribute to increased vitamin C security.

The conceptual strengths and limitations of the research will be discussed throughout and used as a foundation to identify opportunities for future research. Finally, Chapter 7 will conclude with a synthesis of the key findings in relation to the central themes of the thesis.

7.2 Implications for Perceptions of Vitamin C Availability, Accessibility, Utilisation, and Stability in the UK Food System

To start this discussion, it is once again important to restate the aspirations of food security (Chapter 1, Sections 1.1 and 1.2.4). In 1996, the Food and Agriculture Organisation (FAO) defined food security as a state "*when all people, at all times, have physical, economic and social access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life*" (FAO, 1996). A key element of this definition is the aspiration that a state of food security must support a **healthy life**. The concept of vitamin C security, as a sub-component of food security, should follow the same logic: "*a state when all people, at all times, have physical, economic, and social access to sufficient, safe, and varied sources of vitamin C to meet their dietary requirements for an active and* ***healthy life***". It is important, therefore, that assessments of food or nutrient security utilise benchmarks that align with these positive health aspirations.

The benchmark that has been used in contemporary assessments of UK vitamin C security is the UK Government's RNI (Chapter 1, Section 1.2.4; Chapter 2, Section **Error! Reference s ource not found.**; Chapter 3, Section 3.2.3**Error! Reference source not found.**). The UK RNI for vitamin C is 40 mg/day (Public Health England, 2016) and is the joint lowest recommendation set by any national authority (Carr and Lykkesfeldt, 2021). A recommendation of 40-45 mg/day was adopted in the 1990s as an achievable target that would prevent deficiency and scurvy (Carr and Lykkesfeldt, 2021; Department of Health, 1991). However, until now there has been a lack of information related to the vitamin C intakes required for UK citizens to achieve healthy vitamin C status.

The findings of the current research indicate that the use of the RNI as a benchmark may overestimate the UK's position regarding vitamin C security. Analysis of vitamin C intakes and blood biomarkers in the UK's NDNS cohort has shown that the average amount of vitamin C required to achieve healthy status is 73.23 mg/day, 1.8-fold more than the UK RNI (Chapter 3, Section 3.3.1**Error! Reference source not found.**). As such, quantifying the vitamin C r equirements needed to achieve healthy status has key implications for interpreting research on UK vitamin C security.

In previous studies, the amount of available vitamin C from food supplies in 2010 and 2016 was estimated as 150 mg/capita/day and 119 mg/capita/day, respectively (Macdiarmid et al., 2018; Poppy et al., 2022). When benchmarking these supply values against the current UK RNI and the proposed intake (PI) of 73.23 mg/day, vitamin C availability effectively falls from between 300-375% to 163%-205% of population requirements, respectively. Furthermore, domestic food production in 2016 would have been sufficient to meet just 53% of the population's vitamin C requirements if the higher benchmark were applied (Poppy et al., 2022).

Similar shifts in perception also apply to the study of household vitamin C provisions (HCP) presented in Chapter 2. Between 2017 and 2022, HCPs would effectively fall from supporting 108% of the population's requirements to 59% (Figure 7.1; Chapter 2, Section 2.3.1). Here it is important to consider that the HCP estimates did not cover all food sources of vitamin C, a limitation that was discussed in Chapter 2 (Section 2.4.1). Reported dietary intakes in the NDNS can give context to the underestimations of HCP (Chapter 3, Section 3.4), despite having their own limitations with under-reporting (Macdiarmid and Blundell, 1998). The median vitamin C intake reported in the NDNS between 2011 and 2019 was 70 (43, 107) mg/day, equivalent to 96% of the PI.

Taken together, it can be said that there is still sufficient availability of vitamin C to support positive health aspirations at a macro-scale. However, the surplus may not be as great as initially thought when benchmarking supplies against the UK RNI. As a result, the issues concerning the UK's reliance on imports and losses of vitamin C in the food supply chain become more acute. In future research, it may be prudent to give greater consideration to the benchmarks used to assess UK micronutrient security. One approach that could be adopted is to use multiple benchmarks concurrently. Derbyshire (2018), for example, considered both the RNI and the Lower Reference Nutrient Intake (LRNI) as benchmarks in their study of multiple micronutrient intakes. Adding a third benchmark related to positive health aspirations in such assessments would provide an even more holistic perspective.

