The potential contribution of water-soluble vitamins in ready meals to the UK reference nutrient intakes.

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ABSTRACT

The UK ready meal (RM) food industry had an estimated value of £2.7 billion in 2017/18. These meals are a convenient option for many people and are described as a processed food that require reheating before consumption. However, food processing techniques such as heating, irradiation, and freezing can degrade process labile nutrients, especially essential water-soluble vitamins (WSVs), B-vitamins and vitamin C.

Although RMs are an increasingly popular meal option, little is known about the contribution of these meals to the dietary intake of those that are consuming RMs. There is no current guidance available for the recommended nutrient content of RMs, however, Public Health England recommend that meals served as part of public catering should provide at least 30% of the reference nutrient intake (RNI) for all micronutrients for adults when the serving is considered a 'main meal'.

Due to the functional importance of these vitamins, it is imperative to have accurate estimations of nutrients present in the reheated RM. The most susceptible to degradation during food processing are B-group vitamins; thiamine (vitamin B1), riboflavin (vitamin B2) and folate (vitamin B9), and vitamin C. The rise in consumption of RMs mean that it is important to understand the potential contribution of these meals to the UK diet. Therefore, the aim of this research is to assess the thiamine, riboflavin, folate, vitamin B12 and vitamin C content of a popular RM in the UK. This study will highlight opportunities to enhance the WSV quality of meals where these nutrients may be lacking.

A literature review taking a systematic approach was carried out to determine the current patterns of RM consumption in the UK, along with the thiamine, riboflavin, folate, vitamin B12 and vitamin C content of RMs. Five academic articles met the inclusion criteria for consumption, and 15 that examined the nutrient content of RMs, respectively. The review concluded that RMs were consumed 1-2 times per week by the majority of RM consumers, and that those with annual household incomes below £20,000 were the population group most likely to consume RMs.

Analysis of RMs found that vitamin C had the greatest susceptibility to processing including, heating, freezing and storage. Thiamine, riboflavin, folate was liable to processing through hot-holding, irradiation and storage. Vitamin B12 was the least labile of the vitamins reviewed, however only one study analysed the vitamin B12 content of RMs.

Secondary analysis of the National Diet and Nutrition Survey (NDNS) revealed that RMs did not meet the 30% RNI for WSV recommendation for folate and vitamin C. The 30% of the RNI recommendations were met for thiamine, riboflavin and vitamin B12. There was no difference in income between those that consumed RMs and those that did not.

Chemical testing was used to quantify the thiamine, riboflavin, folate and vitamin C content of the RMs. Thiamine method testing revealed that High-Performance Liquid Chromatography (HPLC) was the most accurate method. Vitamin B12 was not analysed because of safety issues associated with quantifying the vitamin.

The thiamine, riboflavin (analysed by HPLC with fluorescence detection), folate (analysed using the Vitafast microbiological assay) and vitamin C (analysed by Campden BRI) content of the sausage and mashed potato RMs were significantly different between five different RMs from four providers. None of the RMs tested met the 30% of the RNI for WSV recommendations for riboflavin, folate, and vitamin C, although they did for thiamine. Lastly, the effect of hot-holding, a method used by catering services to keep food warm, on WSVs was determined. The study found that hot-holding RMs for three-hours at 90^{o}C led to a significant increase in folate concentration in the vegetable portion (peas), but there was no effect on other meal components or vitamins.

This research has shown that there is a need for more accurate information regarding nutrient content of RMs, especially food targeted at older adults, a population group who are at greater risk of nutrient deficiency. Reformulation of RMs, such as incorporating more vegetables into RMs would increase the WSV content of these meals.

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Forgive them anyway.
If you are kind, people may accuse you of selfish, ulterior motives.
Be kind anyway.
If you are successful, you will win some unfaithful friends and some genuine enemies.
Succeed anyway.
If you are honest and sincere, people may deceive you.
Be honest and sincere anyway.
If you find serenity and happiness, some may be jealous.
Be happy anyway.
The good you do today, will often be forgotten.
Do good anyway.
Give the best you have and it will never be enough.
Give your best anyway..." – Mother Teresa

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PUBLICATIONS

Conference Talks

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ABBREVIATIONS

5-FTHF	5-formyltetrahydrofolate
5-MTHF	5-methyltetrahydrofolate
AA	Ascorbic acid
BOP	Back-of-Pack
CO2	Carbon dioxide
DHA	Dehydroascorbic acid
DHAA	dihydroxy ascorbic acid
dTMP	deoxythymidine monophosphate
dUMP	deoxyuridine monophosphates
DW	Dry weight
ELSA	English Longitudinal Study of Ageing
EU	European Union
FAD	Flavin adenine dinucleotide
FCT	Food composition tables
FMN	Flavin mononucleotide
FR	Folate receptor
FOP	Front-of-Pack
FV	Fruit and Vegetables
HPLC	High Performance Liquid Chromatography
LOD	Limit of Detection
MOW	Meals-on-wheels
N_2	Nitrogen
NDNS	National Diet and Nutrition Survey
O_2	Oxygen
PCFT	Proton coupled folate transporter
RFC	Reduce folate carrier
RM	Ready meal
RNI	Reference Nutrient Intake
TAA	Total ascorbic acid
TC1	Haptocorrin
TCII	Transcobalamin

THF	Tetrahydrofolate
UV	Ultraviolet
WFF	Wiltshire Farm Foods
WSV	Water-soluble-vitamins

1 INTRODUCTION

1.1. Defining convenience foods

The UK ready meal (RM) food industry had an estimated value of £2.7 billion in 2017/18 and is described as a convenient food option. Many definitions exist for what convenience foods encompass. The most comprehensive, however, is a food that has been processed or semi-processed, is quick to prepare and/or shelf-stable, and where all or most of the preparation has been carried out by industrial processing (Jackson and Viehoff, 2016). Examples of convenience include RMs, ready-to-eat sandwiches, pre-prepared pizzas, tinned fruit, canned vegetables and bagged salads (Jackson, 2018).

For this dissertation, RMs are defined as foods that are manufactured and prepared outside of the home and preserved - frozen, chilled or tinned - until the meals are re-heated and consumed (Food Standards Agency, 2017, Costa et al., 2001). This dissertation will focus on RMs available at supermarkets, convenience stores or online. Ready-to-cook meals, which are meals that have only been partially cooked and require additional cooking to ensure the meal is safe for consumption, or 'take-away', 'take-out' or fast foods, which are made to order and/or are purchased hot from small independent outlets rather than supermarkets will not be included in this study (Janssen et al., 2018).

1.2. The history of convenience foods

Early food preservation techniques such as pickling, salting and curing were used to increase the convenience of foods by extending their shelf-life, and reducing or removing the need for additional cooking before consumption (Jackson, 2018). Although these techniques are still used today, more modern and industrial food processing methods such as canning, freezing and use of synthetic preservatives have led to an increase in the variety of foods available to the population.

Many of the earliest industrial food production techniques were developed during wartimes due to the need for nutritious foods that were easy to prepare, transport and store for troops on the battlefield. Examples of these large scale food production systems include the use of glass jars to preserve food during the Napoleonic wars (1799-1815)

(Drouard and Oddy, 2016), industrial-scale canning of pork and beans during the US Civil war in 1860, and provision of canned corned beef and Irish stew to the British army in World War I (Jackson, 2018). However, the 'ready meal', or 'TV dinner' as it was initially described, was first produced in the 1950s by C.A. Swanson and Sons in the USA, who served thanksgiving dinner in a segmented aluminium tray that was heated in the oven before consumption (Gust, 2011).

Although it is outside of the scope of this research to understand the reasons for the UK increase in RM uptake, researchers have provided some theories. Gust (2011) suggests that RMs were marketed to housewives as they faced increased time pressures, as many women started to work but were still responsible for preparing food for the family. TV dinners were advertised as being quick to cook, tasted like home cooked recipes and required little to no washing up, which led to a sharp increase in the consumption of these meals (Jackson, 2018). However, the growth in RM consumption during the 1950s was less dramatic in Europe compared to the USA. This lack of European interest was possibly due to the slow transition from coal or petroleum stoves to electrical appliances including hobs, fridges and freezers, which were needed to store and reheat the meals. During the 1960's in the UK, meals manufactured by Vesta such as dried beef curry, Chow Mein and instant mashed potato (Jackson, 2018) were being supplied by major food retailers such as Marks and Spencer and Sainsbury's. Since then, the significant growth in the RM market has led to the UK currently being the largest consumer of RMs in Europe (Jackson, 2018).

1.3. Social influencers of ready meal consumption

The vast range of RMs on offer cater to many different demographics of the population. They include value, luxury, healthy options, different cuisine types, and vegetarian and vegan options (Reed et al., 2003). However, changing lifestyles leading to a reduction in the amount of time available to prepare meals are considered to lead to more convenient meal choices (Celnik et al., 2012, Harris and Shiptsova, 2007). These changing food habits can lead to the normalisation of consuming convenient food options, such as RMs, and as the demand for these products increase, so does the availability in stores and supermarkets.

The setting where RMs are consumed, and the drivers for purchasing a RM, are important factors to consider when trying to understand a person's inclination to eat these foods rather than preparing a meal from individual ingredients at home. These factors were assessed by Ahlgren et al. (2005) using a questionnaire distributed to adult residents of Gothenburg, Sweden; an area where RMs are widely available. They found that RMs are more likely to be consumed alone, or with one other person. This is likely due to these foods being sold as a one-serving meal, are quick to prepare (usually taking between 5-10 minutes to cook) and provide an opportunity to try new foods without the need to buy new ingredients (Geeroms et al., 2008). These factors, coupled with the consistency, the rich taste and appetising appearance of RMs (Olsen et al., 2012), availability of the product, as well as the inclination, skills or perception of time to cook meals from scratch have all been correlated with increased RM consumption (Van Der Horst et al., 2011, Olsen et al., 2010, Howard et al., 2012, Mahon et al., 2006) .

1.4. The ready meal market in the UK

Market research is used to gather information about consumer needs and preferences in relation to a service or product (Cambridge Dictionary, 2019). There are two types of market research; primary and secondary. Primary is the most common and is predominantly conducted by a private market research company to gather consumer preference data in a defined population. The data is collected using a variety of methods such as direct mail questionnaires, telephone and personal interviews; whereas secondary market research is compiled from publicly available data such as white papers, government documents, or reports from industry competitors, and are used to extrapolate consumer preferences and industry trend data (McGivern, 2013, British Library, 2019).

The Department of Environment, Food and Rural Affairs (DEFRA) has gathered annual data on RM purchases in the UK. Data shows that the average consumption of RM containing meat, fish and vegetables has increased during 1974-2017, whereas consumption of fresh food products had decreased over this period (Department for Environment, 2018). Data on RM purchasing in the UK reveal that RMs have become a more prevalent part of food culture in the UK, and therefore have the potential to make a significant contribution to the nutritional intake of the population.

However, this trend was not seen in other food components where consumption of pork, mutton, beef and veal, white fish and fresh green vegetables, which have decreased over the same 43-year period (Department for Environment, 2018). These changes could be due to factors such as the population steering away from red meat, due to the higher prevalence of vegetarianism (Hughes, 1995). Consumption of beef and veal decreased following the Bovine spongiform encephalopathy (BSE) scandal in the early 1990s (Burton and Young, 1996), whereas a decrease in fish purchasing has been associated with a steady increase in the cost along with reduced availability of fish in the UK (Jennings et al., 2016). Furthermore, the increase in chicken purchased is most likely due to changes in industrial chicken rearing practices, which have led to a shorter product cycle, thus reducing the price of chicken available to consumers (Yakovleva and Flynn, 2004).

The increase in a range of vegetables (not including green vegetables) available on the market can be associated with an increased supply through the importation of horticultural crops. The proportion of vegetables on the market that were produced in the UK decreased from 82.7% in 1988 to 52.7% in 2018, with the quantity of horticultural crops being imported into the UK increasing by 316% during the same time period (Department for Environment, 2019). Although there have been some changes in fresh vegetable purchasing in the UK, national diet surveys have shown that there were no changes in the fruit and vegetable (FV) consumption between 2008-2018 (Public Health England, 2019c).

An independent global market research and analysis company; Mintel, collects data through online questionnaires or face-to-face interviews. They have conducted secondary market research on ‘Ready meals and ready-to-cook foods’, and published a report in July 2018 based on the responses from 2,000 participants (Mintel Academic, 2019). The data collection was carried out with internet users aged 16+ in March 2018, with set recruitment quotas including age, socio-economic status and UK region for each group (appendix i) (Mintel Academic, 2018).

Results from Mintel market research shows that there has been a trend towards those aged 16-34 years consuming world cuisine, where 50% of respondents see RMs as an

opportunity to try new cuisine types (Mintel, 2020). The most popular RMs include Italian, Indian, Chinese and British cuisines, however, the most popular meal type per age group is not highlighted in the research. Overall RM consumption is most likely to be 1-2 times per week, and least likely to exceed 6 times a week or more. The most consumed types of RMs are ready-to-cook meals and chilled RMs irrespective of age group. Consumption of ready-to-cook and chilled RMs with a frequency of 1-2 times per week is most common for those aged 16–44-year-olds - 25-30% - compared to 5-23% for those aged 45 years and over. Those aged 35 years and over are more likely to consume ready-to-cook meals once a month or less than those aged 16-34 years old (29-31% v. 21-22%).

For chilled, frozen and non-chilled/non-frozen meals, 11-19% of 16–24-year-olds are more likely to consume meals 3-5 times per week with 12-21% having never consumed any of the RM types. The age group least likely to consume RMs of any kind are 65+ years old, with 29-59% stating that they have never consumed RMs. The RM type least likely to be consumed is non-chilled/non-frozen meals from cans or pouches, with between 21-59% of all age groups never consuming such meals. Furthermore, as the population got older, the frequency of consuming non-chilled/frozen RM decreased, and it was more likely for older adults to consume RMs once a month or less.

Mintel data shows that weekday mealtimes were the most likely time for all age groups to consume a RM, with breakfast being the least likely (71% v. 7%). However, consuming RMs at breakfast is more likely in those aged 16-24 years and 25-34 years - 11% and 15% compared to 1% in those aged 65+. This data also shows that those aged 45 and over are more likely to consume RMs as a weekday dinner (75-79% compared to 63-68% of those aged 16-44 years old). The most likely age group to have a RM for lunch are those aged 16-44 years, compared to 45+ year olds (31-40% v. 21%).

Data from Mintel further shows that 90% of RMs are consumed in the home compared to only 11% in other places such as at work or school. Those aged 16-34 years are more likely to consume RMs outside of the home (25%), such as school or work, compared to 1-17% of those aged 35 years and over. In summary, chilled RMs are most popular in

those aged 34 years or younger, and weekday dinner times are the most common time to consume these meals.

Another research database, EuroMonitor, is a secondary data source which gathers information from macro-economic databases. The data sources include international secondary data from the International Monetary Fund (IMF), Organisation for Economic Co-operation and Development (OECD), United Nations (UN), World Bank, European Commission and central banks. Euromonitor then compiles country reports on a variety of topics based on the available purchase markets (Euromonitor International, 2019). For this research the 'Ready meals in the United Kingdom' country report was used to gather market share information about RM sales and consumption in the UK (Passport Euromonitor, 2018).

Euromonitor data showed that all types of RM sales increased by £500 million to £4750 million in 2017, compared to 2012. Shelf stable RMs and frozen RM sales have both decreased by 14% since 2012 (Figure 1), but there was a 4% increase in dried RM consumption. Figure 2 shows that 14 brands had 1% or more share of sales of RMs. Tesco

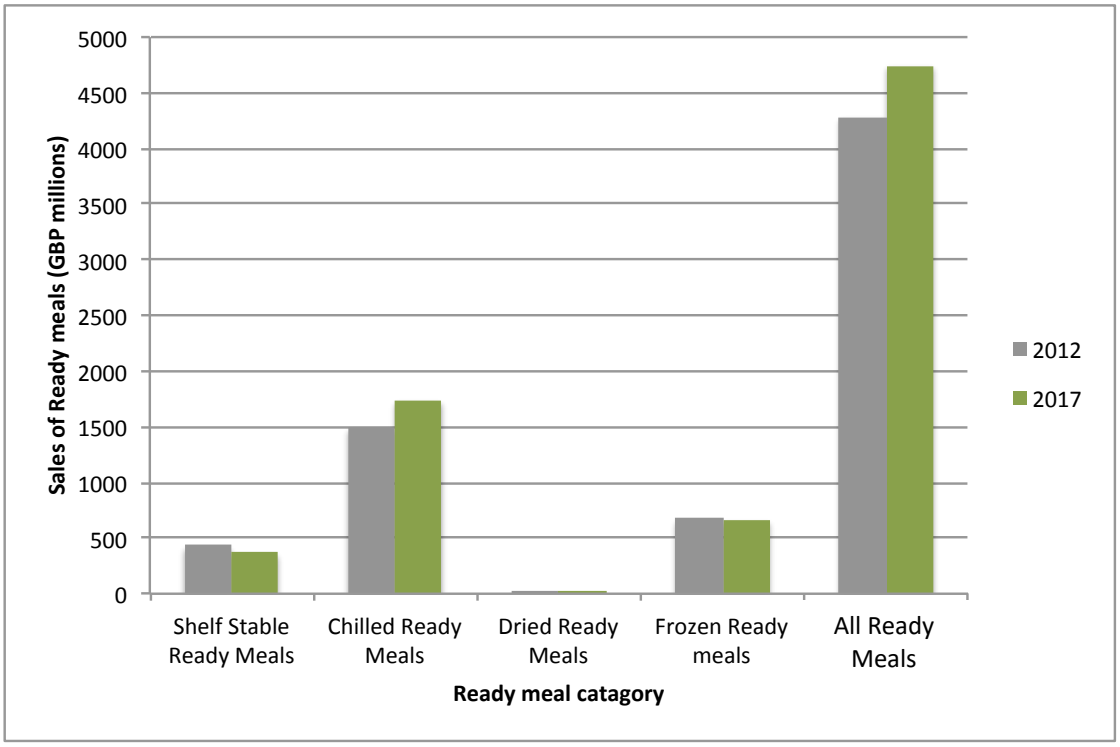


Figure 1: Bar chart to show the sales of RMs by RM category in 2012 compared to 2017. Data compiled from 2017 Euromonitor International - Passport 'Ready Meals in the United Kingdom'.

PLC had the largest share of sales in 2017 at 18%, followed by J Sainsbury PLC, Marks and Spencer PLC and finally ASDA Group Ltd at 11%. However, the largest market shares held by parent companies are Bakkavor Group (11.9%), Northern Foods Ltd (8.4%) ad Birds Eye Ltd (7.1%), as each of these companies supply multiple brands and RM ranges to supermarkets and other food providers (Figure 2).

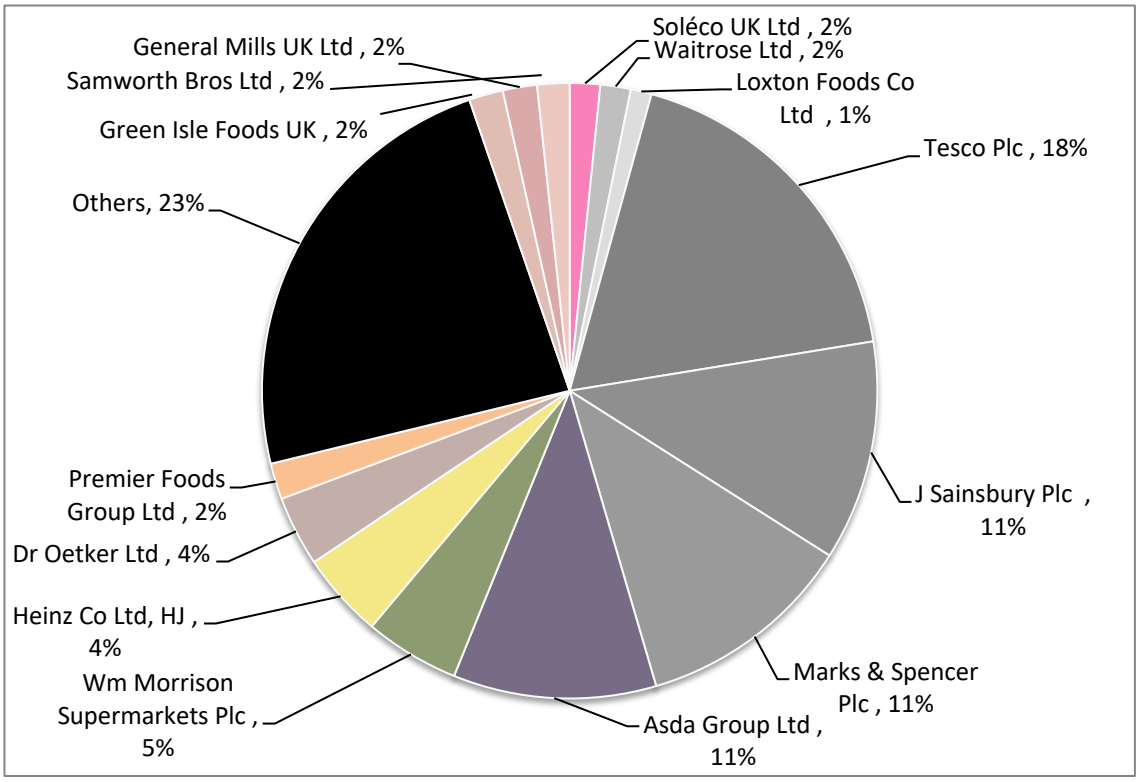


Figure 2: Pie chart to show the sales of RM by brand in 2017. Data compiled from 2017 Euromonitor International - Passport 'Ready Meals in the United Kingdom'.

Another informative data source is IBISWorld, which analyses and provides supply chain information and industry risk rating reports for prepared meals manufacturers. Information is gathered from economic, demographic and government data. These foods include meals that are chilled, frozen, or stored in airtight containers such as cans or jars. The data for this research has been gathered from 'Prepared Meal Manufacturing in the UK' (IBISWorld, 2018). Data from IBISWorld (2017), shown in Figure 3, found that the largest proportion of RM sales were to single adults (34%), followed by single-parent households (27%).

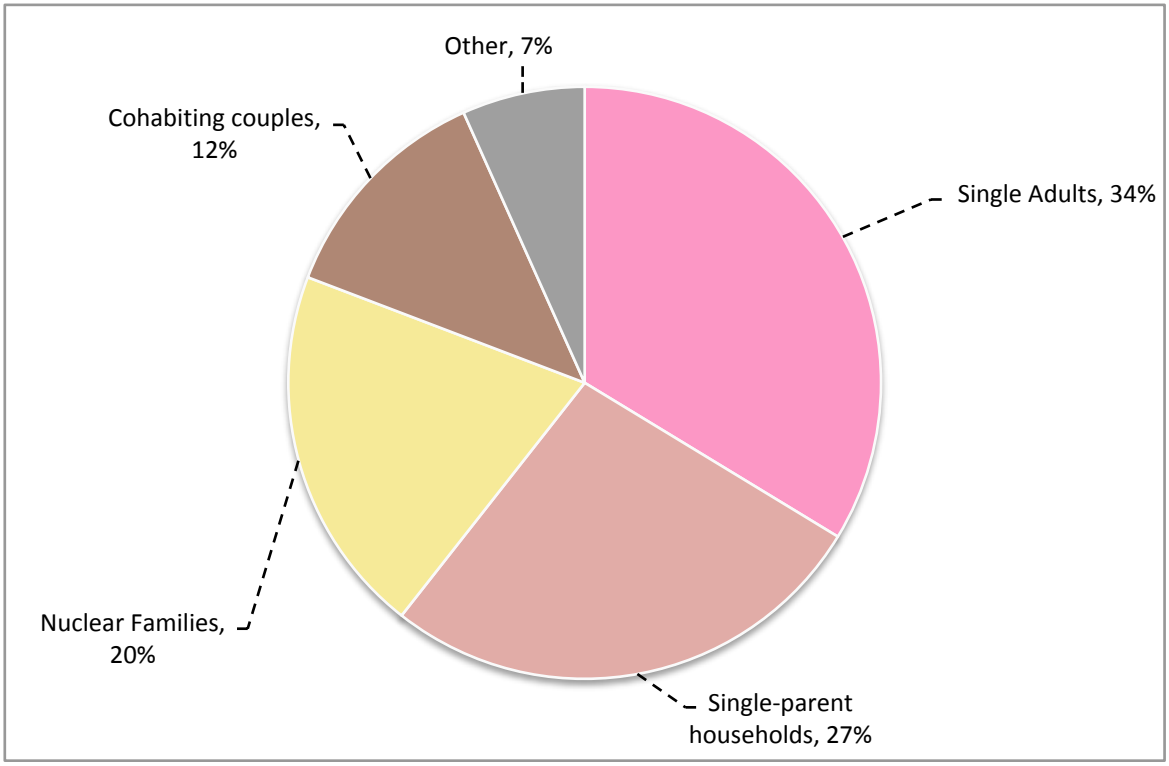


Figure 3: Pie chart to show the major market segmentation of all types of ready meals in 2017. Data compiled from IBISWORLD (2017) 'Prepared meal manufacturing in the UK Industry Report'

1.5. Manufacture and production of ready meals and convenience foods.

As the UK is still governed by the rules of the European Union (EU), food products are highly regulated by law to ensure that consumers are protected against infection or disease. Examples of ways that foods are assessed for quality and safety in the UK include the use of the Red Lion stamp on eggs; indicating that the hens have been vaccinated against salmonella, and the red tractor scheme, which sets standards for animal welfare, food safety, traceability and environmental protection (Department of Health, 2012).

In 2014, the quality of RMs was disputed during the horsemeat scandal, where horsemeat was found in processed foods that were marketed as beef, lamb or pork (Agnoli et al., 2016). Following this, the red tractor assurance company released a new 'Made With' logo which is now used as quality assurance, indicating traceability standards for the ingredients used in RMs such as meat, dairy, fruit, vegetables and oils (NFU Mutual, 2018).

The aim of the 'Made With' logo is to allow consumers to make an informed choice about the food they eat. Another way the population can have a better understanding of food they consume is through the use of nutrition information on the front and back-of-pack labelling (FOP/BOP) (Food Standards Agency, 2017). Retained European law states that BOP labelling must provide information on energy, fat, saturated fat, protein, carbohydrate, free sugars, and salt. Other supplementary nutrients can be detailed if, for example, there is an associated health claim with the food product, but these are optional and in most cases vitamin and mineral content (other than salt) are not provided (European Commission, 2011).

In the UK, the nutritional information shown on BOP labelling is usually calculated by inputting the ingredients into nutrition software such as Nutritics (Nutritics, 2018). This software is populated using food composition tables (FCTs), the most notable being The McCance and Widdowson – Composition of Foods (Finglas et al., 2015). Others include the Composition of Food Integrated Dataset (Public Health England, 2015) and Eurofir (EuroFIR AISBL, 2018). The information within the FCTs are collated through analytical testing to establish the macro and micronutrient information about specific foods prepared in a variety of ways. Using the software is a convenient way to populate nutrition labelling and provide a comprehensive nutrient list by inputting ingredients and quantities used in a given recipe.

Using the information on the BOP labelling, conclusions can be made about the nutritional adequacy of RMs. For example, the nutrient composition of foods that are prepared in similar ways across brands and manufacturers such as bread, butter and sugar may have more consistent BOP labelling. However, due to the nature of composite foods such as RMs, which have a larger variety of ingredients and processing steps, there is a risk of uncertainty regarding nutrient content (Bender, 1978). These differences in the macro and micronutrients highlight the ambiguity of the claimed nutritional quality of RMs, even with strict tolerances specified for nutrients outlined in retained EU regulation No. 1169/2011 (European Commission, 2012).

Studies by Remnant and Adams (2015) and Celnik et al. (2012) investigated the macronutrient content of RMs using the nutrition information provided on the label, and

found that RMs are nutritionally misrepresented. Products represented as 'healthy' were high in saturated fat and salt when the traffic light FOP nutrition guidelines were used. Furthermore, the average RM (400g) was found to contain between 85-195kcal/100g, which means that a portion could contain between 340-780kcal. This may be too few or too many calories for the NHS guidance, which states that lunch or dinner should be approximately 600kcal (Public Health England, 2019a, Celnik et al., 2012).

These findings were also supported by Anderson et al. (2008) and Howard et al. (2012) who found that meals did not meet nutrient based guidelines, where the energy, fat, saturated fat and sodium were all above recommended levels. It should be noted that when the macronutrient quality of RMs were compared to cooking the same meal from scratch using nutrient analysis software, RMs had the same or similar nutrient profiles as home cooked versions of the meal (Naruseviciute et al., 2015). However, the accuracy and reliability of these findings are reliant on the precision of the ingredients and cooking methods that were input into the software.

Chemical analysis of RMs has shown large differences between the macronutrient content provided on the labelling, and the actual nutrient content of the meal quantified using analytical techniques. For food processing, there is an acceptable nutrient tolerance of 10%. A study by Kanzler et al. (2015) found that of the 32 meals tested, 26 of the meals deviated more than 10% for fat, with two meals deviating by more than 100% from stated content. Furthermore, there were 14 meals where the values were greater than the ±10% accepted deviation for total energy content (Celnik et al., 2012, Kanzler et al., 2015). These findings highlight that the method of populating the nutritional information for labels may be inaccurate, or the FCTs that are being utilised to measure other nutrients in the diet may be unreliable for this type of processed food.

Celnik et al (2012) and Anderson et al. (2008) propose a harmonisation of the nutritional standards for macronutrients in RMs, so that consumers can make an informed and accurate decision about the food products they choose to eat. In addition, due to the necessity of having accurate estimates of the nutrient content of foods, more information is required to determine the impact that processing of foods has on the nutritional quality of RMs, as these may not be available using the FCTs.

1.6. The impact of food processing on vitamin content

The term ‘food processing’ describes all types of food handling, from the harvest of produce or rearing of livestock, to the point at which the food is ready to eat. Processing can be carried out in an industrial or in a domestic setting, with many practices having the potential to impact the nutritional quality of the final product.

The impact of domestic and industrial food processing on the nutritional quality of foods has been reviewed (Bender, 1978, Fabbri and Crosby, 2016, Rickman et al., 2007, Mueller, 1990, Hammink, 1978, Sucupira et al., 2012, Weaver et al., 2014, Hotz and Gibson, 2007, Reddy and Love, 1999, Gregory, 1985, Lund, 1988, Stahl, 2014, Henry and Heppell, 2007, Devi, 2015, Prochaska et al., 2000), and concluded that processing food has a range of benefits including increasing bioavailability of nutrients, improving shelf-life, making foods more convenient to prepare and providing more affordable food options. However, loss and degradation of nutrients can occur when ingredients are heated, peeled or chopped (Carmody and Wrangham, 2009, EUFIC, 2006).

For macronutrients, minerals and fat-soluble vitamins; A, D, E and K, cooking and processing has little effect on the nutrient content of the food and mostly remain unchanged. Only vitamin E is susceptible to loss through heating at high temperatures such as frying, or during the process of milling wheat to make flour. However, water-soluble vitamins (WSVs) such as the B-group vitamins and vitamin C are susceptible to loss during both domestic and industrial cooking processes. An overview of how cooking and processing can affect the WSV content of food is displayed in Table 1.

Leaching of nutrients occurs when foods are boiled, leading to the diffusion of hydrophilic vitamins into the cooking water. If the water is discarded after the cooking process has completed, the vitamins will be lost. Alternative cooking processes that have a less detrimental effect on the vitamin content of foods include steaming vegetables, or using the cooking water in the dish such as in a soup or stew (Ryley and Kajda, 1994, Fabbri and Crosby, 2016).

Table 1: Table to describe the essential water-soluble vitamins, function, and how cooking practices can affect them

Name	Food Sources	Susceptibility to food processing	Metabolic function	Reference
Thiamine (B1)	Fortified cereals, beef and pork, legumes, peas, nuts and seeds	Heat liable, cleavage by sodium metabisulphite, alkaline conditions, losses due to leaching into cooking water	Carbohydrate energy metabolise especially in brain and nervous system	(Bender, 1978; Bettendorff, 2012; P. Finglas et al., 2015)
Riboflavin (B2)	Eggs, offal, lean meat, milk and fortified breakfast cereals, green vegetables	Leaching into cooking water. Destroyed under alkaline conditions, degradation when exposed to light	Essential component of coenzymes, which play major roles in energy production; cellular function, growth, and development; and metabolism of fats, drugs, and steroids	(Bender, 1978; McCormick, 2012)
Niacin (B3)	Liver (beef, pork, chicken, turkey), salmon, tuna, yeast extract, peanuts	Milling of cereals causes losses due to discarding bran and germ, leaching into cooking water.	Essential components of nicotinamide adenine dinucleotide phosphate (NADP), a reducing agent in the catabolism of fat, carbohydrate, protein, and alcohol; DNA repair; fatty acid and cholesterol synthesis.	(Bender, 1978; Penberthy & Kirkland, 2012)
Pantothenic acid (B5)	Fortified cereals, dried mushrooms, liver, dried egg yolks and sunflower seeds	Some leaching into cooking water	Required to synthesise coenzyme-A (CoA) and important for synthesis and metabolism of proteins, carbohydrates, and fats.	(Bender, 1978; Miller & Rucker, 2012)
Pyridoxine (B6)	Fortified cereals, pork, turkey, beef, bananas, chickpeas, potatoes	Losses during sterilisation of milk, leaching into cooking water.	Pyridoxal 5'-phosphate; the active form of pyridoxine acts as a coenzyme in more than 100 enzyme reactions in amino acid, glucose and lipid metabolism.	(Bender, 1978; da Silva, Russell, & Gregory, 2012)

Name	Food Sources	Susceptibility to food processing	Metabolic Functions	Reference
Biotin (B_7)	Liver (beef or pork), eggs, yeast extract, whole wheat bread	Inactivated by lipid peroxides from foods that have been heated to high temperatures and then stored e.g. dehydrated eggs	Acts as a coenzyme for gluconeogenesis and carboxylase enzymes involved in synthesis of fatty acids, isoleucine and valine.	(Bender, 1978; Zempleni, Wijeratne, & Kuroishi, 2012)
Folate B_9	Fortified bread, cereals, rice, legumes, green leafy vegetables, orange juice	Unstable below pH 5, losses due to leaching into cooking water, folate is readily oxidised. Destroyed by light	Acts as an initial substrate in spermatogenesis, a cofactor in DNA and RNA synthesis and therefore in the development of the neural tube during pregnancy; amino acid synthesis, regulation of homocysteine levels	(Bailey & Caudill, 2012; Bender, 1978)
Cobalamins B_{12}	Fish, meat, poultry, eggs, milk and milk products.	Leaching into cooking water, destruction due to interaction with vitamin C in gastrointestinal tract.	Cofactor in DNA synthesis; fatty acid and amino acid metabolism, myelinogenesis and red blood cell maturation for bone marrow.	(Bender, 1978; Stabler, 2012)
Ascorbic Acid (vitamin C)	Fruits and vegetables	Heating in slightly acidic solutions (pH6), leaching into cooking water, oxidised when exposed to air. Bruising and handling	Acts as a cofactor for the synthesis of collagen, carnitine and norepinephrine. Immune function.	(Bender, 1978; Johnston, 2012)

Foods used in RMs are processed and cooked to ensure that the meals only need reheating before consumption. These industrial cooking processes could have a detrimental impact on the nutrient profile of the RMs, especially for WSVs. Therefore, it is important to investigate whether those who are consistently consuming RMs are at risk of nutrient deficiency if these meals do not contain essential vitamins and are the main dietary source of WSV.

Some food types are fortified to replace nutrients lost during processing. For example, wheat processing to produce white flour led to the implementation of the UK Bread and Flour regulation of 1998, which specifies that white flour must be fortified to replace nutrients that are lost during the milling process (The Bread and Flour Regulations, 1998). However, the vitamins do not need to be replaced in wholemeal flour as the bran

and germ are retained, and the regulation does not extend to gluten free flours. Another example of mandatory fortification of foods, is the legislation in place which states that margarine, a cheaper butter substitute, is fortified with vitamin A and D so that the nutritional profile of margarine resembles that of butter (O'Connor and Benelam, 2011, Dary and Mora, 2002).

Methods such as drying, curing and salting act to extend shelf life by removing water to create an environment where bacteria cannot grow. Other preservation methods include pickling, brining and fermenting to form a low pH environment, making an undesirable media for the proliferation of harmful bacteria (Thorne, 1986). Although these techniques have been used for more than 5000 years, they are likely to give the food a more acidic or salty taste, and the appearance of the food may change to a duller or browner colour (Thorne, 1986). Nowadays, synthetic preservatives are more widely used as they increase the shelf-life of the food without altering taste or appearance (Gould, 1996). They also prevent decomposition, microbial growth and oxidation which can cause browning, by acting as an antioxidant and antimicrobial.

One of the most popular groups of synthetic preservatives is known as sulphites. They are used in a variety of food and drink including wine, dried fruits, sausages and canned pulses to preserve flavour and colour. However, sulphites, which are also an allergen, have been shown to degrade thiamine in food by breakage of the methyl-bridge within the thiamine molecule (Simon, 2003, Leichter, 1969, Iammarino et al., 2012). Therefore, the use of this preservative is regulated by law (European Commission, 2011).

Freezing is also a well-documented method of preservation, by which the low temperature of the food prevents bacterial growth. Freezing of food in colder climates has been used as early as 1780 BC, while industrial freezing did not start until 1860 in Australia (Wiltshire Farm Foods, 2018). However, much of the modern technology of freezing started through the work of the Birdseye food company, who used the freezing process to preserve fish and peas soon after harvest in order to retain nutrients, taste and increase shelf life (Wiltshire Farm Foods, 2018).

Although some of these preservation methods, particularly freezing, are used to extend the shelf life of RMs, little is understood about the effect that these processes have on the WSV profile of RMs available in the UK. Due to the relative stability of some WSVs it is it outside the scope of this research to examine all WSVs found in foods; therefore, to identify the vitamins that will be the analysed for this research, considerations are made with regards to 1) susceptibility of the vitamin to processing, 2) the risk of vitamin deficiency in the population and 3) the possibility of testing more than one vitamin using the same extraction protocols. For the purposes of the research outlined in this thesis, five vitamins - thiamine, riboflavin, folate, vitamin B12 and vitamin C - have been chosen to form the basis of the analysis of RMs.

Other vitamins, namely niacin, biotin, pantothenic acid and pyridoxine are not included due to the stability of these vitamins in foods (Lawrance and Patel, 2014, Combs, 2012, Lawrance, 2015a, Berry Ottaway, 2009). Additionally, there is lack of evidence to support deficiencies in the UK diet due to the ubiquitous nature of foods that contain these vitamins (Litwack, 2018). It should be noted that although there are some differences between analysed concentrations of nutrient values in FCTs (seen in meat and dairy products) (Gille and Schmid, 2015) any differences in nutrient content can be attributed to seasonality, growing methods, varying processing methods, and different methods of analysis.

In the next section, the justification for the inclusion of the five WSV in this research along with the role these vitamins have within the body and the implications of not meeting the recommended nutrient intake (RNI), along with their susceptibility to industrial food processing techniques is provided.

1.6.1. Thiamine

Thiamine (vitamin B1) is an essential WSV, with the chemical name 2-[3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium-5-yl]ethanol. It is an essential vitamin as humans are not able to produce it endogenously. It is found in many foods, with the highest concentrations in whole grains, meat, fish and yeast, which contain approximately 3mg/kg (wet weight) (Bettendorff, 2012). The UK requirements for thiamine are outlined in Appendix ii (Department Of Health Committee On The Medical Aspects Of Food Policy, 1991). In the UK, the most popular source of thiamine are bread

and cereal products, fortified in milled white flour (The Bread and Flour Regulations, 1998). Long cooking, pasteurisation and leaching caused by boiling vegetables in water also lead to losses of the vitamin (Bettendorff, 2012). Radiation processing (Irawati et al., 2003) and sous vide methods (Tansey et al., 2005) of cooking, however, retain thiamine content of foods.

Thiamine in food is mostly found as free thiamine, but only thiamine diphosphate (ThDP) has been found to be biologically active. Other derivatives include thiamine monophosphate (ThMP), thiamine triphosphate (ThTP), adenosine thiamine triphosphate (AThTP) and adenosine thiamine diphosphate (AThDP) (Bettendorff, 2012), shown in Figure 4. As free thiamine in food is not bioavailable, the enzymatic action of cytosolic pyrophosphatase in the upper small intestine is needed, which phosphorylates thiamine to its diphosphate form; required for absorption (Combs, 2012).

Ingested thiamine is absorbed in the jejunum of the small intestine. The active transport of thiamine between the gut lumen and the blood stream is carried out by thiamine transporter-1 (ThTr1) and thiamine transporter- 2 (ThTr 2) up to 2mM (Combs, 2012). Phosphorylated thiamine can also be transported into the blood by reduced folate carrier 1 (RFC1), as they are highly homologous to ThTr-1 and ThTr 2 (Zhao et al., 2001). At high doses of thiamine (>2.5mg dose), passive diffusion across the membrane can occur and once in the blood stream, thiamine is mostly bound to erythrocytes and taken up into cells via the ThTr-1 and ThTr-2 transporters.

Within the cells, thiamine is phosphorylated by two enzymes; thiamine diphosphokinase in the cytoplasm, which catalyses the formation of thiamine pyrophosphate (ThPP), and ThPP-ATP phosphoryltransferase, which further catalyses the formation of ThTP (Figure 5). ThPP is the metabolically active form of thiamine and acts as a cofactor within the mitochondria for carbohydrate metabolism via glycolysis (the metabolism of glucose), pentose phosphate pathway (NADPH generation and nucleotide synthesis), the citric acid cycle (ATP generation) and breakdown of branched chain amino acids. A schematic of utilisation of ThPP has been shown in Figure 6.