7.3 Population Groups Facing Insecurities in Access and Utilisation of Vitamin C

Beyond macro-scale analyses, it is important to consider that an average requirement or RNI will not meet the needs of every individual. Indeed, in the most current guidance for vitamin C, "*studies of the requirements of different groups within the population*" were identified as a key research gap (Department of Health, 1991). The findings of this thesis have addressed this knowledge gap through an observational study of the NDNS cohort (Chapter 3, Section 3.4.2). Vitamin C requirements were found to be higher than the PI for males (94.64 mg/day), smokers (141.68 mg/day) and obese individuals (108.89 mg/day). Thus, a novel finding of this thesis is that the utilisation of vitamin C is not equal across the whole population and considering only the requirements of an average individual could mask potential threats to broader vitamin C security.

To put these different values in perspective, based on the analysis in Chapter 4 an average 80-gram portion of fresh strawberries would provide 48 mg of vitamin C (Section 0). Thus, the difference between the PI for an average individual and the requirements for individuals who are obese or who smoke are equivalent to an additional 0.74 and 1.43 portions of strawberries each day, respectively.

Concerningly, not only do individuals who are obese or smoke require more vitamin C, but these are population groups for whom consumption of fruits and vegetables and vitamin C intakes are already below average (Chapter 3, Sections 3.4.2.1 and 3.4.2.2). Thus, the findings from this thesis highlight an emerging situation in which the people who need the most vitamin C are also the people who are least able to access vitamin C. To develop effective interventions for these at-risk groups, it is important to consider what barriers may be limiting their access to sufficient vitamin C. Across Chapters 2 and 3, lower household income was observed as a consistent co-variate with reduced access to vitamin C (Sections 2.4.4 and 3.4.1.2) as well as higher levels of obesity and smoking (Chapter 3, Section 3.4.1.2). As such, household income could directly limit the effectiveness of fruit- and vegetable-based interventions in the most at-risk groups.

Further to social and lifestyle factors that can impact access to vitamin C, this thesis has identified consistent and predictable seasonal instabilities affecting the availability, accessibility, and utilisation of vitamin C. In Chapter 2, HCPs were found to consistently fall to their lowest levels between September and November (Section 0). The same trends were corroborated in Chapter 3, with vitamin C intakes and blood biomarkers being lower during the Autumn (September - November) than at any other time of year (Section 3.3.2 and 3.4.1.1). The use of multiple datasets and measures of vitamin C security to verify these seasonal trends highlights a key strength of the holistic approach adopted in the current thesis. These seasonal instabilities further confound access to vitamin C for at-risk population groups.

Government interventions, such as the Healthy Start scheme, already exist with the aim of facilitating greater access to fruits and vegetables for low-income families with young children. Early studies of the scheme found that women who took part in Healthy Start ate more fruits and vegetables than women who didn't and were more likely to meet the RNIs for iron, folate, and vitamin C (Ford et al., 2008). Currently, however, the effectiveness of such schemes has been criticised concerning barriers to accessibility and limited impacts on fruit and vegetable purchases. However, the Healthy Start scheme is highly valued by families and retailers and positive discussions as to how the scheme can be improved are ongoing (Barrett et al., 2024; Parnham et al., 2021). Newer initiatives including Alexander Rose (Alexander Rose Charity, 2023) and Fresh Street (Pan et al., 2025) schemes have sought to explore ways of making voucher schemes more effective. As such schemes continue to improve, the findings of the current thesis support the need to explore and understand if similar schemes could be developed to help low-income adults access more fruits and vegetables and whether this would have sufficient impact to improve vitamin C intakes.

7.4 The Effectiveness of Biofortified Strawberry as an Intervention to Improve UK Vitamin C Security

One of the key strengths of the holistic analyses of vitamin C security is that it enables consideration of who, when, and how much of an impact an intervention strategy could have. The second component of this thesis explored a case study of the potential to increase domestic vitamin C production through biofortification of UK strawberries. The subsequent sections of this Chapter will discuss the how, who, and when questions related to a strawberry-based intervention to improve UK vitamin C security.

7.4.1 The Potential to Biofortify UK Strawberries

In previous reports, the success of biofortified crops has been evaluated against three criteria (Bouis et al., 2013): 1) The increase in micronutrient density is great enough to have a significant impact on nutritional status; 2) the added micronutrients are sufficiently bioavailable to improve micronutrient status; 3) farmers will adopt the cultivation strategies or new varieties and consumers will buy and eat them in sufficient quantities. In relation to UK strawberries, the studies presented in Chapters 4, 5, and 6 have begun to explore the first and third of these criteria. Additionally, a novel aspect of this thesis was the use of genotypes that are relevant to UK breeders and retailers and the use of standardised commercial cultivation systems. Consequently, the conclusions drawn are directly relevant to stakeholders in the UK, in a way that other studies have not been.

Criterion 1 - The increase in micronutrient density is great enough to have a significant impact on nutritional status: Genotypic variation in UK strawberries was repeatedly found to be sufficient to support selective breeding for nutritionally meaningful increases in Vitamin C content (Sections 5.4.1 and 6.4.1). Of the studied genotypes, *Elsanta* was found to have the greatest Vitamin C content: An 80-gram portion of *Elsanta* berries provides 57% more Vitamin C than the mean observed in UK-marketed strawberries (Chapter 4), equivalent to 237% of the current UK RNI, or 130% of the PI discussed earlier in Chapter 7 (Section 7.2). Similar Vitamin C contents were also observed in *Vibrant* and *Malwina* (Section 6.3.1). Therefore, it could be argued that the UK already produces biofortified strawberries, but these perhaps do not currently occupy enough of the retail market to have a meaningful impact for consumers. Indeed, Chapter 4 showed that 3% of the collected samples were *Elsanta* strawberries, compared to 9% for *Malling Centenary* and 11% for *Favori* (Section 4.3.1).

In contrast, agronomic interventions were found to be a less effective method of biofortification of vitamin C content in UK Strawberries; Application of iron-nanoparticles and sodium selenate fertilisers did not produce any nutritionally meaningful changes in the Vitamin C content of *Malling Centenary* or *Vibrant* strawberries (Chapter 5, Section 5.4.1). These findings may be due to the treatments not having been optimised for use in UK cultivation conditions. As such, further studies that optimise treatment doses and timings will be necessary to confirm if the conclusions presented in the current thesis are valid.

Criterion 3 - Farmers will adopt the cultivation strategies or new varieties and consumers will buy and eat them in sufficient quantities: A strength of the approach taken in this thesis was the use of cultivation practices that are representative of standard UK production systems. Moreover, sensory and agronomic traits that are valued by growers, retailers, and consumers were considered alongside vitamin C content when comparing genotypes. This has ensured that conclusions related to nutritional biofortification are considered in the context of what matters to UK growers and retailers.

Due to the large number of available genotypes in UK retailers, the addition of a single biofortified genotype may have a limited impact on Vitamin C intakes. Indeed, a total of 56 unique genotypes were sampled from UK retailers in Chapter 4 (Section 4.3.1). As such, to make a difference to the amount of vitamin C provided by UK strawberries, a genotype like *Elsanta* would either need to become the most abundant, or multiple genotypes like *Elsanta* would need to be adopted at the same time. Either of these scenarios would mean that consumers are more likely to purchase a biofortified product than one of lower nutritional quality.

It is important to consider that all the genotypes studied in Chapters 5 and 6 are already commercially successful. However, *Elsanta* and other genotypes with higher nutritional quality have now been superseded by *Malling Centenary* and *Favori* due to increased productivity and better flavour, both of which are traits favoured by growers and retailers (allmanhall, 2024; Hortidaily, 2016). The current research found no positive associations between vitamin C content and sweetness, colour, or yield (Chapters 4, 5 and 6). Whilst only a limited range of agronomic and sensory qualities were considered here, the findings exemplify the fact that a grower may perceive nutritional quality as a trade-off with productivity (Van Ginkel and Cherfas, 2023). Unless high vitamin C content occurs alongside favourable flavour and productivity, biofortified genotypes are likely to have limited commercial success relative to non-biofortified genotypes. Subsequently, limited commercial success would conceptually translate into limited impacts on vitamin C accessibility and intakes.

Taken together, the evidence from this thesis indicates that selective breeding for increases in vitamin C content in UK strawberries is conceptually a viable strategy. In practice, however, there is currently a lack of incentive for breeders, growers, and retailers to make vitamin C content a priority. From a consumer and public health perspective, it will be important that these incentives to adopt biofortified genotypes do not come at an increased cost for consumers. In recent studies from Germany, survey analysis found that people’s willingness to pay a premium for biofortified fruits and vegetables was up to 25% of normal retail prices (Welk et al., 2021; 2023). However, the people who reported the greatest interest in biofortified fruits and vegetables were people who shopped at farmers markets or organic food shops (Welk et al., 2021). Given the types of people most interested in biofortified fruits and vegetables, marketing biofortified strawberries as niche health-promoting products may limit the extent to which they are accepted by most people. Targeted marketing and premium prices would ultimately undermine the potential for biofortified strawberries to improve accessibility to vitamin C for those most in need.