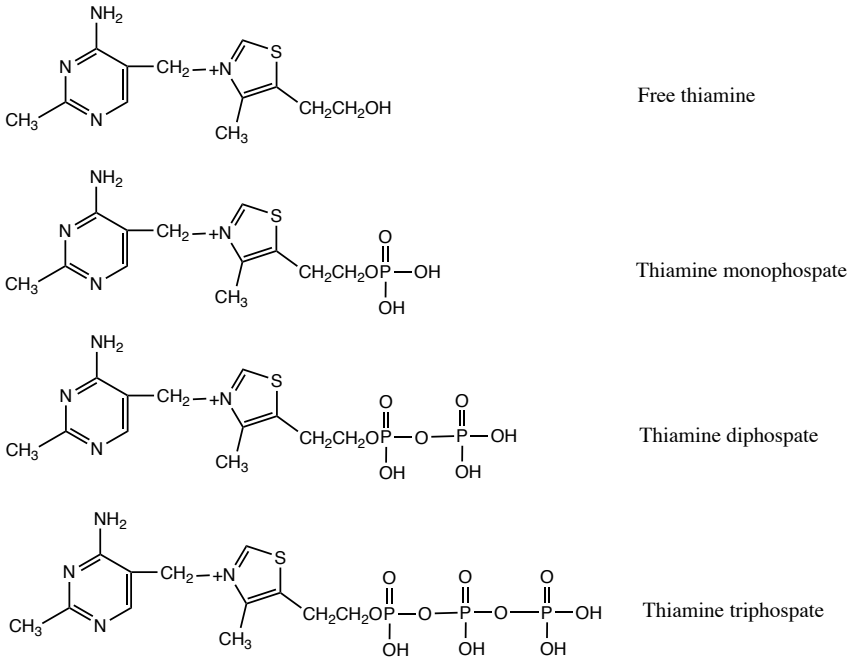


Figure 4: An overview of the chemical structures of free thiamine, and its biologically active derivatives; thiamine monophosphate (TMP), thiamine diphosphate (TDP) and thiamine triphosphate (TTP).

If requirements for thiamine are not met in the diet, deficiency symptoms include anorexia, cardiac enlargement, paraesthesia and muscle weakness (Frank, 2015). The syndrome associated with thiamine deficiency is known as Beriberi and has three clinical types; wet, where cardiac symptoms are more prevalent; dry, which is more prevalent in

adults and is characterised by impaired sensory and nerve stimulation, and infantile, usually the result of breastfeeding mothers who are thiamine deficient (Combs, 2012). Those at increased risk of thiamine deficiency include individuals who are alcohol dependent due to reduced intake of thiamine-containing foods and impairment of the gut lining (Frank, 2015).

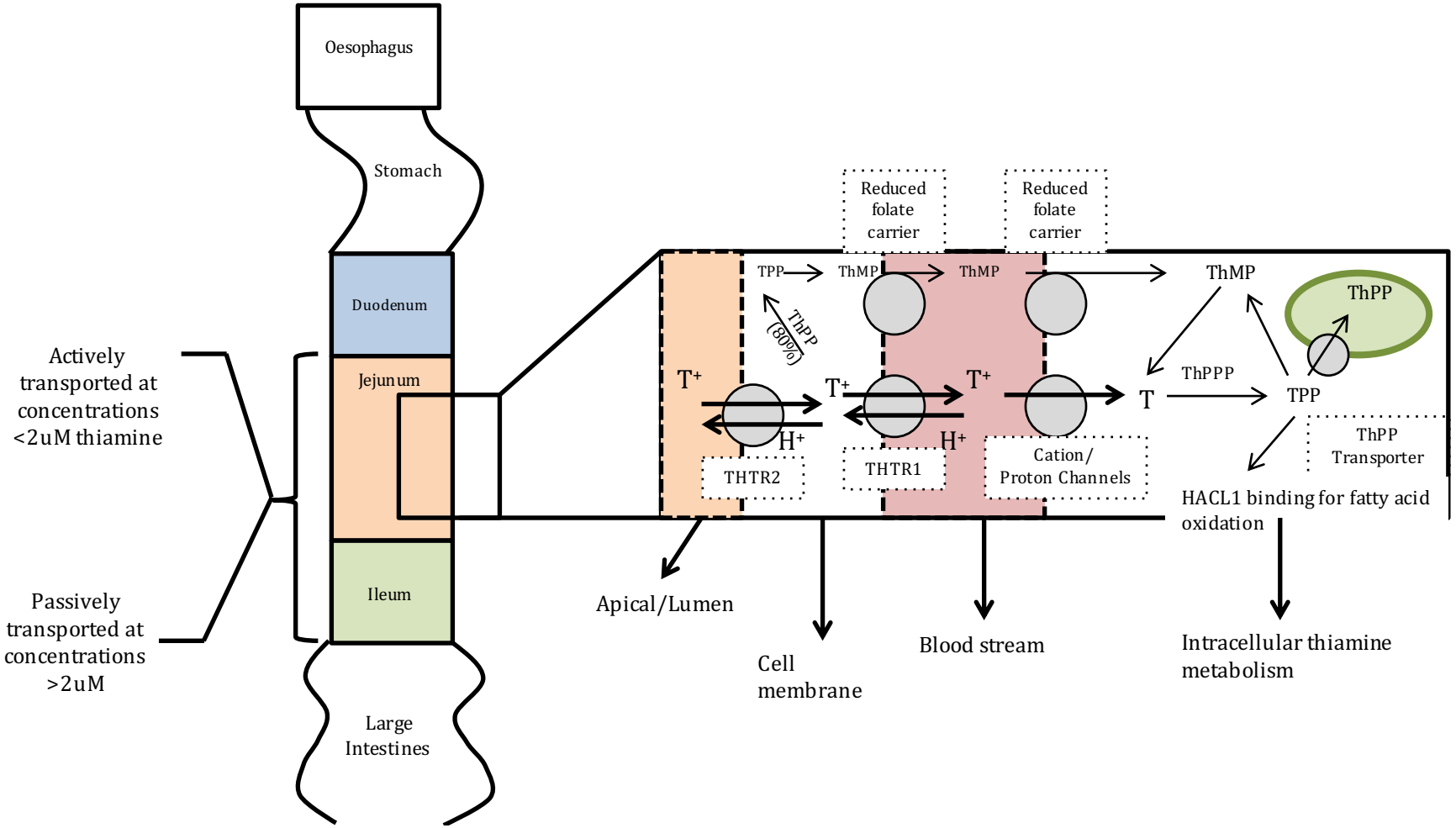


Figure 5: Site of intestinal absorption of thiamine. The pathway of thiamine transport from the jejunal lumen to cells. ThTr1 (thiamine transporter-1) and (ThTr 2) thiamine transporter- 2, ThPP (thiamine phosphokinase), ThPPP (thiamine pyrophosphokinase), HACL1 (2-hydroxyacyl-CoA lyase) ThMP (thiamine monophosphate), ThPP Transporter (Thiamine phosphate transporter).

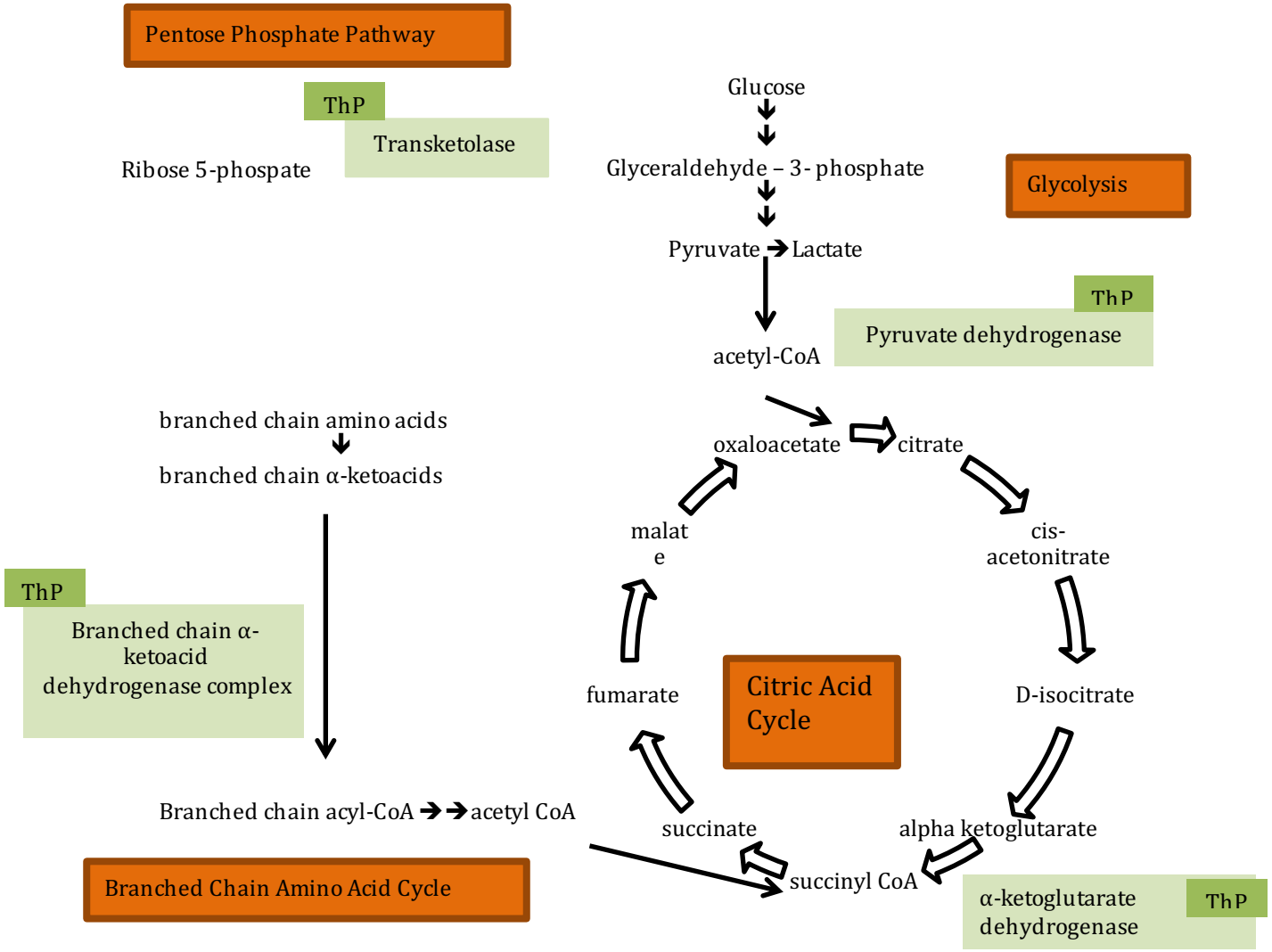


Figure 6: A schematic of the metabolic pathways requiring Thiamine Pyrophosphate (ThPP).

Thiamine is most stable at pH 2-4, whilst unstable at alkaline pH (>pH7) and is broken down in the presence of anti-thiamines. The most common anti-thiamines found in food are sulphites, which cleave thiamine at the methyl bridge, and thiaminase enzymes that are found in some fish (eel, carp or Baltic herring) and shellfish, which enzymatically breakdown thiamine. There are several methods that have been used to analyse the thiamine concentration in food including colorimetry, electrochemistry, high-performance liquid chromatography (HPLC), mass spectrometry, fluorescence and ultraviolet (UV), which have been reviewed extensively by Edwards et al. (2017). In summary, analytical methods using chemical activity use the cationic charge of thiamine via exchange for different isolation and separation techniques, the activity of thiamine in the UV range, and its ability to be oxidised and detected using fluorometric methods.

Other biological methods use thiamine dependent organisms, or organisms where thiamine has a role as a co-factor for enzymatic reactions. However, due to the interference of other WSVs using UV, lack of progression and specificity of the electrochemical methods and expense of the mass spectrometry method, the most common ways of thiamine measurement in food are the microbiological assay and HPLC methods.

1.6.2. Riboflavin

Riboflavin (vitamin B2), 7,8-dimethyl-10-ribityl-isoalloxazine, is an essential water-soluble B-group vitamin (Zempleni et al., 2013). The main sources of riboflavin in the western diet are milk and dairy products, with the richest being beef and yeast extract, meat, dairy and fortified breakfast cereals (Finglas et al., 2015), all which provide between 1.1-11.9mg/100g fresh weight. The UK requirements for riboflavin are outlined in Appendix ii (Department Of Health Committee On The Medical Aspects Of Food Policy, 1991). In the UK, bread and cereals are fortified with riboflavin as it can be lost during the milling process (The Bread and Flour Regulations, 1998).

The biologically active forms of riboflavin are flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are important cofactors in redox reactions within aerobic cells (Powers, 2003). During intermediary metabolism including β-oxidation of

fatty acids, carbohydrate metabolism and amino acid metabolism, FAD and FMN act as coenzymes in electron transfer during energy metabolism.

Free riboflavin (Figure 7a) found in food is converted to FMN (Figure 7b) and FAD (Figure 7c) in the small intestine. The structure of free riboflavin is comprised of an isoalloxazine ring bound to a ribitol side chain (Powers, 2003). The main site of absorption is in the duodenum and jejunum. However, before absorption, the vitamin must be hydrolysed to free it by nonspecific hydrolases within the enterocytes of the brush border membrane. The absorption limit of riboflavin is estimated to be approximately 25mg, greater than the requirement, where uptake is enhanced during times of vitamin deficiency and the presence of bile salts (Combs, 2012), shown in Figure 8. Once transported into membrane brush border cells, riboflavin is phosphorylated to FMN by ATP-dependent flavokinase, and thereafter actively transported into portal circulation as both FMN and free riboflavin via riboflavin transport proteins, RFVT1, RFVT2 and RFTVT 3 (Combs, 2012, Ghosal and Said, 2012) (figure 8).

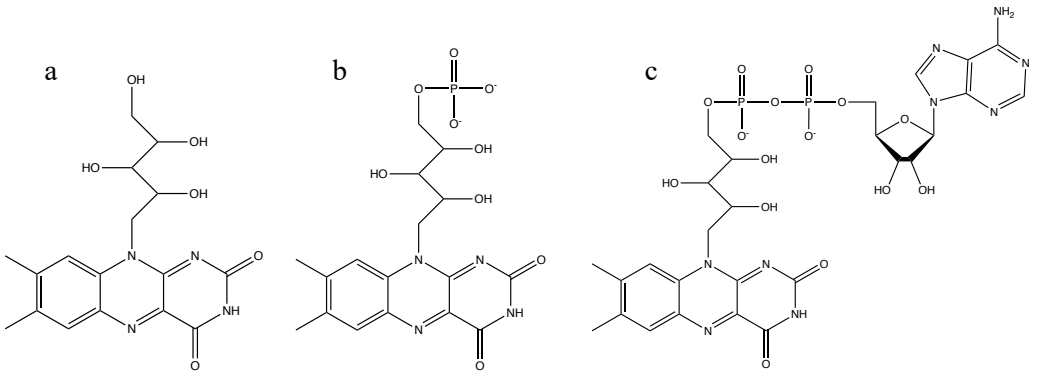


Figure 7: Chemical structures of riboflavin (a), flavin mononucleotide (FMN) (b), flavin adenine dinucleotide (FAD) (c).

While obvious clinical signs of riboflavin deficiency are uncommon due to the many food sources of riboflavin, the risk of subclinical riboflavin deficiency is increasing in prevalence, which can lead to a reduction in function of key metabolic pathways (Combs, 2012). Subclinical deficiency, possibly due to a decline in milk consumption in the UK (Mosegaard et al., 2020), may manifest as impairments of carbohydrate, fatty acid and amino acid metabolism. This malfunction in metabolism leads to the loss of absorption

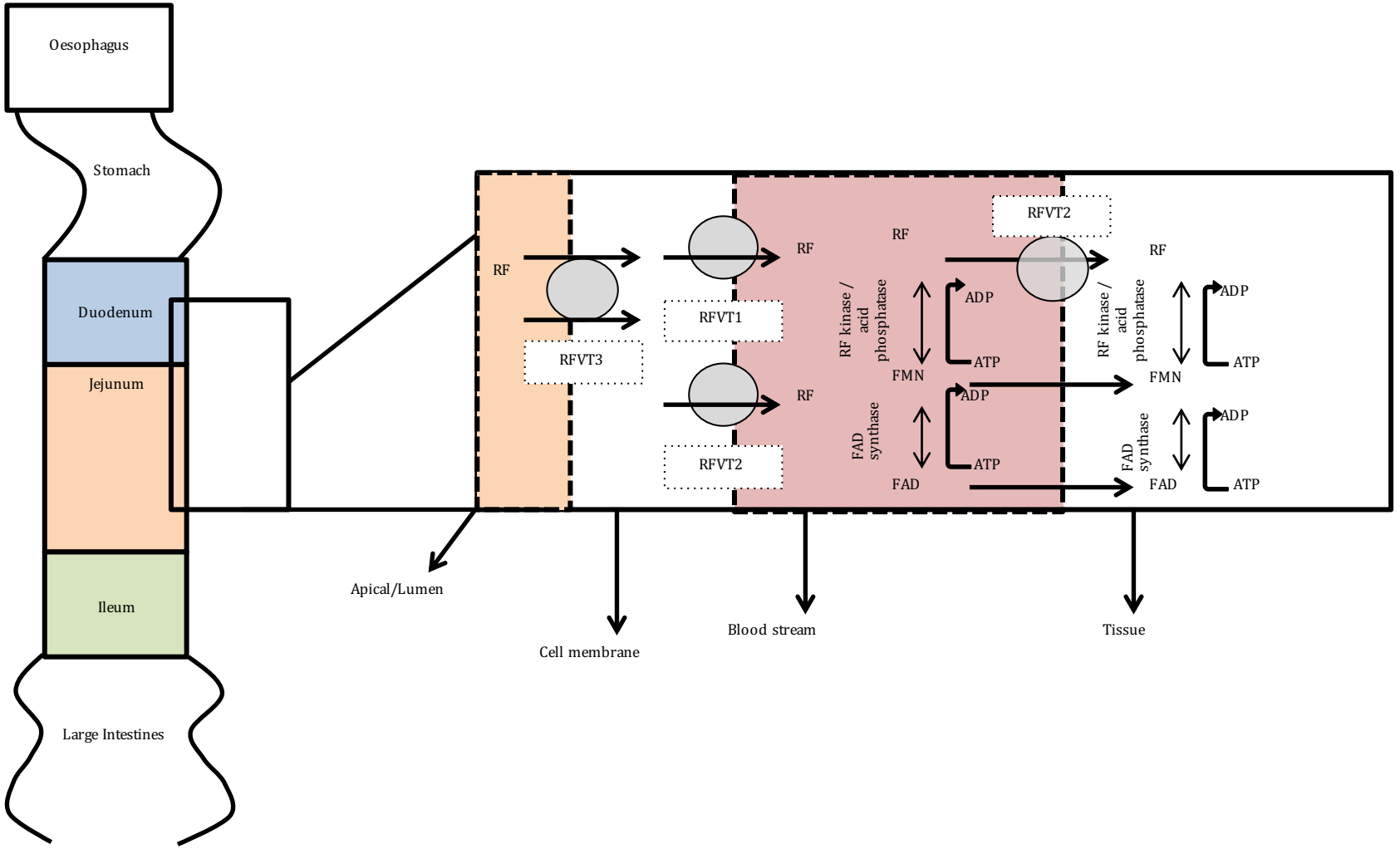


Figure 8: A schematic of riboflavin absorption in the duodenum and the jejunum and metabolism in tissue. RFVT1 (riboflavin transporter-1), RFVT-2 (riboflavin transporter-2, RFVT3 (riboflavin transporter-3), RF (riboflavin), FAD (flavin adenine dinucleotide), FMN (flavin mononucleotide), ADP (adenosine diphosphate) and ATP (adenosine triphosphate).

of dietary iron due to disruption of microvilli function in the gut, and can further lead to loss of appetite and impaired growth (Combs, 2012).

Although riboflavin deficiency is associated with deficiency of other WSV's, populations that have been found to be at risk of riboflavin deficiency include those with malabsorptive diseases such as coeliac disease (Coeliac UK, 2017), or those who are alcohol dependant (Golbach et al., 2014). Therefore, with coeliac disease affecting at least 1% of the UK population and the increased risk of subclinical deficiency, the study of riboflavin is justified.

While riboflavin is stable under γ-irradiation and temperatures up to 100°C in nitrogen (N_2) or carbon dioxide (CO_2) environments, the rate of degradation is increased in oxygen (O_2) rich environments at temperatures as low as 36°C. The presence of water and metal sulphates in food during storage also decreases the riboflavin content. Riboflavin present in food is more stable under high temperatures compared to thiamine (Choe et al., 2005).

Light can also degrade riboflavin, where >50% of riboflavin was lost in dried pasta and milk within one day of being exposed to light (Choe et al., 2005). However, when riboflavin degradation is measured in fresh food, degradation only occurs in part of the food. For example, the riboflavin concentration in the centre of a block of cheese would remain unchanged, whereas the outer regions of the cheese would be affected (Choe et al., 2005).

Riboflavin is degraded by light through the process of Type I and Type II photosensitisation, which leads to fluorometric excitation of the vitamin, resulting in the formation of radicals. These reduced riboflavin and anionic riboflavin radicals can be oxidised to produce oxidised riboflavin, which is no longer bioavailable. Furthermore, the oxidised form of riboflavin can also degrade other vitamins present in food including vitamin A, vitamin C, vitamin D and vitamin E (Choe et al., 2005).

To quantify riboflavin, several methods have been used including fluorometry, HPLC, immunoassays and microbiological assays. However, as riboflavin fluoresces strongly at wavelengths between 440-500nm, this is the most commonly used method of analysis

when used with HPLC (Golbach et al., 2014). Other methods including immunoassays such as ELISA, and a microbiological assay utilising the riboflavin dependent *Lactobacillus rhamnosus* has also been reviewed (Golbach et al., 2014). However, due to the lack of specific antibodies to be used in the ELISA and the long period of incubation in the microbiological assay (Golbach et al., 2014), riboflavin analysis is usually carried out using HPLC with fluorescence detection, as outlined by Finglas and Faulks (1984). This method is also used for food analysis to provide data for McCance and Widdowsons Composition of Food database (Finglas et al., 2015) and will be used for this research.

1.6.3. Folate

The term 'folate', also known as vitamin B9, represents all folic acid derivatives which includes naturally occurring polyglutamates and folic acid; the synthetic folate form that is used for food fortification and supplementation (Iyer and Tomar, 2009). The derivatives of folate include 5-methyltetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-FTHF or folinic acid), 10-formyl-THF, 5,10-methylene- THF, unsubstituted THF and folic acid, shown in Figure 9. The basic structure of folic acid is a pteroyl group linked to a glutamic acid residue, which needs to be metabolised by dihydrofolate reductase in a series of pathways within the cell to be converted into the metabolically active tetrahydrofolate (THF) form. Folinic acid is the naturally occurring form of folate and is readily converted to the bioavailable THF.

For absorption of folate to occur in the proximal intestinal lumen, specifically the duodenum and jejunum, polyglutamate forms must be converted to the monoglutamate form. This absorption can occur passively or actively, where active absorption occurs mainly in the proximal small intestine (Scaglione and Panzavolta, 2014). The main folate transporters are reduced folate carriers (RFC), proton coupled folate transporters (PCFT) and folate-receptors (FR). RFC has a high affinity for 5-methylTHF and 5-formylTHF, and is readily transported into to the circulatory system where PCFT has increased folate absorption in low pH, and has a higher affinity for folic acid (Visentin et al., 2014). Folate receptors, also known as folate binding proteins, assist in folate uptake into cells and aid movement of folate across the apical membrane into the cell (Combs, 2012).

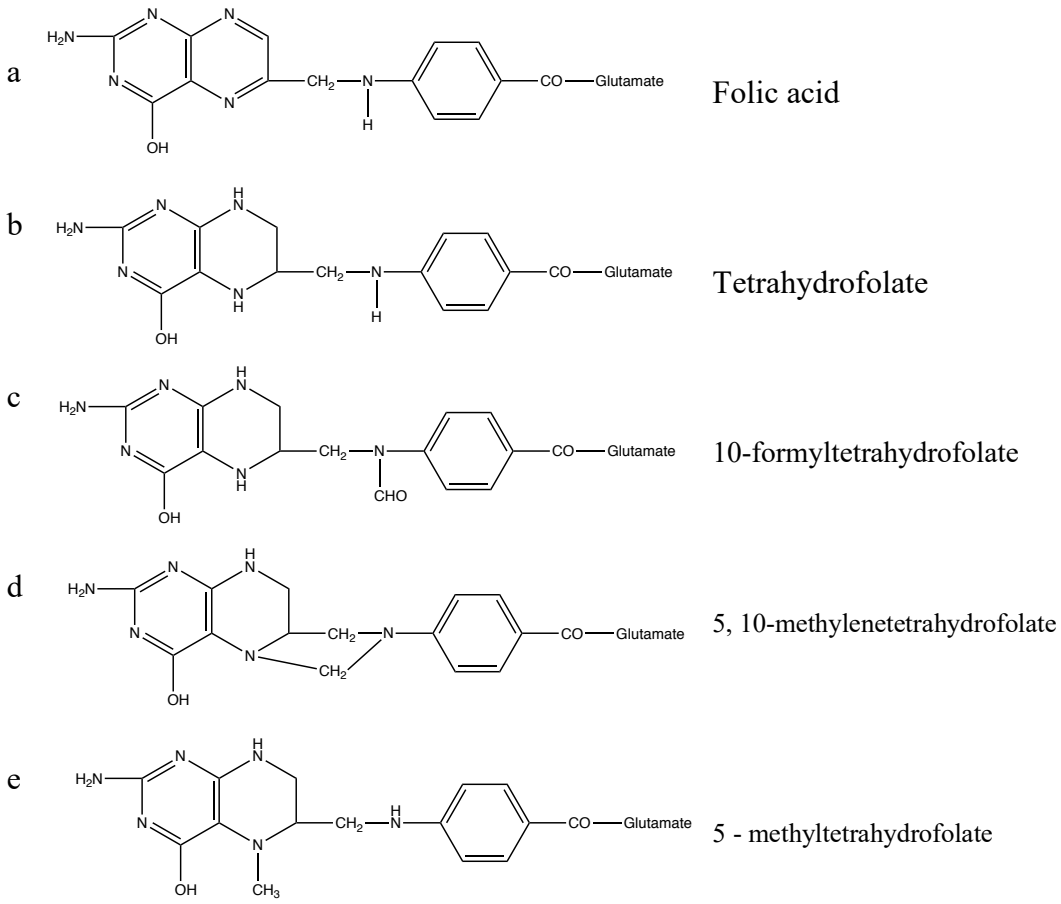


Figure 9: Chemical structure of folate and its derivatives; folic acid (a), Tetrahydrofolate (b), 10-formyltetrahydrofolate (c), 5, 10-methylenetetrahydrofolate (d), 5 – methyltetrahydrofolate (e).

Folate is an essential component in one carbon metabolism and the breakdown of amino acids, with the products of this used to carry out gene regulation, especially methylation and DNA synthesis processes which involves the transfer of the methyl group from folate to homocysteine, catalysed by methionine synthase. This formation of homocysteine is important for DNA methylation during transcription (Neidhart, 2016). Furthermore, 5-10-methylene THF is important for the production of RNA (Combs, 2012). A schematic of folate dependent mechanisms is shown in Figure 10.

The richest food sources of folate include yeast and beef extract, black eye and pinto beans, fortified breakfast cereals and offal meat, which can supply between 300 and 4000µg/100g fresh weight of food. The UK requirements for folate are outlined in Appendix ii (Department Of Health Committee On The Medical Aspects Of Food Policy, 1991).

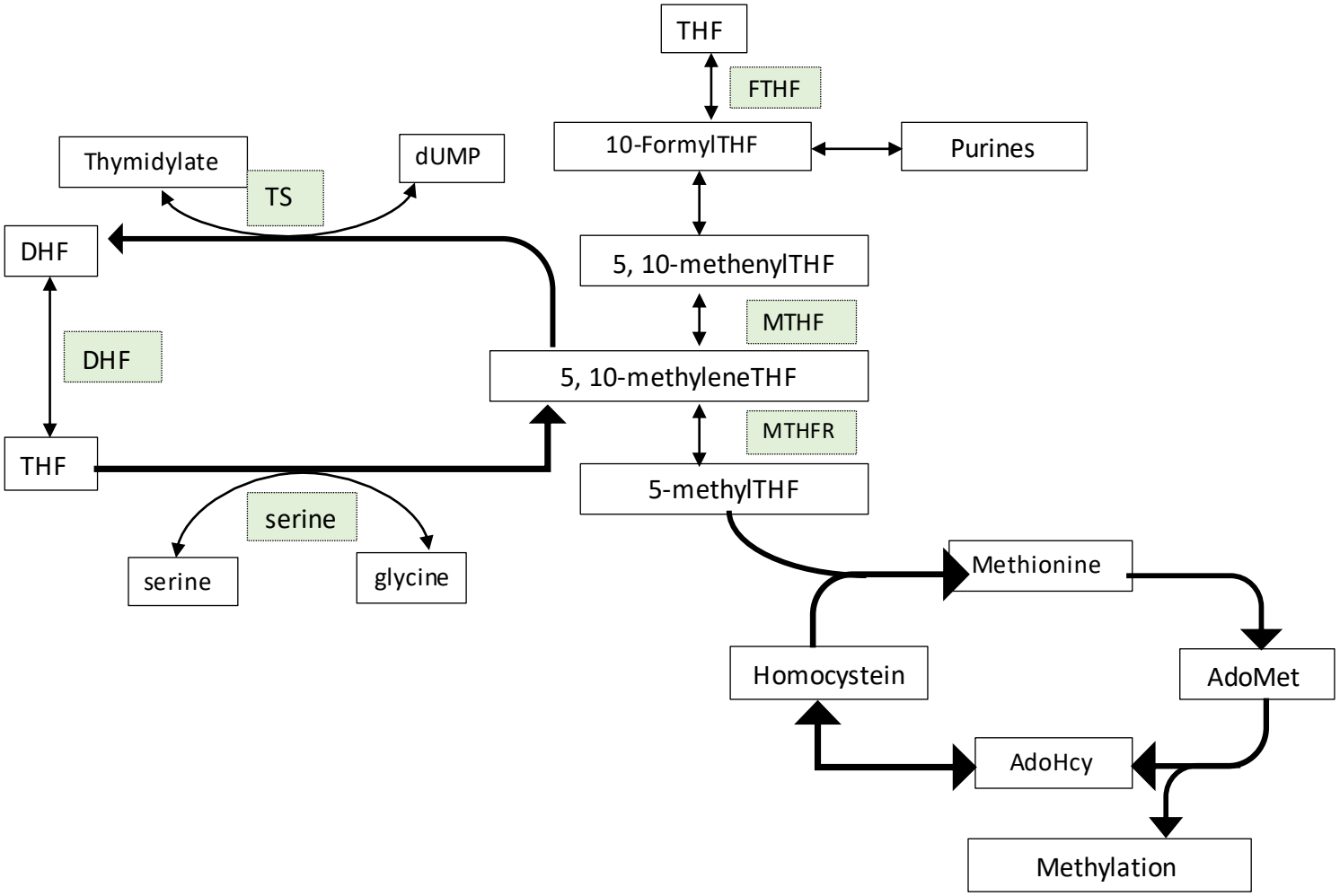


Figure 10: A schematic of folate metabolism. TS (Thymidylate synthase), DHFR (dihydrofolate reductase), DHF (dihydrofolate), THF (tetrahydrofolate), FTHFS (formate tetrahydrofolate synthase), MTHFD (methylenetetrahydrofolates dehydrogenase), MTHFR (methylenetetrahydrofolates reductase), AdoHcy (S-Adenosyl-L-Homocysteine), AdoMet(S-Adenosyl methionine).

Deficiency disease associated with low intake of folate is known as folate-deficiency anaemia. Due to the role of folate in DNA synthesis, one symptom of the disease is impaired biosynthesis leading to reduced cell division. Biochemical signs of deficiency are known as megaloblastic anaemia, which is characterised by large, nucleated, erythrocyte-precursor cells; macrocytes. Symptoms include dermatologic lesions, weakness, fatigue, depression, and shortness of breath. Furthermore, due to the reduction in cell division, megaloblastosis occurs in enterocytes leading to malabsorption and diarrhoea (Combs, 2012).

In pregnant women, folate is vital for brain and spinal cord development in the first trimester of pregnancy (Scott et al., 1994). The importance of this has been highlighted in a consultation with the UK government on the mandatory fortification of flour with folic acid (Department of Health and Social Care, 2019), due to the link between low folate intake in pregnant women and the increased risk of neural tube defects (Combs, 2012).

Although the recommendation that there should be mandatory fortification of folate to flour is being disputed, due to the ability of high doses used in fortification to mask the deficiency of vitamin B12, especially in older adults, the need to explore the folate content of RMs is justified as RMs are a possible sources of folate, and could contribute to a reduction in the risk of neural tube defects in infants (Craig et al., 1985, Mills et al., 2018a).

Folates can be oxidised by light, heat and oxygen, which causes the splitting of the folate molecule into biologically inactive forms that cannot be absorbed. However, the presence of antioxidants such as ascorbic acid and thiols can protect folate from oxidation (Johansson et al., 2008)

There are two validated methods for the determination of folate in food. Firstly, the competitive binding assay, which uses competitive binding between radiolabelled folate and that which is present in the food. The amount of radiolabelled bound protein is inversely correlated to the amount of vitamin present in the food. Although this method assumes that all derivatives of the nutrient have the same affinity for the folate-binding

protein, different products have various affinities for the protein. Thus, the derivative present in the food must be used for the calibration (Strandler, 2012).

Other assays that can be used to determine folate concentration in food include HPLC, liquid chromatography and immunoassays, but these methods require extensive clean-up and expensive analytical equipment. An alternative validated method for measuring the total folate content in food is by using a microbiological assay. The most commonly used organism for this assay is the lactic acid bacteria *Lactobacillus rhamnosus*, with other organisms including *Streptococcus lactis, Streptococcus faecalis, Pediococcus cerevisiae, Tetrahymena pyriformis* (geleii) and *Bacillus coagulans*, although the latter are used less frequently due to lack of sensitivity to all derivatives of folate (Arcot and Shrestha, 2005).

1.6.4. Vitamin B12

Vitamin B12 is the collective term for several related compounds that have cobalt as the central ion in a corrin ring (Figure 11, A). This group of related compounds are also called cobalamins, with hydroxycobalamins and cyanocobalamin being used for fortification, supplementation and pharmaceutical products. Other derivatives include deoxyadenosylcobalamin and methylcobalamin transformation of hydroxycobalamin (Figure 11, D) and cyanocobalamin (Figure 11, E). Methylcobalamin (Figure 11, C) and 5′-deoxyadenosylcobalamin (Figure 11, B), which are both utilised in the body during endogenous metabolism (Combs, 2012).

The metabolism of vitamin B12 has been outlined in Figure 12. Vitamin B12 in the diet is bound to protein; once the protein-bound vitamin enters the stomach, it is released from the complex due to the action of hydrochloric acid and pepsin in the stomach. To further stabilise vitamin B12, R-protein that was released from the salivary glands in the mouth binds to the vitamin B12 (Combs, 2012). Once the R-protein-vitamin B12 complex has reached the proximal small intestine, pancreatic protease act on the complex to release the R-protein. This allows intrinsic factor (IF), a glycoprotein, released from the parietal cells in the stomach to form a complex that allows vitamin B12 to associate with site-specific membranal transport proteins called cubulin, in the distal ileum. The IF-vitamin B12 complex is dissociated within the ileum cell and IF is degraded; Haptocorrin (TCI)

and transcobalamin (TCII) are then used to transport vitamin B12 into circulation via the portal system (Combs, 2012).

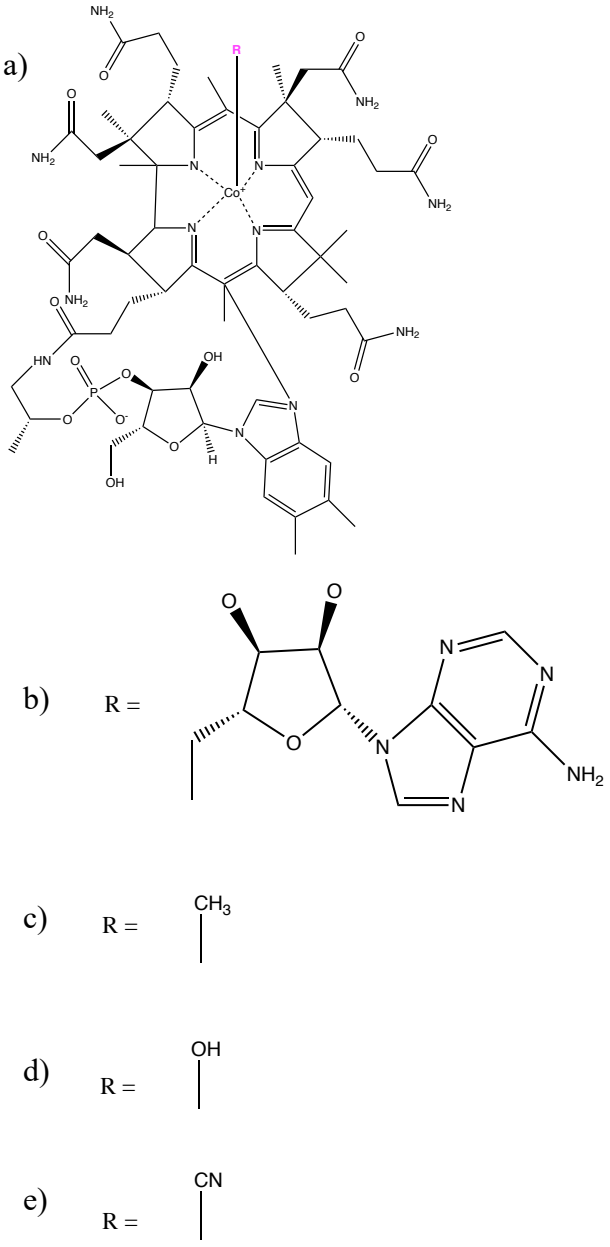


Figure 11: Chemical structure of vitamin B12 (a). The basic structure of vitamin B12 is shown above with the R groups below; b) 5´-deoxyadenosylcobalamin, c) methylcobalamin, d) hydroxycobalamin and e) cyanocobalamin.

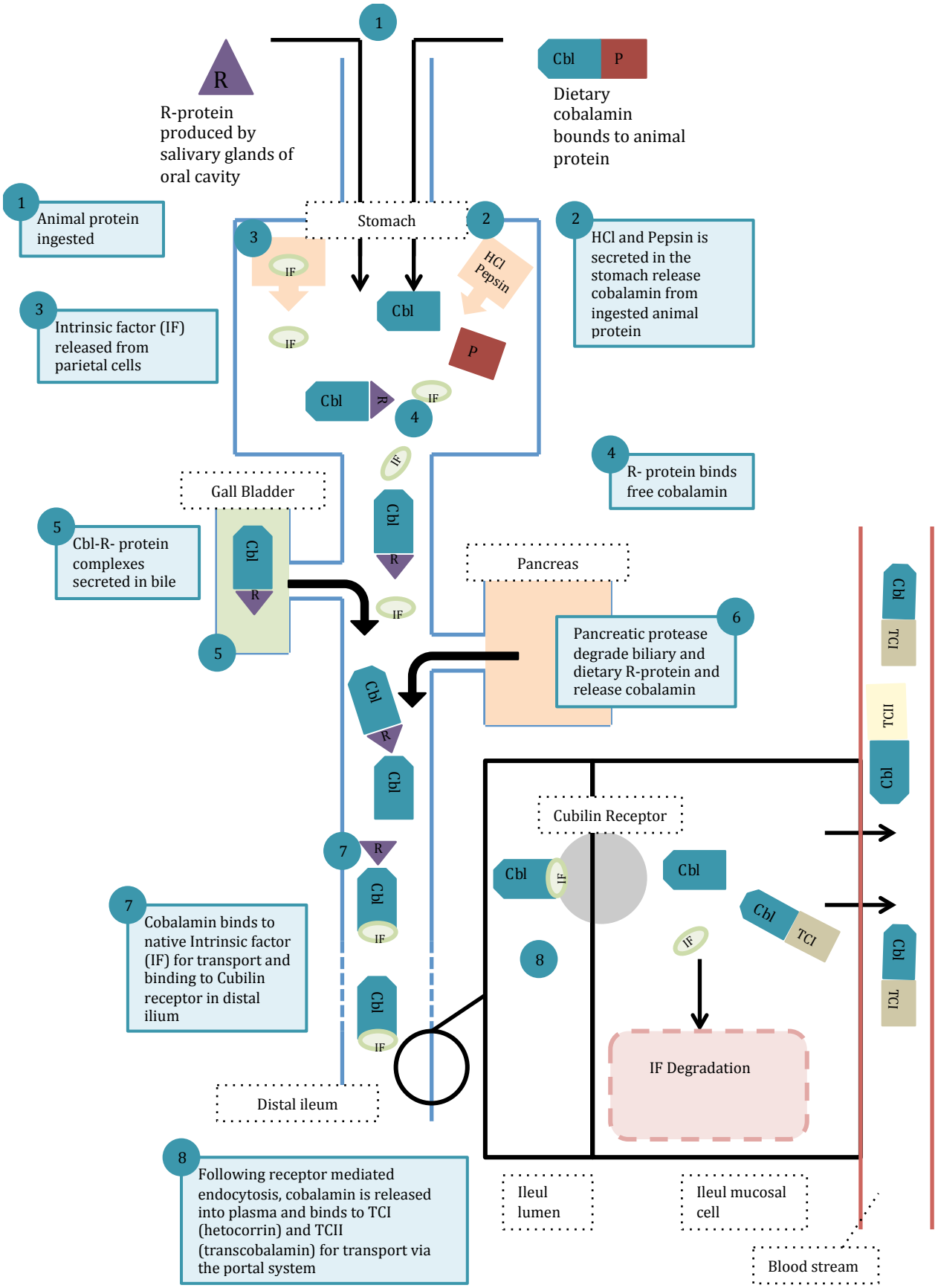


Figure 12: A schematic of vitamin B12 (cobalamin) absorption. CBL (cobamlamin), IF (intrinsic Factor, TCI (haptocorrin), TCII (Transcobalamin).

Vitamin B12 contributes to the function of two coenzymes, adenosylcobalamin and methylcobalamin, which carry out metabolism of single carbon, propionate and amino acids. Vitamin B12 is transported to cells as hydrocob(III)alamin, an oxidised compound, which is then reduced to cob(I)alamin. Methylcobalamin and adenosylcobalamin are the coenzyme forms utilised within methionine synthase and methylmalonyl-CoA Mutase respectively. Methylmalonyl synthase catalyses the conversion of methylmalonyl CoA to Succinyl CoA in the metabolism of fatty acids, while methionine synthase catalyses the metabolism of homocysteine to regenerate methionine, an important step in protein and polyamine synthesis (Combs, 2012).

The UK requirements for vitamin B12 are outlined in Appendix ii (Department Of Health Committee On The Medical Aspects Of Food Policy, 1991). There are some communities that may need to supplement their diets with vitamin B12 including vegetarians, vegans, or older adults due to the decreased function of the parietal cells.