7.4.2 Improving UK Vitamin C Security Through Strawberry-based Interventions

One of the key drivers of the strawberry case study presented in this thesis was to evaluate the potential for biofortified UK strawberries to increase domestic vitamin C production, thus making the UK's available vitamin C more secure against global supply chain disruptions. The collective findings of the strawberry case study indicate that a strawberry-based intervention could have a positive impact on domestic vitamin C supplies, but it would have maximum impact during the Summer. This is due to the coincidence of peak purchasing patterns (Chapter 2, Section 0), peak vitamin C content and lowest prices (Chapter 4, Section 4.3.3) all occurring between June and August. If a biofortified variety or varieties with similar vitamin C contents to *Elsanta* (Chapter 6, Section 6.3.1.1) were to be broadly adopted by growers in the UK, the contribution of strawberries to vitamin C availability would be amplified even further during this seasonal period.

Outside of the Summer, however, increases in the vitamin C content of strawberries would have a limited impact on vitamin C availability due to lower overall availability (Chapter 4, Section 4.3.1) and consumer purchases (Chapter 2, Section 0). Therefore, the seasonality of strawberry consumption and nutritional quality highlights the limitations of only considering a single crop in the context of macro-scale increases in vitamin C production. For biofortification to have an overall effect on domestic vitamin C supplies, future research must consider the potential to biofortify multiple crops that have complementary seasonal consumption patterns.

It is the view of the author that, the most effective approach to improving vitamin C intake in the UK population is not a choice between biofortification or behavioural change, but a coordinated strategy that leverages both. One of the major conceptual advantages of biofortification lies in its potential to deliver "health by stealth": enhancing the nutritional quality of commonly consumed foods without requiring any change in consumer behaviour.

If the current barriers to biofortifying UK-grown fruits and vegetables can be overcome, and nutrient-dense produce made widely available and affordable, then population-level vitamin C intakes could be improved passively, without reliance on consumer education or motivation. For example, biofortification of strawberries, coupled with targeted campaigns promoting increased access and consumption during the autumn, could amplify vitamin C intakes for at-risk groups during an unstable seasonal period. Given that widespread biofortification may not be realised for many years, campaigns and outreach to promote and improve access to healthy fruit and veg would be especially critical in the short term to address the acute vitamin C insecurities that UK is currently facing.

Ultimately, a combined and persistent approach would be most likely to succeed. Biofortification of multiple popular local crops should be pursued alongside public health messaging and community engagement to create a supportive food environment in which both the quality of the food supply and the public's dietary behaviours are improved in tandem. Addressing vitamin C insecurities effectively will requires this kind of long-term, co-developed strategy to both enhance the nutritional value of our food and ensures equitable access and utilisation.

7.5 Perspectives for Future Research

Regarding vitamin C availability, further investigation is needed to identify incentives for breeders, growers and retailers to adopt biofortified genotypes. To avoid the development of niche and premium biofortified products, future studies should explore how nutritional quality can be positively linked with crop traits that are important to growers and retailers. Conceptually, such benefits could include increased stress tolerance resulting in reduced production costs for growers, or increased shelf-life resulting in reduced waste for retailers. The analysis of sensory traits (sugar/acid ratios) presented in Chapter 4 and 6 could also be expanded to more analytes, such as amino acid and anthocyanin content, as well as sensory panels, to ensure that any inferences about relationships between nutritional and sensory qualities are supported by robust and holistic assessments of sensory quality. Interdisciplinary life-cycle assessments could be conducted to investigate the holistic benefits and limitations of increased nutritional quality in relation to the needs of different stakeholders. Ultimately, if vitamin C and nutritional quality can be coupled to other desirable traits, biofortification may be seen as a route to increased profitability without the need for niche or premium marketing. Broad adoption of biofortified fruits and vegetables would not only increase the overall amount of available vitamin C but would also ensure that biofortified fruits and vegetables are accessible to a broad range of consumers. It is important to consider, however, that the current thesis has only considered the potential to biofortify strawberries in a UK context. Understanding if biofortification of UK crops would work as a larger-scale intervention will also require evaluations of variability in other crops such as sweet peppers and broccoli.