Due to the fact that the synthesis of vitamin B12 mostly occurs in bacteria found in animals, the major sources of vitamin B12 are from animal origin particularly beef liver, dairy products, fish and seafood, where concentrations range between 0.1-122mg/100g. However, nori, a variety of seaweed mostly consumed in Japan has vitamin B12 concentrations ranging from 32-78mg/100g. It should be noted that only a small portion of this seaweed is usually consumed - between 2-4g - and therefore requirements may not be met if only nori is added to the diet.

For individuals that do not consume foods of animal origin such as vegans, supplementation of vitamin B12 should be advised, as it can be produced using bacteria and archaea through aerobic and anaerobic synthesis respectively (Combs, 2012). The most common bacteria used in the industrial production of vitamin B12 are *Pseudomonas denitrificans, Propionibacterium shermanii*, or *Sinorhizobium meliloti,* which utilise bacterial fermentation.

If the requirements for vitamin B12 are not met, the deficiency disease pernicious anaemia may occur. This is a result of the reduction in function of pathways associated with cell division, and the production of single carbon units. This reduces the rate of

mitosis and cells formed are abnormally large with large cytoplasmic volume, known as megaloblastic transformations. Clinical presentations may include gastritis, mild personality changes, or memory loss (Toh et al., 1997), however, many of these symptoms can be masked when there is a high intake of folate due to the interdependency of the two vitamins (Mills et al., 2018a).

A number of methods have been utilised to measure vitamin B12 in human tissue, which have been reviewed by the National Measurement Office (Lawrance, 2015b). The most used method of analysis is the microbiological assay, which utilises the growth vitamin B12 dependent *Lactobacillus delbreueckii* or *Ochromonas malhamensis* (Finglas and Morgan, 1994, Gille and Schmid, 2015). The turbidity of the organism can then be measured using spectrophotometry, and the concentration calculated using a calibration curve. The most popular assay is Vitafast, which is a simplified assay, however, the method still requires careful handling and is time consuming as it requires long incubation times for extraction and analysis.

A review by Lawrance (2015b) explains that fortified foods require fewer steps in the extraction process compared to naturally occurring forms of vitamin B12, as the latter are bound to protein within the food matrix. The food matrix can be broken down using heat, protease enzyme or pepsin, but to analyse the naturally occurring vitamin B12, cyanide must be used to convert all vitamin B12 to the cyanocobalamin form for analysis. Furthermore, naturally occurring levels of vitamin B12 in food products can be hard to analyse due to the low levels present and the existence of other compounds within the food matrix which may interfere with the analysis.

Other methods used for analysis of biological samples include immune assays such as ELISA, HPLC, capillary electrophoresis, mass spectrometry and various optical detection techniques (Tsiminis et al., 2017). However, these methods have not been validated in food to detect fortified vitamin B12. Cyanocobalamin is the most stable derivative of vitamin B12, where the compound favours an acidic environment (pH 4-4.5). Nevertheless, strong acidic or alkali environments and the presence of oxidising agents such as ascorbic acid, sulphite and iron salts (Gille and Schmid, 2015) will destroy the vitamin (Lawrance, 2015b).

As vitamin B12 is a WSV, it is susceptible to leaching when cooking food in boiling water, though washing has little effect (Reddy and Love, 1999). However, conventional processing such as boiling, roasting or frying may have an effect on the vitamin concentration in a variety of ways. For example, due to reduction of water in meat upon heating, vitamin B12 may become more concentrated within the food. However, if water is used as part of the cooking method such as boiling, leaching may occur and vitamin B12 may be lost. Cooking at high temperatures for a long duration can cause losses of between 10-40% of the vitamin, but if cooked at a lower temperature resulted in negligible losses (Gille and Schmid, 2015). Microwaving foods containing vitamin B12 such as beef, pork and milk for six minutes decreases vitamin B12 content by 30-40%, a larger reduction compared to when milk was boiled at 100^{o}C. Furthermore, the study by Watanabe et al. (1998) found that the amount of biologically inactive vitamin B12 increased upon heating in the microwave.

A study by Ueta et al. (2011) found canned clams to be a good source of bioavailable vitamin B12 in the diet, providing 1-4.8mg of free vitamin B12. The concentration found in the clams were lower than the values provided in the Japan FCTs (17.4-39.4mg/100g compared to 63.8mg/100g). However, the canning method was not outlined in the paper and could vary between providers. In addition, the finding of this study may not be transferrable to the UK diet, as fish consumption (not including shellfish) in the UK is low at 21g/d (Food Standards Agency.; Public Health England, 2018) compared to the recommendation of 40g/d (NHS, 2019). Lastly, there was no cooking or reheating before testing occurred, therefore further losses may occur if the food is further processed.

1.6.5. Inter dependency of folate and vitamin B12

Due to the co-dependant functions of folate and vitamin B12, the deficiency of one vitamin also has an impact on the functionality of the other. During DNA synthesis and repair, deoxyuridine monophosphate (dUMP), which requires folate and vitamin B12 as co-factors, is converted to deoxythymidine monophosphate (dTMP) (Frewin, 2014). If folate requirements are not being met, dUMP can accumulate. This accumulation of dUMP causes uracil to be incorporated into the DNA sequence, rather than thymine. Incorrect coding of the genetic sequence can lead to mutation, generation of single and double stranded breaks in the DNA (stopping the DNA from being replicated) (Lee and

Orr-Weaver, 2001), chromosome breakage (breakage of the DNA backbone strand) and micronucleus formation (malformation of the nucleus leading to chromosomal instability) (Fenech, 2012) (Figure 13).

Furthermore, as vitamin B12 is a cofactor for methylmalonyl CoA mutase, its deficiency can lead to the disruption of mitochondrial metabolism. This compounded effect of deficiency has been implicated in increased cancer risk and cardiovascular disease due to accumulation of uracil and homocysteine, respectively (Fenech, 2012).

The inter relationship of folate and vitamin B12 is especially important in older adults, as vitamin B12 deficiency can be masked by folate intake. Studies have shown that high doses of folate (>400mg/day) can mask the short-term symptoms of megaloblastic anaemia in those that are vitamin B12 deficient. However, long-term symptoms due to the absence of the vitamin within the diet are not masked, and vitamin supplementation of folate in those people who are vitamin B12 deficient will exacerbate the symptoms (Combs, 2012). Therefore, it is important to consider the possible effects of folate supplementation on vitamin B12 status in older populations.

1.6.1. Vitamin C

Vitamin C, also known as L-ascorbic acid (2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol) is an essential WSV. In nature, vitamin C occurs as L-ascorbic acid, D-ascorbic acid and dehydroascorbic acid (DHA), the oxidized form of ascorbic acid. However, D-ascorbic acid has little biological function (Naidu, 2003), therefore for this dissertation ascorbic acid (AA) will only refer to L-ascorbic acid. DHA can be reduced to AA and is a reversible reaction, but if DHA is irreversibly reduced to diketogulonic acid it becomes biologically inactive, shown in Figure 14. This reaction occurs in both aerobic and anaerobic conditions and particularly during heating (Erdman and Klein, 1982).

The main function of vitamin C is as an antioxidant in the cells. Other functions include the role as a co-factor for monooxygenase and hydroxylase enzymes. These enzymes are involved in the synthesis of carnitine, fatty acid metabolism, and collagen formation; the main component in connective tissue and neurotransmitters (Naidu, 2003). Due to the tendency of vitamin C to lose electrons easily, it can serve as a biochemical redox system

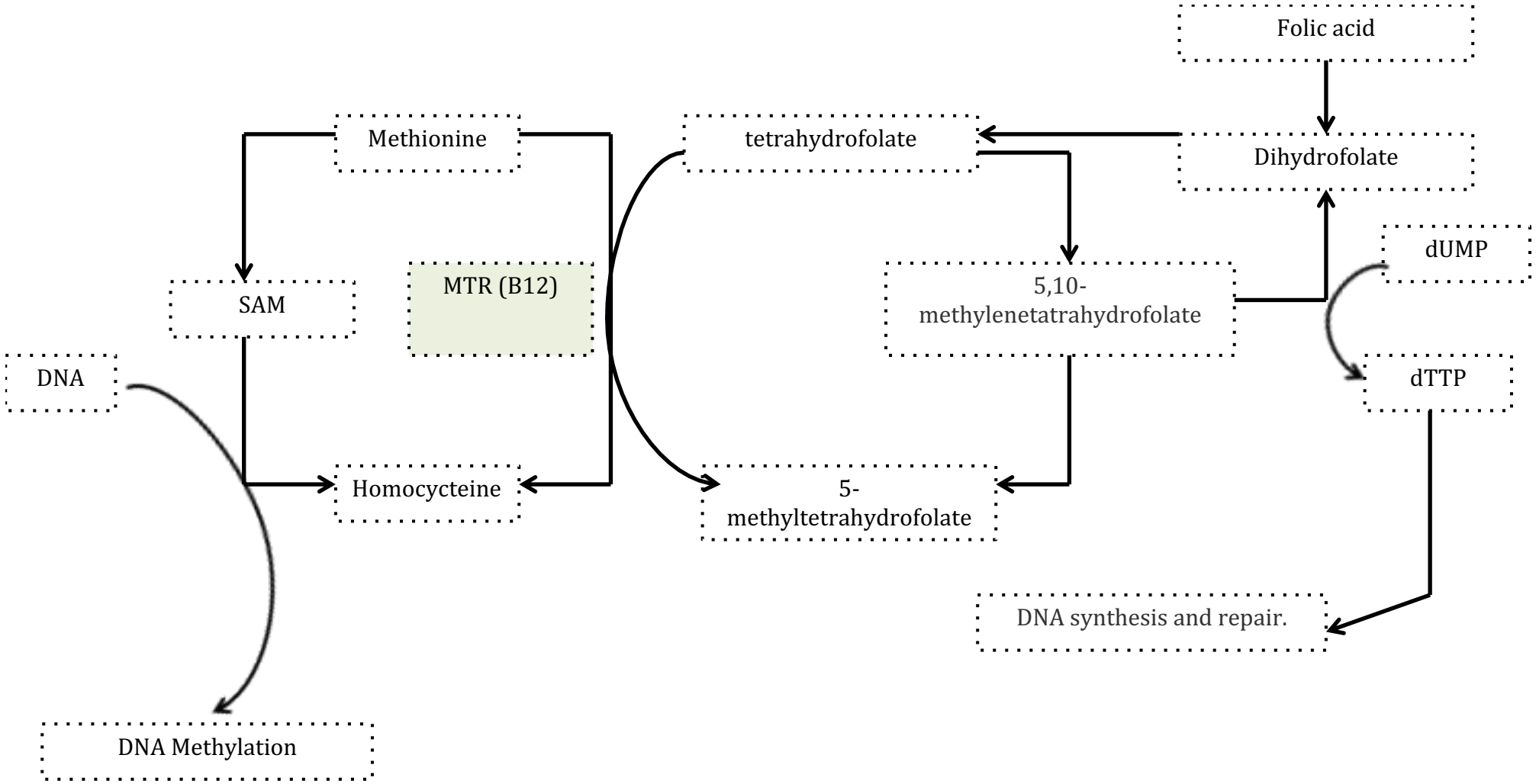


Figure 13: Schematic of B12 metabolism. SAM (S-adenosylmethionine), MTR (methionine synthase), dUMP (deoxyuridine monophosphate), dTTP(deoxythymidine triphosphate

and can react readily with reactive oxygen species to yield a stable intermediary compound - an ascorbyl radical - and provide antioxidant protection to the cells. This redox characteristic of vitamin C can also increase the enteric absorption of non-haem iron. Vitamin C reduces the ferric form of iron (Fe^{3+}) to the ferrous form (Fe^{2+}), which can form an alkaline soluble and stable chelate that is more easily absorbed in the small intestine (Combs, 2012).

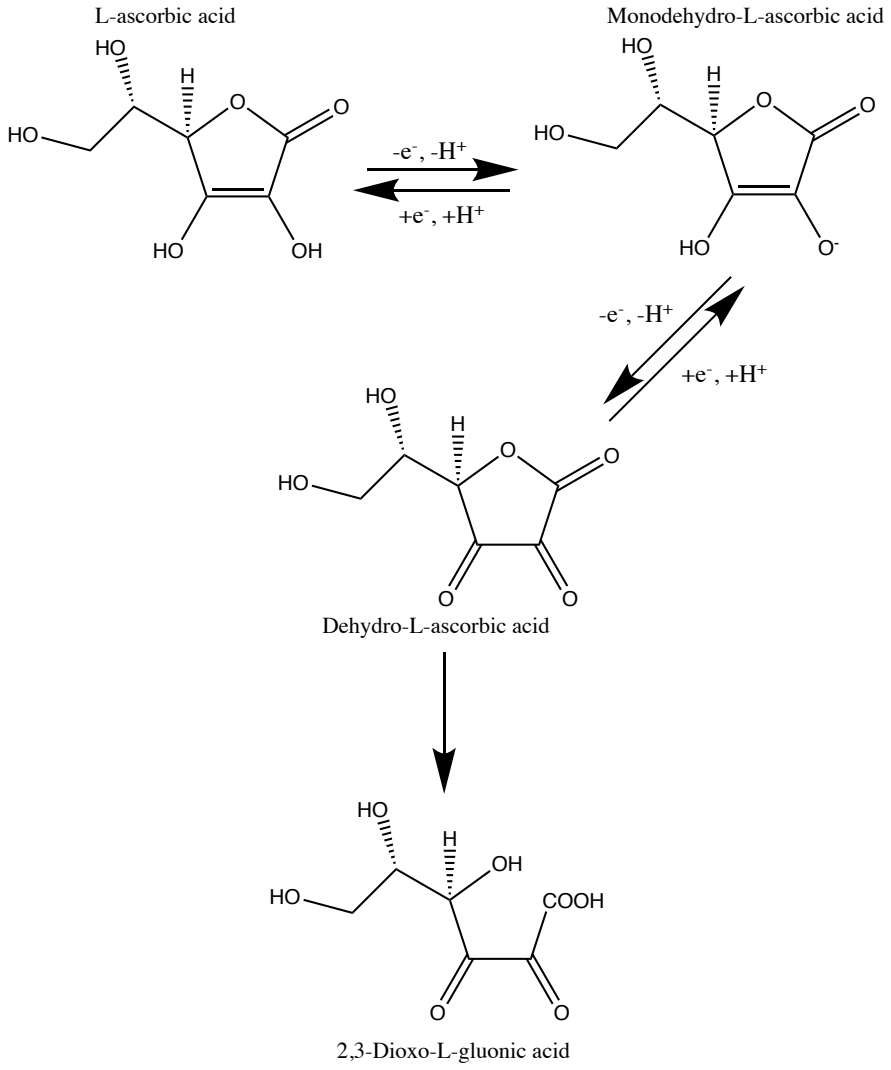


Figure 14: Chemical structure of L-ascorbic acid and the reversible oxidation to monodehydro – L – ascorbic acid followed by the irreversible reaction to 2,3-dioxo-L-gluonic acid.

Vitamin C is both actively and passively transported into cells. Passive transport occurs at higher doses of vitamin C intake, however physiological doses are transported across the brush border membrane by sodium dependent vitamin C transporters (SVCT1), and glucose transporters; GLUT1 and GLUT3. Uptake via glucose transports are more

efficient than SVCT1 transporters and have a higher affinity for DHA compared to AA. Once dehydroascorbic acid is transported into the cell, it is rapidly reduced to AA by thioredoxin reductase or glutareductase (Combs, 2012, Bohndiek et al., 2011).

The UK requirements for vitamin C are outlined in Appendix ii (Department Of Health Committee On The Medical Aspects Of Food Policy, 1991), with the richest food sources of the vitamin being FVs such as blackcurrant, lemons, Brussel sprouts and parsley, which can contain 40-300 mg/100g fresh weight. If the recommendations are not being met for vitamin C, there is an increased risk of deficiency, which presents as scurvy. The first symptoms of scurvy occur in the connective tissue of the body and cause impaired wound healing, oedema and haemorrhage of the skin, internal organs, mucous membranes, and muscles. Weakening of collagen-rich structures such as the teeth and bone may also occur. Subclinical symptoms include susceptibility to infection, fatigue and muscular weakness (Combs, 2012).

Cooking and industrial processing can also affect the vitamin C concentration of food, where heat-induced oxidation and leaching into water can cause substantial losses of the vitamin. Erdman and Klein (1982) discuss the thermal processing of green peas and found that there was up to 68% cumulative loss of vitamin C, with the largest losses occurring at the blanching stage of the canning process. Microwave heating has been found to be least destructive method of cooking (Lee et al., 2018). Moreover, sous-vide, the method of cooking under vacuum at low temperature, has been shown to retain vitamin C by up to 96% due to the gentler cooking method (Tansey et al., 2005).

As vitamin C is sensitive to light, heat and oxidation, samples analysed for vitamin C content require careful handling and minimal time between sample preparation, extraction and analysis (Spínola et al., 2014). The most common method of vitamin C analysis is by titration, especially for fruit and juices, as it is low-cost and simple. When analysing food with a more complex food matrix, HPLC is employed (Spínola et al., 2014). Importantly, Spínola et al. (2014) explains the matrix effect which arises due to the variation of foods. Failure to account for this phenomena (Tarrago-Trani et al., 2012) during the extraction process of the foods can lead to over- or underestimation of the vitamin C content of the sample (Spínola et al., 2014). One way to overcome this is to

use certified reference materials, but these can be very expensive and are not always available in a range of foods (Spínola et al., 2014).

Considering the importance of thiamine, riboflavin, folate, vitamin B12 and vitamin C in healthy development and the proven susceptibility to loss during processing of foods, there is a need to assess the nutritional adequacy of RMs, especially as they have the potential to be a major source of WSV in the diet. To understand the current knowledge about WSV content of RMs, the consumption patterns of RMs in the UK and identify gaps in the knowledge a review of literature will be carried out. The findings of the review will form the rationale of this research and seek to contribute to the exiting understanding of RMs in the UK.

THESIS STRUCTURE

To defend this thesis, the following dissertation is organised into seven chapters. Firstly, chapter 1: Introduction has described the history of ready meals (RMs), and the current RM industry in the UK. The chapter presented the current market research and considered possible societal influences on RM consumption. This chapter presented a detailed overview of the most process-labile micronutrients that are essential for health. The justification for the focus on thiamine, riboflavin, folate, vitamin B12 and vitamin C was also provided.

Chapter 2 presents the literature review, which used systematic approaches to identify relevant academic literature and provide an overview of the current knowledge of RM consumption in the UK, and the water-soluble vitamin (WSV) content of RMs. The review identified gaps in the literature and was used to formulate the rationale for this study.

Chapter 3 presents a secondary analysis of data from the National Diet and Nutrition Survey (NDNS) to quantitively evaluate the current RM consumption in the UK and the contribution RMs make to the WSV intake of the population. There chapter explores RMs consumption by the cuisine type and the amount eaten by different age groups. The investigation also assessed whether the RMs consumed by NDNS participants met the 30% RNI recommendation for thiamine, riboflavin, folate, vitamin B12 and vitamin C.

To explore the best method to use for the analysis of thiamine in sulphite-containing foods, a comparison of a high-performance liquid chromatography (HPLC) method and microbiological method were assessed using Bland-Altman analysis. The most precise and accurate method was used for the remaining studies of the research. This is discussed in chapter 4.

Once the most suitable analytical methods were found for riboflavin, folate and vitamin C, five different sausage and mash RM components were analysed and presented in chapter 5. The vitamin content was compared to the recommendation that RMs should

provide 30% of the RNI for micronutrients. Following this Chapter 6 describes the resulting WSV content of meals being subjected to hot-holding for three-hours at 90ºC.

Each of the chapters ends with an independent discussion highlighting the main findings from each of the studies. The seventh and final chapter presents a general discussion bringing together the data from all the studies and makes recommendations for future research and policy.

2. LITERATURE REVIEW

2.1. Introduction

This chapter presents a review of the literature regarding the riboflavin, thiamine, folate vitamin B12 and vitamin C content of RMs, and consumption patterns of RMs in the UK using systematic approaches. The use of a systematic approach for this literature review was chosen due to its rigorous, methodological process which aims to provide transparent and reproducible methods and ensure that all available literature is reviewed and included (Bruce and Mollison, 2004). The aim of this literature review is to analyse the current state of knowledge and identify gaps in the literature regarding water-soluble vitamin (WSV) content of RMs and consumption patterns of RMs in the UK.

2.2. Scope of the systematic literature review

The objectives of this literature review were to identify, summarise and critique 1) studies relating to thiamine, riboflavin, folate, vitamin B12 and vitamin C in RMs and 2) studies analysing consumption patterns of RMs in the UK. The review focusses on analytical literature based on experiments that used nutritional biochemical assays rather than food compositional data, which have been found to be inaccurate when calculating nutrient concentrations (Egan et al., 2007). The critique of consumption pattern data was carried out in studies from the UK only as dietary patterns can vary depending on cultural norms.

2.2.1. Searching the literature: Consumption of ready meals in the UK

Although consumption patterns in the UK are variable over time, no date restriction was added to the searches to ensure that changes to consumption patterns over time could be identified. A database search was carried out on platforms that were available through the University of Hertfordshire. These were EBSCOHost, Pubmed and Scopus; which provide historical and current science texts, biological and technological literature. The search was carried out in March 2021 using the key search terms (“Ready meals”) AND ("eating habit" OR consum* OR freq*) AND (UK or United Kingdom or Britain or England or Wales or Scotland or Northern Ireland) NOT (micro* OR cereal).

Similarly, a combination of terms was used (shown in Table 2) where terms from column one, followed by "AND", along with one term from column two, and three was searched in the three databases, until all possible search combinations were completed. Specific search terms can be found in appendix iii. An initial assessment of title and abstracts was undertaken to excluded papers that did not meet the pre-defined inclusion criteria– these included those that did not assess consumption patterns, were not written in English, were review papers, or those that were protocols. Inclusion and exclusion criteria for the literature search is shown in Table 3.

A total of 94 papers that were identified from the initial database searches. Once relevant papers were identified from a title and abstract search, lateral searched involving checking the reference list identified one further paper yielding five papers for review that met all inclusion criteria and were relevant to the aims and objectives of the project. Data were extracted and summarised and reasons given for inclusion and exclusion of papers. A summary of identified literature identified is presented in appendix iv. A Prisma flow diagram is shown in figure 15.

Table 2: Literature Review of search terms for ready meal consumption patterns in the UK

Column one	Column two	Column three
	Eating habit	UK
"ready meal"	Consum*	United Kingdom
"ready meals"	Freq*	England
"ready-meals"		Wales
"convenience food"		Scotland
"ready to eat"		Northern Ireland
"ready-to-eat"		Britain
"ready prepared"		
"ready-prepared"		

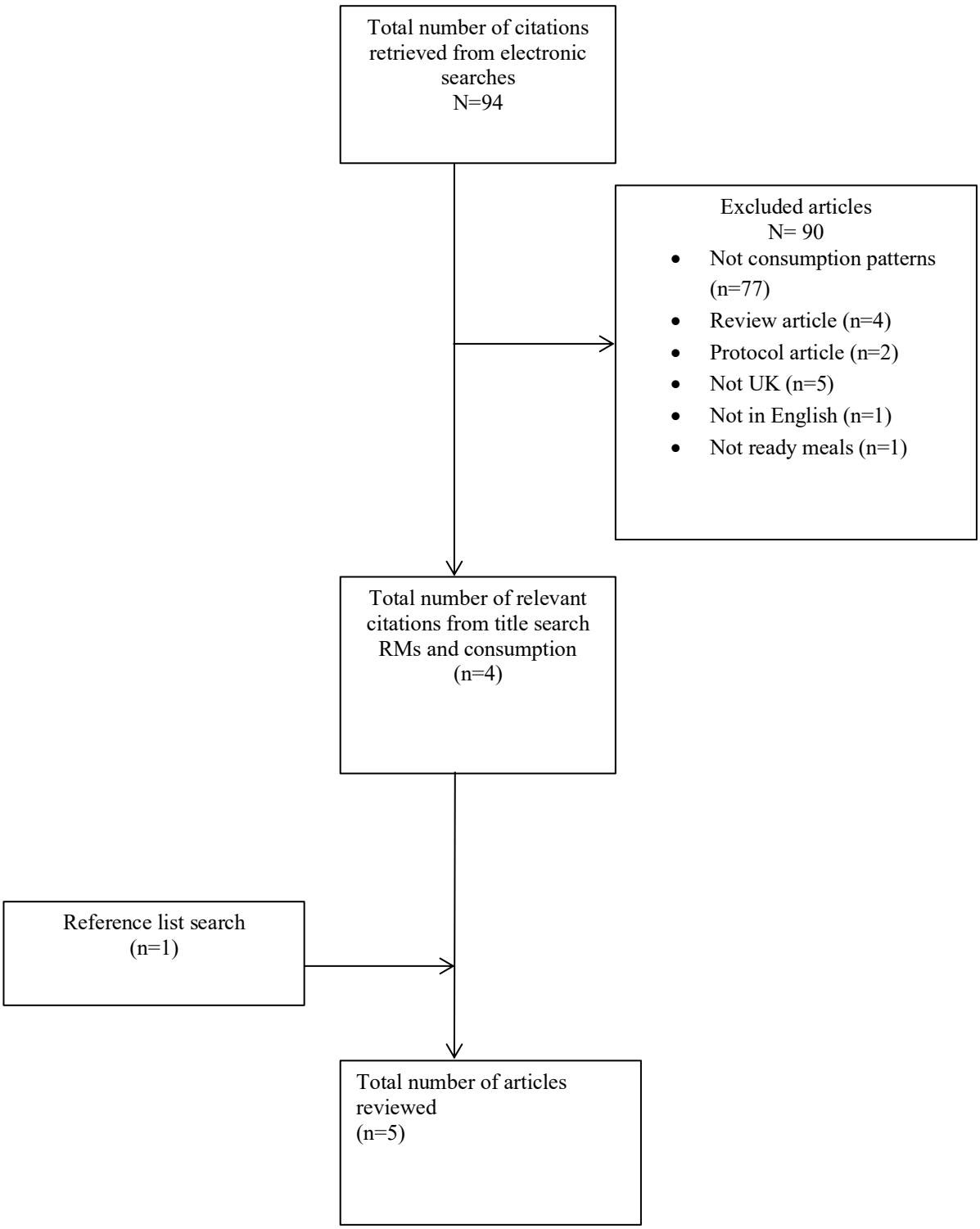


Figure 15: A Prisma flow diagram of the papers identified (n=5)

Table 3: Inclusion and exclusion criteria for selection of papers for consumption frequency ready meals in the UK.

Inclusion Criteria for RM consumption pattern	Exclusion Criteria for Rm consumption data
Frequency of RM consumption was provided	Frequency of RM consumption was not provided
Studies written in English	Studies not written in English
Ready-meals consumption specifically assessed	Ready meals were not assessed i.e. only takeaways or snacks assessed
If there was an intervention, baseline has been provided	Baseline RM consumption not provided
Data collected in the UK only	Assessment occurred outside of the UK

2.2.2. Searching the literature: water-soluble vitamin content of ready meals

Although processing practises have changed over time, there was no date restriction on the literature search carried out. This was to ensure that developmental work in the field of vitamin content in RMs would be represented. A database search was carried out in EBSCOHost, Pubmed and Scopus in June 2019 using the key search terms ("Ready meals") AND ("l-ascorbic acid" OR "ascorbic acid" OR "vitamin c" OR cobalamin OR "vitamin b12" OR folate OR "folic acid" OR "vitamin b9" OR "vitamin b2" OR riboflavin OR "vitamin b1" OR thiamin OR thiamine).

For the database search, a combination of terms was used (shown in Table 4) where one term from column one, followed by "AND", along with one term from column two was searched in all databases, until all possible searches were completed. A detailed overview of search terms used is summarised in appendix v. An initial assessment of title and abstracts was undertaken to exclude irrelevant papers - these included narrative reviews, papers that used food composition tables (FCTs) to quantify nutrient content, and those not written in English due to the high cost of translation. Studies that were excluded from this review include those that used FCTs to estimate the WSV content of the RMs as this may lead to inaccurate calculation (Leclercq et al., 2001), especially for folate (Fajardo et al., 2012) and vitamin C (Jones et al., 1988). Inaccuracies in the FCTs could be due to the differences in food production or composition, variation in food types between countries, or the differences in analytical techniques used to determine vitamin content (Deharveng et al., 1999). To assist the search, keywords were used to identify further

relevant literature. Experimental studies were included, and articles were reviewed to understand their possible contribution to our existing knowledge about the WSV content of RMs.

Table 4: Literature Review search terms

Column one	Column two
"ready meal"	thiamine
"ready meals"	thiamin
"ready-meals"	"vitamin b1"
"convenience food"	riboflavin
"ready to eat"	"vitamin b2"
"ready-to-eat"	"vitamin b9"
"ready prepared"	"folic acid"
"ready-prepared"	folate
	"vitamin b12"
	cobalamin
	"vitamin C"
	"Ascorbic acid"
	"l-ascorbic acid"

The most relevant papers initially identified, followed by a further lateral search that checked reference lists of identified papers, are shown in appendix v. The Prisma flow diagram of papers is shown in Figure 16. Relevant papers yielded (n=15) from a total of 250 papers found in the initial title and abstract search. Inclusion and exclusion criteria are shown in Table 5. A summary of the papers included in the review are shown in appendix vi. Data were extracted from papers and stored in a spreadsheet developed for this literature review. This approach was used to ensure that all studies could be annotated throughout the review to provide information regarding reasons for inclusion or exclusion.

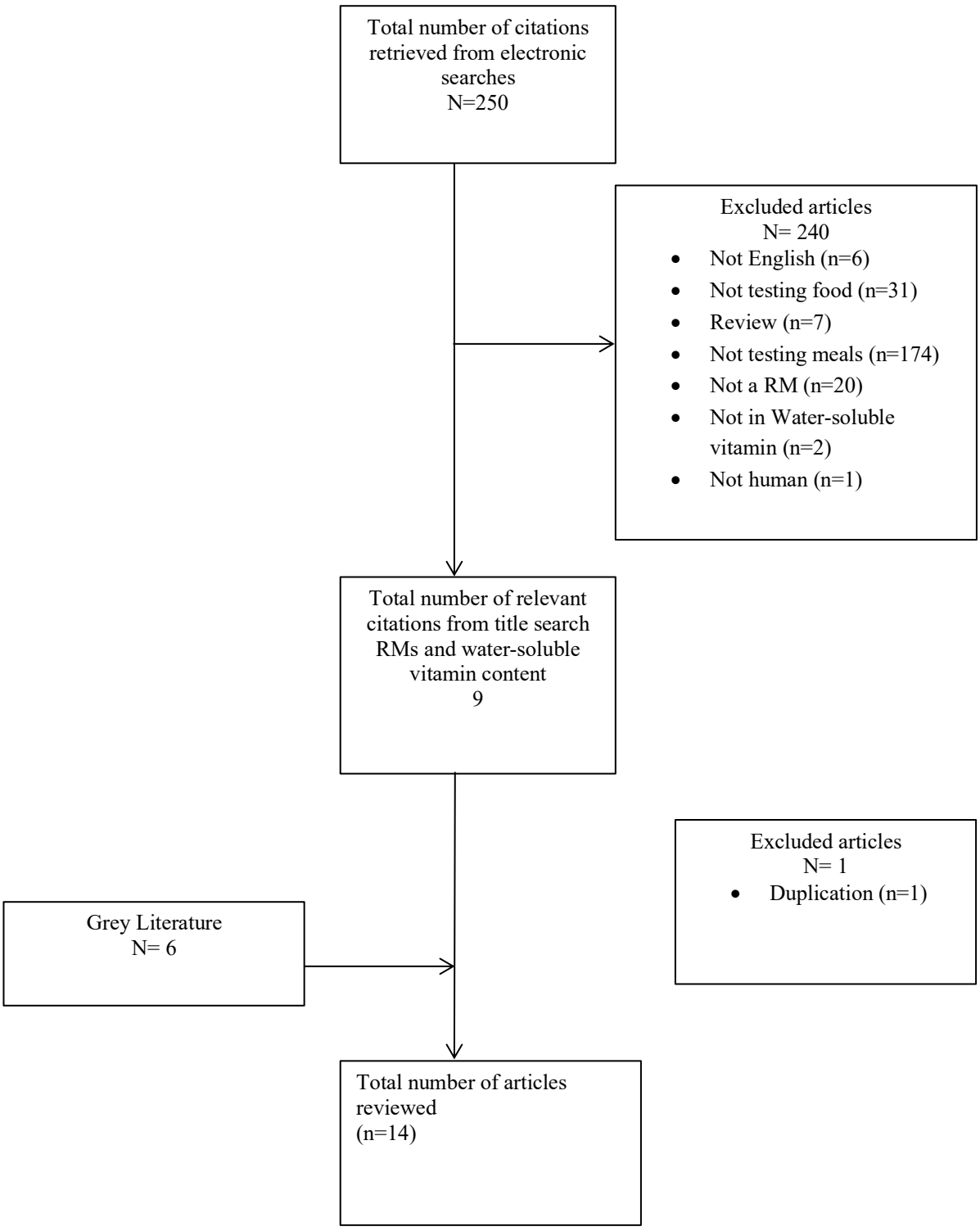


Figure 16: A flow diagram of the papers identified, those that were included and excluded, and the total number of articles reviewed (n=16)

Table 5: Inclusion and exclusion criteria for selection of papers for the effect of processing on the thiamine, riboflavin, folate and vitamin B12 content of ready meals

Inclusion Criteria for Effect of processing on WSV content of RMs	Exclusion Criteria for Effect of processing on WSV content of RMs
Main focus of the study concerns the effect of food processing on WSV content	Main focus of the study does not concern the effect of food processing on WSV content
Studies written in English	Studies not written in English
Individual foods tested, and not whole meal tested	Studies that did not provide pre and post intervention WSV content data
Main focus of the study concerns in effect of freezing, drying, canning or preservative use on WSV content	Studies that used invalid analytical methodology e.g. animal nutrient deficiency models.
Studies evaluated pre and post processing on WSV content	Nutrient data was collated using food composition tables
Studies that used validated analytical methods	Food items/ meal description was not specified
Fresh weight of foods provided in study	Narrative review articles
WSV content was analysed after each processing method	Not a product that needs heating before consuming

2.3. Overview of the studies identified

2.3.1. Ready Meal Consumption in the UK

Studies that investigated the consumption patterns of RMs in the UK were included in the current literature review. Five papers, which were identified through primary database and reference list searches, met the inclusion criteria. All studies measured frequency of RM consumption using self-reported food frequency style questioning where participants were asked about the frequency of habitual consumption.

Studied included a variety of demographic groups including parents of nursery aged children in Scotland (Garcia et al., 2014, Garcia et al., 2020), adults in England (Mills et al., 2018b, Pettinger et al., 2006) and Slimming World members who were university students across the UK (Sprake et al., 2017).

Survey methods used included online surveys (Sprake et al., 2017) and postal surveys (Garcia et al., 2020, Garcia et al., 2014, Mills et al., 2018b, Pettinger et al., 2006). Three out of the five studies (Sprake et al., 2017, Garcia et al., 2020, Mills et al., 2018b) were consistent and found that RMs were consumed 1-2 times per week. Furthermore, daily consumption was the least common consumption pattern on RMs with between 0.5-18.2% of the sample populations stating that they consume RMs more than 5 times per week (Garcia et al., 2020, Pettinger et al., 2006, Sprake et al., 2017).

There were higher odds of consuming RMs more than twice a week in those with household incomes below £20,000 per annum compared to households with an income of £40,000 or higher (Mills et al., 2018b). Additionally, Sprake et al. (2017) found that as the frequency of RM consumption increased, so did the risk of weight gain in Slimming World members.

Strengths of the studies included stratification of population samples (Pettinger et al., 2006, Mills et al., 2018b), the use of validated questionnaires (Pettinger et al., 2006, Garcia et al., 2020) and generalisability of the findings to the UK population (Mills et al., 2018b). Limitations of the studies included being self-reported data (possible underreporting), majority female (>80%) (Garcia et al., 2020, Garcia et al., 2014) or female only populations (Sprake et al., 2017) and high dropout rate for the studies that included a follow-up (Garcia et al., 2014, Garcia et al., 2020, Pettinger et al., 2006).

2.3.2. Water Soluble Vitamins in Ready Meals

Studies that chemically analysed the thiamine, riboflavin, folate, vitamin B12 and/or vitamin C content of RMs were included in this literature review. Of the studies found, vitamin C was the most consistently tested nutrient, where 11 out of the 14 papers investigated the effect of different processing techniques on the vitamin C content of the meal. Eleven of the studies analysed whole RMs, while the remainder of the studies looked at components of the meals, specifically vegetable portions. However, some studies did not report on all components within the meals tested (appendix vi).

This literature review has provided insight into the WSV content of RMs, but considerations should be given to their applicability when applying these findings to the UK setting, as meal types, ingredients and recipes may vary. There were three papers published by research groups in the UK (Gatherer, 1971, Faulks, 1991, Patterson and Stewart, 2003), the remaining 11 were carried out in Sweden (n=1) (Johansson et al., 2008), Spain (n=1) (Fajardo et al., 2017), India (n=1) (Agte et al., 2002), the Republic of Ireland (n=3) (O'Leary et al., 2000, Redmond et al., 2002, Redmond et al., 2004), the USA (n=3) (Salunkhe et al., 1979, Hoppner et al., 1973, De Ritter et al., 1974), the Netherlands (n=1) (Hammink, 1978) and Germany (n=1) (Bognar, 1980).

In four of the studies (Gatherer, 1971, O'Leary et al., 2000, Redmond et al., 2002, Redmond et al., 2004), the complete meals were not specified and only components of the meals were tested, therefore the total vitamin content of the meal was not stated. Furthermore, the method of cooking was not indicated in three of the studies (Salunkhe et al., 1979, Gatherer, 1971, Agte et al., 2002), and could not be taken into account when determining what stage of the industrial cooking process could have caused the biggest losses in vitamin content.

The most common methodology used was the 2, 6-dichloroindophenol titrimetric method for vitamin C (Salunkhe et al., 1979, Gatherer, 1971, O'Leary et al., 2000, Agte et al., 2002, Redmond et al., 2002, Redmond et al., 2004). For thiamine, derivatisation to thiochrome with fluorometric detection was used (Salunkhe et al., 1979, Gatherer, 1971, Agte et al., 2002, Patterson and Stewart, 2003). For riboflavin, fluorometric detection was the method that was most popular (Salunkhe et al., 1979, Agte et al., 2002) and the HPLC method for folic acid (Agte et al., 2002, Johansson et al., 2008). Total folate concentrations were calculated using the microbiological assay (Fajardo et al., 2017).

2.3.3. Thiamine

Five studies were identified that measured thiamine content. In a study that analysed 260 RMs and ready-to-eat foods from Asia, Africa, Europe, USA and Latin America, and were found to contain approximately 0.025mg/100g of thiamine by Agte et al. (2002), and contained between 0.003-0.13mg/100g in meals tested by De Ritter et al. (1974). Analysis showed that the process of heating thiamine-containing foods such as pork in chilled RMs caused the largest reduction in thiamine content, compared to preceding

processing techniques such as chopping or cooking (Patterson and Stewart, 2003, De Ritter et al., 1974). However, lower pH found in some meals (not specified in the study) may result in better retention of thiamine (De Ritter et al., 1974).

One study found that the techniques used to increase the shelf-life of RMs, such as irradiation, led to a dose dependent reduction in thiamine content of between 12-34% in pork, with further losses of between 20-26% occurring after 7-14 days of storage at 3ºC. Storage of other irradiated sterile meals such as army rations retained more thiamine when stored at 4.4ºC than those meals kept at 37.8ºC, where there were losses of 7% and 70% respectively (Salunkhe et al., 1979).

One study compared different RMs used in hospital provision, and showed a higher thiamine content compared to meals cooked from scratch for catered services (Hammink, 1978). The losses were found to be between 0-55% compared to up to 100% in meals cooked from scratch, with minced meat having the largest loss of 100% after keeping the meal hot during delivery of up to 80 minutes (Gatherer, 1971). However, Bognar (1980) found no significant differences between chilled meals and meals made from scratch that were not kept heated for long periods of time. As there is no information provided as to how the homemade meals were cooked in these studies, it could be speculated that the differences in the thiamine retention between the meals could have been due to varying cooking techniques.

2.3.4. Riboflavin

Six studies analysed riboflavin. One study found that the riboflavin content of RMs and ready-to-eat foods was approximately 0.01mg/100g (Agte et al., 2002), with riboflavin in vegetables and meat being mostly unaffected by heating (Finglas and Faulks, 1984, De Ritter et al., 1974), and no differences between the riboflavin content of chilled RMs and cooking the meals from scratch (Bognar, 1980). However, storage temperature of sterile meal rations found that storing these foods at 4.4ºC retained 30% more riboflavin than storing at 37.8ºC (Salunkhe et al., 1979). Lastly, for those RMs that are provided as part of a hospital catering service, Hammink (1978) found that RMs contain more riboflavin than those meals that were cooked from scratch. However, the vitamin concentrations that were found were not provided in the report of the study.

2.3.5. Folate

Five studies were identified that measured folate. One study by Agte et al. (2002) analysing RMs and ready-to-eat foods found that they contained approximately 4mg/100g of folate, similar findings to Johansson et al. (2008) and Fajardo et al. (2017). However, heating was found to cause the largest reduction in vitamin content, where microwaving or cooking baked potatoes in a conventional oven led to a 10-40% reduction in folate (Faulks, 1991), and fried potatoes retained more folate than boiled potatoes (Hoppner et al., 1973).

Analysis of RMs by Hoppner et al. (1973) found that meals which included a source of protein, vegetables and potatoes with a minimum calorie content of 340kcal, met folate requirements by containing 15.4mg/100g of the food product. Those meals that did not include these vegetables and potatoes contained significantly lower amounts of folate, between 5.9mg/100g and 10.3mg/100g. The study also highlighted that the presence of a vegetable portion within a meal improved the folate content of the RM overall. This finding was echoed by Fajardo et al. (2017), who found that the vegetables in Spanish RMs were able to retain up to 93% the folate content through the action of antioxidants, which are present in vegetables, and protect the vitamin from oxidation during the cooking process.

Fajardo et al. (2017) further found that the folate content of RMs can vary, where content was found to be between 4.6-103.8mg/100g fresh weight, and upon reheating of the RMs, folate retention varied vastly between the meals, (between 12.5-97%). However, Johansson et al. (2008) found that there was a significant reduction in folate content of between 40-45% upon reheating. These differences are more likely due to the cooking method used to reheat the RM, as Johansson et al. (2008) heated the meal in a saucepan for 10 minutes, compared to three minutes in the microwave in the Fajardo et al. (2017) study. Microwaving has been shown to retain nutrients better due to the shorter cooking times and less chance of leaching (Lee et al., 2017).