Improving access to fresh fruits and vegetables presents a major challenge to vitamin C security in the UK. Analysis of the NDNS cohort has identified several population groups that have an increased risk of inadequate access, including individuals with low incomes, as well as individuals who are obese or smoke due to their greatly increased vitamin C requirements. Chapter 3 highlighted the need to develop a stronger understanding of the factors that limit access to vitamin C-rich fruits and vegetables in each of these population groups, and how these factors relate to vitamin C intakes and status (Section 3.4). Classic barriers to be explored include physical and financial inaccessibility, but future studies could also explore the ways in which improving education and awareness of vitamin C requirements could impact people's behaviour in relation to consumption of fruits and vegetables. As such, a logical next step would be to co-develop social outreach and surveys to at-risk groups to gain a first-hand understanding of people's knowledge of the importance of vitamin C, as well as the practical and perceived barriers to accessing sources of vitamin C. This outreach work would be most effective when co-developed with social scientists, medical practitioners, local councils, and charitable organisations that are already delivering community wellness outreach programmes. From here, it would be possible to work with communities to develop targeted interventions that are most likely to have a positive impact in the population groups currently at risk of vitamin C insecurities.

Considering the population groups that have been identified as being at risk of inadequate access to sufficient vitamin C, it may also be prudent to explore the potential to fortify staple foods. Currently, there is a lack of research regarding the viability of vitamin C fortification in both practical and commercial contexts. Future studies could address these gaps by investigating the stability of vitamin C in fortified staple foods and whether the addition of significant amounts of vitamin C has any negative impact on consumer preference. Whilst fortification should not replace efforts to increase access to fruits and vegetables, it may help to ensure that those with low-incomes or with increased vitamin C requirements are still able to access sufficient vitamin C.

In relation to vitamin C utilisation, further research will be needed to validate the predicted vitamin C requirements for specific population groups with higher utilisation, including males, individuals with obesity, and smokers. A key limitation associated with the predicted vitamin C requirements reported in Chapter 3 was that they were based solely on observational data. Whilst the predicted requirements align with other observational and pharmacokinetic studies, the nature of the study means that the predicted intakes may not be completely accurate. To address this limitation, further pharmacokinetic studies could help to validate the increased vitamin C requirements for males, individuals who are obese, and smokers.

The need to have reliable predictions of vitamin C requirements is especially strong in relation to the increased utilisation of vitamin C in individuals who are obese. Given the increased utilisation of vitamin C associated with obesity, there will be a need to understand how rising levels of obesity in the general population may impact future vitamin C demands. Scenario-based modelling, informed by epidemiological and pharmacokinetic data, could provide insights into how much more vitamin C may be required to support the future UK population and whether the UK food system would be able to support the increased demands. These insights could facilitate policy discussions on the necessity, scale, and urgency of interventions to ensure adequate vitamin C availability.

As explored in the current thesis, future interventions could include the development of biofortified crops. However, there is currently limited understanding of the bioavailability of vitamin C in biofortified crops. Future studies could utilise in-vitro digestion experiments and randomised controlled trials with strawberry genotypes of varying vitamin C content. Such studies would help to determine whether the differences in vitamin C content found in this thesis significantly impact the utilisation of vitamin C. The evidence generated by such studies, in conjunction with the findings of the current thesis, would be useful in evaluating the cost-effectiveness of biofortifying UK crops as an approach to improving vitamin C security.

With respect to the seasonal instabilities identified in Chapters 2 and 3, there remains a need to understand why accessibility and utilisation of vitamin C were worse in the Autumn. Future research could explore several potential factors in relation to the observed seasonal instabilities: Behavioural or socioeconomic factors that could be influencing the overall reductions in purchases and consumption of fruits and vegetables; A lack of awareness relating to vitamin C requirements and the foods that provide vitamin C; A potential gap in the availability of locally grown produce; Decreased utilisation of vitamin C from seasonal vegetables due to losses during cooking. Ultimately, developing a better understanding as to why Autumnal instabilities exist in the UK will help to support the development of targeted interventions in the future.

As a broader approach to mitigating seasonal instabilities in vitamin C, further research could seek to optimise controlled-environment agriculture for year-round production of nutrient-rich fruits and vegetables. These studies would effectively aim to demonstrate that fruits and vegetables with consistently high levels of vitamin C could be produced year-round in the UK. However, such studies must also consider whether the increased availability of local fresh fruits and vegetables will have any impact on the seasonal consumption habits of consumers. Consideration must also be given to the cost of controlled environment produce, as keeping prices low will be a key factor in ensuring that the produce is accessible by those who need it most.