2.3.6. Vitamin B12

The literature searches yielded two studies testing the vitamin B12 content of RMs. In summary, five out of the nine meals tested by De Ritter et al. (1974) contained vitamin B12 which is most likely due to the presence of meat components in these RMs.

Furthermore, it was stated that for this study, conventional cooking of meals in hospitals provided more vitamin B12 than industrial cooking. However, no further conclusions about the vitamin B12 content of RMs could be drawn due to the lack of numerical data on the vitamin content of each meal analysed in the study by Hammink (1978).

2.3.7. Vitamin C

There were 11 studies that measured vitamin C content. RMs (n=205) tested by Agte et al. (2002) provided approximately 6mg/100g of vitamin C, compared to fresh fruits and vegetables which contained 8-16mg/100g. Those RMs with the highest vegetable content were found to be highest in vitamin C after cooking (De Ritter et al., 1974). Due to vitamin C being readily oxidised in temperatures above 0°C (Rickman et al., 2007), O'Leary et al. (2000) and Redmond et al. (2002) showed that the freeze-chill method had the most detrimental impact on the vitamin C content of mashed potato and broccoli, more so than freshly prepared and frozen mashed potato (Redmond et al., 2004).

Heating of vegetable-based RMs caused substantial losses of vitamin C and led to a reduction of vitamin C of between 67% and 71% when using a microwave and a conventional oven, respectively. The largest losses of vitamin C were in the vegetable lasagne (41-43% retention) compared to prepared vegetables. However, cooking vegetables such as carrots and potatoes whole retained the most vitamin C (Faulks, 1991).

Irradiation led to a dose-dependent loss in total ascorbic acid (TAA) of between 51%-54%, and larger losses of ascorbic acid (AA) of between 91-94%, whereas the dihydroxyascorbic acid (DHAA) increased by up to 51%, a result of the conversion of AA to DHAA during irradiation (Patterson and Stewart, 2003). However, cooking resulted in the largest losses of 61% and 97% of AA and DHAA, respectively (Patterson and Stewart, 2003). Furthermore, the storage of fruit meal rations, which were sterilised to preserve the food product, led to a reduction of between 4-78% of vitamin C over 0-18 months when they were held at 4.4°C up to 37.8°C, where higher temperatures resulted in an increased loss of vitamin C over time (Salunkhe et al., 1979).

Storage environment of RMs can affect the vitamin C content of the food. However, pre-blanching vegetable ingredients protects the vitamin C content compared to fully cooking

vegetables such as broccoli (O'Leary et al., 2000). A study by Bognar (1980) found up to 100% loss of AA in boiled potatoes and spinach stored at 2ºC for up to 10 days.

For meals produced as part of a hospital service, there was better retention of AA in conventionally cooked meals compared to end-cooked frozen meals, where 45-100%, and 0-52% was retained for conventionally cooked and end-cooked frozen meals, respectively (Gatherer, 1971, Hammink, 1978). However, Bognar (1980) found that meals that were chilled had 30-90% less vitamin C compared to fresh cooked meals.

2.4. Discussion

This literature review explored the knowledge base related to the frequency of consumption of RMs in the UK, and the WSV content of RMs. To the knowledge of the researchers, this is the first review to investigate the body of literature around the consumption pattern of RMs in the UK. As discussed in chapter 1 of this dissertation, market research has demonstrated the substantial contribution of RMs to the UK food industry and showed consistent consumption of RMs in the UK. In the current review, RMs were found to be consumed 1-2 times per week, with the likelihood of consuming RMs increasing as household income decreases or body weight increases.

These findings are consistent with Mintel report findings, which state that chilled RMs are usually consumed 1-2 times per week (Mintel, 2017). However, market research findings, which included Mintel data, are not population-based studies, and therefore generalisability of the findings are challenging due to factors such as requiring internet access to complete the surveys (reducing access for older individuals) (Office for National Statistics, 2017), and using self-reported consumption (where under-reporting of RM consumption could occur) (Burrows et al., 2019).

This review found that RM consumption was associated with a lower household income (Mills et al., 2018b). This is contrary to findings from Caraher et al. (1999) and Reed et al. (2003), where it was found that RM consumption was associated with managerial or higher occupations; an indicator of higher household income. However, it should be noted, that those studies are almost 20 years old, and consumption patterns of RMs have changed over time, as the availability, and consequently the consumption of RMs in the

population has increased. This review suggests that those in households with incomes below £20,000 per annum and students frequently consume RMs.

The finding that consumption of RMs is linked to increase body weight was found in other studies (Van Der Horst et al., 2011, Alkerwi et al., 2015). Reasons for this could be the nutrient composition of RMs, where studies have shown that RMs are high in energy, saturated fat and sugar. Furthermore, RMs are seen as balanced by consumers who were overweight, and therefore were more inclined to eat them and that weight status was positively associated with RM consumption (Van Der Horst et al., 2011). It should be noted, however, that the Van Der Horst et al. (2011) study was carried out in Switzerland, not the UK, and therefore the RMs consumed may have a different macronutrient nutrient composition.

With regards to the WSV content of RMs, this review found studies focused on a range of processes which could impact the vitamin content of RMs. Generally, losses of WSV occur during the freezing and reheating process of RM production, with some methods allowing better protection of the most labile vitamins. This review showed that although vitamin B12 was retained in RMs, there are no recent studies to support this in the UK, and due to the higher risk of vitamin B12 deficiency in older adults, more research is required to ensure that meals are meeting nutritional requirements of this population group.

An extensive review exploring the effect of processing on the vitamin C content of foods by Armstrong et al. (2019) found that the all types of processing will cause varying degrees of vitamin degradation. However, the current review found that riboflavin and vitamin B12 showed the least amount of degradation (De Ritter et al., 1974, Faulks, 1991), where thiamine, folate and vitamin C were most susceptible to manufacturing methods. However, the rate of vitamin degradation was associated with the type of processing, where boiling cut vegetables was the most detrimental (Bognar, 1980), and cooking whole vegetables was the least detrimental method of preparation (Faulks, 1991).

Food service methods commonly used in hospitals such as cook-chill is a popular large-scale food process, where food is fully cooked and then chilled before being reheated for

service. However, Charlton et al. (2004) found that the production of meals using the cook-chill food system led to between 87-89% loss of vitamin C, with the largest losses occurring during cooking and reheating of the vegetables. De Ritter et al. (1974) and Bognar (1980) found that this could be countered with the addition of high vitamin C foods such as cranberry sauce or lemon juice and the fortification of foods before sterilisation enhanced the AA content of the meal.

Chilling and storage of meals can further impact the vitamin content of RMs through the degradation of vitamins. Bognar (1980) found that vitamin C content fluctuated between RM components and meal providers. Furthermore, there was a positive correlation between the amount of vitamin C lost and the rate of chilling where there were losses of 2-17% when chilled within 0.5 hours, using a blast chiller, compared to 10-38% losses when chilled in a conventional fridge, which can take up to 5 hours. The biggest losses of vitamin C occurred in mashed potato, which is most likely due to the larger surface area available for oxidation to occur compared to boiling potatoes whole or preparing baked potatoes (Kincal and Giray, 1987).

The best retention of AA in food has been shown by using 'freezing only' methods, where the foods were not thawed, or kept fresh which had the highest retention of AA in potatoes and other fruits and vegetables (Rickman et al., 2007, Favell, 1998). Another way that the AA content of these foods could be enhanced or protected is through the use of encapsulated AA in foods (Redmond et al., 2004), where ascorbic acid is packaged within a liposome to prolong bioavailability of a supplement (Davis et al., 2016). Mashed potato with added encapsulated AA had a higher retention of vitamin C after chilling and freeze-chilling treatments, whereas mashed potato fortified with non-encapsulated vitamin C had approximately 2mg/100g, compared to 15mg/100g cooked weight in encapsulated vitamin C. However, the current cost of using encapsulated vitamins and the lack of enquiry into consumer preference of AA fortified food are prohibitive and require more investigation before they can be used regularly in food production (Nedovic et al., 2011).

Cooking or heating has been found to be one of the most destructive processes in food production, as many of the WSVs are heat sensitive (Ryley and Kajda, 1994). However, the type of cooking can impact the rate of degradation. An example of this is folate, where the hydrophilic nature of folate means that frying potatoes retains more than boiled

potatoes (Hoppner et al., 1973). Frying allows the retention of the vitamin within the food matrix due to the low affinity of WSV to non-polar environments. Further protection of folate in RMs has been shown when the meal contains vegetables that are rich in antioxidants, which stop the oxidation of folate during storage (Johansson et al., 2008).

Storage conditions can also affect WSVs, especially thiamine and riboflavin. Storage of beef stew army rations for up to 54 months retained AA and riboflavin, but caused a slight degradation in thiamine levels when stored at 4.4ºC (Salunkhe et al., 1979). Bognar (1980) found further reduction in thiamine and riboflavin content in RMs of up to 50% and 30% respectively, when chilled for up to 10 days at 2ºC, and up to 50% on reheating meals. These differences could have been the result of variation in packaging, where the presence of oxygen may lead to increased degradation of vitamins, or preparation techniques, where leaching has occurred during boiling or blanching (Kramer, 1977).

Other processes carried out to improve the shelf-life of foods such as irradiation have been found to be detrimental to the thiamine, riboflavin and vitamin C content of foods. This is an important step in the manufacturing process to ensure the safety of RMs over time. However, Patterson and Stewart (2003) found that when roast pork and vegetable RMs were irradiated using 1-3kGy, there was a dose-dependent reduction in the thiamine and vitamin C content of up to 21% and 54% respectively, compared to 13% and 24% in non-irradiated samples. Although this reduction of vitamin C is substantial, the largest loss of vitamin occurred during the reheating of the meal ready for consumption with an additional 83% reduction of vitamin C. For thiamine, the retention was 90%, but there were significant overall losses during the irradiation followed by reheating of the meal. Alternative methods have been identified that could protect the vitamin content of fruit juices, including the use of ultrasound or gas treatment (Jiménez-Sánchez et al., 2017). These methods should be employed with RMs to investigate how alternative methods could protect the WSV content of food components within them.

2.4.1. Limitations

There are several limitations to the findings of this literature review; in three out of the five RM consumption studies, the majority of participants surveyed about their RM consumption were female (Garcia et al., 2014, Garcia et al., 2020, Sprake et al., 2017),

however, Mills et al. (2018b) found that females were more likely to consume home cooked meals than RMs or take-aways. Furthermore, as highlighted previously, self-reported recall data is not the most robust method of dietary analysis, and is more susceptible to under-reporting of dietary intake (Burrows et al., 2019). Moreover, the data gathered from this literature review does not provide insight into how much of the meals are being consumed, the types of meals that are eaten, or the current demographic of RM eaters, or restrictive inclusion criteria for the sample populations.

With regards to the studies that explored the vitamin content of RMs, there were a number of different analytical methods (Appendix vi) used to calculate the vitamin contents of the foods, which could have led to variation in the values that were found (Puwastien et al., 2005). Furthermore, 50% of the studies were carried out between 1970-2000, where there were vast changes in the processing of chilled RMs and which could have led to changes in the losses attributed to RM production (Welch and Mitchell, 2000). Moreover, there were many different recipes used; for example, pork may have been minced or cooked as a joint, or potatoes may have been mashed or whole, which would have impacted the vitamins susceptibility to degradation. This was not considered in many of the studies. Lastly, only two studies provided information about the vitamin content of the total meal rather than just per 100g.

2.4.2. Conclusion

This literature review has shown that whilst RMs are increasingly popular among UK consumers, the literature that has been identified in this review show gaps in academic knowledge about accurate estimates about general consumption patterns of RMs across the UK population. Furthermore, there is a lack of robust data about the potential contribution of RMs to the nutrient intake of thiamine, riboflavin, folate, vitamin B12, vitamin C of the UK population. It is important to consider the nutrient quality of foods that are commonly eaten in the diet, especially foods that are marketed as a meal, to ensure that these can be eaten to support a balanced diet, and consumers can make a more informed choice about the food that they consume. The review findings will also inform the meals used for lab-based nutrient analysis of popular RMs available to purchase to explore the WSV content of these meals.

2.5. Research aims and objectives

The literature review has identified numerous areas where further research is required to determine the consumption patterns of RMs of the UK population and the WSV content of RMs available on the market. Therefore, the aims of this research are:

1. To explore the contribution that UK RM consumption makes to the intake of selected process labile WSVs as a proportion of current UK Dietary recommendations.

2. To determine the actual vitamin content of a popular UK RM with a focus on selected process labile vitamins.

3. To gain deeper understanding of the effects of additional food processing on the WSV content of RMs i.e. how methods of food service delivery could impact selected process labile vitamin content of the RMs.

2.5.1. Objectives

- To extract and analyse RM consumption data from UK dietary cohort studies.
- To explore which UK demographic groups are consuming RMs using a secondary analysis of diet study data of consumers and non-consumers of RMs
- To analyse the quantity and cuisine types of RMs consumed by the UK population
- To identify, select and utilise the most appropriate analytical methods to measure process labile vitamins in individual food components of a popular RM
- To quantify the contribution of RMs to process labile vitamin intake and compare to the UK dietary recommendation values
- Utilising RM consumption data, with focus on the adult population, compare use of RM cuisine types between different age groups in the UK to identify a popular RM in the UK for laboratory analysis
- To assess how the hot holding associated with service delivery could affect the content of selected process labile vitamins in a selected popular RM

3. CONTRIBUTION OF READY MEALS TO THIAMINE, RIBOFLAVIN, FOLATE, VITAMIN B12 AND VITAMIN C TO THE UK DIET AND MEALS ON WHEELS USE IN THE UK: SECONDARY ANALYSIS OF NATIONAL SURVEY DATA.

3.1. Introduction

This chapter reviews the contribution of ready meals (RMs) to the UK diet, specifically with regards to the dietary intake of thiamine, riboflavin, folate, vitamin B12 and vitamin C. In the UK there are a variety of ways that information is gathered about the diet, health, spending habits and consumer behaviours of the population. Health surveys carried out regularly in the UK include Eurostat, Living Cost and Food Survey, the National Diet and Nutrition Survey (NDNS), Health Survey for England and the English Longitudinal Study of Ageing (ELSA) (UK Data Service, 2019). These are expensive, large-scale studies that are carried out over many years to identify trends in health, and monitor the health status of the population (Soucie, 2012). This data is used to inform health policy development and for media reports, marketing and branding (UK Data Service, 2019).

Studies such as Eurostat and the Living cost and Food survey only report on household expenditure, and not food composition or intake, whereas the Health Survey for England only provides data on health status of the population and not food consumption (NHS Digital, 2020, Eurostat, 2021, Office for National Statistics, 2021). Another study, ELSA, does provide information of portions of fruit and vegetable intake, but does not provide data on other food intake such as RMs, and is therefore not suitable for this study (ELSA, 2019). Data regarding the habitual eating habits and health of the UK population over time are collected using rolling cross-sectional studies. The most comprehensive population study regarding the eating habits of the UK, which also provides the nutrient composition of foods is the NDNS. Therefore, the NDNS survey will be used to investigate the contribution of RMs to specific micronutrients in the UK.

The literature review in chapter 2 identified gaps in knowledge with regards to the consumption patterns of RMs and the demographic of RM users in the UK. Due to the growing RM market highlighted in chapter 1, it is important to understand patterns of consumption and the contribution these meals make to the diet. This data can then be

used to inform public health policy. This chapter will analyse the dietary intake of thiamine, riboflavin, folate, vitamin B12 and vitamin C from RMs using NDNS data.

3.1.1. The National Diet and Nutrition Survey (NDNS)

The NDNS is a private household survey providing an assessment of the diet and nutritional status of a representative sample of people aged 1.5 years and over in England, Scotland, Wales and Northern Ireland. The NDNS survey is a collaborative survey, currently carried out by NatCen Social Research and the Medical Research Council Epidemiology unit in the UK, and the Northern Ireland Statistics and Research Agency in Northern Ireland (Whitton et al., 2011).

Department of Health (2019) explains that the study began in 1992 as a series of representative cross-sectional surveys carried out across preschool children (aged 1.5 to 4.5 years), young people (4 to 18 years), adults (19-64 years) and older adults (aged 65 and over). The survey is a rolling programme with methods for nutrient analysis and coding of food products to ensure consistency of data input by researchers (Department of Health, 2019). Sampling for the NDNS occurs annually with the aim of collecting data from a representative sample of 1000 people per year from across all age groups (Whitton et al., 2011, Department of Health, 2019). The NDNS is used to inform policy and monitor progress of nutritional objectives in the UK, such as tackling obesity (Roberts et al., 2018).

Data collection for the NDNS survey is carried out in various ways including the completion of a food diary, lifestyle questionnaire and blood and urine samples to assess nutrient status (Department of Health, 2019). Participants are asked to document all food and drinks consumed, including brand names, in and outside of the home over four consecutive days, which can be during the week or over the weekend. All participants are provided with training on how to complete the food diary, as well as how to estimate portion size using a food photograph atlas and an equivalents food list (Whitton et al., 2011).

Once all food diaries are collected, the information is coded into nutritional assessment software DINO (Diet In Nutrition Out) by trained coders and editors at Human Nutrition

Research, Cambridge. The food composition data are populated using the Department of Health's Nutrient Databank (Whitton et al., 2011). The aim of coding is to match the food item that has been provided in the 4-day food diary to a specific code from the Nutrient Databank, and a portion code to understand nutrient intake. If the item is not available on the system, the manufacturer is contacted to collect nutritional information (Whitton et al., 2011).

Auditing and feasibility checking of the coding process is carried out to maintain a high level of quality control throughout the data analysis (Whitton et al., 2011). Although the NDNS data provides insight into the composition and consumption of RMs in the UK. It can also provide information about how these meals contribute to the dietary intake of specific nutrients, as information about food based nutrient data, including the macro and micronutrient content is collected. Therefore, its use in the current study is justified.

3.1.2. Defining a 'meal'.

Understanding the nutrient content of RMs is important due to the use of the word 'meal' in the product name. Leech et al. (2015) have highlighted that there are numerous ways to describe a 'meal'. These include the pattern of eating (frequency, regularity or skipping), the context of eating (eating with friends or family and eating at home or out of the house) and the format of eating (the sequence of foods, the food/nutrient composition). For the purpose of this study, a 'meal' is defined by the format of eating and the pattern of eating, rather than its context.

The literature indicates that RMs are described by the food industry as a 'main meal' (Howard et al., 2012), or main course; a meal that has been found to be the main contributors of thiamine, vitamin B6, vitamin B12, vitamin C, vitamin D and vitamin E (Leech et al., 2015). This further justifies the need to investigate RMs as a potentially important source of essential nutrients. Furthermore, if there is a reliance on these foods to meet nutrient requirements, consumers could be at risk of deficiency if these meals do not provide a minimum amount of nutrients (Louzada et al., 2015).

3.1.3. Types of ready meals consumed

An overarching objective of this research is to carry out lab-based analysis of RMs. This analysis will inform which meal will be tested as part of the analysis presented in Chapter

5. To ensure that the choice regarding which RM is tested is informed, the most popular cuisine types within the UK will be explored. Mintel (2020) findings summarised in Chapter 1.4 showed that Chinese, Indian, Italian and British cuisines were the most popular cuisine types, but the most popular cuisines were not ranked in the dataset. Therefore, this study will utilise the NDNS to investigate the cuisine types of RMs consumed by the UK population.

3.1.4. Nutrient recommendations for ready meals.

One of the aims of this current study is to understand the contribution of RMs to the intake of water-soluble vitamins (WSVs) within the UK population. To give context to the findings, it is important to consider the concentration of nutrients within RMs in relation to the UK nutrient recommendations. This provides an opportunity to understand where recommendations are met, or where the nutritional profile of the meal could be improved.

Although there is no guidance that stipulates the amount of nutrients that RMs should provide, there are public health guidelines that summarise the nutrient requirements for 'meal' provision across the population, which will be summarised in this section. The summary of these guidelines will focus on the nutrients that are being investigated as part of this study; thiamine, riboflavin, folate, Vitamin B12 and Vitamin C, as justified in Chapter 1.

Currently, there are a number of age specific recommendations for those working in public catering. For infants under five years of age in childcare, where the main meal is lunch, the reference nutrient intake (RNI) states that the meal should provide at least 40% of the RNI for micronutrients (Crawley, 2006).

The Caroline Walker Trust school-based recommendations state that, where food is provided all day (such as boarding schools), that the main meal (lunch) and evening meal should provide 40% and 20%, respectively, of the RNI for vitamin C and folate. However, there are no guidelines for the other WSVs (Crawley, 2005). It is worth noting that although this recommendation is more than 15 years old, there have been no new nutrient-based recommendations to date. For meal planning within the population, at

establishments such as hospitals and care facilities, which cater for adults aged 19-74 years old, the UK government recommend that an evening meal (the main meal) should provide 30% of the recommendation for vitamins and minerals (Public Health England, 2017b).

A similar recommendation is made for meal replacements (European Union, 2016). These are nutritionally regulated products used, for example, to aid weight loss. Although the energy content of these products may be restricted, the micronutrient content within them needs to support a balanced diet; therefore, the products should meet minimum nutrient concentrations to reduce the risk of nutrient deficiency. Therefore, meal replacement products must provide at least 30% of the RNI for micronutrients per meal (European Union, 2016). Furthermore, the Caroline Walker Trust also provide recommendations for meals that are provided to those that are 65 years and older. These recommend that main meals, including delivered meals or those provided at a lunch club, should provide at least 50% of folate and vitamin C, and 33% of thiamine, riboflavin and vitamin B12 (Caroline Walker Trust, 2004).

Although the Public Health England (2017b) guidance was originally used for establishments such as care homes and hospitals, in the absence of other guidance, it was decided to use the minimum nutritional recommendation of 30% across all the population. The specific age group of 19-74years olds have also been identified in chapter 1 as the demographic with the largest RM consumption. It should be noted however, that the use of the 30% recommendation has limitations. Some groups in the population have higher micronutrient recommendations (Crawley, 2005; Crawley, 2004, Crawley, 2006). The recommendation of 30% of the RNI is suitable for most of the population and should be considered the minimum concentration of WSV that should be provided by RMs. The Public Health England (2017b) recommendations are for publicly procured meals and the origin of the meals within the NDNS survey that are being assessed as part of this study is not specified and, therefore, there may be differences in meal composition as a result of difference in food processing techniques used in the home compared to a catered institution (Davis et al., 2018).

Despite the issues presented, and in the absence of other guidance, for the purpose of this research, it will be assumed that a meal should provide 30% of the RNI for thiamine,

riboflavin, folate, vitamin B12 and vitamin C. This study will provide a point of discussion by investigating the frequency of RM consumption in the UK and exploring nutrients that RMs provide in relation to dietary recommendations. The RNIs for the WSVs assessed is outlined in Appendix vii.

3.2. Aim

To identify patterns of RM consumption in the UK and calculate the contribution that RMs make to the intake of nutrients that are liable to food processing; specifically, thiamine riboflavin, folate, vitamin B12 and vitamin C, using NDNS datasets.

3.2.1. Objectives

- To describe the demographics of RM consumers
- To analyse the quantity of RMs consumed across the NDNS age groups and understand consumption patterns across the age groups.
- To analyse the different cuisine types that are consumed across the age groups to establish representative meals for lab testing.
- To determine the thiamine, riboflavin, folate, vitamin B12 and vitamin C content in the RMs consumed by age group
- To compare the thiamine, riboflavin, folate, vitamin B12 and vitamin C intake from RMs in relation to dietary requirements by age group

3.3. Methods

3.3.1. National Diet and Nutrition survey

Data were retrieved from UK Data Services for the NDNS for Years 1-8, which comprises data collected between 2008-2017 and included food diary and personal data. All participants (n=12,097) who consumed RMs were identified within the 'SubFoodGroupDesc' column in the food level data file and were filtered out by those cases that contained the term 'ready meal' within each wave. The participants consuming RMs were identified by unique code and then cross referenced in the 'indiv' level data and any duplicates were excluded. The participant numbers where RMs were identified were located in personal level data file to identify RM 'users' and 'non-users'. Initially, all RMs consumed during the study were identified and coded (1 = RM, 2 = Not RM

consumer). Following this, all participants that stated that they consumed one or more RMs during the study period were identified. RM user data was compiled and the frequency of 'MealTimeDescription' were analysed to explore the number of times RMs were consumed during the study period. All data was coded in SPSS Version 23 as all NDNS data are provided in this format.

Using the food level data file, the weight of RM consumed was calculated per person (total g of RMs consumed/ days food diary was analysed for [n=4 days]). The contribution of RMs to the intake of vitamin C, thiamine, riboflavin, vitamin B12 and folate was also calculated using the food level data. The average amount of each nutrient was calculated per person, and then, for each nutrient, an overall average was gathered within the RM 'user' group. This data was then split by NDNS age groups; 1.5-3 years, 4-10 years, 11-18 years, 19-64 years, and 65 years and over. The percentage of RM contribution to the diet was calculated as follows: (amount of vitamin provided by RM/vitamin intake over the day)*100. An average was then taken over the RM consumers in each of the age groups.

To compare the vitamin content in RM to the 30% of the RNI, the nutrient data was gathered from the food level data, and an average was taken across the meals consumed. The concentration of vitamin C, thiamine, riboflavin, and vitamin B12 was compared to the 30% RNI for each of the age groups (Appendix vii).

To analyse the RMs by cuisine type, all RMs consumed were allocated to a cuisine type (British, Italian, Chinese, Indian, Mexican or others) and a count of RMs in each cuisine type was taken, and then stratified by age group. This information will be used to identify commonly consumed RMs in the UK and used to inform the laboratory-based testing presented in chapter 5.

3.3.2. Statistical analysis

Data were represented using summary statistics. For the NDNS data analysis a Kruskal-Wallis test with Dunn's pairwise comparison post-hoc testing was used to compare the quantity of RM consumed by age group (1.5-3 years, 4-10 years, 11-18 years, 19-64 years and 65+ years), contribution of RMs to overall intake of thiamine, riboflavin, folate,

vitamin B12 and vitamin C in RM consumers by age group. A Chi-Square test for trend was used to identify trends in cuisine types consumed between age groups. A one-sample t-test was carried out to compare the RNI values with the theoretical nutrient values provided by the food-level data.

All statistical analysis was carried out using SPSS statistical software version 23.

3.4. Results

3.4.1. Demographic of ready meal consumers

The NDNS data showed that 10% of participants consumed RMs during the 4-day study period. The age of consumers was significantly higher in the RM group than non-consumers, and the mean difference in age = 3.2 years, 95% confidence interval (1.7, 4.7, $p<0.001$), as shown in Table 6. There was no significant difference between equivalised household income and RM consumption (0.402, $p=0.172$).

Table 6: Table showing the age and gender of ready meal consumers and non-ready meal consumers from the National Diet and Nutrition Survey (NDNS), year 1-8 compiled data, n=12,096.

	Ready meal consumers	
	Yes (n= 1131)	No (n=10966)
Age (n, [SD])	32.9 (24.9)	29.7 (24.0)
Male (n, %)	523 (4.1)	5048 (41.7)
Female (n, %)	608 (5.0)	6526 (54.0)
Annual Income (salary, SD)	£28,102 (21,245)	£29,061(20,937)

3.4.2. Frequency of consumption

The data showed that there were between one and four instances of RM consumption during the food diary data collection period of 4 days. Data showed that 98% of RM consumers ate these food products 1-2 times.

3.4.3. Quantity of ready meal consumed by age group

Figure 17 showed that there was a significant difference between age groups ($p<0.001$), with those aged between 0-10 years consuming fewer RMs per portion (g) compared to those aged 11 years and above. Descriptive statistics are provided in Appendix viii

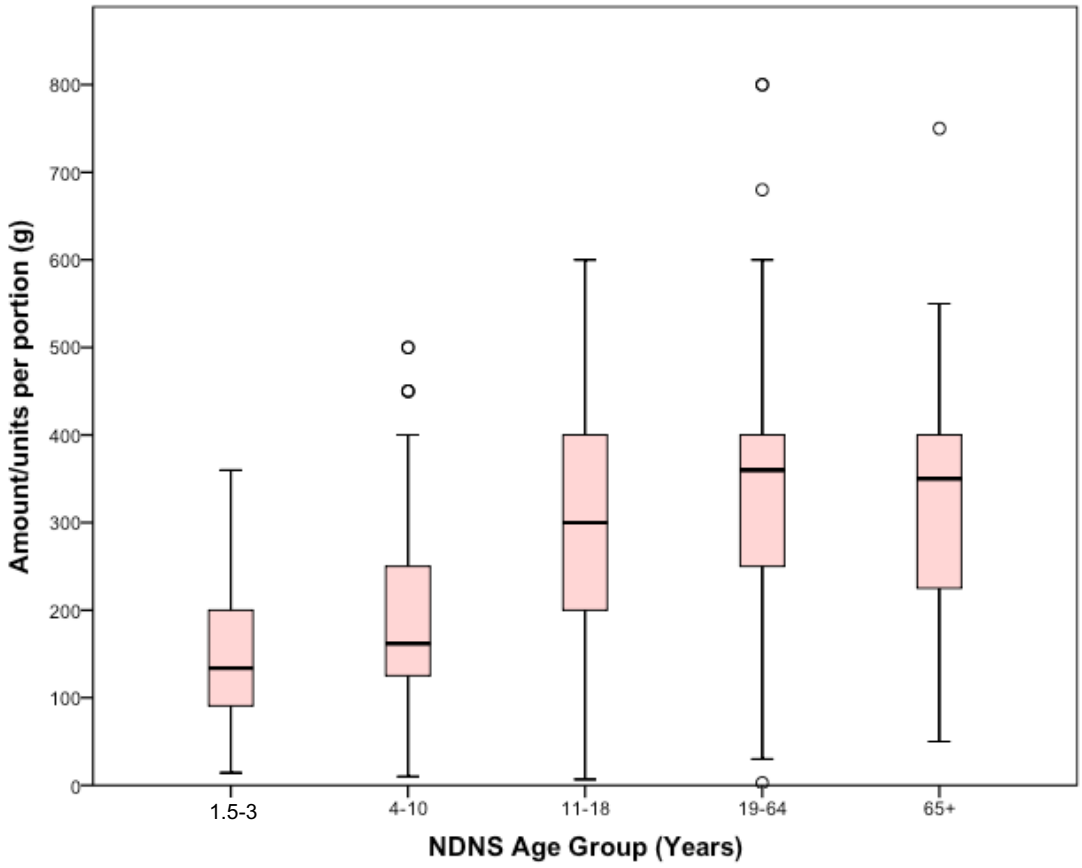


Figure 17: Boxplot of mean amount/units (g) of ready meal portion consumed by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8. Boxplots – median [lower quartile, upper quartile], interquartile range; whiskers, 1.5* interquartile range; o (outliers), >1.5*interquartile range; *(outliers), >3*interquartile range.

3.4.4. Cuisine types consumed by age group

A chi-square test for trend found that there is a significant positive linear trend between age group and the proportion of meals that were British cuisine, and significant negative trend between age group and the proportion of meals that were Italian cuisine ($p<0.001$) (figure 18). Descriptive statistics are provided in appendix ix.

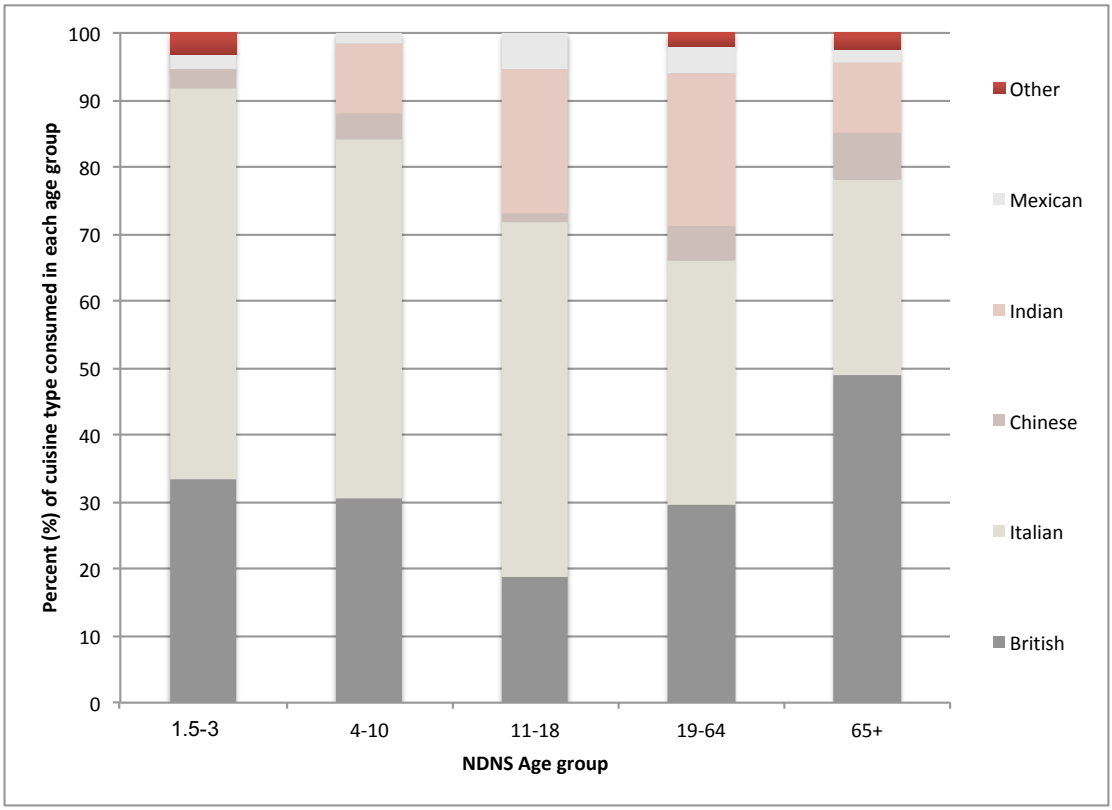


Figure 18: Stacked 100% bar chart to show the percentage (%) of ready meal cuisine type consumed by ready meal consumers by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8.

3.4.5. Percentage of thiamine, riboflavin, Vitamin B12 and folate intake provided by ready meals by age group

A Kruskal- Wallis test showed a significant difference between age groups for all vitamins ($p<0.001$). Descriptive statistics are provided in Appendix xi. A Dunn's post hoc test showed that there was significantly higher percent contribution of RMs to vitamin intake in 11-18 years , 19-64 years and 65+ years of age compared to 1.5-3 years and 4-10 years ($P<0.05$), but there was no significant difference between 1.5-3 years and 4-10 years for the percentage contribution of RM to riboflavin, thiamine, vitamin B12, folate and vitamin C intake (Figure 19-23). Descriptive statistics are provided in appendix xi.

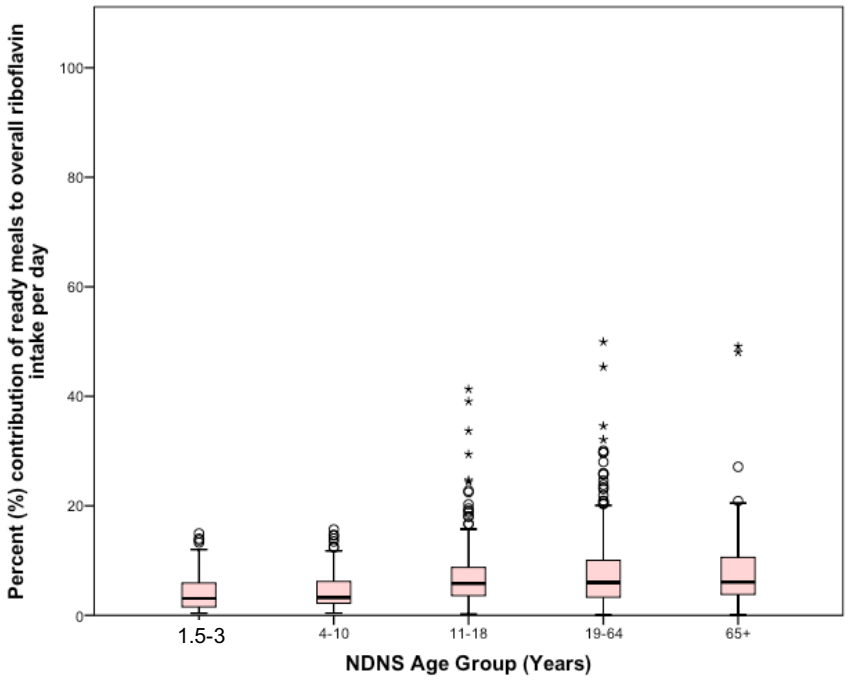


Figure 19: boxplots of the contribution of ready meals to the overall riboflavin (%) intake per day by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8.

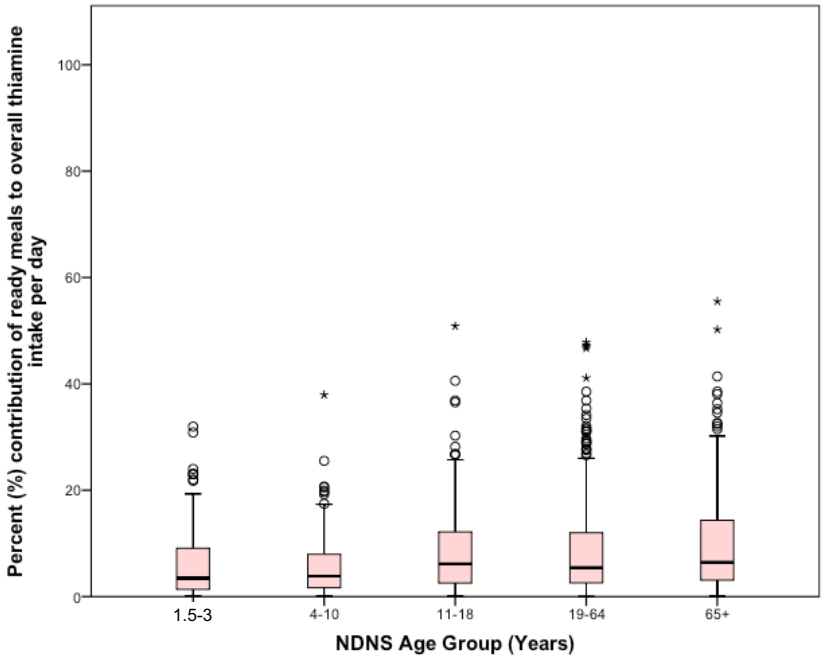


Figure 20: boxplots of the contribution of ready meals to the overall thiamine (%) intake per day by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8.

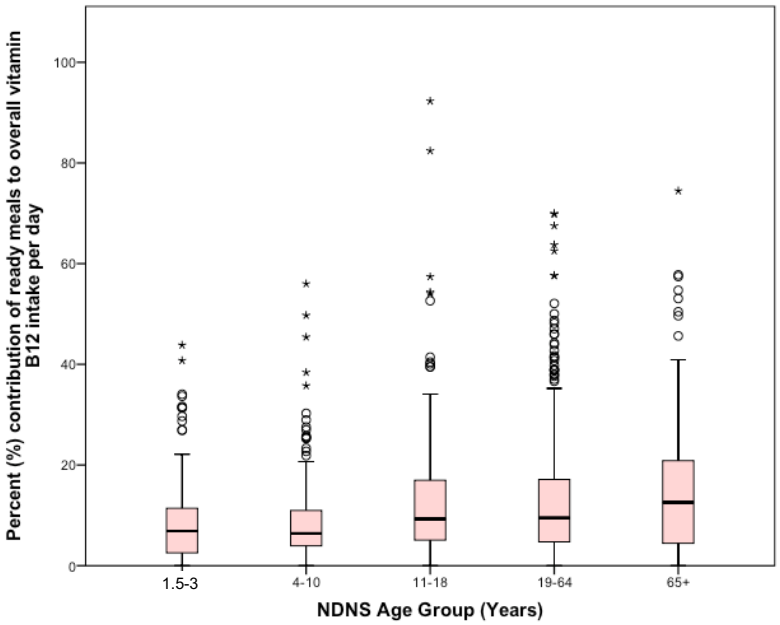


Figure 21: Boxplots of the contribution of ready meals to the overall vitamin B12 (%) intake per day by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8.

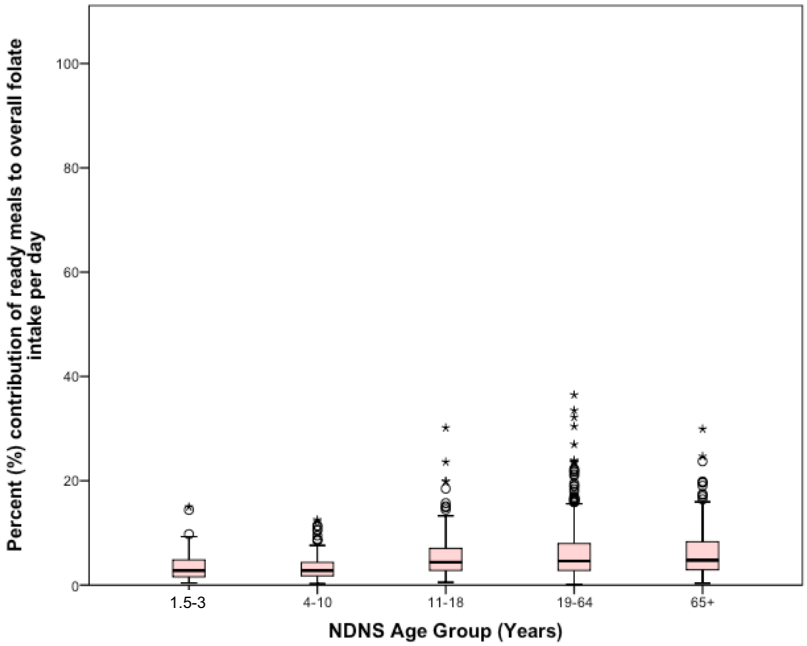


Figure 22: boxplots of the contribution of ready meals to the overall folate (%) intake per day by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8.

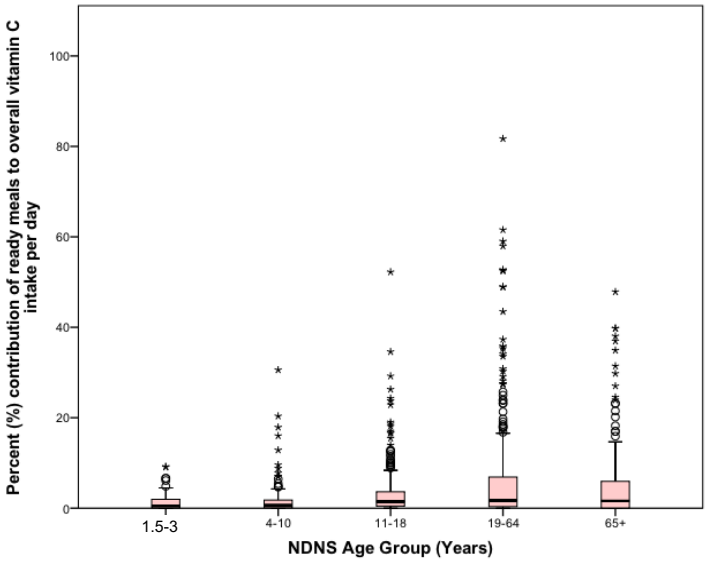


Figure 23: Boxplots of the contribution of ready meals to the overall vitamin C (%) intake per day by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8.

3.4.6. Average concentration of thiamine, riboflavin, vitamin B12, folate and vitamin C in ready meals consumed by age group

A Kruskal-Wallis test found that there was a significant difference across the age groups for the concentration of vitamins within all RMs consumed ($P<0.001$). A one-sample t-test was carried out to determine the vitamin concentration of the RMs consumed to 30% of the RNI for each age group. The results show that there was no difference or significantly more of thiamine, riboflavin and vitamin B12 in the meals consumed compared to the RNI for each age group.

There was significantly less folate in 1.5-3 years ($p<0.000$), 4-10 years ($p<0.001$), 11-18 years ($p<0.001$), 19-64 years ($p<0.001$) and 65+ years ($p<0.001$), and in vitamin C in 1.5-3 years ($p<0.001$), 4-10 years ($p<0.001$) and 11-18 years ($p<0.001$) (Figure 24-28). Descriptive statistics are provided in appendix xii.

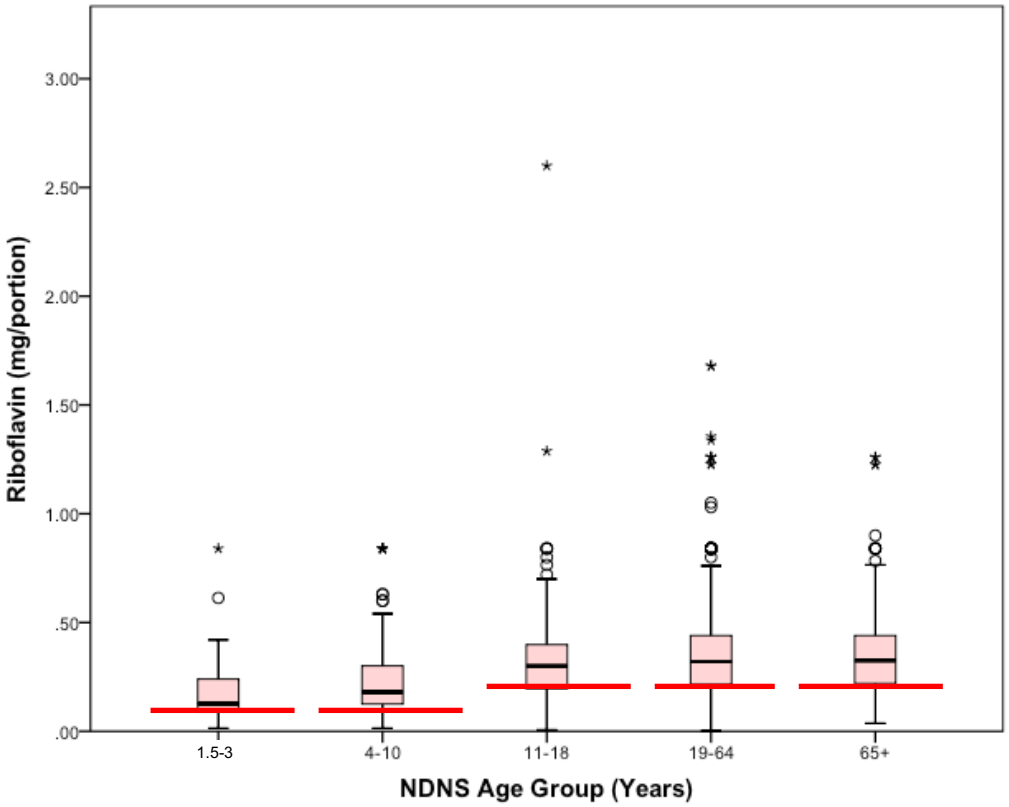


Figure 24: Boxplots of the mean riboflavin (mg/portion) content of RMs consumed by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8. The red line above each box plot identifies 30% of the reference nutrient intake (RNI) for that age group.

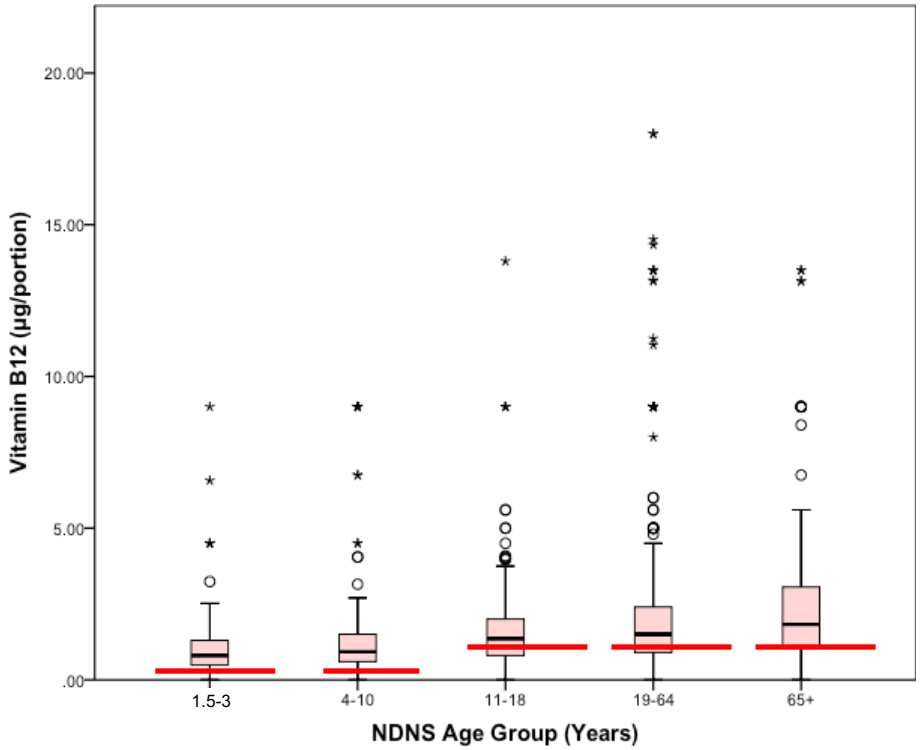


Figure 25: Boxplots of the mean vitamin B12 (µg/portion) content of ready meals consumed by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8. The red line above each box plot identifies 30% of the reference nutrient intake (RNI) for that age group.

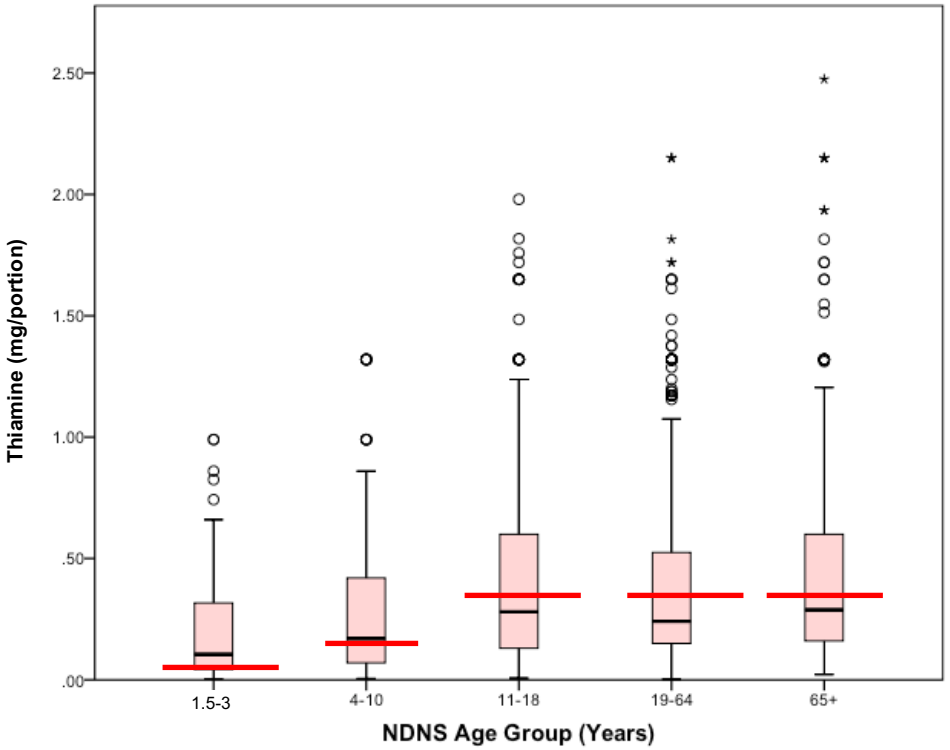


Figure 26: Boxplots of the mean thiamine (mg/portion) content of ready meals consumed by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8. The red line above each box plot identifies 30% of the reference nutrient intake (RNI) for that age gr

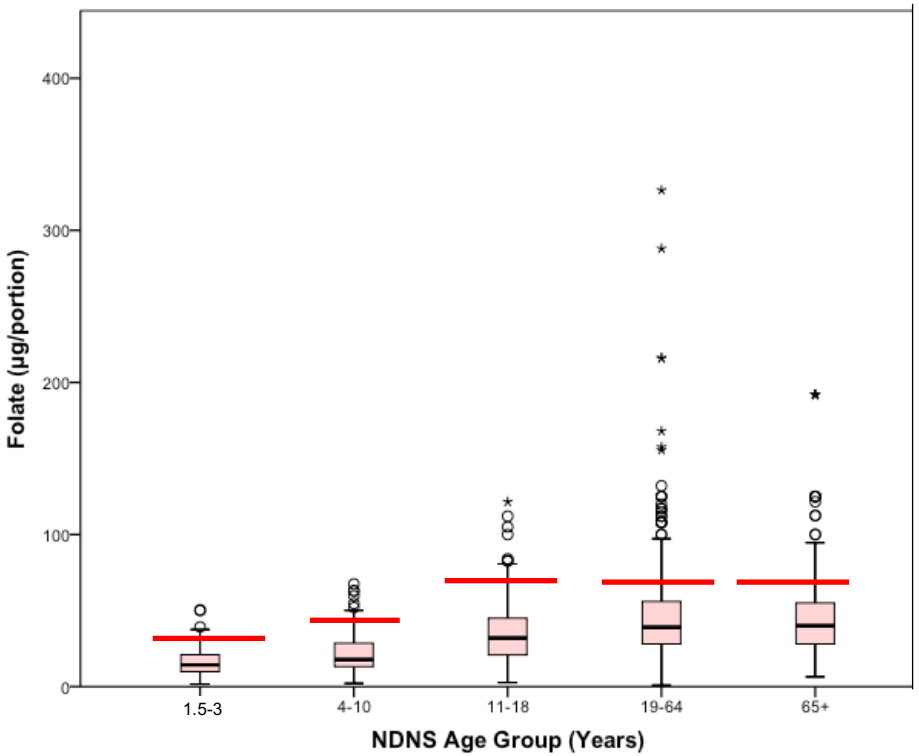


Figure 27: Boxplots of the mean folate (µg/portion) content of ready meals consumed by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8. The red line above each box plot identifies 30% of the reference nutrient intake (RNI) for that age group.

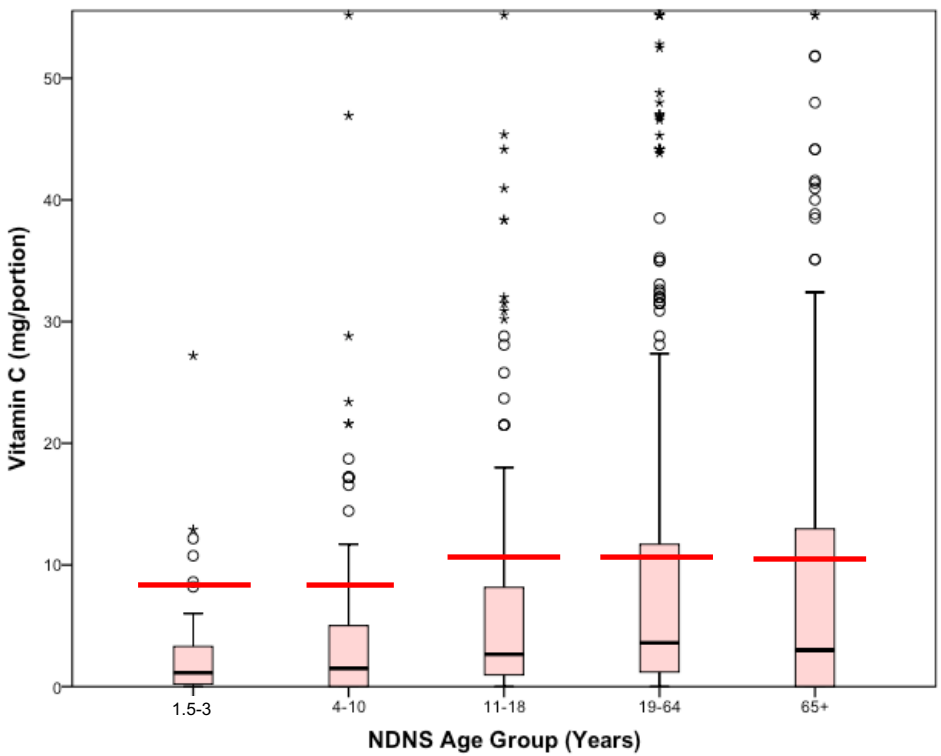


Figure 28: Boxplots of the mean vitamin C (mg/portion) content of ready meals consumed by the age groups defined in the National Diet and Nutrition Survey (NDNS). Data compiled from NDNS data from years 1-8. The red line above each box plot identifies 30% of the reference nutrient intake (RNI) for that age group

3.5. Discussion

This study found that those that consumed RMs were significantly older than those that did not. When using the 30% of the RNI cut off, the recommendation for folate were not met for any age groups, and vitamin C were not being met by RMs for 1.5-3 years, 4-10 years and 11-18 years. However, the requirements for thiamine, riboflavin and vitamin B12 were met for all ages. British followed by Italian RMs were the most popular cuisine types consumed during the study periods and there was no significant difference in household income between RM consumers and non-consumers. This study has highlighted the need to understand the current intake of RMs in the population, especially given the contribution of these meals to the diets of individuals that are at risk of nutrient deficiency.

3.5.1. Frequency of ready meal consumption

The current study found that RMs were consumed on average, between one and four times during the 4-day food diary. 98% of the participants that consumed RMs ate them 1-2 times during the period of data collection. Although this information shows that RMs are being consumed regularly by those eating these meals, this data cannot provide information on habitual consumption. The data that was reviewed in Chapter 2 was summarised in weekly consumption, however, the NDNS data is collected over four days, and therefore cannot be extrapolated to weekly consumption (Medical Research Council, 2021). Furthermore, as RMs are perceived as a less healthy option, they are foods that are susceptible to under reporting (Geeroms et al., 2008).

3.5.2. Quantity of ready meals consumed

Data from this study revealed that the amount of RMs consumed varied across the age groups, where those aged over 11 years old consumed significantly more than those <11 years. This result is to be expected, as the nutritional requirements increase as we age, which means that more food needs to be consumed to meet these requirements (British Nutrition Foundation, 2019), and therefore dietary intake may need to increase to meet demand (Change4Life, 2019). As the growth rate begins to decrease into adulthood, food intake plateaus at 18 years and over which can be seen in figure 17. There are however periods during the life cycle such as during pregnancy, lactation or during times of illness, where requirements may increase to enable healthy growth and repair (Lutz et al., 2014),

but these have not been identified in this study. This research has shown that older adults seem to consume slightly less food than younger adults, a phenomenon that has been seen previously (Scientific Advisory Committee on Nutrition, 2021), and is most likely due to reduced appetite (Giezenaar et al., 2016).

A position statement by Scientific Advisory Committee on Nutrition (2021), which reviewed 30 systematic reviews (of which 15 included a meta-analysis) about the role of nutrition in older adults living within the community, found that although there were only a small number of randomised controlled trials, which were of short duration, folate status was poor. This current study has highlighted that RMs could contribute to lower intakes of folate, and therefore a lower folate status and highlights a need for further investigation into how meal quality can be improved for those at higher risk of micronutrient deficiency.

Changes in appetite as people age is well documented and has been linked to changes in gut function, olfactory and gustatory function and medication side effects (Pilgrim et al, 2015). Furthermore, impaired dexterity may mean that food takes longer to eat, causing the feeling of fullness to occur before the meal is finished (Pilgrim et al., 2015). However, this reduction in food intake may mean that older populations may be at an increased risk of deficiency (Hickson, 2006).

Recently, a number of organisations including Apetito, a RM provider, have introduced smaller, enriched meals that have the same amount of some macronutrients; especially energy (kcal/kJ) and protein, as standard portions. These meals provide a smaller portion for individuals with reduced appetites (Apetito, 2019b), which have been found to improve the energy and protein intake of the meal (Ziylan et al., 2016). However, processing can change the flavour of the meals leading to low acceptance (Höglund et al., 2018, Tsikritzi et al., 2015). This is due to enrichment being carried out through the addition of seasoning or sauces (Best and Appleton, 2011). Other ways to increase nutrient intake in older populations may be to eat smaller meals more frequently throughout the day (Caroline Walker Trust, 2004).

3.5.3. Ready meal cuisine types

Cuisine preference differed over the age groups, where Italian and continental style meals were favoured in ages 1-18 years old, and British meals were favoured in adults aged 65 years and over ($p<005$). This is corroborated by YouGov (2019) and other survey data (Reed et al., 2003, Mintel, 2017), which found that Italian meals were the most popular in the UK. There was no difference in preference between British and Italian meals between 19-64-year-olds.

The popularity of Italian RMs in the UK could be attributed to the food being perceived as more authentic, or higher quality (Reed, 2001). However, the marketing of Italian or Mediterranean style meals heightens the perception of healthiness compared to other cuisines. This is due to the Mediterranean diet being recommended by the UK government and health professionals to improve health outcomes and reduce the risk of chronic disease progression (Cannon, 2005, Reed et al., 2003).

The findings that >65-year-olds seem to prefer British meals, compared to other cuisines, could be linked to familiarity and overall preference for meals that they know and enjoy, with hedonistic appeal being the basis for the majority of food choices (Whitelock and Ensaff, 2018). However, British meals are also preferred by 30% of adults aged between 19 and 64 years. Italian meals were found to be popular in older Dutch adults and were generally chosen as the most popular ethnic option outside traditional meal option (Frongillo et al., 2010). However, Mintel (2011) found that people over-55s may be less likely to choose unfamiliar foods or new cuisines to eat.

The concept of familiarity with food choice in older adults has also been explored in a review by McKie (1999), who found that ‘proper meals’ were conceptualised as ‘healthy meals’, and tended to be a more traditional style cuisine that was consistent with what people had eaten throughout their lives. These meals were made up of two courses; one that consisted of fish or meat, potatoes and vegetables, and another course with pudding, soup or a piece of fruit. However, researchers also found that where there were diverse food options, a variety of different foods and cuisine types were chosen (Costa et al., 2001). Ultimately, this study has identified British and Mediterranean as the most popular RMs across all adult age groups in the UK, and therefore will be used to inform the meal identified for testing in Chapter 5.

3.5.4. Percentage contribution of ready meals to the overall intake of water-soluble vitamin and RNI

The percentage (%) contribution of RMs to the overall intake of thiamine, riboflavin, folate, vitamin B12 and vitamin C was significantly different across the age groups for all vitamins ($p<0.05$). 20-40% of the total daily intake of these vitamins was provided by RMs in those who consumed them at least once during the 4-day food diary. This is the first study that has investigated the contribution of RMs to the intake of WSVs. Therefore, the findings of this study could be used to understand how the nutritional quality of RMs could be improved to ensure that if they are being provided as a main meal and are the source of most of the daily intake of nutrients, that they are providing adequate nutrition.

3.5.5. Water-soluble vitamin content of meals consumed

This study found that the WSV content of the meals consumed met the recommended 30% of the RNI nutrient cut-off for thiamine, riboflavin and vitamin B12, but this was not achieved for vitamin C or folate. With regards to thiamine, this study found that the meals contained between 0.01-0.43mg/100g of RM. These findings are supported by previous studies of the WSV content of RMs (Bognar, 1980, Agte et al., 2002, De Ritter et al., 1974), where meals provided between 0.04 – 0.6mg/100g. Furthermore, for riboflavin, the current study found that RMs contained between 0.02-1.3mg/100g, which is similar to previous studies where a concentration of 0.006-0.26mg/100g was found (Salunkhe et al., 1979, Agte et al., 2002, De Ritter et al., 1974).

Unfortunately, the studies outlined here do not provide the portion weights of the meals, and therefore cannot be compared to these findings to assess if they meet the 30% of the RNI recommendations. Although the studies identified through the literature review were older, these, more recent findings seem to suggest that the thiamine content of these meals are adequate.

Lastly, for vitamin B12, this study found a content of between 0.0-6µg/100g and 0-18µg/portion (mean = 1.86µg/portion), which is above the daily requirement for vitamin B12. These findings are supported by De Ritter et al. (1974), however, a study by Hammink (1978) found that frozen meat and gravy components from a frozen meal did not provide as much vitamin B12 compared to freshly prepared meals. Conversely, the concentration data was not provided, therefore, there is not enough information to

validate this study. It should be noted that the study by De Ritter et al. (1974) is an old study and based in the U.S.A and therefore tastes may have changed since its publication. Furthermore, the meals that were tested are not ones commonly consumed in the UK, such as fried chicken dinner or fried shrimp dinner.

With regards to vitamin C, this study found that the meals did not meet the 30% RNI cut-off for all age groups, with meals containing 0-29mg/100g or 0-159mg/portion (mean =8.74mg) or 26-83% of the RNI. These findings were consistent with others (De Ritter et al., 1974, Agte et al., 2002, O'Leary et al., 2000, Redmond et al., 2002, Redmond et al., 2004, Salunkhe et al., 1979, Patterson and Stewart, 2003, Faulks, 1991, Gatherer, 1971, Bognar, 1980). Although the findings of these studies support the conclusions of this investigation, it is worth highlighting that only four out of the 10 studies were from the UK and were at least 18 years old and therefore meal types and processing may have changed. Furthermore, Hammink (1978) did not provide concentrations of vitamin C within the meal. Finally, as the methods of assessment differed between the studies, this could be a source of variation in the results.

Folate concentration in this study found that RMs contained between 3-54µg/100g or 1-326µg/portion (mean = 37µg/portion) or 38-67% of the RNI. These findings are consistent with other studies (Fajardo et al., 2017, Agte et al., 2002, Johansson et al., 2008) which found that the folate content of RMs was between 5- 13µg/100g. Similarly, the studies investigating the vitamin C content of RMs were not carried out in the UK, and therefore the processing and ingredients of the meals tested will differ.

The findings with regards to folate and vitamin C are a cause for concern. This is due to the recommendations for school age children and older adults, which states that the main meal should provide 40% and 50% of the RNI for vitamin C in school aged children and older adults respectively, and 50% of the RNI for folate in older adults (Caroline Walker Trust, 2004, Crawley, 2005). This higher recommendation is provided due to lower intakes of folate values shown in the NDNS survey, and due to vitamin C not being stored effectively in the body (Public Health England, 2019b). Furthermore, due to the importance of folic acid in neural tube development during pregnancy (Combs, 2012), if these meals are being consumed during times of increased dietary requirement, then dietary supplementation should be advised (Greenberg et al., 2011).

3.5.6. Limitations

Although there are strengths to this study, particularly the inclusion of a wide range of RMs and the nutrient content of the meals identified, the nutrient information relied on the data gathering methods used during the NDNS, and the manner in which the micronutrient information (if the meal was not in the DINO database) was collected is not provided. Therefore, the WSV content of the meals is not an indication of actual individual meals, but the average nutrient content of meals that were consumed overall. The NDNS collects a 4-day food diary, and therefore weekly consumption for comparison to the market research data and the literature review data could not be carried out. Furthermore, due to preconceptions of RMs, this may have caused underreporting of RM consumption (Geeroms et al., 2008). High respondent burden of the NDNS survey may cause under representation of demographics within the population that have low literacy skills, are isolated or are unmotivated to complete the survey (Medical Research Council, 2021). Therefore, further research should look to explore the use of RMs in institutions such as schools, hospitals and meals on wheels (MOW) services to understand the contribution of RMs to public meal provision.

3.5.7. Conclusion

This study has shown that for those who regularly consume RMs, these meals could be a major source of WSVs in the diet, including vitamin B12 and thiamine. The data suggests that around 10% of the population consume RMs, and can contribute up to 50% of the overall dietary intake for thiamine, riboflavin, folate, vitamin B12 and vitamin C. Furthermore, RMs consumed did not meet the 30% nutrient content recommendation used in this study for folate and vitamin C.

Preference for cuisine types changed across the age groups, however, the most popular cuisines for adults of all ages were British and Mediterranean. Moreover, there is a lack of data available concerning the nutrient content of RMs, yet this current study indicates that they are becoming a more substantial part of the UK diet. This study highlights the need to investigate the actual WSV content of RMs using validated techniques to ensure required levels of process-labile nutrients are being provided.

4. A COMPARISON OF VITAMIN B1 (THIAMINE) DETECTION IN PULSES AND SAUSAGES USING A MICROBIOLOGICAL VITAFAST ASSAY AGAINST THE GOLD STANDARD METHOD; HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC).

4.1. Introduction

The literature review (Chapter 2), recognised two methods of thiamine quantification; the microbiological assay and the HPLC assay with fluorescence detection. There are benefits and disadvantages in both methods - the microbiological assay is useful to analyse the free/biologically active derivatives of the vitamins, and is a more cost effective method; while the fluorescence method using HPLC, which uses derivatisation of thiamine to thiochrome, uses expensive equipment but is cheaper to run when analysing a large number of samples (Edwards et al., 2017). Both methods are validated; the microbiological assay is used as a method of thiamine quantification in the UK food composition tables (Finglas et al., 2015), and the thiochrome method is widely employed by the Association of Official Analytical Chemists (Edwards et al., 2017). However, no literature was found investigating the precision or accuracy of either method when used to analyse foods containing sodium metabisulphite, a powerful antioxidant.

4.1.1. Use of sulphites in food

Sulphites is a collective term used to describe food preservatives that contain a sulphite group and include sodium sulphite, sodium bisulphite, sodium metabisulphite, potassium bisulphite, potassium metabisulphite and sulphur dioxide. Sulphites are widely used in a variety of foods including dried fruit, wine, breakfast sausages, processed meat, and canned foods. They act as a powerful antioxidant to prevent the discolouration and degradation of food, especially in meat and canned white beans (Garcia-Fuentes et al., 2015).

One of the main benefits of using sulphites in the food industry is that it has enabled the storing of food for longer, whilst maintaining their organoleptic and aesthetic characteristics. However, due to sulphites being an allergen, foods containing this preservative must have it detailed on the back of pack labelling. The level of sulphites used in the food industry are highly regulated

by retained European Commission law (European Commission, 2011). As previously described in Chapter 1, thiamine can be degraded by sulphites, causing it to no longer be bioavailable within the gut. The rate of thiamine degradation by sulphites is dependent on temperature and pH, where higher temperatures and increased pH environments increase the rate of thiamine cleavage (Leichter, 1969, Hermus, 1969).

When establishing the best methods to use for the analysis of foods, validation is used to ensure that a method is reliable and reproducible. However, validity of a method for use under certain conditions may not be transferable. For example, different storage or cooking conditions. (Taylor, 1983). The rationale for this investigation into the comparison of two common methods for analysing thiamine foods, with and without sulphites, is due to the action of sulphites in different environments (Leichter, 1969). The extraction techniques used in each method differs, therefore sterilisation is required for the microbiological assay, but not for the fluorescence assay.

Sterilisation in the presence of thiamine has been shown to increase the rate of degradation of thiamine in foods containing sulphites (Mapson and Wager, 1961, Luh et al., 1978). These factors may influence the rate of thiamine breakdown during the extraction process, and therefore analysis should be carried out to establish what methods could be susceptible to error due to methodological interferences. It is important to understand these differences to ensure that an appropriate detection method, which will produce accurate estimations of thiamine content in foods, is employed.

4.1.2. Use of the microbiological assay and HPLC for the determination of thiamine in food.

Sarett and Cheldelin (1944) first explained the microbiological assay, which utilises the growth of bacteria to determine the concentration of thiamine. Microbiological assays have been found to be sensitive at very low levels of thiamine (approximately 2ng/L) (Edwards et al., 2017). However, some disadvantages in using these assays include long incubation times, accessibility of the growth organism and the ability for some organisms to use derivatives of thiamine that are not biologically active in humans. Commercially available microbiological assays use *Lactobacillus fermentum*, a thiamine-deficient bacterium, that is coated on microtitre plate wells. Once the extract and growth media are added to the wells and incubated, the turbidity can be measured using spectrophotometry (Edwards et al., 2017). However, due to the inherent

presence of bacterium in food and the environment that could begin to grow during the incubation period, the extract must be sterilised at a high temperature. This protects against false positive results (Merck, 2019).

The enhanced degradation of thiamine in the presence of sulphites during the extraction process, caused by high temperatures and pH, may lead to inaccurate measurement of thiamine content within the foods. The main benefit of using the microbiological assay is that the kit is affordable, and there is less requirement for expensive equipment for a small sample size, for example testing foods within the food industry. Therefore, it is still a commonly used method for thiamine determination in foods.

4.1.3. Use of the HPLC with fluorescence detection assay for the determination of thiamine in food.

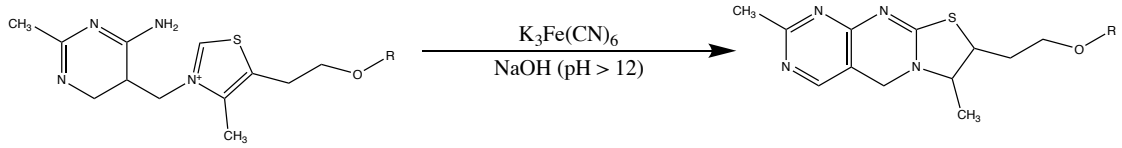
HPLC is an automated liquid chromatographic technique. In summary, reverse phase liquid chromatography is the separation of different compounds within a sample using a polar solvent, known as the mobile phase, which is pumped though tightly packed particles that are contained within a column. The most commonly used particles are silica; however, this can differ based on the analytical requirements. The particles contain cylindrical pores that are lined with non-polar compounds, most commonly C18 groups, known as the stationary phase. The rate at which a compound moves through the stationary phase is determined by its affinity to the stationary phase. As different compounds have distinct polarity, similar compounds will group together within the column forming bands, which then move through the column collectively. As the groups of compounds within the sample have different polarity, more polar compounds will elute quicker than less polar compounds (Snyder et al., 2009).

As long as the environment of the system (temperature, pH and flow rate) remains consistent, the time taken to move through the column can be used as an identifier of different compounds. This is known as the retention time. Once the compound has passed through the column it can be detected and quantified by a detector. For the purpose of this study, a fluorescent detector was used (Chemguide, 2016), however, ultraviolet and mass spectrometry detection can also utilised as alternatives (Snyder et al., 2009).

As there are six derivatives of thiamine found in food, quantification of each may require the use of multiple assays and techniques (Bettendorff, 2012). A way to quantify all esters at the

same time is through the use of derivatisation. Derivatisation is the transformation of a chemical compound, in this case thiamine derivatives, into a product with similar chemical characteristics (Royal Society of Chemistry, 2019), yielding similar elution times, when HPLC is used to determine analyte concentration. Derivatisation of thiamine to thiochrome has been shown in equation 1 and was first explained by Jansen (1936). In the production of thiochrome, thiamine and its esters are oxidised with potassium ferricyanide to form thiochrome, a group of fluorescent compounds producing one peak on the HPLC chromatogram. The benefit of the HPLC assay over the microbiological assay is that there is no requirement for aseptic conditions. Sterilisation of the samples within the microbiological assay require the sample to reach >80ºC, which may result in degradation. The use of HPLC to determine thiamine in samples containing sulphites may reduce the risk of error occurring during the extraction step.

There are many benefits of using HPLC, one being that it exists as the gold standard approach, but the equipment required to carry out the testing is expensive and requires technical expertise to run and maintain the system. Although the longer-term running costs are reduced, the initial cost of setting up the system can be prohibitively expensive, and researchers may favour the microbiological assay if they are testing smaller sample sizes.



Thiamine Thiochrome

Equation 1: A chemical equation of the alkali oxidation of thiamine derivative to the fluorescent compound thiochrome thorough oxidation with potassium ferricyanide (K_3[Fe(CN)6] in alkali conditions (Lu and Frank, 2008)

4.2. Aim

To assess the validity and precision of the VitaFast microbiological assay and the HPLC assay methods to determine thiamine concentration in canned pulses and cooked sausages, which are sold with or without sulphites.

4.2.1. Objectives

Analyse the validity and precision of the Vitafast microbiological assay compared to the HPLC assay when analysing foods with and without sodium metabisulphite.

4.3. Method

To assess the validity and precision of the VitaFast Microbiological assay and the HPLC assay methods to determine thiamine concentration in canned pulses and cooked sausages that are sold with and without sulphites.

4.3.1. Chemicals and reagents

Analytical grade chemicals were used throughout the experiment. Thiamine hydrochloride, Taka-diastase from *Aspergillus oryzae*, sodium citrate, and potassium ferricyanide were obtained from Sigma. Citric acid, sodium hydroxide, HPLC grade methanol, glacial acetic acid, sodium acetate anhydrous were obtained from Fisher.

4.3.2. Sample Preparation

Six different commercially available canned chickpeas and butterbeans were purchased from a supermarket and categorised into two groups consisting of three samples without the presence of sodium metabisulphite and three which contained sulphate as indicated on the food label. The samples were weighed, drained and re-weighed. Four different commercially available chilled sausage products were purchased from a supermarket. The sausage was grilled for five min. on each side until cooked. The sausages were weighed before and after cooking. All samples were lyophilised (Edwards, Micro Modulo, RV5) until dry, re-weighed and stored at -20^{o}C until analysis. All samples were prepared in duplicate.

4.3.3. Thiamine analysis

Thiamine analysis was carried out on canned, lyophilised chickpea and butter bean sample using VitaFast Microbiological assay, and HPLC with fluorescence detection.

4.3.4. VitaFast Microbiological assay

Aseptic conditions

All handling of extracted material was carried out in a sterile hood (Thermo Scientific, MSC Advantage). Disposable latex gloves were worn at all times. Sterile hood, Gilson pipettes, tip boxes and scalpel were cleaned with 70% Industrial Methylated Spirit (IMS) to minimise the contamination of the food extract prior to incubation.

Standards

Addition of 1.8ml sterile water to 1.44µg of thiamine hydrochloride bottle made up a stock solution of 0.08mg/100ml thiamine hydrochloride. The following dilutions were made: 0, 0.012, 0.024, 0.036, 0.048 and 0.060 mg/100ml thiamine hydrochloride into sterile 2ml Eppendorf tubes. A calibration curve was plotted and was used to determine the thiamine hydrochloride equivalent concentration (mg/100ml). The peak area of the absorbance at 486nm was used.

Determination of Thiamine Concentration using a Vitafast Microbiological Assay with Lactobacillus Fermentum.

The method was carried out as described in the kit instructions.

Assay medium

To the assay medium bottle supplied in the kit, 10ml of sterile water was added, sealed and heated in a water bath (Grant, SBB6) for 5 minutes at 95ºC, shaking every minute. The assay medium was then chilled down to below 30ºC in an ice bucket, and then filtered through a 0.22micron filter into sterile 15ml Falcon tubes.

Citrate Buffer

Five hundred millilitres of 1M NaOH was prepared. Citric acid monohydrate (15g) was dissolved in 500ml of deionised water in a 1L beaker with a magnetic stirrer. One hundred and twenty millilitres of 1M NaOH was added to the dissolved citric acid, quantitatively transferred to a 1L volumetric flask, and filled up with deionised water. The pH was adjusted to 4.5 with 1M NaOH and 1M HCl. The buffer was stored for up to 3 days at 2-8ºC.

Sample Extraction

To 50ml Falcon tubes, 1±0.1g of lyophilised sample, 300mg of Taka Diastase and 20ml of citrate buffer was added. The samples were shaken until fully mixed and the samples were extracted overnight in an orbital shaker (Thermo Scientific Orbital Shaker, Max Q 8000) at 100 rpm at 37ºC for 18-24 hr.

Distilled water was added to a final sample volume of 40ml and heated in a water bath at 95ºC for 30 minutes, shaking every five minutes. The samples were then cooled down quickly in ice below 30ºC, centrifuged at 13,000rpm for 5 minutes, filtered through 0,22µM sterile filters and diluted to within the calibration range with sterile water provided in the test kit into sterile 2ml Eppendorf tubes.

Test Implementation

In aseptic conditions, 150µl of assay medium, and 150µl of diluted sample or standard was pipetted into the 96-well microtiter plate provided in the test kit, which contained *Lactobacillus fermentum*. The wells were covered with adhesive foil, ensuring all wells were sealed and then incubated (VWR, Incu-line cooled Premium) at 37°C in the dark for 44-48 hours.

Following the incubation, the adhesive foil was removed, bubbles were removed using a sterile scalpel and turbidity was read using a microtitre plate reader (Thermo Scientific, Multiscan FC) set at 620nm. All samples and standards were carried out in triplicate. The following equation 2 was used to determine the active vitamin B1 concentration.

Equation 2: An equation to yield the active thiamine (vitamin B1) using the standard curve concentration, dilution factor, and sample weight (g)

$$Active\ Vitamin\ B1\ (mg/100g)\ or\ (mg/100mL)\ = \frac{conc.using\ standard\ curve\ \times\ dilution\ factor\ \times\ 0.787}{sample\ weight\ in\ g\ or\ ml}$$

4.3.5. HPLC determination of thiamine

Acetate Buffer

In a 1L Duran bottle, 47±0.1ml of glacial acetic acid and 14.7±0.01g of sodium acetate anhydrous were added and made up to 1L with deionised water. The pH was adjusted to 4.6 with 1M NaOH and 1M HCl.

Extraction method

Finglas and Faulks (1984) have previously detailed an extraction and measurement method used to determine thiamine concentration. Lyophilised samples were placed in a conical flask with 65ml of 0.1M HCl, and a magnetic stirrer. The mixture was then agitated for 30 minutes. Once agitated, 16ml of 1.25% (w/v) Taka-Diastase in 1M acetate buffer (pH4.6) was added to the sample mixture. The sample was then incubated at 50±1°C in a shaking water bath set at 100 rpm. After incubation the sample was left to cool on the bench to room temperature, transferred to a 100ml volumetric flask and made to volume with distilled water. The sample was mixed and then filtered through Whatman 541 filter paper into 50ml falcon tubes. The filtrate was kept at -20°C until analysis.

Derivatisation of Thiamine

The thiamine present in the filtrate was derivatised to thiochrome as described by Valls et al. (1999). In summary, 1ml of 0.03M potassium ferricyanide in 3.75M aqueous NaOH to 2ml of sample filtrate in a thick-walled glass bottle. The reaction was then stopped with 0.2ml of 5M

phosphoric acid. A C18 Sep-Pak Cartridge was activated with 0.4ml methanol and 0.4ml deionised water, followed by 3.2ml of extract. Interfering compounds were removed with 5% methanol/95% 5mM phosphate buffer at pH 7.0. Thiamine was eluted with 0.6ml methanol and made up to 1mlwith methanol. The elution was passed through a 0.22µm Millipore filter into amber vials before HPLC analysis.

HPLC analysis

The spectra of thiochrome is shown in figure 29. Standards (0, 0.5, 1, 2.5, 4, 7.5µg/ml) (figure 30), blanks (0.1M HCl), and filtered samples were analysed for thiamine. A sample/standard/blank injection volume of 10µL was used with a Shimpack GIST, 5µm, C18, 4.6 x 150mm column in isocratic conditions at 35^{o}C. A mobile phase of 70:30 (v/v) phosphate buffer (5mM, pH 7.0): acetonitrile was used at a flow rate of 2ml/min using a ShimadzuProminence-I LC20-30 with Shimadzu fluorescence detector (RF-20A/RF-20Axs). Peak detection was carried out using a Shimadzu fitted with an 8µL flow cell. Excitation and emission wavelengths (nm) were 360nm and 430nm for thiamine. Data was analysed using Shimadzu LabSolutions Lite.

Calibration curves were created using the area of the curves from standard solutions (figure 30).

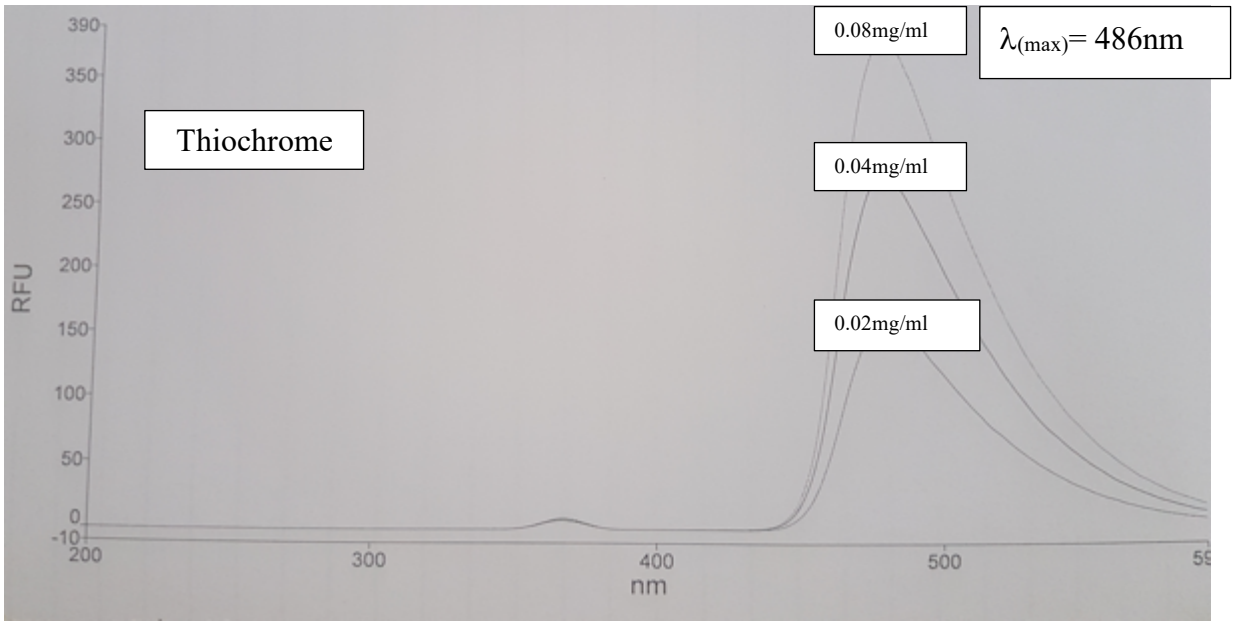


Figure 29: A spectra of 0.08mg/ml, 0.04mg/ml and 0.02mg/ml thiamine hydrochloride derivatised to thiochrome measured on a fluorometer, l(max) =486nm.