Finally, this thesis highlights the utility of the four-pillar framework in making holistic assessments of micronutrient security. What is clear from applying this framework to vitamin C is that effective interventions cannot consider any one pillar in isolation. Effective interventions must consider the interactions between biological, policy, business, and consumer behaviour. As such, all the suggestions for future research should be framed in the context of broader vitamin C security and would benefit from interdisciplinary collaborations. Future research should also extend the four-pillar framework beyond vitamin C to identify cross-cutting micronutrient insecurities that could identify opportunities for coordinated multi-nutrient interventions.

7.6 Conclusions

The primary aims of this thesis were to conduct a holistic assessment of UK vitamin C security and to explore the biofortification of UK strawberries as a case study for improving domestic vitamin C production.

The study of the relationship between vitamin C intake and blood biomarker levels highlights the need to refine the benchmarks used to assess vitamin C security in the UK. While assessments based on the UK RNI of 40 mg/day suggest a substantial surplus of available vitamin C, findings from this thesis indicate that average intakes would need to be **1.8-fold higher** to support adequate vitamin C status in the UK population. By reframing vitamin C security in alignment with positive health aspirations, the perceived surplus of vitamin C in the UK is effectively reduced. In this context, concerns regarding the UK's dependence on imported fruits and vegetables to maintain sufficient vitamin C availability are amplified, emphasising the importance of future work to ensure the UK food system is resilient to global supply chain shocks.

Furthermore, this thesis has identified smokers, individuals with obesity, and those from low-income households as population groups at heightened risk of vitamin C insecurity. The challenges faced by these groups are twofold: smoking and obesity are associated with increased vitamin C utilisation, while lower socioeconomic status is linked to reduced dietary access and intake. Given the overlap between these demographic factors, an emerging concern is that individuals with the highest vitamin C requirements also encounter the greatest barriers to adequate intake. Seasonal fluctuations in vitamin C availability further compound these risks, disproportionately affecting the most vulnerable populations. Therefore, strategies aimed at increasing overall vitamin C availability must be implemented alongside targeted interventions designed to improve accessibility and stability for at-risk groups.

In the case of strawberry, selective breeding presents a promising strategy for increasing the UK's self-sufficiency in relation to vitamin C availability. However, further research is needed to establish tangible incentives that could facilitate the adoption of biofortified strawberries and to understand whether the enhanced vitamin C contents would have any meaningful impact on accessibility and utilisation of vitamin C in the wider population.

The collective findings of this thesis also emphasise the importance of developing multifaceted interventions to address micronutrient insecurities. As demonstrated in the current thesis, a strawberry-based intervention in isolation is unlikely to improve vitamin C accessibility and stability for the most vulnerable population groups. This exemplifies the importance of future interdisciplinary research to develop comprehensive strategies that integrate agricultural innovation with public health and socioeconomic policies to enhance vitamin C security in the UK.

Therefore, it is believed that this thesis has achieved its purpose. The collective studies have provided valuable insights for researchers, politicians, consumers, farmers, and retailers as to the vitamin C requirements of the UK population and the challenges that might be faced in securing sufficient vitamin C to support a healthy population.

Appendix A Additional Tables of NDNS Cohort Characteristics

A.1 Seasonal Consumption of Fruits And Vegetables, Vitamin C Intake, and Plasma Ascorbic Acid Concentrations in The Filtered NDNS Cohort.

Characteristic	Winter N = 828[1]	Autumn N = 799[1]	Spring N = 779[1]	Summer N = 824[1]	p-value[2]
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	3.99 (2.47, 5.53)	3.60 (2.24, 5.14)	3.82 (2.51, 5.38)	3.85 (2.34, 5.51)	0.11
Vitamin C Intake (mg/day)	74 (45, 112)	65 (40, 97)	69 (45, 105)	72 (44, 112)	**0.007**
Plasma Ascorbic Acid (µmol/L)	51 (30, 66)	50 (30, 66)	49 (32, 64)	50 (35, 63)	0.8

[1]Median (Q1, Q3)

[2]Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

A.2 Characteristics of the Filtered NDNS Cohort by Age-Group Tertile.