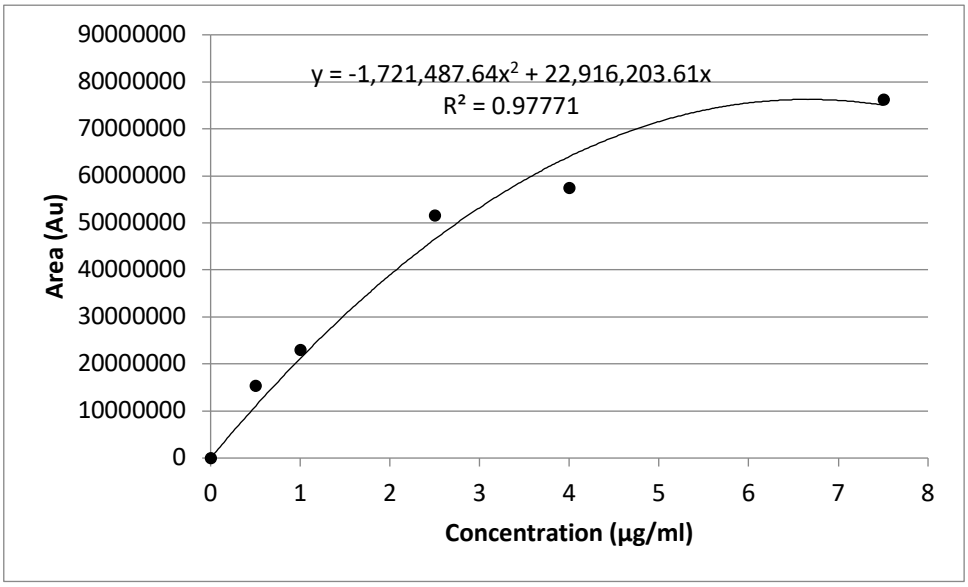


Figure 30: A calibration curve of concentration (mg/ml) to area of the curve on the chromatogram (Au) for derivatised thiamine hydrochloride to thiochrome and measured by HPLC with fluorescence detection (excitation and emission 360nm and 430 nm, respectively); y=-1,721,487.64x + 22,916,203.61x2=0.98

4.3.6. Statistical analysis

A Bland-Altman (Bland and Altman, 1986) plot was used to compare thiamine concentration measured using the microbiological (VitaFast®) assay and the HPLC assay in canned beans (n=12). A one-sample t-test was used to determine the differences of the measured thiamine concentrations compared to zero. For the purpose of this investigation, the criteria for agreement of methods is that at least 95% of data points must fall within 2SD of the mean (Giavarina, 2015). Analysis was performed using Microsoft Excel and SPSS version 23.

4.4. Results

The analysis showed that the HPLC assay yielded better precision compared to the microbiological assay in all samples. Bland-Altman analysis showed no agreement between the two methods in beans that contained sulphites, as less than 95% of the values were within 2SD of the mean difference. However, for beans with sulphites and cooked sausages all values were within 2SD of the mean difference. A systematic error was discovered, where the HPLC method consistently measured 0.28-0.29mg per100g dry weight (DW) higher thiamine concentrations than the microbiological method (figure 31). A one-sample t-test found that the difference of measured values was significantly different to 0 ($p<0.001$), where 0 represents total agreement (figure 31). A regression analysis showed that there is proportional bias for uncooked sausages, but not for beans (figure 32). If there was pre-existence of proportional

bias, it would be appropriate to transform the data by taking logs (Bland and Altman, 1986). However, there was only a negligible change in the mean value.

This was then compared with the data gathered form the cooked sausage data, which found that the two methods agreed, and not significantly different (p=0.81) (figure 33). A one-sample t-test found that there was no proportional bias between the methods (P>0.05) (figure 34).

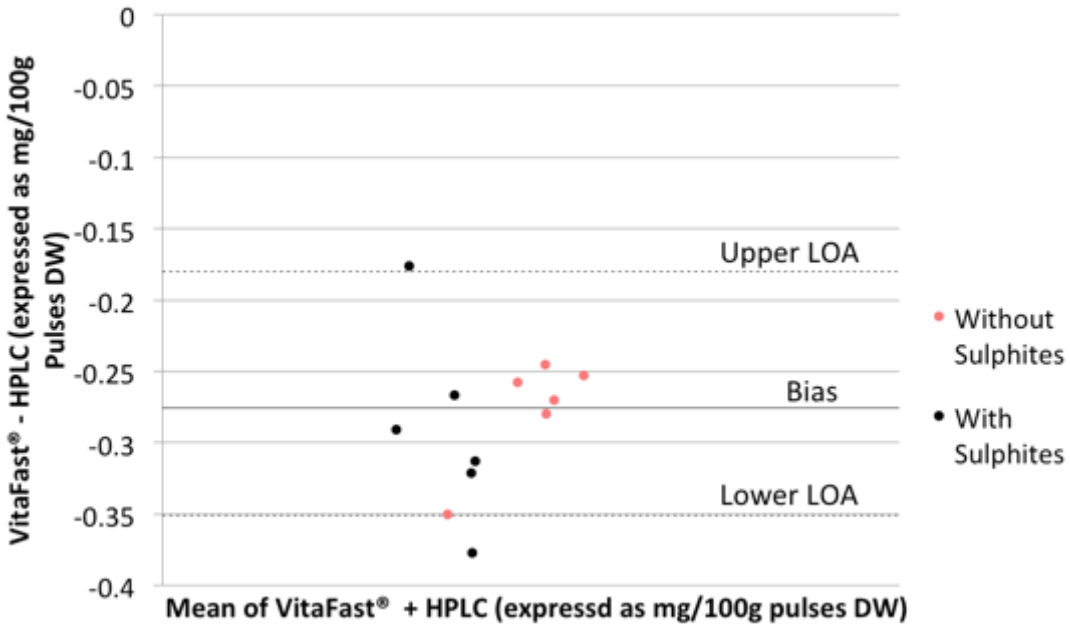


Figure 31: A Bland-Altman analysis plot to assess the agreement between the measurement of thiamine in canned pulses containing sodium metabisulphite. Bias = -0.275, 95% limits of agreement (LOA) = [0.180, 0.351], indicated by solid and dashed lines respectively. '·' represent canned samples that contained sulphites. '·' represent canned samples that did not contain sulphites. A one-sample t-test showed a significant difference between methods (p<0.001)

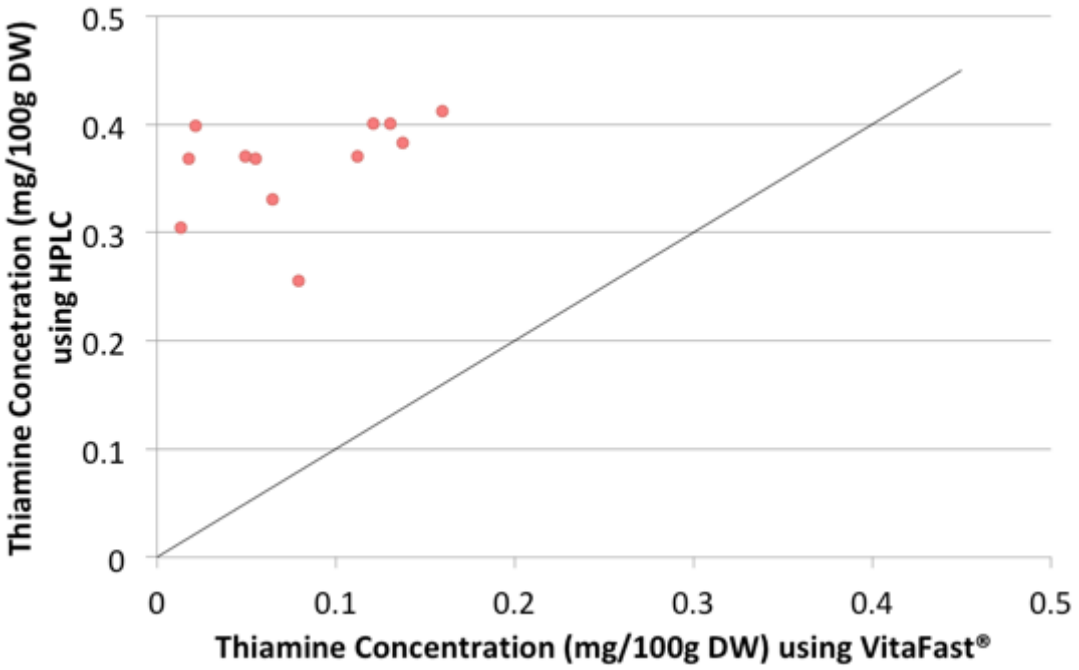


Figure 32: A scatter plot to show the agreement between the HPLC and Vitafast method when measuring thiamine in canned pulses using two methods on the same samples alongside total agreement of measurements shown by the line of identity. Regression analysis showed no proportional bias (p=0.727).

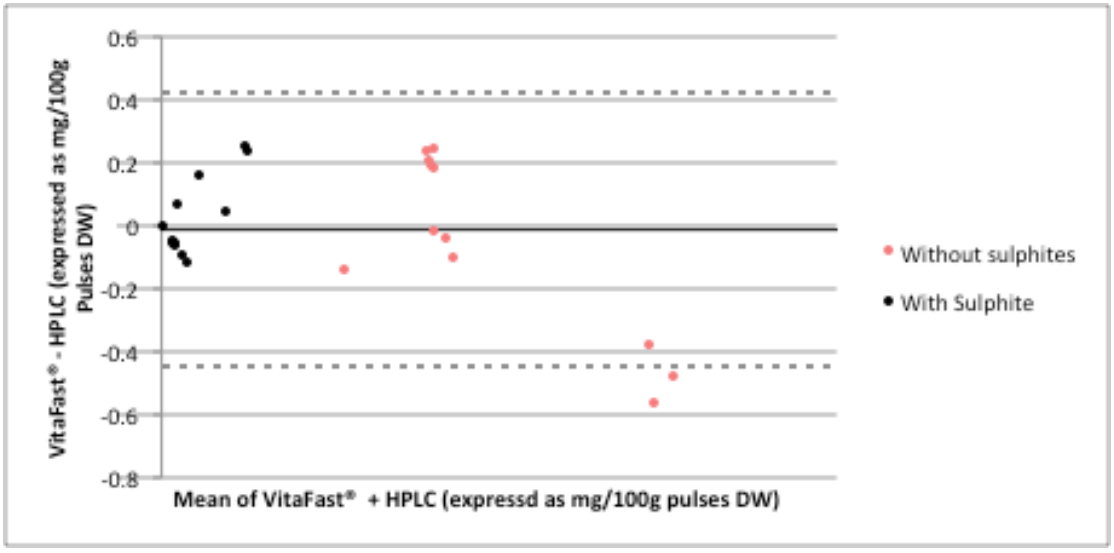


Figure 33: A Bland-Altman analysis plot to assess the agreement between the HPLC and VitaFast method when measuring thiamine in cooked sausages sodium metabisulphite. The '·' identify those samples that contained sulphites. The '·' identify those samples that did not contain sulphites. Bias =-0.013, 95% limits of agreement = [-0.447, 0.421] indicated by solid and dashed lines respectively. A one-sample t-test showed no significant difference between methods (p=0.781).

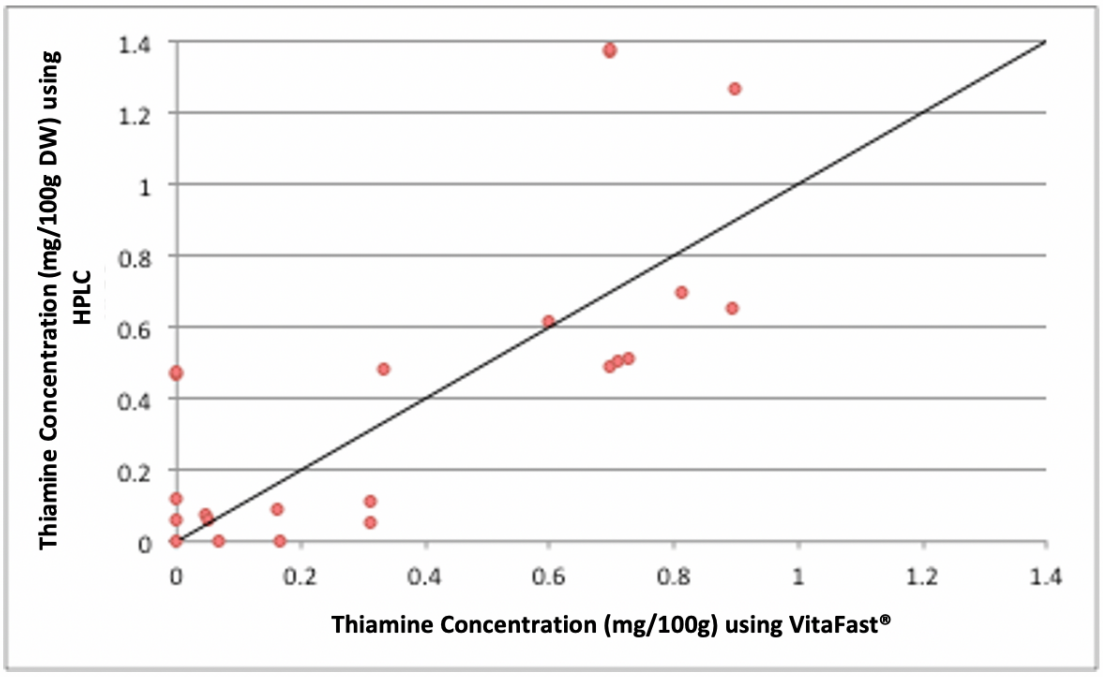


Figure 34: A scatter plot to show the agreement between HPLC and Vitafast method when measuring thiamine in cooked sausages using two methods on the same samples alongside total agreement of measurements shown by the line of identity. Regression analysis showed proportional bias ($p<0.05$).

4.4.1. Validation of HPLC method: Limit of detection

The limit of detection (LOD) was carried out on different samples. The LOD is the lowest amount of analyte that can be detected in a sample, but it may not be possible to be quantified. The LOD of thiamine was 0.5ng/ml (Table 7)

The signal to noise ratio was also quantified. It is an established norm that a signal to noise ratio between 2-3:1 is considered acceptable for estimation of the LOD (Figure 35). Samples were analysed in triplicate. The mean intra-assay coefficient of variation was 5.53%, with mean inter-assay coefficient of variation being 5.11%. The recovery rate was 90%.

Table 7: Summary of validation data for the quantification of thiamine using Shimpack GIST, 5µm, C18, 4.6 x 150mm column, 70:30 (v/v) phosphate buffer (5mM, pH 7.0): acetonitrile, excitation and emission wavelengths (nm) were 360nm and 430nm.

	tR (min)	LOD (ng/ml)	LOQ (µg/ml)
Thiamine	3.4	0.5	0.03

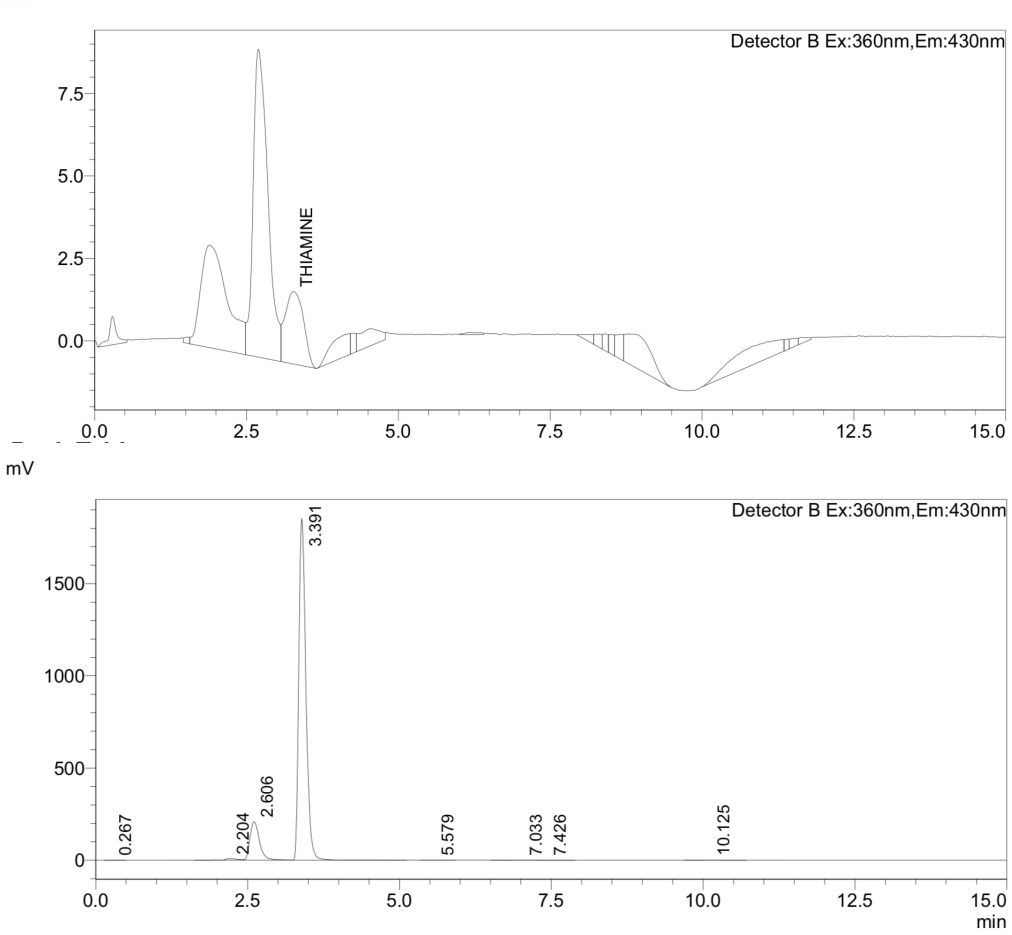


Figure 35: The chromatogram for the limit of detection (LOD) for thiamine.

4.5. Discussion

This study tested the precision and accuracy of two methods to quantify thiamine using Bland-Altman analysis. This method compared the vitamin concentration of a sample using validated HPLC and microbiological methods, where the limits of the agreement for the comparison were defined before the study.

Although the microbiological assay is a widely used method and has been validated (R-Biopharm, 2007), the method is time consuming due to long incubation times and is not suitable for all foods (Edwards et al., 2017). Alternatively, thiamine can be analysed by derivatising free thiamine and its derivatives to thiochrome in alkaline conditions and measured using HPLC with fluorescence detection. The assay was shown to be reproducible, but there was no agreement with foods that had not been cooked before extraction.

There have been studies that successfully evaluate the use of the microbiological assay in fruit juices (Niemeijer et al, 2009), white flour and dehydrated pork (Sarett and Cheldelin, 1944), Alfalfa (Radu et al., 2010), sprouted grain flours (Pîrvulescu et al., 2014), and sweet potato flour (Feng Godinez, 2015), and is the standard method for thiamine determination in the McCance and Widdowson's Composition of Food (Finglas et al., 2015). However, this study found that the microbiological method is not suitable for foods containing sulphites, especially those foods that are stored in sulphites and then heated during the extraction process, such as canned white beans. One possible explanation for this is that thiamine contained within the food is not available for sulphites to breakdown whilst the food is being held at room temperature. Once the food is heated, however, the sulphites are able break down the thiamine. High temperatures have been shown to increase the rate of thiamine degradation (Leichter, 1969).

During the microbiological assay and the sterilisation of the sample at >80ºC, thiamine was likely to have been degraded during the extraction, and therefore not quantified. The pH of the buffer that keeps thiamine stable during the extraction and incubation stages (pH4) is also the optimum pH for thiamine degradation by sulphites. However, as shown with the results in sausages, if the foods are cooked before the measurement occurs, the sulphites can degrade the available thiamine during the high temperatures of the cooking process. Therefore, the sterilisation process of the microbiological assay did not influence the thiamine content of the sample, as there was complete saturation of the sulphites within the food.

The use of HPLC to determine thiamine concentration in food by derivatisation of thiamine to a fluorescent compound using the Finglas and Faults (1984) method has been carried out in vegetables; potatoes (Finglas and Faulks, 1984), mushrooms (Çağlarırmak, 2007), soybeans, tofu (Fernando and Murphy, 1990), cereal products (Reyes and Subryan, 1989), and meat and fish; catfish (Ersoy and Özeren, 2009), mackerel (Erkan et al., 2010) and chicken (Graham et al., 1998). This study found that there were differences between thiamine content measured using the HPLC and the VitaFast microbiological assay. These differences could be explained by the lower temperatures used in HPLC extraction method, which was <50ºC, whereas the microbiological assay extraction method reached temperatures of up to 100ºC. Research has shown that heating thiamine over 50ºC will increase the degradation of the vitamin in a buffer solution (Farrer and Morrison, 1949). Therefore, the heating of the sample extract during the

microbiological assay may have accelerated the destruction of thiamine in solution, leading to reduced accuracy of the assay.

4.5.1. Conclusion

This comparison between two established methods of measuring thiamine has shown variation and systematic error, where the HPLC method consistently measures higher amounts of thiamine. Experiments to analyse the kinetics of thiamine destruction in foods containing sulphites should be carried out to establish the conditions that could explain this variation, and to ensure accurate dietary advice can be given. Cooked sausages containing sulphites have a lower thiamine content compared to sausages without the preservative. This difference may be explained by increased action of the sulphites on thiamine as the food was heated, where the action of sulphites on thiamine contained in the food had already occurred, so no further reactions could take place. Therefore, for the purpose of this dissertation, the HPLC method will be used for the determination of thiamine.

5. THE THIAMINE, RIBOFLAVIN, FOLATE, AND VITAMIN C CONTENT OF SAUSAGE AND MASH READY MEALS FROM DIFFERENT RM PROVIDERS.

5.1. Introduction

Due to the lack of recommendations for the nutrient requirements of ready meals (RMs), this research has identified and reviewed recommendations for a variety of age groups which are used for meals provided in public institutions such as schools and hospitals. For the purpose of this research it has been recommended that RMs should provide a minimum of 30% of the reference nutrient intake (RNI) for thiamine, riboflavin, folate, vitamin B12 and vitamin C as this was the requirement for the majority of the UK population, and corresponded to the age demographic of RM consumers discussed in Chapter 1 and 3. It should be noted that for some populations, i.e. those younger than 19 years, and older than 64 years of age have additional requirements for vitamin C and folate, and though therefore the 30% recommendation is being used here to determine the minimum requirement for nutrients in RMs. It should be noted that where needs for some population groups are increased, this level will be inadequate.

Results from the NDNS analysis in Chapter 3 revealed that although RMs met the 30% of the RNI nutrient recommendations (Public Health England, 2017a) for thiamine, riboflavin and vitamin B12, they did not meet the recommended nutrient requirement for folate and vitamin C. Research from Chapter 3 indicates that approximately 10% of the UK population consume RMs, with the most likely consumers being young working adults who live alone (IBISWorld, 2018). Those who are most at risk of nutritional deficiency are young children during infancy or childhood, women during pregnancy or lactation (Bruins et al., 2018) and older adults who have some frailty, or live alone and are socially isolated (Coulston et al., 1996, Russell and Elia, 2010, Tilston, 1993).

Although the majority of RM consumers were adults aged 19-64 years old, results from Chapter 3 found that all age groups (1.5 years and over) consumed RMs. For children, adolescence and during pregnancy and lactation, there is an increased risk of nutrient deficiency due to higher nutritional needs as a result of rapid growth and development (Dewey, 2013, Marangoni et al., 2016), some may not get the nutrients they need due to picky eating (Taylor and Emmett, 2019) or fad dieting (Whyte et al., 2004). Whereas for older adults,

physical and physiological changes (Leslie and Hankey, 2015, Csapó et al., 2017) may mean that that they are not be able to prepare a full meal in their own home from scratch and are more likely to purchase RMs from supermarkets or other specialist providers as they are cheaper (van der Pols-Vijlbrief et al., 2017), there is more variety, shopping is less bulky (McKie, 1999, Sidenvall et al., 2000), there is less washing up, and usually require only a microwave to prepare (Kendall et al., 2013), making RMs a convenient option.

In the UK, there are a large variety of different RM providers (IBISWorld, 2018), with all major supermarkets in the UK having their own brands which cover a vast range of cuisine types and dietary requirements. RMs can be purchased frozen, chilled or canned and vary by brands and prices (Remnant and Adams, 2015). Consumers base their choices on a number of different factors including price, food preference, availability, animal welfare, the use of British produce, brand or nutrition (Mahon et al., 2006, Mintel Academic, 2018, Mintel, 2017).

Some RM types are found ubiquitously across providers such as British or Italian cuisines, which, as shown in chapter3, are the most popular cuisines across the age groups. However, the composition of these meals, such as the individual ingredients and the vegetables or meat content can impact the vitamin content of the product. The composition of the meal may also have an effect on its price; luxury ranges may have more expensive ingredients, but have also been found to contain higher energy, salt and saturated fat content compared to cheaper RM varieties (Remnant and Adams, 2015). A lack of information on the back-of-pack (BOP) labelling (European Commission, 2012) can make it difficult for consumers to make an informed decision with regards to process-labile water-soluble vitamins (WSVs), about what meals would be the most nutritionally adequate.

RMs tend to be considered as an unhealthy meal option, and have been described as dissatisfying and unhealthy by 18-25 year olds in a study carried out in the USA and Canada by Labrecque et al. (2011). This perception was also held in Belgium (Geeroms et al., 2008), and across Europe, where RMs are perceived as less healthy than cooking meals from scratch using fresh products, particularly when preparing meals containing fish (Vanhonacker et al., 2013). To further support these consumer perceptions, studies by Celnik et al. (2012), Kanzler et al. (2015), Anderson et al. (2008) and Fajardo et al. (2012) found that energy, salt (Thomas and McCabe, 2007) and saturated fat content were inconsistent between RM ranges, and therefore it is difficult for consumers to know which meals are healthier than others; many

meals provided more calories that are stated on the label. Furthermore, many meals did not constitute a balanced meal, with Fajardo et al. (2012) concluding that in the Spanish RMs, they did not meet the requirement for folate as a result of processing. These studies indicate that those who frequently consume such meals as a major part of their diet, over the long-term, may not achieve the recommended intake of some nutrients.

In the UK, initiatives have been put in place to try and improve the nutrient quality of RMs, where major RM providers have signed up to the 'Veg Pledge' (The Food Foundation, 2019), pledging to increase the vegetable content of their meals. The annual report from The Food Foundation (2018) stated that one of the supermarkets, Lidl, explicitly stated that they would ensure their RMs would contain at least one portion of vegetables. However, given that RMs have shown to contribute to increased salt (Thomas and McCabe, 2007) and fat intake in the diet of older adults, and that RMs may not be providing adequate nutrients (as seen in the NDNS analysis), it is important to chemically analyse these meals to understand whether they meet the nutrient requirements for those who consistently consume them.

The large number of different RMs available on the market means that testing all RMs is not possible within the scope of this research. A meal type that can be found across different supermarket ranges, brands and providers will be used to evaluate the thiamine, riboflavin, folate, vitamin B12 and vitamin C content, price and meal composition.

Supermarkets with the largest market share for RMs include Tesco, Sainsbury, Marks and Spencer, Asda and Morrison's, where these retailers make up 56% of RM sales in the UK (Passport Euromonitor, 2018). Furthermore, there are other popular low-budget supermarkets, such as Aldi, that should be considered. Aldi is one of the main purchasers of RMs from Northern Foods UK, a RM manufacturer which has the third largest market share of RM production (4.8%) (IbisWorld, 2021). Aldi have experienced substantial growth since 2014 and, within the supermarket industry, hold the largest overall market share when compared to Lidl and other low-budget stores (Wareing, 2017, IbisWorld, 2021) and therefore should be represented in this research as a provider of RMs. Furthermore, meals provided directly to customers such as Wiltshire Farm Foods (WFF) and Oakhouse foods, deliver frozen RMs to client's homes. These meals are targeted at older and isolated adults as a main meal, and should be represented here due to the increased risk of nutrient deficiency to older adults (Apetito, 2019a).

With regards to the type of meal that will be tested, data analysis in Chapter 3 found that British and Mediterranean meals were the most popular cuisine types across the age groups. Although Mediterranean meals were more popular in those aged 19-64, there were no meal types that were available across the supermarkets in Hatfield, Hertfordshire; the location of this study, and via online providers. However, a traditional British meal, sausage and mashed potato RMs have been found to be popular in the UK by other researchers (Hopkins and Thomas, 2008, Read and Worsfold, 1998) and are available from a range of RM providers, and across ranges; luxury and budget-friendly, in the local area. Furthermore, communication with Apetito, the producers of Wiltshire Farm Foods meals, showed that sausage and mashed potato were the most popular meal (Apetito, 2016). Therefore, sausage and mash was identified from RM providers in Hatfield, Hertfordshire, UK from Tesco's (finest range, and standard brand), Asda, Aldi and WFF (an Apetito brand) and used for testing.

Due to the large amount of variation of nutrient content in the food composition tables (Deharveng et al., 1999, Leclercq et al., 2001, Burlingame, 2004), and due to the lack of ingredient quantities on the BOP (European Commission, 2012), chemical analysis will be used to quantify the actual vitamin content of the RMs. The five vitamins, thiamine, riboflavin, folate, vitamin B12 and vitamin C, are all process-labile essential vitamins that need to be investigated. Due to safety issues associated with the analysis of vitamin B12, this vitamin will not be analysed for this dissertation.

5.2. Aim

The aim of this study is to analyse the thiamine, riboflavin, folate and vitamin C content of five different 'sausage and mash' RMs from four different RM providers, compare the vitamin content to the recommendation that meals should meet 30% of the RNI and carry out price comparison between meals.

5.2.1. Objectives

- Determine the thiamine, riboflavin, folate and vitamin C content, in five sausage and mash RMs from four RM providers using lab-based assays
- To compare thiamine, riboflavin, folate and vitamin C content in sausage and mashed potato RM components (sausages, mashed potatoes and gravy) between different RM providers

- To determine whether thiamine, riboflavin, folate and vitamin C content on the specified RM, meet the 30% of UK RNI recommendations for 1.5-4 years, 5-10 years, 11-18 years, 19-64 years and 64+ years
- To carry out price and food portion composition comparison between the five RMs.

5.3. Methods

5.3.1. Sample preparation

Five 'sausage and mash' supermarket RMs from four different providers, RMs of two different batches from each provider were weighed (+/- 0.1g) and cooked in the microwave using the package instructions (table 9). The meals were then weighed after cooking, meal components weighed (±0.1g), homogenised using hand blender (Russell Hobbs, Model 14452, 200W), and quickly frozen in a freezer (-20^{o}C). The product trays and film were also weighed. The samples were kept in subdued light, with minimal exposure to air.

5.3.2. Reagents and standards

Analytical grade chemicals were used throughout the experiment. Sodium ascorbate and pig pancreatin were purchased from Sigma. Sodium dihydrogen phosphate dehydrate, HPLC grade acetonitrile, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, sodium hydroxide, hydrochloric acid, riboflavin standard and HPLC grade methanol were all purchased from Fisher.

5.3.3. Thiamine determination

Thiamine was analysed and quantified as explained in Chapter 4.3.4

5.3.4. Riboflavin determination

For determination of riboflavin concentration, extraction was carried out as described for thiamine in chapter 4. Samples were then filtered through a 0.22-micron syringe into 2ml amber HPLC vials.

HPLC conditions for riboflavin determination.

Standards (0, 0.02, 0.04, 0.08, 0.1µg/ml), blanks (0.1M HCl), and filtered samples were analysed for riboflavin (Figure 36). A high-performance liquid chromatograph (Shimadzu

Prominence-I LC 2030C) equipped with a Prominence fluorescence detector (RF-20A). Peak detection was carried out using a Shimadzu fitted with an 8µL flow cell. The chromatographic column was a 15cm x 4.6mm (Shimadzu) packed with Shimpack Gist C18 5µm. A blank/sample/standard injection volume was 10µL, followed by separation and analysed isocratically at 25°C in a mobile phase consisting of 70:30, water:methanol (v/v) at 2ml/min. Excitation and emission wavelengths were 450nm and 510nm, respectively. Chromatograms were analysed using LabSolutions software.

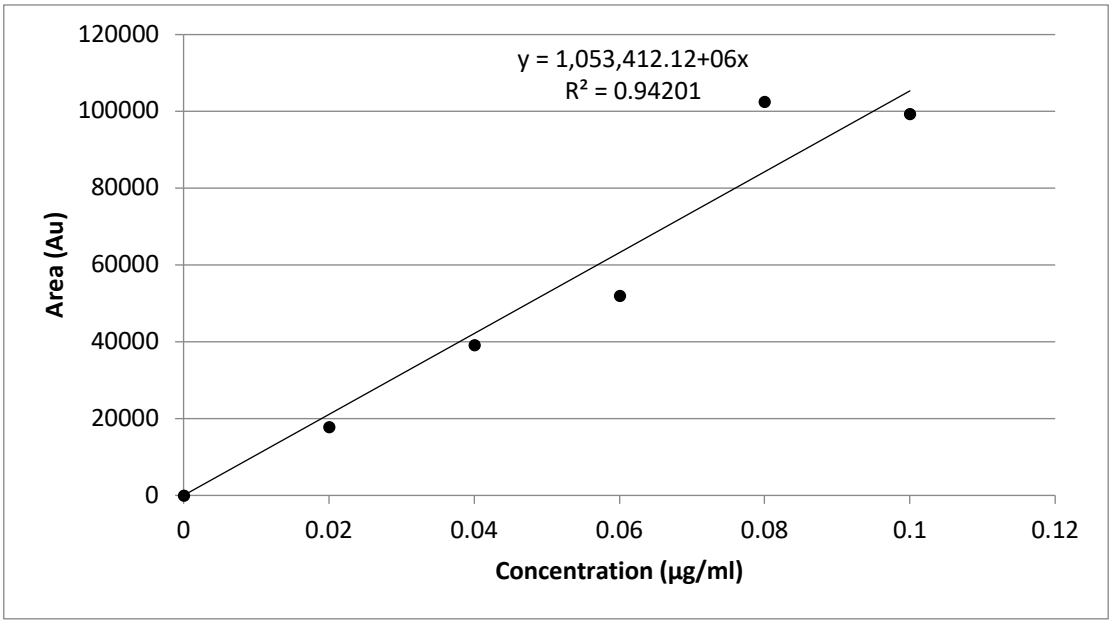


Figure 36: A calibration curve of concentration (µg/ml) to area of the curve on the chromatogram (Au) for riboflavin and measured by HPLC with fluorescence detection (excitation and emission 450nm and 510 nm, respectively); y=1,053,412.12 + 6x . r2=0.94

5.3.5. Folate determination

Folate concentration was determined using VitaFast microbiological assay with *Lactobacillus rhamnosus* coated 96-well plate. The kit also contained sterile water to solubilise standard (1.6µg/100g stock solution) and medium, and folate buffer solution. Phosphate buffer (0.5mol/L, 0.1% sodium ascorbate, pH7.2) was prepared daily and was used as extraction buffer.

Folate extraction

A sample extraction protocol was provided in the VitaFast vitamin assay kit. In summary, 1±0.1g of homogenised food samples were extracted in a sterile 50ml falcon tube with 20mg of pig pancreatin, approximately 30ml of phosphate buffer and shaken and filled up to exactly

40ml. The sample was then extracted in an orbital incubator (Thermo Scientific Orbital Shaker, Max Q 8000) at 37°C, 110rpm for 30 min., followed by heating at 95°C for 30 mins in a water bath (Grant, SBB6), shaking at least twice. After that, the centrifuge tubes chilled quickly to below 30°C in an ice bucket, followed by centrifugation at 8000 rpm for five min. (Thermo Scientific IEC CL31 Multispeed centrifuge). For analysis, the samples were diluted with sterile water in sterile 2ml Eppendorf tubes to ensure that the sample concentration matched the calibration curve.

Folate analysis

The protocol for analysis was followed as provided in the VitaFast kit. The test procedure was carried out as follows: 150µL of medium was pipetted into wells followed by 150µL of standard or sample, in triplicate. The wells were then covered with adhesive foil and incubated at 37±0.1°C in the dark for 44-48 hours (VWR Incu-line Cooled Premium). Once incubated, the adhesive was pressed down, shaken on the bench surface, foil removed, and any bubbles removed with a pipette tip. The turbidity of samples was measured read on a bench-top plate reader (Thermo Scientific, Multiscan FC) at 620nm. A calibration curve was used to calculate the concentrations between 0.16-1.28µg/100g(ml) (Figure 37).

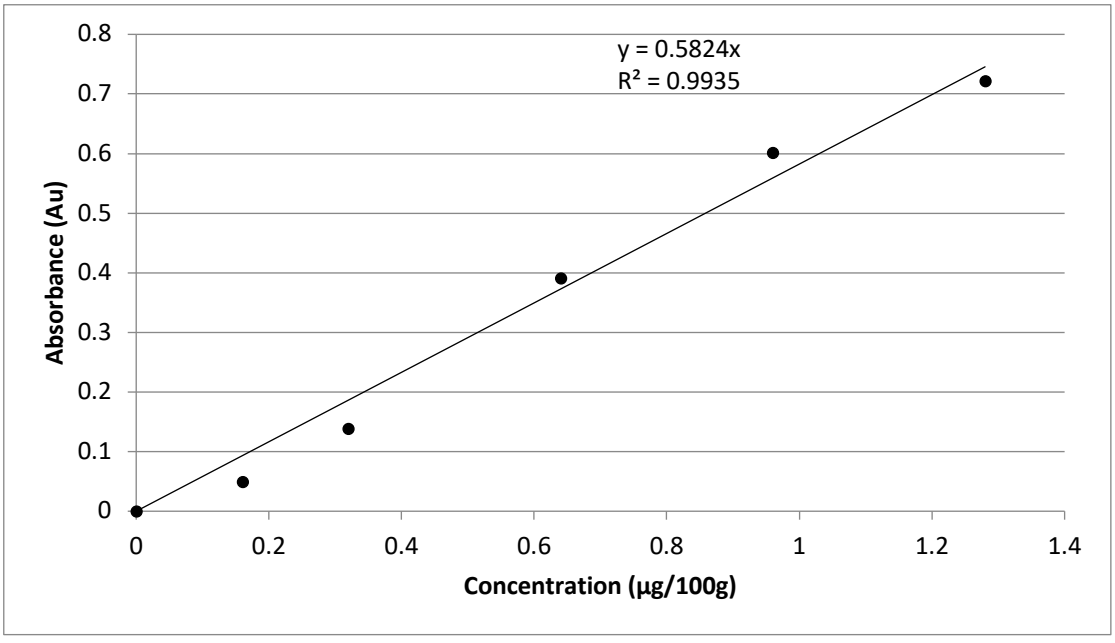


Figure 37: A calibration curve of concentration (µg/ml) to absorbance (Au) for folate read on a microtiter plate at 620nm; y=0.5824x. r2=0.98

5.3.6. Vitamin C analysis.

The samples were prepared as described in section 5.3.1. Samples were protected from light in foil covered zip-lock bags and frozen. The samples were then packaged in an insulted box at delivered to Campden BRI (Chipping Campden) Limited (registered no. 3836922) using same-day delivery to protect the samples from deterioration. and Vitamin C was analysed by Campden BRI using test reference TES-AC-745 (non UKAS) using an ISO 9001 certified assay between 10th June 2019 and 5th July 2019. Samples were corrected for recovery where required. Results from the analysis carried out by Campden BRI was then returned by email correspondence. Vitamin C content was provided in mg/100g of food sample.

5.3.7. Statistical analysis

Data were represented using summary statistics. Due to the small sample sizes and possible non-normality of the data, nonparametric methods were used. Providers were compared on RM content of thiamine, riboflavin, folate and vitamin C using the exact form of the Kruskal-Wallis analysis of variance. The sign test was used to compare the difference between the RM and RNI recommendations for thiamine, riboflavin, folate and vitamin C in 1.5-3 years, 4-10 years, 11-18 years, 19-64years and 64+ years. ANOVA was used to compare the differences between thiamine, riboflavin and folate between meal providers. The Kruskal Wallis test was used to compare the vitamin content in sausage, mashed potato and gravy from different providers. Associations between vitamin content, price, meat content and mashed potato content were examined using Spearman's correlation coefficient. The analyses were carried out using SPSS version 23.

5.4. Results

Descriptive statistics for the total thiamine, riboflavin, folate and vitamin C are shown in Table 8An overview of the sample used are provided in Table 9. Results from the exact form of the Kruskal-Wallis analysis of variance shows that there was a significant difference in the vitamin concentration between RMs for thiamine ($p<0.05$) (Figure 38), riboflavin ($p<0.05$) (Figure 39), folate ($p<0.05$) (Figure 40) and vitamin C ($p<0.01$) (Figure 41) (appendix xiv). The sign test showed that the vitamin content of vitamin C ($p<0.002$) (Figure 41) and folate ($p<0002$) (Figure 40), did not meet the recommendation of 30% of the RNI. Furthermore, riboflavin (Figure 40) did not meet the 30% of the RNI recommendation (Figure 41) ($p<0.002$), whereas thiamine met the 30% of the RNI recommendation ($p<0.002$) (Figure 38) (appendix xv). The

mean thiamine concentrations of each of the meals met 400-1958% of the recommendation, whereas riboflavin, folate and vitamin C met 10-52%, 8-60% and 1-7% of the recommendation, respectively.

Table 8: A table of descriptive statistics of the thiamine (mg/meal portion), folate (µg/meal portion), riboflavin content (mg/meal portion) and vitamin C (mg/meal portion) of sausage and mash ready meal from five different ready meal providers.

Vitamin	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
Thiamine (mg/meal)	10	2.22	0.86	1.14	3.73	1.50	1.97	3.01
Riboflavin (mg/meal)	10	0.06	0.02	.030	.10	0.04	0.06	0.09
Folate (µg/meal)	10	8.88	2.03	5.89	12.85	7.46	8.58	10.09
Vitamin C (mg/meal)	10	0.58	0.35	0.13	1.07	0.27	0.55	1.00

Table 9:A table of the sample information and preparation for the ready meals tested (Wiltshire Farm Foods, ASDA, ALDI, Tesco's Finest and Tesco's) including the component weights (g), date purchased (DD/MM/YY), cost (£) and microwave cooking method

Provider	Range	N	Weight on packaging (g)	Average weight before cooking (g)	Average weight after cooking (g)	Mean sausage weight (g)	Mean Mashed Potato Weight (g)	Mean Gravy Weight (g)	Mean Peas Weight (g)	Date Purchased	Cost (£)	Cooking method
Wiltshire Farm Foods	Own Brand	2	380	417	384	95	105	56	126	10.07.2018	3.15	Microwave 900W, 2.5 min., stand 1 min., 2 min.
ASDA	Own Brand	2	450	439.5	430.5	116	231	71	Not applicable	31.01.19	2.20	Microwave 900W, 5.5 min.
ALDI	Own Brand	2	450	446	426.5	101.5	227	90.5	Not applicable	04.02.19	1.89	Microwave 900W, 5 min., stand 1 min., 2.5 min.
Tesco's	Tesco's Value Range	2	450	449.5	439.5	90.5	225.5	111.5	Not applicable	04.02.19	2.50	Microwave 900W, 5 min.
	Tesco's Finest	2	500	494	457.5	158	183	103.5	Not applicable	04.02.19	3.50	Microwave 900W, 5 min.

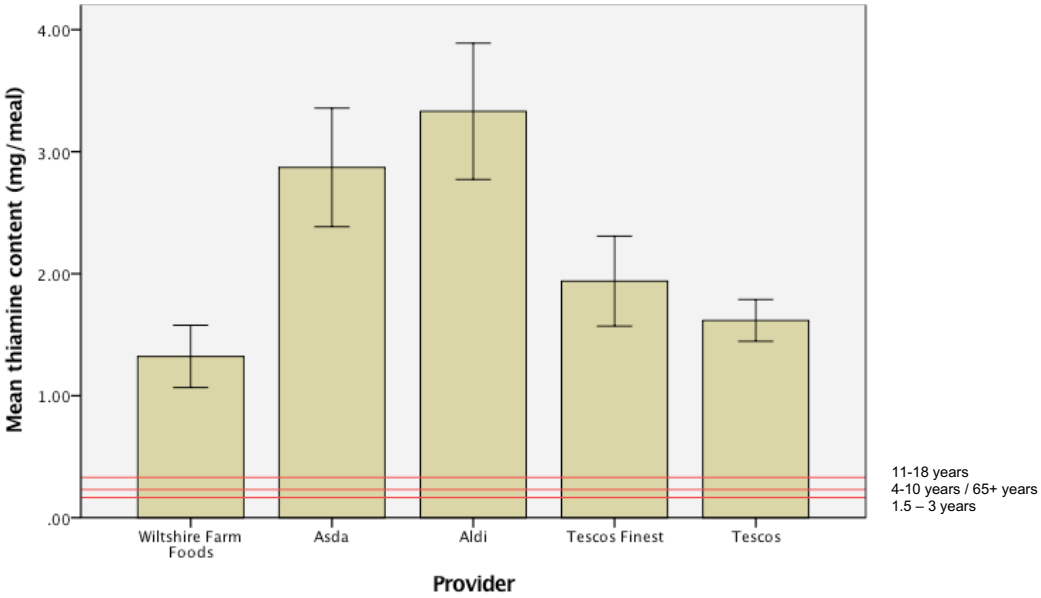


Figure 38: A bar chart to show the thiamine content (mg/meal) in five different providers of sausage and mash ready meals have met 30% RNI for different age groups. The red line indicates 30% of the reference nutrient intake for different age groups; 1.5-3 years, 4-10 years/65 years + and11-18 years old. Error bars represent 1 standard deviation of the mean. Chi-square (H=7.64, p<0.05). Sign test sig. (exact) p<0.001 (1 tailed), p< 0.002 (2 tailed)

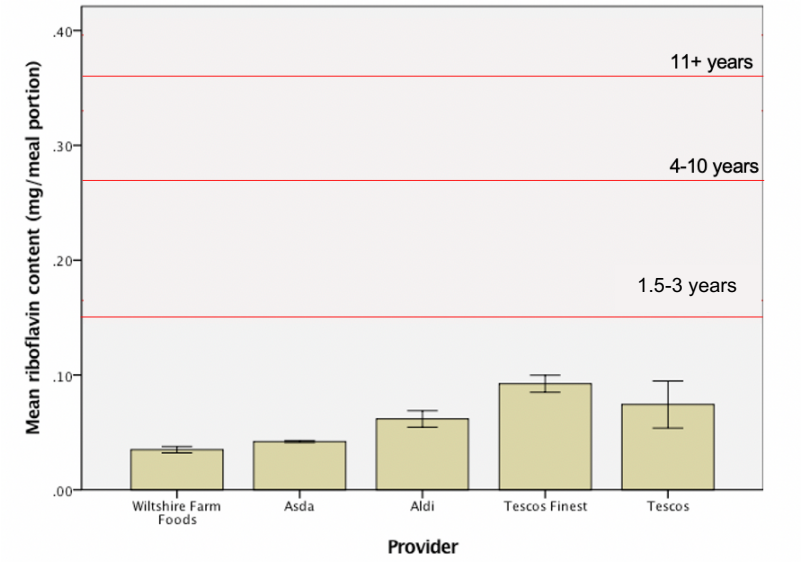


Figure 39: A bar chart to show the riboflavin content (mg/meal) in five different providers of sausage and mash ready meals has not met 30% the RNI for different age groups. The red line indicates 30% of the reference nutrient intake for different age groups; 1.5-3 years, 4-10 years, 11+ years old. Error bars represent 1 standard deviation of the mean. Chi-square (H=7.96, p<0.05). Sign test sig. (exact) p<0.001 (1 tailed), p< 0.002 (2 tailed)

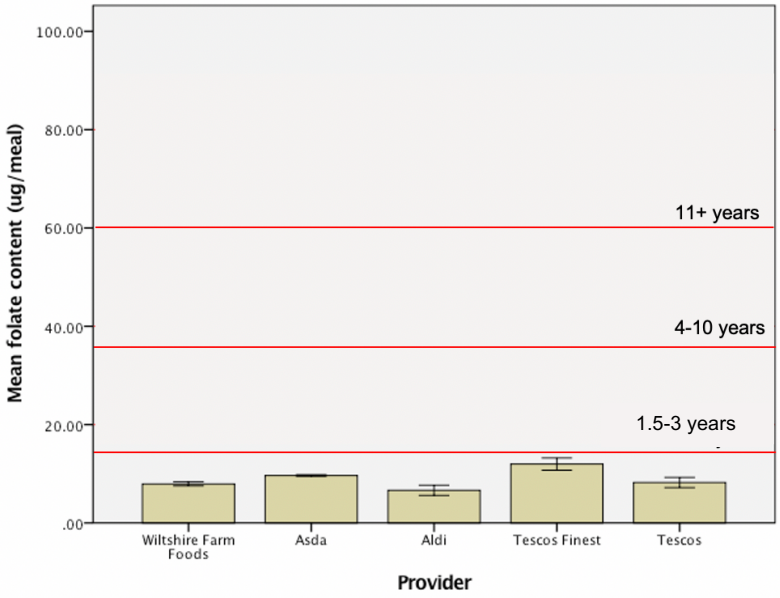


Figure 40: A bar chart to show the folate content (µg/meal) in five different providers of sausage and mash ready meals did not meet the RNI of 30% for different age groups. The red line indicates 30% of the reference nutrient intake for different age groups; 1.5-3 years, 4-10 years, 11+ years old. Error bars represent 1 standard deviation of the mean. Chi-square (H=8.29, p<0.05). Sign test sig. (exact) p<0.001 (1 tailed), p< 0.002 (2 tailed)

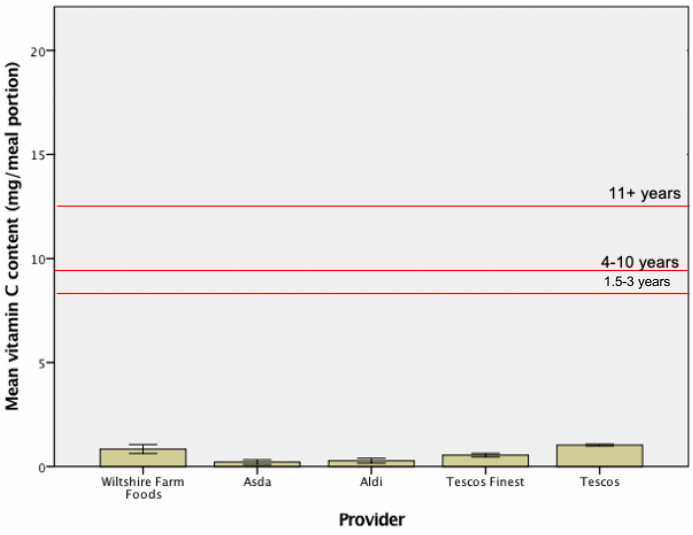
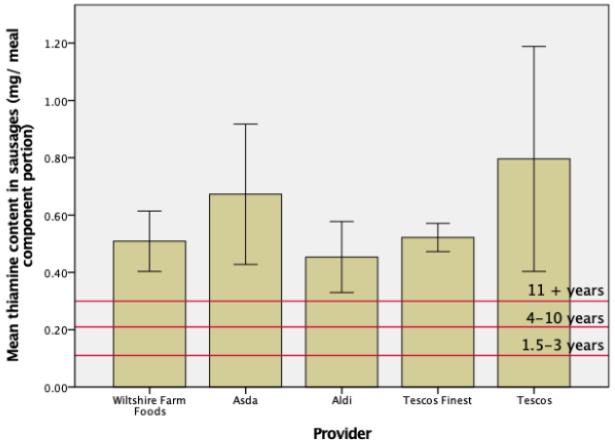


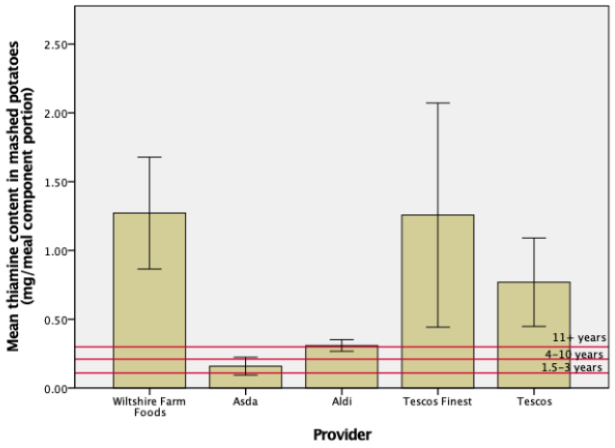
Figure 41: A bar chart to show the vitamin C content (mg/meal) in five different providers of sausage and mash ready meals did not meet the RNI for 30% for different age groups. The red line indicates 30% of the reference nutrient intake for different age groups; 1.5-3 years, 4-10 years, 11+ years old. Error bars represent 1 standard deviation of the mean. Chi-square (H=8.40, p<0.01). Sign test sig. (exact) p<0.001 (1 tailed), p< 0.002 (2 tailed).

Descriptive statistics for the meal components including the price, and meal composition are provided in Table 9. Results showed that the price ranged from £1.89-£2.50 per meal, and weighed between 417 – 494g, with the most expensive meal also being the heaviest; Tesco's Finest range. Only one meal, WFF, contained a vegetable portion of green peas. The ingredients used in the meals are provided in appendix xvi.

The descriptive statistics of the mean thiamine, riboflavin, folate and vitamin C content of RMs are provided in Table 8. Descriptive statistics for the concentration of thiamine, riboflavin, folate and vitamin C in RM components across the different sausage and mash RMs are shown in appendix xvii, appendix xviii, Appendix xix and Appendix xx, respectively. Results from Kruskal-Wallis testing revealed that there were significant differences in riboflavin (Figure 43) and folate (Figure 44) content of the sausage ($p<0.001$), mashed potato ($p<0.001$) and gravy ($p<0.001$) portions across the RM providers (appendix xxi).

Riboflavin content was between 0.00-0.05mg/portion, 0.01-0.04mg/portion and 0.01-0.07mg/portion for sausage, mashed potato and gravy, respectively. Folate content was between 0.83-2.66µg/portion, 1.38-9.97µg/portion and 0.00-1.43µg/portion for sausage, mashed potato and gravy respectively. Data analysis further showed that the thiamine content (Figure 42) of mashed potato and gravy were significantly different ($p<0.001$). Thiamine content of mashed potato ranged from 0.10-2.19mg/portion, and 0.11-2.36mg/portion for gravy. Vitamin C (Figure 45) content of sausage and mashed potato were significantly different ($p<0.05$) across RM providers (appendix xxi). Vitamin C content of sausages ranged from 0.00-0.71mg/portion and 0.00-0.38mg/portion.





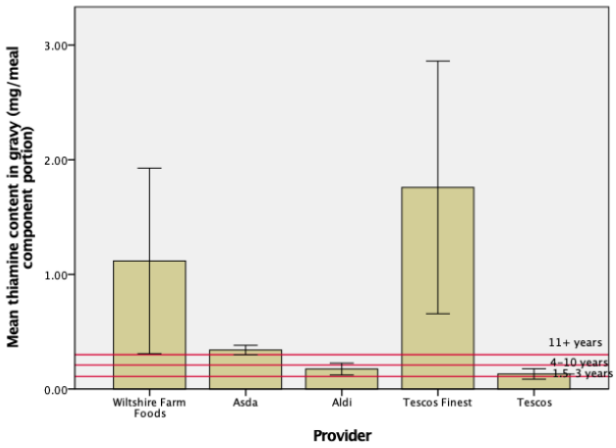
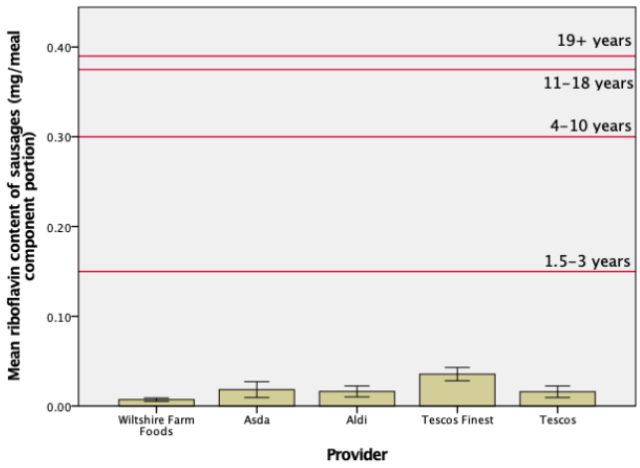
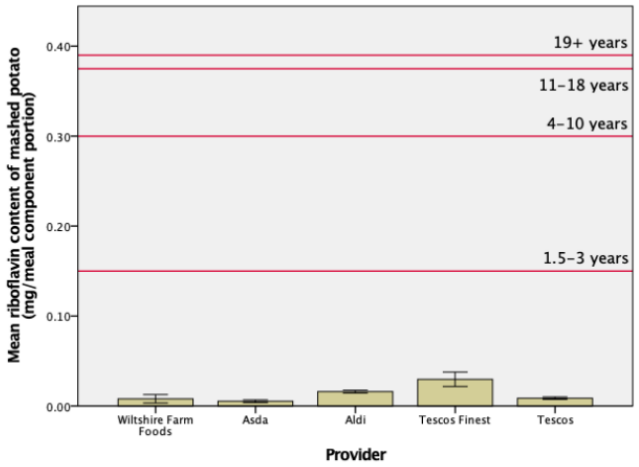


Figure 42: Bar charts to show the mean thiamine content of sausage, mashed potatoes and gravy (mg/meal component portion) in five difference 'sausage and mash' ready meals. Error bars depict 1 SD of the mean.





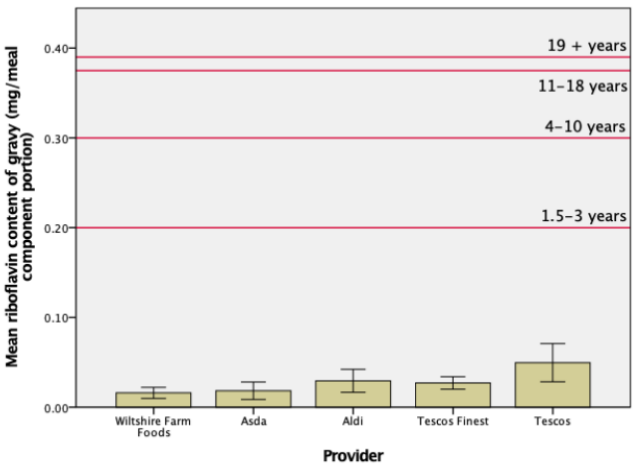


Figure 43: Bar charts to show the mean riboflavin content of sausage (top), mashed potatoes (middle) and gravy (bottom) (mg/meal component portion) in five difference 'sausage and mash' ready meals. Error bars depict 1 SD of the mean.

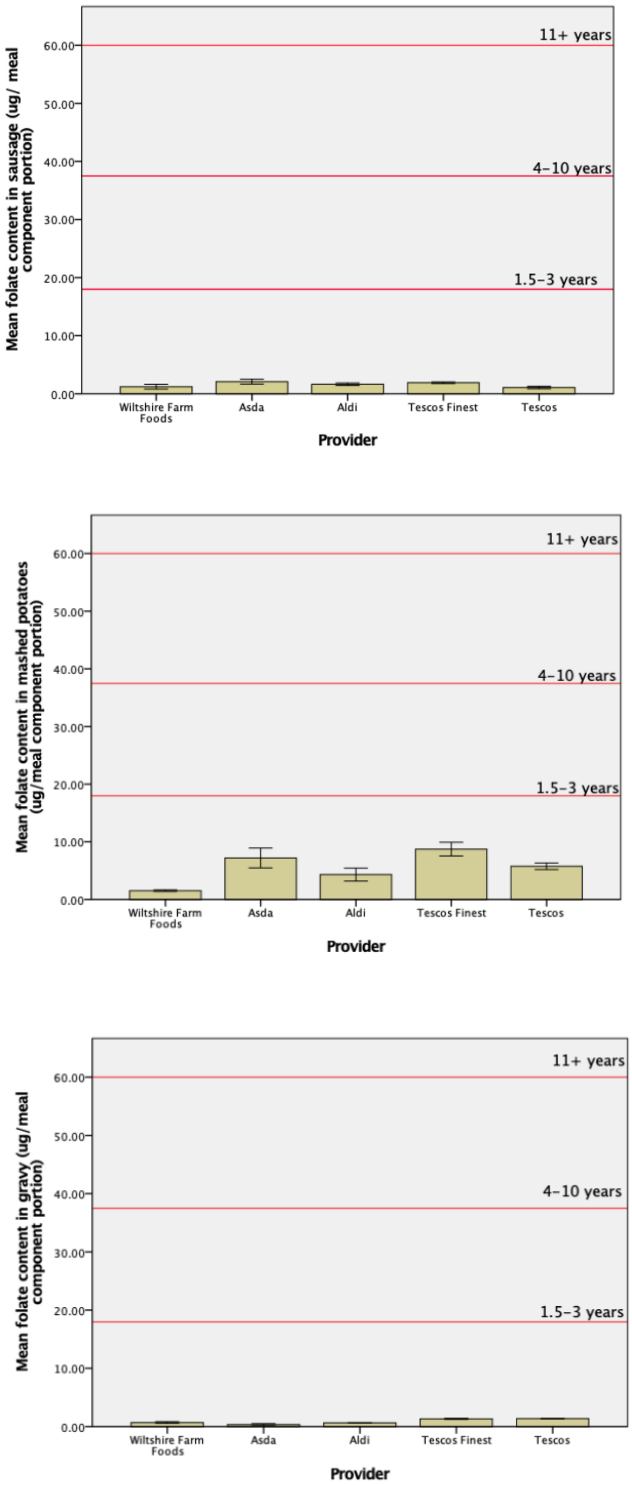


Figure 44: Bar charts to show the mean folate content of sausage (top), mashed potatoes (middle) and gravy (bottom) (µg/meal component portion) in five difference 'sausage and mash' ready meals. Error bars depict 1 SD of the mean.

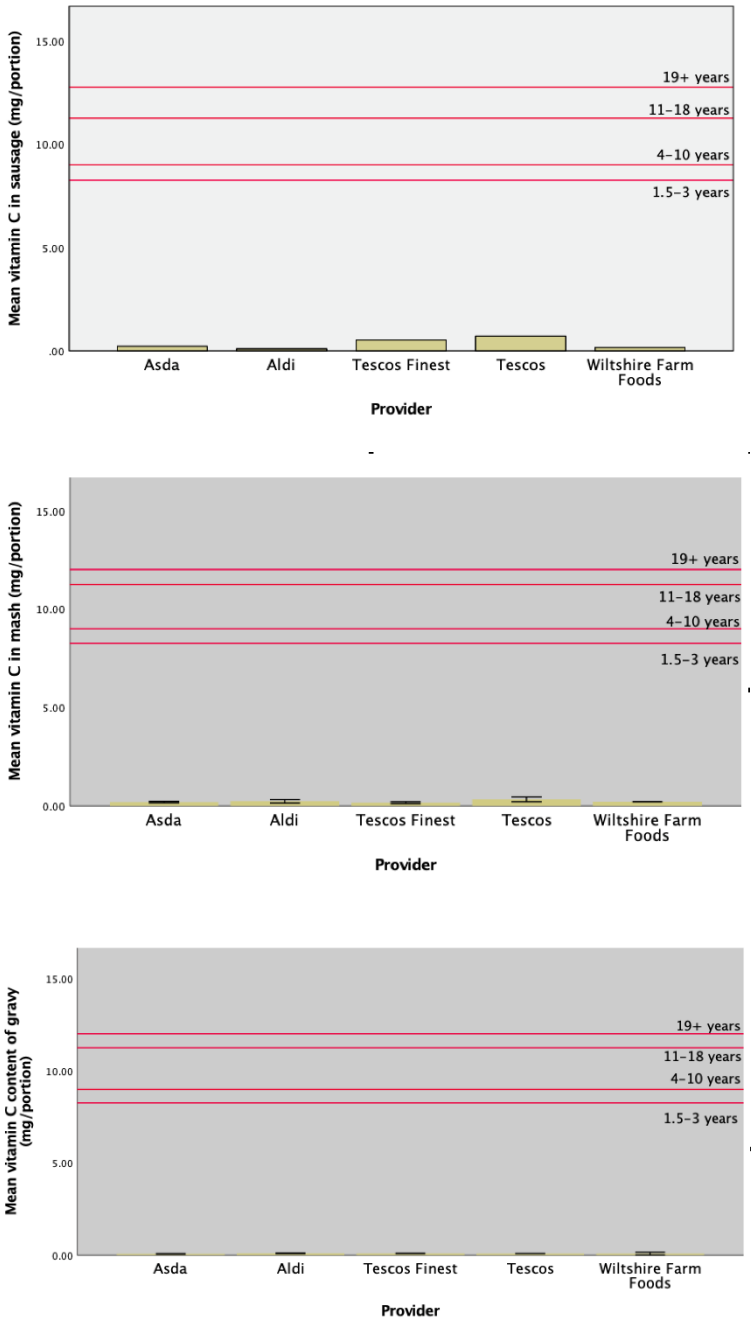


Figure 45: Bar charts to show the mean vitamin C content (mg/meal component portion) of sausage (top), mashed potatoes (middle) and gravy (bottom) (mg/meal component portion) in five difference 'sausage and mash' ready meals. Error bars depict 1 SD of the mean.

Descriptive statistics were carried out to determine the correlation between riboflavin (mg/meal portion), folate (µg/meal portion), thiamine (mg/meal portion), price (£), meat content in mean (%) and vitamin C (mg/meal portion) (appendix xxii). Pearson correlation analysis revealed that there was a strong positive correlation between folate content (µg/meal portion) and price ($p<0.05$), and between vitamin C (mg/meal portion)

and mashed potato in meal (%). There was a very strong positive correlation between price (£) and mashed potato content of the meals (%) ($p<0.01$). There was a strong negative correlation between thiamine (mg/meal portion), price (£) ($p<0.05$) and mashed potato content of the meal (%) ($p<0.01$). In addition, there was a negative correlation also noted in meat in the meal (%) and mashed potato in the meals (%) tested, and meat in meal (%) and vitamin C content of the meal (mg/meal portion). There was a very strong negative correlation between price (£) and meat content of the meal (%), and thiamine content of meal (mg/meal portion) and vitamin C content in meal portion (mg/meal portion) (appendix xxiii).

5.4.1. Validation of Vitafast for Folate determination

Samples were analysed in triplicate on the microtitre plate and the mean intra-assay coefficient of variation was 1.96%, with mean inter-assay coefficient of variation being 8.93%. The recovery rate was 105%.

5.4.2. Validation of HPLC for Riboflavin determination

LOD of several different samples were detected using HPLC to demonstrate the applicability of this analytical method for the detection of riboflavin. The LOD of is the lowest amount of analyte that can be detected in a sample, but it may not be possible to be quantified. The signal to noise ratio was also quantified. It is an established norm that a signal to noise ratio between 2-3:1 is considered acceptable for estimation of the LOD (Figure 46).

Based on signal-to-noise (S/N) approach, determination of signal-to-noise ratio was performed by comparing measured signal from riboflavin with 0.005ng/ml and 0.01ng/ml concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected. LOD of riboflavin was detected by fluorescence at 4.16 min for the concentration of 0.02 and 0.01 (Table 10). This showed a 2.55 S/N ratio compared to blank samples. The LOD was carried out on different samples.

Samples were analysed in triplicate and the mean intra-assay coefficient of variation was 11.38%, with mean inter-assay coefficient of variation being 18.26%. The recovery rate was 88%.

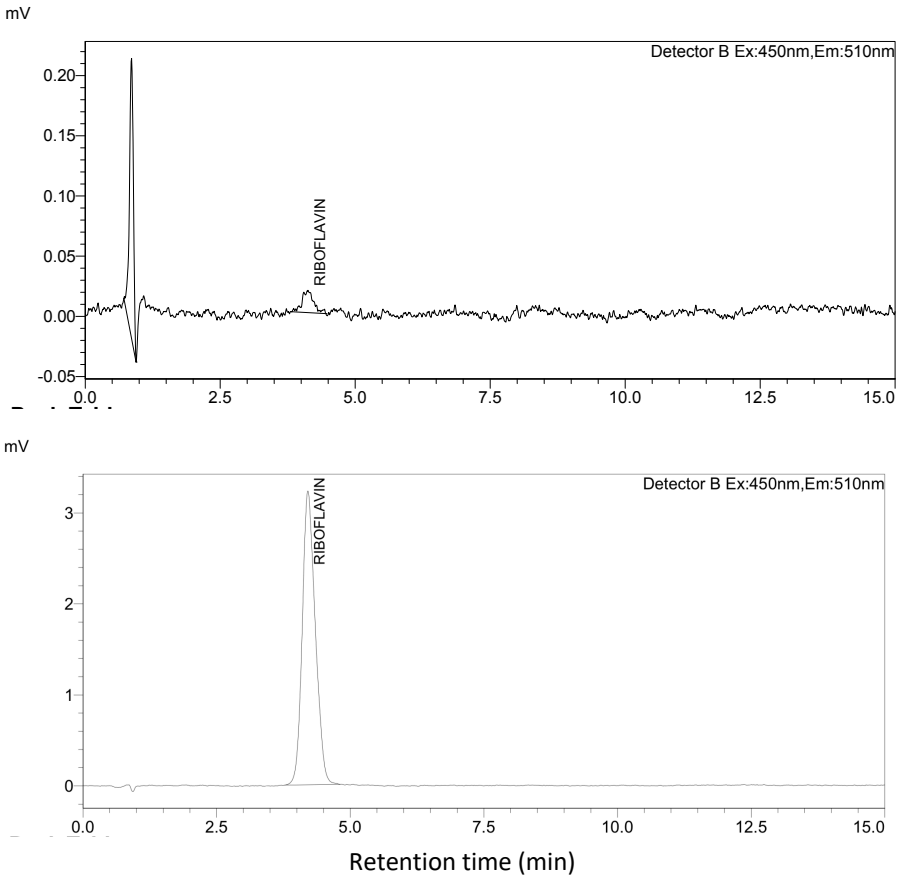


Figure 46: Chromatograms of limit of detection (LOD) (top figure) and standard for riboflavin (bottom figure). The x-axis is the retention time and the y is mV.

Table 10: Summary of validation data for the quantification of riboflavin using Shimpack GIST, 5µm, C18, 4.6 x 150mm column, 70:30 (v/v) methanol: water, excitation and emission wavelengths were 450 and 510.

	tR (min)	LOD (ng/ml)	LOQ (µg/ml)
Riboflavin	4.2	0.1	0.01

5.5. Discussion

The NDNS analysis data in Chapter 3 showed that up to 10% of the population aged over 1.5 years are consuming RMs (Table 6), and for some individuals (0.08-2.9% of the population that consumed RMs) these meals RMs contribute a large proportion (>50%) of dietary intake. Therefore, it is important to understand the nutritional contribution of

these meals to nutrient intake, especially as macronutrient content has been shown to vary considerably between providers and ranges (Celnik et al., 2012). However, there has been no research carried out on the content of process-labile vitamins such as thiamine, riboflavin, folate and vitamin C, across different RM providers.

5.5.1. Vitamin content of sausage and mashed potato ready meals between ranges.

This study has shown that the thiamine, riboflavin, folate and vitamin C content of sausage and mashed potato RMs vary significantly between providers and ranges. The recommendation used in this research states that meals should contain 30% of the RNI for micronutrients. The recommendation was met for thiamine, but not for riboflavin, folate and vitamin C, where 8.97-60%, 11-66% and 1.75-5.72% of the RNI was provided, respectively. However, this study found that the recommendation for riboflavin was not met in these meals and although this analysis was only carried out on one RM type, this is a popular meal for consumers, and shows that they may not be providing adequate nutrients to constitute a balanced meal (Hopkins and Thomas, 2008, Read and Worsfold, 1998).

5.5.2. Comparison of vitamin content between different providers

Sausage and mashed potato meals across the providers were significantly different for all vitamins tested ($p<0.05$). Although this is the first study to investigate the differences between WSV content of RMs purchased from different providers, Cleanthous et al. (2011) found that there was a significant difference between the fat and saturated fat content between RMs; private label products (own-brand meals) were 70% higher in total fat (g) and 85% higher in saturated fat (g) in comparison to branded meals. However, the study did not specify the meals analysed, and nutrient labels were used to calculate the concentrations, not chemical analysis (Cleanthous et al., 2011). This current study found that Tesco's finest meals had the highest vitamin content per portion for all vitamins, however, this is likely due to the larger portion size of the meal.

A study by Cowper (2015), which investigated the difference in nutrient quality of individual foods across different providers, found that there were significant differences in energy, fat, fibre and protein between Wholefoods (a higher priced food store) and

Safeway (a budget food store) vegetables. However, fat content in lamb from different supermarkets did not differ significantly (Angood et al., 2008). Therefore, these differences could be the result of various factors including disparities between manufacturing processes, seasonality and ingredients (Reddy and Love, 1999), rather than being dependent on the place where the meal is purchased.

This study found that there were differences in nutrient content between the providers, that could be due to a variety of reasons not tested as part of this study; including freshness and production methods, which warrant further investigation.

5.5.3. Comparison of vitamin content of RMs to dietary recommendations

Although thiamine recommendations were met in the sausage and mashed potato RMs, the riboflavin, folate and vitamin C content of the meals did not meet the nutrient recommendation. While there has been little research carried out using the chemical analysis of vitamins, analysis of the folate content in Swedish frozen vegetarian RMs by Johansson et al. (2008) and Spanish chilled vegetarian RMs by Fajardo et al. (2017) provided between 6-27% and 2-60% of the recommended daily intake of 300-400µg. These meals focused on vegetable based RMs that were expected to be good sources of folate; however, when both studies are combined, 14 meals out of the 45 tested met the 30% of the RNI recommendation. It should be noted that the meals in the current study were not the same tested as in other literature, and recipes vary.

The findings for the vitamin C content of meals was in agreement with studies identified in the literature review (De Ritter et al., 1974, Agte et al., 2002, O'Leary et al., 2000, Redmond et al., 2002, Redmond et al., 2004, Salunkhe et al., 1979, Patterson and Stewart, 2003, Faulks, 1991, Gatherer, 1971, Bognar, 1980), and findings from Chapter 3, where vitamin C content of meals were lower than the recommendation. The highest vitamin C content in the meal analysis in this study was the Tesco's finest meal, which also had the highest weight of mashed potato; a source of vitamin C (Redmond et al., 2004). Furthermore, the WFF meal had a vitamin C content that was similar to the Tesco's finest product, most likely due to the inclusion of frozen green peas in the meal; a better source of vitamin C compared to fresh peas (Hammink, 1978, Nursal and Yücecan, 2000).

These findings highlight the importance of vegetable portions in RMs, and supports the need for initiatives such as the 'veg pledge' to improve the vegetable content of these food products (The Food Foundation, 2019). Other opportunities to improve the vitamin C content of RM components such as mashed potato have been investigated by Redmond et al. (2004), which includes the use of encapsulated vitamin C. However, changes to the characteristics of food should be minimised (Redmond et al., 2004, Nizori et al., 2020).

An interesting finding from this analysis, which was contrary to previous investigations, was that the 30% RNI cut-off for riboflavin was not met. The NDNS analysis in Chapter 3 and findings from the literature review (Agte et al., 2002, De Ritter et al., 1974, Salunkhe et al., 1979) found an adequate riboflavin content in RMs and that riboflavin is, other than when exposed to ultraviolet light, mostly retained after processing (Choe et al., 2005). A potential cause for this outcome is due to the composition of the dish rather than because of processing where the analysed meal did not contain any of the main sources of riboflavin, which include milk, eggs and fortified breakfast cereals (Finglas et al., 2015)(Table 1).

The riboflavin content of the sausage and mashed potato meals consumed in the NDNS survey was consistent with the findings of this study, where NDNS sausage and mashed potato meals contained approximately 0.2mg/portion. These findings highlight the importance of variety in the diet, where a balanced diet should include a variety of foods to meet dietary requirements (Foote et al., 2004). This study has investigated one meal as a representation of RM nutrient content; however, the diet should contain a variety of foods to meet nutrient requirements (Kennedy, 2004), and this study did not investigate the total diet.

It should be recognised that this study assumes that 100% of the meal is eaten and therefore is reflective of intake. However, Fogler-Levitt et al. (1995) found that due to reduced appetite of some older adults and the effect of eating alone, the total meal may not be eaten, with only 75% - 81% of the meal being consumed on average (Owen et al., 1992). This leads to a lower nutrient intake, and therefore may increase further the risk of nutrient deficiency in those individuals consistently consuming RMs.

Several interventions have been shown to improve the nutritional status of consumers. These include the provision of 100% of the dietary recommendation in a meal service for older adults (Kretser et al., 2003) and dietary supplementation (Lipschitz et al., 1985). Others suggest the implementation of legislation that sets out minimum guidelines for meals provided to vulnerable adults (O'Dwyer et al., 2009) and the fortification of sauces within the meals to improve nutrient profile of the meals (Best and Appleton, 2011, Höglund et al., 2018, Redmond et al., 2003). Lastly, food processing methods could be improved to protect the micronutrient quality of the meals during reheating or hot-holding (Gatherer, 1971).

5.5.4. Price and meal composition comparison between meal providers

The meals tested varied by price (£1.89-£3.50) and vitamin content. This analysis provided valuable insight into how brands, price and formulation of RMs can impact the nutritional quality of the meal and could provide consumers with more information about nutrient quality for the RMs on offer. Currently, there is no mandatory micronutrient information, other than salt, provided on BOP (European Commission, 2012), however, consumers do have price information and the overall composition of the meal (percentage of mashed potato and meat content), which could be utilised when choosing the most nutritionally adequate meals.

As expected, where there was a higher percentage of meat (pork sausages), there was a lower percentage of mashed potato. Furthermore, a lower percentage of meat was indicative of a higher vitamin C content, most likely due to the higher mashed potato content, which has been shown to be a source of the vitamin (Redmond et al., 2003, Love and Pavek, 2008, Williams, 1996, Pelletier et al., 1977). This was confirmed through analysis, as the higher the mashed potato content, the higher the overall vitamin C content of the meal. A lower mashed potato proportion in the cheapest RMs (ALDI and ASDA) was also correlated with a higher thiamine content, as there was a higher pork content (a source of thiamine) in the cheaper meals compared to the other three providers (Tesco's and WFF).

Price was also an indication of meal composition, where the higher the meat content, the lower the price. This would seem counter-intuitive, as meat is perceived as being a more

expensive ingredient in the UK (Richardson, 1994, Fousekis and Revell, 2000). However, it has been shown that for ultra-processed food such as sausages (Monteiro, 2009), the price will be lower, with vegetables being more expensive in terms of price of food per calorie (Monsivais and Drewnowski, 2007, Moubarac et al., 2013).

Price was positively correlated with folate content, where the most expensive meals (Tesco's Finest and WFF) had the highest folate content. This was due to the presence of peas in the WFF meal, and the addition of ale (containing yeast, a source of folate) to the gravy which led to a higher folate content compared to the other brands. However, those meals with a higher thiamine content, most likely due to the higher proportion of sausages, had lower vitamin C content, a consequence of lower proportions of mashed potato within the meal. Subsequently, meals with the highest vitamin C content (WFF and Tesco's) had a lower thiamine content, a result of the higher mashed potato content of these meals and presence of peas in the WFF meal.

In previous research, more expensive meals have shown to have a higher total fat, saturated fat, and salt content than cheaper or value meals. However, this study has shown that higher priced meals also correlate with high vitamin C and folate content. Although the vitamin C and folate content of sausage and mashed potato RMs are lower than the recommendation, there is reason to investigate how the composition of meals can have an impact on the overall nutritional quality of the RM, and how this affects the price of the meals. One way to improve the vitamin content of meals is through the addition of antioxidant-rich foods, which can protect vitamins such as folate from degradation during heating (Johansson et al., 2008).

When deciding if the meals are 'healthy' or not, care needs to be taken to understand the population that is being catered for. Considering the obesity epidemic in the UK (NHS Digital, 2019), meals with a higher calorie content would be considered unfavourable to eat for younger populations. However, for older populations that are at a higher risk of energy, protein and micronutrient malnutrition (BAPEN, 2016), these meals may be a preferred meal option. It should be noted, however, that salt reduction in these meals is important for everyone in the population in order to reduce the risk of hypertension (Schorling et al., 2017).

5.5.5. Limitations

Although this study provided insight into the process-labile vitamins within RMs in the UK, one limitation is the low sample size of the meals; it would be beneficial to look at a larger sample to account for batch-to-batch variations, and differences between the same ranges throughout various stores. Furthermore, the analysis was only carried out on one type of RM, sausage and mash, and the composition of other meals would vary and consequently have a different vitamin profile. This confirms the need for there to be more accurate data on the vitamin content of these RMs that are sold by different providers. This is especially important for meals that are being regularly consumed by individuals who are at higher risk of vitamin deficiency, such as older adults, adolescents and during pregnancy and lactation. Lastly, further research should be carried out into the vitamin B12 content in RMs, especially as the absorption capacity of the vitamin can decrease in older adults, as described in Chapter 1.

5.5.6. Conclusion

Although the thiamine content of the meals met the 30% of the RNI nutrient recommendation, riboflavin, folate and vitamin C levels were significantly lower than the recommended vitamin content meeting between 1-60% of 30% of the RNI recommendation. As RMs could be the main meal of the day, they should provide an adequate amount of daily essential nutrients. Therefore, it is important to have accurate estimations of these nutrients to ensure that the diet can be supplemented and that nutrient requirements are met. This study further found that there is a large amount of variation in the vitamin content of RMs between providers and ranges, but consumers currently cannot make an informed decision about which meal may be the most nutritionally adequate due to lack of labelling. This uncertainty is increased when the price or composition could further impact the nutrient quality of the meals. Further research should focus on how to improve the process-labile vitamin content, especially for riboflavin, folate and vitamin C, to ensure that they meet the 30% of the RNI recommendation.

6. THE EFFECT OF 'HOT-HOLDING' ON THIAMINE, RIBOFLAVIN, FOLATE AND VITAMIN C IN COOKED SAUSAGE AND MASH READY MEALS.

6.1. Introduction

Results from analysis of the National Diet and Nutrition Survey (NDNS) survey in Chapter 3 and ready meal (RM) analysis in Chapter 5 have found that RMs consumed in the population are not meeting the 30% of the reference nutrient intake (RNI) for vitamin C and folate. Furthermore, the composition of meals and price can contribute to the variability of the nutrient content of RMs. Given these findings it is important to undertake chemical analysis of meals in order to understand if the micronutrient quality may be further impacted by the procedures utilised during meal provision within the community, such as delivery of hot meals or keeping meals hot to be consumed later; a process known as hot-holding.

6.1.1. Hot holding of ready meals

During the early stages of this research, a visit to a meals-on-wheels (MOW) provider was organised. This visit revealed that meals were delivered between 11am and 2pm (Hertfordshire County Council, 2019), which meant that the food could be kept hot for up to three hours after initial reheating. Other meal providers also deliver meals in a similar time frame (Apetito, 2019a, Sodexo, 2019).

When MOW provision was initially introduced, meals were cooked fresh in kitchens and then delivered out to customers in insulated containers. In recent times, however, MOW meals are mostly regenerated in an oven or microwave until cooked, and then continuously heated at 90^{o}C in a heated bag until delivery (Winterton et al., 2013, Sustain, 2018). This process is known as 'hot-holding', and describes the practice of cooking food until ready for consumption, followed by keeping it hot until the meal is served/delivered (Williams, 1996). Although many MOW services use this method, they aim to deliver the meals as soon after initial cooking as possible (Gatherer, 1971), but delivery times can vary.

Hot-holding is commonly used in hospital catering (Creed, 2010); where in 2019 42% of hospitals had delivered meals (Sellen et al., 2018) (an increase from 33% in 2005)

(Rimmington and Carlton-Smith, 2005) and in schools (Nelson et al., 2010); where 40% of schools surveyed (Sellen et al., 2018) purchased pre-prepared RMs, delivered to the premises and kept hot before service.