Characteristic	**Youngest** N = 1,320[1]	**Middle** N = 949[1]	**Oldest** N = 974[1]	**p-value**[2]
Smoking Status				**<0.001**
Non-current Smoker	903 (77)	730 (86)	778 (90)	
Current Smoker	267 (23)	122 (14)	90 (10)	
Age	31 (25, 37)	50 (46, 54)	69 (63, 75)	**<0.001**
Body Weight (Kg)	74 (64, 86)	79 (68, 91)	76 (66, 89)	**<0.001**
Waist Circumference (cm)	86 (77, 95)	94 (85, 104)	97 (87, 106)	**<0.001**
Body Mass Index (Kg/m^2)	25.2 (22.3, 28.7)	27.2 (24.4, 30.9)	27.8 (24.8, 31.0)	**<0.001**
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	3.25 (2.07, 4.79)	4.09 (2.75, 5.80)	4.27 (2.73, 5.94)	**<0.001**
Vitamin C Intake (mg/day)	63 (38, 98)	74 (45, 110)	77 (49, 113)	**<0.001**
Plasma Ascorbic Acid (µmol/L)	50 (34, 65)	52 (33, 65)	49 (30, 64)	0.2

[1]n (%); Median (Q1, Q3)

[2]Pearson's X^2: Rao & Scott adjustment; Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

A.3 Characteristics of the Filtered NDNS Cohort by Geographic Region.

Characteristic	**South** N = 708[1]	**East of England** N = 319[1]	**London** N = 393[1]	**Midlands** N = 522[1]	**North** N = 789[1]	**Scotland** N = 296[1]	**Wales** N = 164[1]	**p-value**[2]
Household Income Tertile								**0.008**
Highest	179 (44)	81 (41)	103 (53)	114 (37)	148 (32)	51 (39)	26 (29)	
Middle	99 (24)	66 (34)	51 (27)	91 (30)	152 (33)	35 (26)	30 (32)	
Lowest	129 (32)	49 (25)	39 (20)	99 (33)	165 (36)	46 (35)	36 (39)	
Smoking Status								0.13
Non-current Smoker	534 (84)	250 (86)	297 (88)	377 (80)	578 (84)	212 (79)	124 (80)	
Current Smoker	99 (16)	39 (14)	41 (12)	92 (20)	109 (16)	57 (21)	32 (20)	
Age	48 (35, 63)	48 (31, 60)	43 (31, 54)	45 (32, 59)	50 (35, 64)	47 (34, 61)	52 (38, 64)	**0.002**
Body Weight (Kg)	74 (64, 86)	78 (68, 91)	73 (63, 82)	79 (67, 92)	77 (66, 90)	76 (65, 87)	76 (67, 88)	**<0.001**
Waist Circumference (cm)	89 (81, 100)	93 (85, 103)	88 (81, 97)	92 (82, 103)	93 (83, 104)	92 (84, 102)	93 (85, 105)	**<0.001**
Body Mass Index (Kg/m^2)	25.9 (23.2, 29.1)	26.5 (23.4, 30.2)	25.2 (22.6, 28.8)	27.0 (24.0, 30.7)	27.5 (24.1, 30.8)	26.7 (23.6, 30.5)	27.4 (24.0, 31.5)	**<0.001**
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	4.02 (2.62, 5.64)	4.41 (3.19, 6.21)	4.13 (2.58, 5.49)	3.58 (2.15, 5.30)	3.37 (2.17, 5.15)	3.50 (2.12, 4.93)	3.53 (2.21, 5.38)	**<0.001**
Vitamin C Intake (mg/day)	75 (47, 109)	79 (52, 115)	72 (43, 115)	70 (44, 111)	66 (39, 99)	63 (38, 91)	66 (44, 105)	**<0.001**
Plasma Ascorbic Acid (μmol/L)	54 (34, 68)	53 (36, 66)	52 (35, 68)	48 (31, 63)	48 (29, 63)	48 (31, 63)	49 (30, 64)	**0.007**

Characteristic	**South** N = 708[1]	**East of England** N = 319[1]	**London** N = 393[1]	**Midlands** N = 522[1]	**North** N = 789[1]	**Scotland** N = 296[1]	**Wales** N = 164[1]	**p-value**[2]

[1]n (%); Median (Q1, Q3)

[2]Pearson's X^2: Rao & Scott adjustment; Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

A.4 Characteristics of the Filtered NDNS Cohort by Household Income Tertile.

Characteristic	**Highest** N = 706[1]	**Middle** N = 528[1]	**Lowest** N = 570[1]	**p-value**[2]
Smoking Status				**<0.001**
Non-current Smoker	531 (89)	362 (82)	368 (75)	
Current Smoker	63 (11)	78 (18)	123 (25)	
Age	47 (35, 57)	47 (32, 61)	47 (31, 66)	0.8
Body Weight (Kg)	77 (67, 89)	76 (66, 89)	75 (65, 88)	0.3
Waist Circumference (cm)	90 (82, 100)	92 (83, 103)	91 (82, 102)	0.2
Body Mass Index (Kg/m^2)	26.2 (23.3, 29.4)	26.9 (23.6, 30.2)	26.9 (23.5, 30.7)	0.2
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	4.43 (2.98, 6.19)	3.87 (2.50, 5.16)	3.02 (1.81, 4.62)	**<0.001**
Vitamin C Intake (mg/day)	83 (55, 119)	67 (42, 97)	53 (35, 91)	**<0.001**
Plasma Ascorbic Acid (µmol/L)	57 (42, 68)	50 (31, 67)	42 (23, 60)	**<0.001**

[1]n (%); Median (Q1, Q3)

[2]Pearson's X^2: Rao & Scott adjustment; Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

A.5 Characteristics of the Filtered NDNS Cohort by Biological Sex.

Characteristic	Female N = 1,657[1]	Male N = 1,586[1]	p-value[2]
Smoking Status			0.2
Non-current Smoker	1,244 (84)	1,167 (82)	
Current Smoker	230 (16)	250 (18)	
Age	48 (34, 63)	47 (33, 60)	0.088
Body Weight (Kg)	68 (61, 80)	83 (74, 94)	**<0.001**
Waist Circumference (cm)	86 (78, 97)	96 (88, 106)	**<0.001**
Body Mass Index (Kg/m^2)	26.1 (22.9, 30.4)	27.0 (24.2, 30.0)	**0.015**
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	3.86 (2.42, 5.44)	3.73 (2.34, 5.38)	0.5
Vitamin C Intake (mg/day)	70 (44, 107)	70 (42, 107)	0.7
Plasma Ascorbic Acid (µmol/L)	55 (36, 68)	46 (30, 61)	**<0.001**

[1]n (%); Median (Q1, Q3)

[2]Pearson's X^2: Rao & Scott adjustment; Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

A.6 Characteristics of the Filtered NDNS Cohort by Weight Category.

Characteristic	Healthy Weight N = 1,067[1]	Overweight N = 1,150[1]	Obese N = 812[1]	p-value[2]
Smoking Status				0.3
Non-current Smoker	770 (82)	875 (85)	617 (84)	
Current Smoker	171 (18)	152 (15)	121 (16)	
Age	39 (28, 55)	49 (37, 63)	52 (39, 64)	**<0.001**
Body Weight (Kg)	64 (58, 71)	78 (71, 85)	95 (87, 105)	**<0.001**
Waist Circumference (cm)	80 (74, 86)	94 (88, 99)	107 (101, 115)	**<0.001**
Body Mass Index (Kg/m^2)	22.7 (21.5, 23.8)	27.2 (26.1, 28.5)	33.1 (31.3, 36.2)	**<0.001**
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	4.01 (2.38, 5.64)	3.89 (2.58, 5.42)	3.53 (2.27, 5.07)	**0.017**
Vitamin C Intake (mg/day)	75 (47, 111)	68 (45, 104)	66 (43, 103)	**0.019**
Plasma Ascorbic Acid (µmol/L)	59 (40, 71)	50 (34, 63)	43 (26, 59)	**<0.001**

[1]n (%); Median (Q1, Q3)

[2]Pearson's X^2: Rao & Scott adjustment; Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

A.7 Characteristics of the Filtered NDNS Cohort by Smoking Status.

Characteristic	**Non-current Smoker** N = 2,411[1]	**Current Smoker** N = 480[1]	**p-value**[2]
Age	49 (35, 63)	40 (28, 53)	**<0.001**
Body Weight (Kg)	76 (66, 89)	76 (64, 89)	0.7
Waist Circumference (cm)	91 (83, 102)	92 (81, 102)	0.8
Body Mass Index (Kg/m^2)	26.7 (23.7, 30.3)	26.2 (23.0, 30.2)	0.2
Fruit, Vegetable, and Juice Consumption (portions/day)[3]	4.05 (2.65, 5.68)	2.38 (1.52, 3.63)	**<0.001**
Vitamin C Intake (mg/day)	76 (48, 111)	48 (29, 74)	**<0.001**
Plasma Ascorbic Acid (µmol/L)	52 (36, 65)	34 (16, 51)	**<0.001**

[1]Median (Q1, Q3)

[2]Design-based KruskalWallis test

[3]A portion of fruits and vegetables is 80g and a portion of Juice is 150ml.

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