Previous research in the field of food processing has found that hot-holding meals can reduce vitamin C content, by as much as 80%, and thiamine by 19% in vegetables and potatoes, if kept at temperatures greater than 85ºC for up to 4 hours. The cause of this degradation is due to the ability of heat to decrease vitamin C and thiamine concentrations over time (Williams, 1996). A study by Ang et al. (1975) found further evidence that heating meals including frankfurters, beans and pot-roast at 93-99ºC for 0-3 hours caused a significant thiamine reduction of between 18-19%, which was significant. Riboflavin, however, was stable during these experiments, with approximately 90% retention throughout. Nevertheless, it should be noted that these meals were made fresh and then hot-held for the duration of the experiment, whereas many RMs are cooked, frozen or chilled, reheated and then hot-held before delivery (Winterton et al., 2013).

In a more recent study by Johansson et al. (2008), it was found that vegetable RMs which had been heated on a stove or in an oven/microwave had a total folate retention of between 50-93%. Furthermore, those with the highest vegetable content retained the highest amount of nutrients, probably due to protective properties of antioxidants within them. Although this shows that the meal composition can impact the nutrient content, these were ‘one-pot’ meals, like a soup or stew. In many RMs the vegetable and meat portions are kept separate (figure 47) and therefore the protective antioxidant capacity of the vegetables may not be utilised.

Due to food safety requirements outlined in The Food Safety and Hygiene (England) Regulations 2013 (2013), food that is stored by hot-holding needs to be above 63ºC to ensure that there is a restriction of harmful microbial growth or toxin formation. In this case, food is kept above 63ºC in a box heated at 90ºC. However, as discussed, this may mean that valuable nutrients are being lost during the transport of these foods (Albrecht et al., 2009).

Figure 47: A picture of a sausage and mash RM.

The nature of the processing and potential degradation of heat-labile vitamins mean that it is important to understand how the transport and service of such meals may impact the micronutrient content when they are delivered, or are hot held in institutions such as hospitals or schools (Hunt, 1982). This is especially vital when meals are being delivered to individuals who are at a higher risk of nutritional deficiency (Krassie et al., 2000), such as young children, adolescents and older adults.

Sausage and mash RMs were found to be the most popular meal by other researchers (Hopkins and Thomas, 2008, Read and Worsfold, 1998), and was chosen for analysis in Chapter 5. For the purpose of this study, one meal brand will be chosen to investigate the impact of hot holding. Due to the WFF meal also containing a vegetable portion, a good source of vitamin C, it would be important to assess the effect of hot holding on vegetable portions over time. Therefore, this meal will be used for testing the process-labile vitamin retention. It should be noted, that the meal tested here has the same sausage and mashed potato component, but varying vegetable portions compared to the meals delivered as part of the MOW service; this study is looking at the effects of hot-holding on meal components overall, rather than specifically for this MOW service.

6.2. Aim

The aim of the experiment was to investigate the effect of 'hot-holding' (0-3 hours) at 90ºC on thiamine, riboflavin, folate and vitamin C content in sausage and mash RMs.

6.2.1. Objectives:

- Analyse the thiamine, riboflavin, folate and vitamin C content of sausage and mashed potato components and in the RMs after reheating and hot-holding RM for 0-3 hours at 90°C
- Measurement of weight changes of meals after reheating and hot-holding for 0-3 hours at 90°C for sausage and mashed potato RMs.
- Measurement of vitamin retention (% of reheated vitamin content) of meal components after reheating and hot-holding at 90°C for 0-3 hours.

6.3. Method

6.3.1. Sample preparation

Sausage and mash meals were purchased frozen from an online RM provider; Wiltshire Farm Foods (WFF). Whole packages of two different commercial batches were weighed and cooked in the microwave using the package instructions (900W/category E for seven minutes, left to stand for two minutes) and then kept in an oven at 90±1°C for 0h, 1h, 2h and 3h, shown in appendix xxiv. The meals were then weighed (±0.1g) after cooking, meal components weighed, homogenised using a hand blender (Russell Hobbs, Model 14452, 200W), and frozen at -20°C. The trays and film were also weighed. The samples were kept in subdued light, with minimal exposure to air before being analysed.

6.3.2. Reagents and standards

Analytical grade chemicals were used throughout the experiment. Sodium ascorbate and pig pancreatin were purchased from Sigma. Sodium dihydrogen phosphate dehydrate, HPLC grade acetonitrile, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, sodium hydroxide, hydrochloric acid, riboflavin standard and HPLC grade methanol were purchased from Fisher.

6.3.3. Thiamine Determination

Thiamine was analysed and quantified as explained in chapter 4.3

6.3.4. Riboflavin determination

Riboflavin was analysed and quantified as explained in chapter 5.3.4

6.3.5. Folate determination

Folate was analysed and quantified as explained in chapter 5.3.5

6.3.6. Vitamin C determination

Vitamin C was analysed and quantified as explained in chapter 5.3.6

6.3.7. Statistical analysis

Data were represented using summary statistics. A Kruskall-Wallis test with exact sampling distribution testing was used due to the small sample size to test the difference between the mean vitamin content (thiamine, riboflavin, and folate) of meal components and total vitamin content of meals over time (t=0, t=1, t=2, t=3). The analysis was carried out using SPSS version 23.

6.4. Results

Images of the sausage and mash RMs at each time point are shown in Figure 48. There was a 3.5-6% decrease in the weight of the RM after reheating. Reheating caused an average decrease of 1.8%, 4.7% and 10.3% in weight at t=1, t=2 and t=3, respectively (appendix xxiv). Results from the time lapse analysis showed that there was a significant increase from 4.49mg/portion to 10.11mg/portion in riboflavin content of peas over time ($p<0.05$), however, there was no significant difference in vitamin content over time for any other vitamins within the RM components tested (figure 49- 52; appendix xxv)

6.4.1. Effect of hot holding at 90°C on vitamin in sausage and mash ready meals

There were no significant differences between the time points for the total vitamin content of the meals for thiamine (p=0.881), riboflavin (p=0.418), folate (p=0.244) or vitamin C (p=0.212) (Figure 49-52). Data regarding the retention of vitamins over time are quantified are provided in Appendix xxvi and appendix xxvii

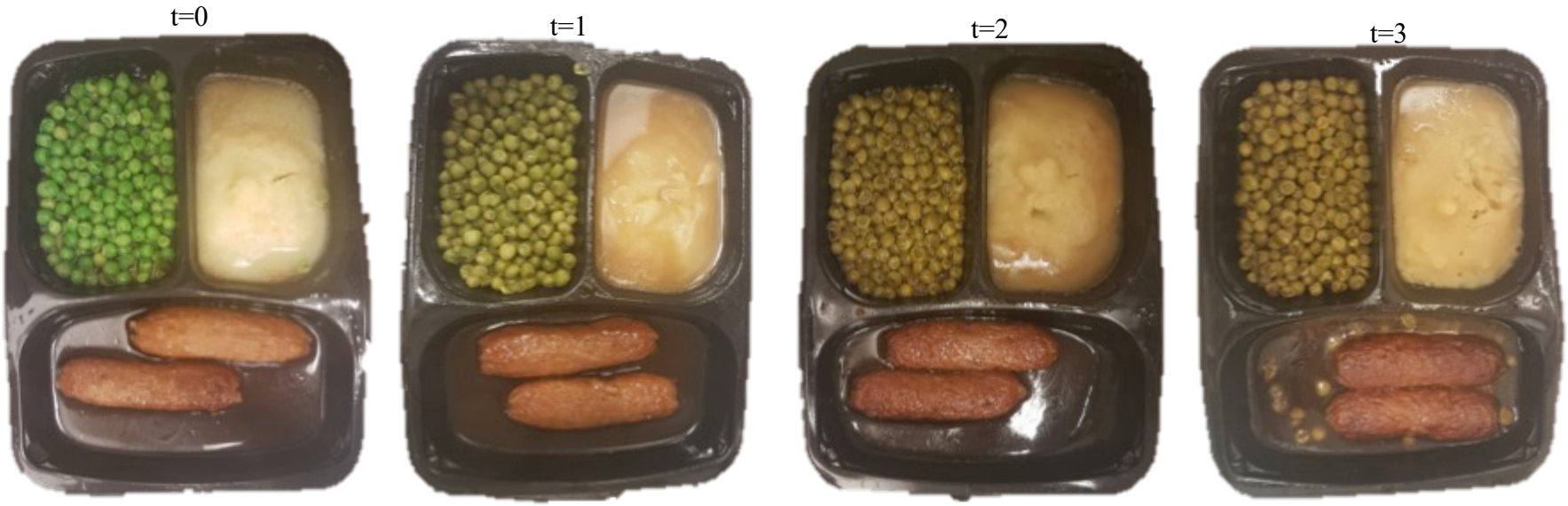


Figure 48: Pictures of the Wiltshire Farm Food meals at t=0; microwave cooked for 7 minutes (900W) only, t=1; microwave cooked for 7 minutes (900W) then held at 90±1°C for 1 hour, t=2; microwave cooked for 7 minutes (900W) then held at 90±1°C for 2 hours, t=3; microwave cooked for 7 minutes (900W) then held at 90±1°C for 3 hours.

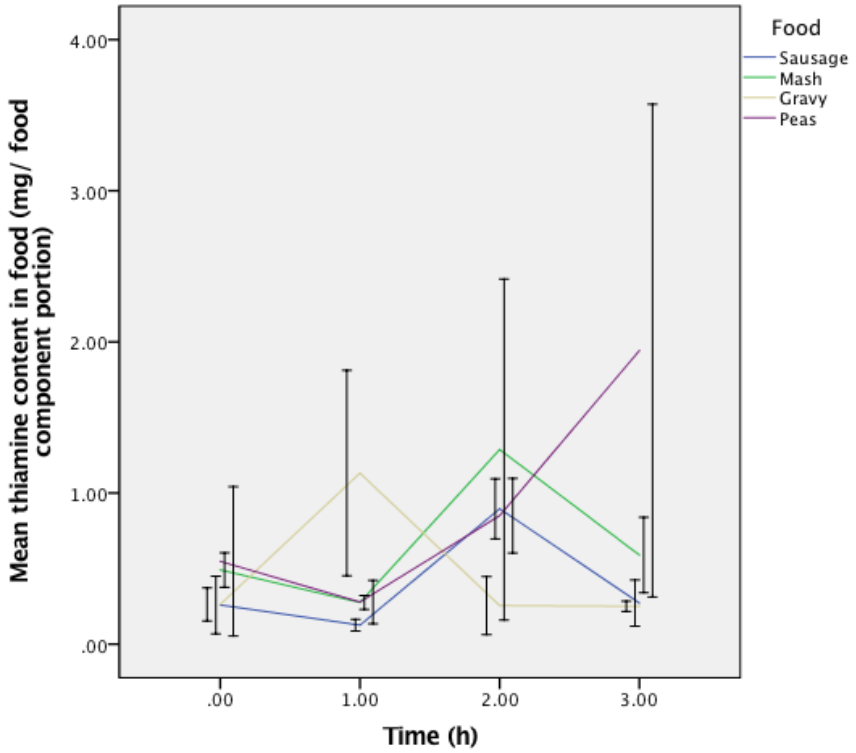


Figure 49: A line graph of mean thiamine content (mg/food component portion) of sausage, mash, gravy and peas over time (h), where t=0; microwave cooked for 7 minutes (900W) only, t=1; microwave cooked for 7 minutes (900W) then held at 90±1°C for 1 hour, t=2; microwave cooked for 7 minutes (900W) then held at 90±1°C for 2 hours, t=3; microwave cooked for 7 minutes (900W) then held at 90±1°C for 3 hours. Error bars depict 1 standard deviation of the mean.

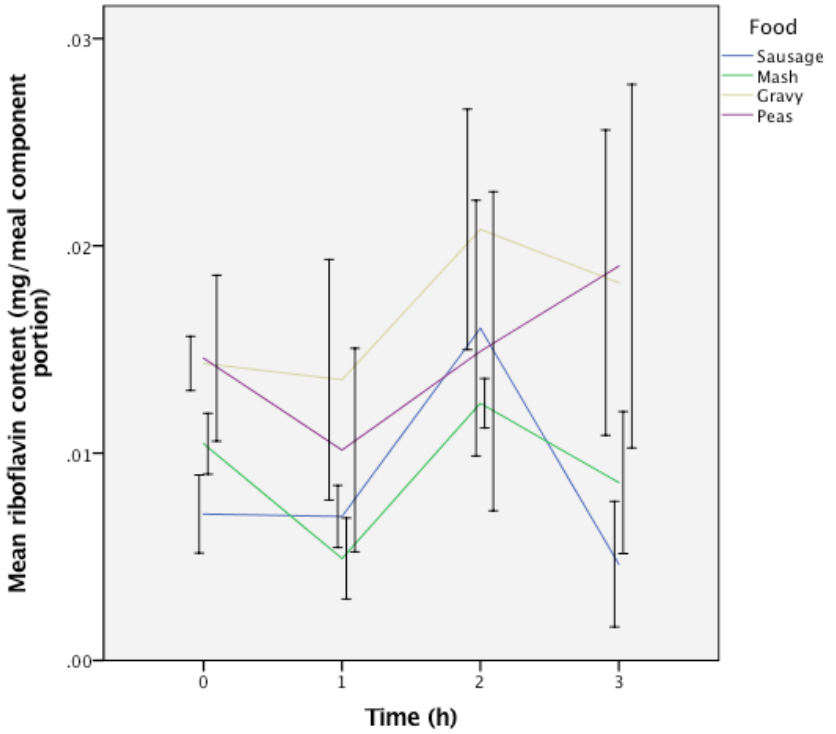


Figure 50: A line graph of mean riboflavin content (mg/food component portion) of sausage, mash, gravy and peas over time (h), where t=0; microwave cooked for 7 minutes (900W) only, t=1; microwave cooked for 7 minutes (900W) then held at 90±1°C for 1 hour, t=2; microwave cooked for 7 minutes (900W) then held at 90±1°C for 2 hours, t=3; microwave cooked for 7 minutes (900W) then held at 90±1°C for 3 hours. Error bars depict 1 standard deviation of the mean.

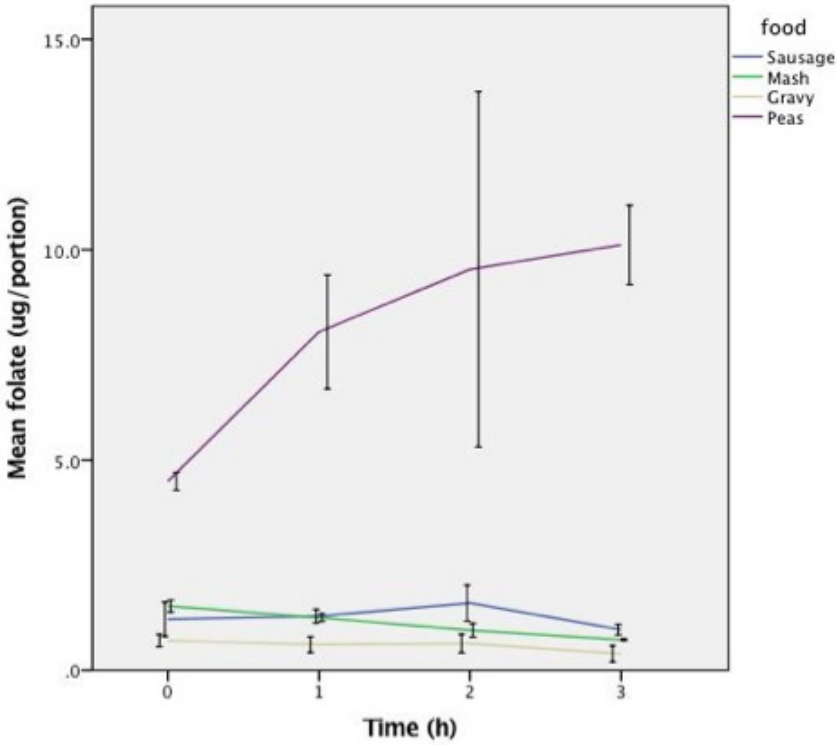


Figure 51: A line graph of mean folate content (mg/food component portion) of sausage, mash, gravy and peas over time (h), where t=0; microwave cooked for 7 minutes (900W) only, t=1; microwave cooked for 7 minutes (900W) then held at 90±1°C for 1 hour, t=2; microwave cooked for 7 minutes (900W) then held at 90±1°C for 2 hours, t=3; microwave cooked for 7 minutes (900W) then held at 90±1°C for 3 hours. Error bars depict 1 standard deviation of the mean.

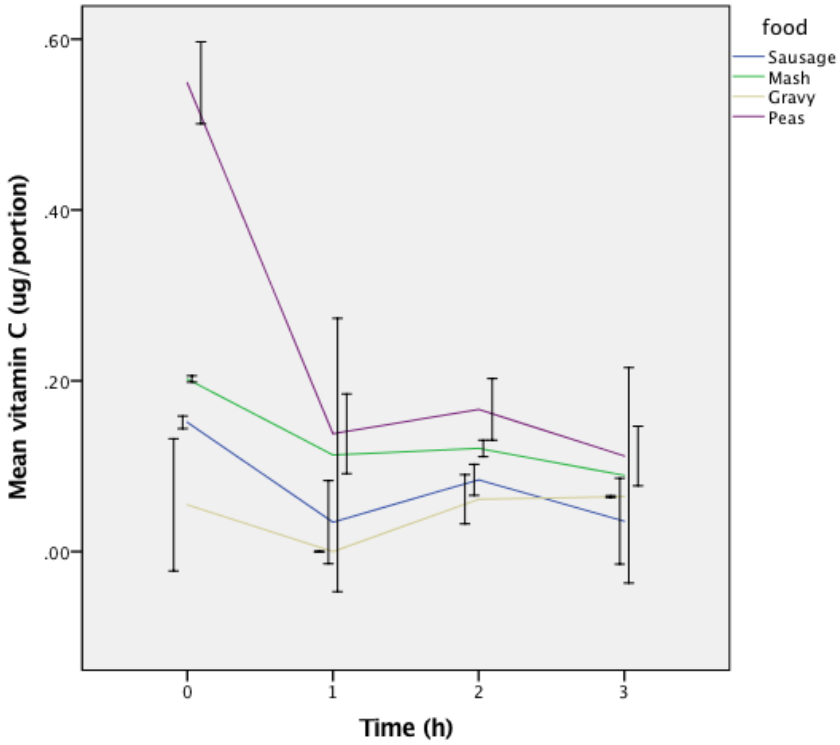


Figure 52: A line graph of mean vitamin C content (mg/food component portion) of sausage, mash, gravy and peas over time (h), where t=0; microwave cooked for 7 minutes (900W) only, t=1; microwave cooked for 7 minutes (900W) then held at 90±1°C for 1 hour, t=2; microwave cooked for 7 minutes (900W) then held at 90±1°C for 2 hours, t=3; microwave cooked for 7 minutes (900W) then held at 90±1°C for 3 hours. Error bars depict 1 standard deviation of the mean.

6.5. Discussion

This study investigated the effect of a 90ºC, three-hour hot-holding process on vitamin content of reheated RMs and examined the effect of hot-holding on thiamine, riboflavin, folate and vitamin C in RM components. The research intended to understand the impact of hot-holding on the overall micronutrient quality of meals (Hickson, 2006). Results from this study showed that the only significant change in vitamin content was the riboflavin content of peas, where vitamin concentration increased over time by up to 45% at two hours but reduced by 23% when the meal was heated for three hours. This increase could have been due to water loss during the heating of the meal rather than improvement of the vitamin content (Johansson et al., 2008).

Hot-holding is a popular method for keeping food heated to a safe temperature and ready for service in a number of care settings including hospitals, care homes, during MOW deliveries, and sometimes in the home. There are several advantages of hot-holding including being able to bulk cook foods before delivery (Creed, 2010, Sustain, 2018), and ensuring the food has been heated to the correct temperature to comply with food safety regulations before service, where food needs to be kept above 63ºC to prevent the growth of harmful bacteria (Khan et al., 1983, The Food Safety and Hygiene (England) Regulations 2013, 2013).

Gatherer (1971) examined the effect of holding MOW meals in hot lockers during delivery on the vitamin C and thiamine content of vegetable meal components. The meals were prepared and cooked just prior to delivery (conventionally cooked), and included end-cooked frozen meals. Results showed that end-cooked frozen peas, carrots and mashed potato retained more ascorbic acid (vitamin C) than conventionally cooked meals, whereas the latter were better at retaining thiamine during the hot-holding, rather than end-cooked frozen meals. Although it should be noted that due to the age of the study, the methods used by Gatherer (1971) have changed, although, the effect of heat on vitamin C content is similar in more recent literature (Bureau et al., 2015). Gatherer (1971) used hot lockers, which would likely have varying temperatures due to being heated by coals at the bottom of the lockers, whereas nowadays it is more common to use an insulated heated bag (Sustain, 2018), which allows the food to be kept at a more constant temperature.

A review by Williams (1996) discussed the effect of hot-holding on the vitamin content of food, and found that vitamin C and folate in peas, potatoes and mashed potatoes decreased by more

than 10% when held for two hours at temperatures above 70°C. Furthermore, meals that were cooked and hot-held for 90 minutes or less retained more vitamin C, compared to completely cooking the meal followed by chilling and then reheating, also known as cook-chill (Williams, 1996). It should be noted that these were foods being cooked from fresh, not RMs or frozen RMs, and therefore the impact of initial production of the RM was not considered.

The method of cooking the meal before delivery could affect the retention of process-labile vitamins such as thiamine, riboflavin, folate and vitamin C differently. A study by Ang et al. (1975) found that reheating frozen meals using different methods (microwave oven, infrared oven, steamer and convection oven), followed by hot-holding for 30 minutes, led to a retention of vitamin C (23.8-41.4%), riboflavin (92.6-96.5%), and thiamine (88.3-92.0%) in frozen mashed potato, fish and meat-based RMs. The study found that for all foods tested, the frozen meals that were reheated using a steamer lost more thiamine, riboflavin and vitamin C compared to all other methods, while heating in an infrared oven or microwave caused less degradation of the vitamins. This study also found that thiamine and riboflavin were retained better than vitamin C after hot-holding.

The current study found that heating led to a decrease in the weight of the meals by up to 10% after 3 hours, due to the loss of water during the heating (Nelson et al., 2001, Creed, 2010). The lack of difference in vitamin content between the time points could have been due to vitamin concentration resulting from water evaporation during cooking. Ways to minimise the loss of heat-labile vitamins in meals that are hold-held would be to reduce the amount of time that the food is kept hot; batch cooking vegetables so that they are not overcooked or reheat the meals individually rather than all at the same time (Williams, 1996). Apetito have reduced the time that meals are kept waiting by creating special delivery vehicles known as ‘ChefMobil’, where meals are cooked en-route to the client (Apetito, 2019c).

Studies have modelled the best delivery system to maintain quality of MOW and created technology such as the ‘ChefMobil’, but these are not available to all public catered meal providers (Apetito, 2019c). These meals are a vital source of macro and micronutrients for MOW recipients and school pupils, and there are a number of ways that the process-labile water-soluble vitamin (WSV) could be enhanced in these meals including the fortification of meals, the addition of vitamin rich sauces such as cranberry sauce, or the provision of freshly prepared fruit or vegetables with meals (Best and Appleton, 2011, Dunne and Dahl, 2007).

6.5.1. Limitations

There were some limitations of this study. Due to the small sample size of the experiment (n=2) for vitamin C and (n=8) for all other vitamins tested, there was a large amount of variation between the thiamine, riboflavin, folate and vitamin C. Furthermore, the testing was carried out in sausage and mashed potato RMs only, so the changes in vitamin content may vary due to difference in sauce content and meal composition. The vitamin retention of the meal components did not consider the weight loss in the meals. This was due to not being able to remove the sleeve of the meal to weigh the food components before hot-holding was carried out.

6.5.2. Conclusion

This study has shown that hot-holding significantly increased the concentration of riboflavin in peas, but not riboflavin in sausage and mashed potato or any meal components for thiamine, folate and vitamin C. However, this lack of significance could be due to large variations in composition in the meals analysed. These findings could have implications for individuals who consume meals after hot-holding, or keep meals hot within the home, as the content of the meal is already below the 30% of the RNI for folate and vitamin C, and riboflavin in some RMs, after reheating alone. Further research should be carried out on the impact of hot-holding on the WSV content of the meals, alongside ways to improve the WSV content, possibly through the inclusion of fortified sauces, side dishes or freshly prepared fruit and vegetables.

7. DISCUSSION

This research confirms that ready meals (RMs) are a popular convenience food in the UK, with more than 10% of the population consuming them at least once per week. Ready prepared foods are frequently used as part of catering provision services; meals-on-wheels (MOW), school catering and hospital catering, and thus may be the main source of essential nutrients for some individuals in the population, especially those who may not be able to prepare a hot meal themselves (Krassie et al., 2000). The study found that those eating RMs may be at a higher risk of vitamin C, riboflavin and folate deficiency. Consumption trends indicate a greater reliance on RMs as the main meal, and consequently, these meals are increasingly making a substantial contribution to the overall intake of essential micronutrients. This research has reviewed the importance of these essential nutrients (chapter 1.6) and highlighted a greater risk of vitamin deficiency in those individuals where RMs provide up to 50% of dietary intake for thiamine, riboflavin, folate, vitamin C and vitamin B12, especially as the study found that for folate and vitamin C, RMs do not provide enough to meet 30% of the reference nutrient intake (RNI).

This investigation showed that although the recommended nutrient content of the RMs for thiamine was met, folate, riboflavin and vitamin C did not meet the suggested levels. Therefore, the rise in popularity of RMs in the UK indicates a need to understand the role that RMs play in the intake of process-labile water-soluble vitamins (WSVs). This research has identified vitamins that are at low concentrations in these meals and, therefore, has implications for RM manufacturers; particularly those who provide foods targeted at consumers already at a higher risk of nutrient deficiency. This discussion will explore the potential role of food manufacturers and providers in ensuring that meals are more nutritionally adequate for consumers.

7.1. Water-soluble vitamin content of ready meals compared to nutrient recommendations

In both the analysis of the NDNS data shown in Chapter 3 and the lab-based analytical testing presented in Chapter 5, thiamine was found to meet the Public Health England (2017a) recommendations which states that meals should provide 30% of the RNI. National Diet and Nutrition Survey (NDNS) analysis showed that RMs consumed by participants met the recommendation for riboflavin, however, in testing of these meals in the laboratory (Chapter 5)

riboflavin did not meet the minimum nutrient content, and folate and vitamin C was not met in either the NDNS analysis or the lab testing.

The thiamine, riboflavin, folate and vitamin C content of sausage and mashed potato meals across ranges varied considerably, providing up to three times the RNI for thiamine, and between 1-60% of the 30% of the RNI recommendation for riboflavin, folate and vitamin C. The lack of on-pack information about micronutrients currently prohibits consumers from making an informed choice, however, a reliance on these meals to provide adequate micronutrient intake is questionable. Previous research has shown that luxury ranges of RMs are higher in salt, total fat and saturated fat compared to cheaper ranges (Remnant and Adams, 2015, Celnik et al., 2012), however this research found that luxury ranges have a higher vitamin C and folate content than cheaper ranges.

Research by Johansson et al. (2008) and Fajardo et al. (2017) similarly found that the folate content of RMs did not meet the recommended nutrient content. The addition of antioxidant rich vegetables to meals, such as carrot, broccoli and onions protected the folate content during the reheating of the RMs (Johansson et al., 2008). However, the meals that were tested in this study had individual items separated in different compartments in the RM tray. Sausages and mashed potato were separate in the meal, and therefore the protective effect of the vegetables could not be utilised. Further research should be carried out on other popular UK RMs including soups and stews to evaluate how the addition of antioxidant-rich vegetables could help to preserve the overall vitamin content of the meal.

7.2. Factors affecting the nutrient composition of ready meals.

Price was found to have an impact on both the composition and the vitamin content of the meal, where the more expensive meals had higher vitamin C concentrations, most likely due to there being more mashed potato or vegetables within the meal portion. This finding was interesting, as other research has concluded that luxury meals should be avoided due to being higher in fat and salt, contributing to an increased risk of cardiovascular disease (Celnik et al., 2012). The cheaper meals, however, were higher in thiamine due to a larger portion of meat within the meal, but they had a lower vitamin C and folate content. Therefore, it is important to understand the population being catered for and the meals that are being consumed when making recommendations for meal choice, as a higher calorie option is beneficial for older adults at risk

of malnutrition, with the added benefit being the contribution of more vitamin C compared to cheaper meals.

Furthermore, meal composition can mean that nutrients are not contained within the meal due to differences in ingredients used, or recipes. The meal tested in this research lacked ingredients that are a good source of riboflavin, which resulted in the recommendation for riboflavin not being met. This further supports the need for a variety of foods to support a balanced diet (Foote et al., 2004).

7.3. Expectations for the nutrient quality of ready meals

The lack of strict guidelines about the nutrient content of RMs has led to inconsistencies about the perception of how healthy they are. It is generally understood by RM consumers that these meals are good quality and they have an adequate vitamin content, but these meals are seen as less healthy by non-consumers (Van Der Horst et al., 2011). Therefore, there is a need for more research to be carried out into the perception of nutrient quality within these meals, and the expectation that they provide essential nutrients to consumers.

This lack of information about nutrient quality could be overcome using a marque on products. An example of how labelling can be used on food products can be seen in France, where in 2017 the NutriScore was introduced, which assigns a rating to processed food based on the overall nutrient quality of the product (Julia et al., 2018). The score evaluates the favourable components of a food such as the vegetable, fibre and protein content, compared to the less healthful components such as energy, sugar, saturated fat and salt content, to give an overall score. This score is then printed on the front of pack (FOP) labelling where ‘A’ is the most healthy option, and ‘E’ is the least healthy (Chantal et al., 2017). In a study by de Edelenyi et al. (2019) where the NutriScore of RMs were evaluated, the majority (48%) of the meals were in group C, neither healthy or unhealthy, with only 1.5% of the meals being classified as group E, the most unhealthy category. However, it should be noted that only the proportion of vegetables are quantified, and the micronutrient content and processing techniques used are not considered, therefore the actual vitamin content may not be accurately reflected in the score.

Another labelling system that takes into account vitamin content of foods is the ‘Guiding Star’, which is used in Canada and the USA and provides an on-shelf rating system for food products

(Fischer et al., 2011). This system rates processed food from 0-3 stars, with 3 stars being the healthiest. The ratings are based on products that are over 5kcal per serving, and foods are credited for their content of vitamins, minerals and macronutrient balance. Although this is a more sensitive measure using a scientific algorithm (Fischer et al., 2011), a study by Hobin et al. (2017) found that using the Guiding Star on-shelf labelling led to increased purchasing of healthier cereals, fruits and vegetables and meat products, but had no effect on purchasing of 'mixed' products such as RMs. This may mean that an alternative labelling system may be required to assess the healthiness of a RM, for example an enhanced NutriScore.

Front-of-pack labelling has been shown to be favourable for consumers and provide helpful information that supports public health guidelines (Tarabella and Voinea, 2013). The limitation of the 'traffic-light' system currently used in the UK is that it only focuses on the nutrients that need to be limited and does not provide any information on the beneficial nutrients that are present in the food product. A more comprehensive labelling system for foods such as RMs would give consumers a better understanding of the overall nutrient quality of the meal (Tarabella and Voinea, 2013). However, the challenge of implementing food labelling should be highlighted. A review (Grunert et al., 2010) of current understand of FOP and BOP food labels in the UK found that only 27% of consumers used labelling when considering what food they choose to purchase, and furthermore, shoppers were less likely to consider the labelling when indulgent or convenient foods were being purchased compared to other products that have 'healthier' ranges. Therefore, there may need to be alternative labelling options for foods such as RMs.

Grunert et al. (2010) highlights that nutrition education plays a role in use of labelling, where those who had a higher education level, or had an interest in healthy eating were more likely to use nutrition labels to inform food choice. Therefore, there may be opportunities to improve the usage of FOP information, by improving public understanding of food and nutrition and then embedding this into the broader public health policy. An example of this would be assessing the effect of marketing the five-a-day message in the UK, along with highlighting how much a food contributes to 5-a-day on food labelling, to a person's intake of fruit and vegetables (FVs) (Tobi et al., 2019). Research by García et al. (2019) has shown that although marketing can be used to increase the consumption of more healthy foods, such as the 5-a-day message, labelling does not always accurately reflect the content of the meals; where those foods marketed as

contributing to an adults 5-a-day did not meet the recommended portion size of 80g. Therefore, regulation of labelling for RMs would need to be considered if initiatives were introduced.

7.4. Improving nutrient quality of pre-prepared foods

The current research shows that there is a trend for RMs having low concentrations of vitamin C and folate. Hence, reformulation of meals through the addition of fresh FVs, fortification of food components such as the addition of vitamin C to mashed potato, or through the use of addition meal components, for example sauces such as apple or cranberry sauce is recommended (Rodgers, 2004).

Although not currently available, research into the potential impact of functional meals, such as through fortification to improve the nutrient quality of RMs, should be carried out as an opportunity to improve the nutrient profile of RMs (Rodgers, 2004). The low availability of functional meals could be due to the challenges associated with creating a stable, palatable and aesthetically pleasing product, as the addition of functional ingredients could lead to unwanted changes in taste. Examples are the use of water dispersible vitamins in milk, which created unwanted flavour changes (Yeh et al., 2017), or colour change of salt with the addition of WSVs (Hurrell, 2002).

Although reformulation is a potential technique to improve nutrient quality, it does not always lead to changes in purchasing habits. A study in Norway by Olsen et al. (2012) investigated the likelihood of buying 'healthier' RM alternatives, and found that the probability of choosing a healthier salmon meal was significantly related to age and education, but not related to choosing an alternative chicken-based RM (Geeroms et al., 2008, Mahon et al., 2006). Interestingly, this shows that there are potential barriers to the introduction of 'healthier' RMs without the promotion of nutrition education to the population (Drichoutis et al., 2005), and a shift in the belief that RMs can be considered an appropriate choice as a main meal. Another initiative uses the marketing of vegetables alongside RMs which has shown to improve the attractiveness of fresh salads and vegetables to consumers, as they are more accessible compared to RMs being sold alone (Prim et al., 2007). This could be incorporated into the 'Veg Pledge' scheme, described in chapter 5.1, to improve the variety of foods that are consumed with RMs, with possible meal-deal options that include FVs to improve consumer interest (Prim et al., 2007, The Food Foundation, 2019).

7.5. The effect of hot holding on water-soluble vitamin composition of ready meals

Analysis of the effect of hot-holding on the vitamin content of sausage and mashed potato RMs found that the riboflavin content of peas increased over time. This is likely due to the breakdown of the food matrix during the hot-holding process (Parada and Aguilera, 2007), and the evaporation of water causing the concentration of vitamins during cooking. There were no significant differences between any of the other meal components tested, which could have been due to the large variation in vitamin content between the samples.

The findings of the hot-holding experiment have shown that we need to increase our understanding of how hot-holding could impact other meals that are provided as part of catered services, which use RMs, in order to ensure that the vitamin content is not reduced during transport, or during the holding of food before service. Future research should investigate ways to reduce the impact of hot-holding, such as heating the meal at the point of delivery or during transport. As previously described in section 6.5, methods currently being used such as ChefMobil should be evaluated to show any differences between hot holding and heating during the journey on vitamin content (Apetito, 2019c). Another technique known as Steamplicity has been introduced to hospital catering facilities, and uses a specialised type of steaming to retain texture, WSVs and colour of the food (Dillon et al., 2012). This method has additionally been shown to reduce the amount of food waste and the amount of energy required to prepare the meal.

7.6. Analysis of thiamine in foods containing sulphites

Prior to conducting analysis of RM components, different analytical methods were reviewed to determine which are considered the most accurate in the analysis of vitamin content in a range of foods. This novel study found that microbiological assay and HPLC methods concur when the food is cooked, or when thiamine has been degraded by high temperature, or in the presence of sulphites at high temperatures. However, for raw food products, there was no agreement between the assays. The results showed that there were lower concentrations quantified in the

microbiological assay compared to the HPLC method, which was most likely due to extraction procedures (Ramaswamy et al., 1990).

Validation of methods for food analysis is an important step in understanding the vitamin concentration in food consumed nationally, as this can be used to inform the health of the population. These include the National Diet and Nutrition Survey, the provision of food based dietary guidelines, the Eat-well plate in the UK, and therapeutic nutrition, for example the control of diabetes (Elmadfa and Meyer, 2010). However, the precision and accuracy of a method should be based on a range of foods. This is due to the effects that the food matrix, additives or other ingredients within the food product may have on the extraction phase or on analysis of the nutrient (Greenfield and Southgate, 2003, Ramos, 2012). Previous research (Edwards et al., 2017) has shown that growth of *Lactobacillus fermentum* in the microbiological assay can be affected by the sample matrix, but there have been no other studies showing the effects of sulphites within the sample matrix on the quantification of the vitamin in food. Due to the degradation in the sample during the microbiological assay, this study has shown that the HPLC method should be used to quantify thiamine in foods that are not cooked and contain sulphites.

7.7. Strengths and Limitations

This research is the first to investigate the WSV content of RMs in the UK using secondary analysis, and provided insight into the WSV content of a popular UK RM. However, this research was not able to understand consumption patterns of RMs due to the method of data collection in the NDNS survey. Further investigations should seek to understand patterns of consumption more accurately.

The studies conducted in this research used the most accurate methods available to assess the vitamin content rather than relying on food composition tables (FCTs), which have shown to be inaccurate for composite foods such as RMs. A limitation of the research was that although it was one of the most popular RMs in the UK, only one type, sausage and mash, was tested. The nutrient content would vary across different types of meals and recipes. Furthermore, due to safety issues, vitamin B12 could not be tested, and as there is a potential for this vitamin to be mal-absorbed in older adults (Baik and Russell, 1999) it is important to understand the contribution of RMs to the intake of vitamin B12 using lab-based testing.

Lastly, the large variation between the meals meant that few conclusions could be reached about the impact of hot-holding on meal components. Further replications could provide more insight into the effect of hot-holding on the vitamin content of RMs. Furthermore, although there may be an increased risk of deficiency, this research does not investigate the whole diet, and therefore, nutrients not provided in the RMs could be consumed elsewhere.

7.8. Recommendations for Policy and Practice

Quality assurance practices such as validation of chemical analysis methods used for populating the FCTs are an important step when analysing the nutrient content of food. FCTs are an invaluable source of information used by government, practitioners and the food industry to provide information to the population, and therefore need to be as accurate as possible. Therefore, Association of Official Analytical Chemists or Composition of Foods integrated dataset databases should provide food specific methods where necessary to ensure that research on the nutrient analysis of food is carried out using validated and accurate methods, especially where the food matrix may interfere with analysis, such as with sulphite-containing foods.

This research found that the vitamin content of a popular RM, sausage and mash was below the riboflavin, folate and vitamin C recommendations set by Public Health England (2017a). Improvement of nutrient content could be made at various stages of production, preparation or provision of RMs, and reformulation of the meals to include more antioxidant-rich vegetables or sauces within the meal itself could protect folate content (Fajardo et al., 2017). This could also increase the marketing potential for the product if it was to be associated with the 5-a-day campaign, if one or more portions of vegetables are provided by the meal (Capacci, 2010).

Other ways to improve nutrient quality include using alternative cooking techniques such as sous-vide (Creed, 1995) for soups or stews rather than simmering or boiling. Changing cooking techniques would be less detrimental to the WSV content of the meal, and the use of technology such as the ‘ChefMobil’ allows meals to be cooked on route rather than being hot-held during delivery (Apetito, 2019a). Moreover, the introduction and promotion of prepared fresh fruit or fruit-based desserts within catering services would improve the quality of these RMs to those who may not be meeting nutrient recommendations. This would be particularly important for those who rely on RMs for a major part of their diet.

7.9. Further research

The findings of this research showed that the microbiological assay was not suitable for testing foods containing sulphites. Foods containing sulphites that have been tested using the microbiological method should be retested using HPLC method or using a validated matrix specific assay to quantify the vitamin content of these items.

As only one type of RM was tested as part of this study, further studies should include testing a wider variety of meals to see if these findings are similar across all RMs with regards to WSVs. Additionally, this research was unable to assess the vitamin B12 concentration of the sausage and mashed potato RMs. This analysis should be carried out to ensure that these meals are meeting the recommendations due to the increased risk of folate and vitamin B12 deficiency in older adults (Scientific Advisory Committee on Nutrition, 2021).

Due to the Public Health England (2017a) recommendation that meals should meet 30% of the RNI for micronutrients, further research should be carried out to assess how the presence of antioxidant-rich vegetables in RMs could protect the folate content of food (Johansson et al., 2008). Further research exploring how RMs could be reformulated to improve the WSV content, and how alternative cooking methods or addition of WSV rich foods to improve the nutrient quality, should be carried out.

As stated in the 'implications for policy and practice' section of this chapter, although the use of labelling to show the nutrient quality of RMs may be a possible strategy to improve the information provided to the consumer, more research is required to understand if this could be useful to consumers or not. There are font and layout restrictions already in place that are outlined by retained EU legislation (European Commission, 2012). There is a lot of information included on the BOP and FOP labelling, and the diverse range of colours and layouts used to provide this information can reduce their effectiveness (Goiana-da-Silva et al., 2019).

7.10. Conclusion

This research shows that large numbers of the UK population are consuming RMs, but there is little research carried out as to how the manufacturing of these meals may have an impact on the vitamin content of foods, especially with regard to those vitamins that are labile to processing such as WSVs.

The dissertation has shown that RMs may not be meeting the Public Health England (2017a) recommendations for riboflavin, folate and vitamin C, and therefore may put people at risk of nutrient deficiency if RMs are the main source of these essential vitamins within the diet. There are a number of ways that the food industry could make changes to products that contribute to population health as well as providing more information to consumers about the nutrient quality of RMs, such as through the use of nutrition labelling. Measures should be taken by the food service industry to ensure that further processing of RMs does not diminish nutrient quality further. ChefMobil and Steamplicity are examples of how these challenges can be overcome. More research is needed to better understand the demographic of RM users, and therefore how reformulation, product development and marketing can suit the population, especially those who are at risk of nutrient deficiency.

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APPENDIX

i. Mintel participants demographic. (a) The percentage and number of men and women in each age group, (b) the percentage and number of participants by region, (c) the percentage and number of people by socio-economic grade.

(A)

Age groups by gender	**%**	**N**
16-19 Men	3.1	63
16-19 Women	3.0	60
20-24 Men	4.4	88
20-24 Women	4.2	84
25-34 Men	9.2	184
25-34 Women	9.1	182
35-44 Men	8.4	168
35-44 Women	8.5	171
45-54 Men	9.1	183
45-54 Women	9.5	189
55-64 Men	7.4	147
55-64 Women	7.6	152
65+ Men	8.0	159
65+ Women	8.5	170
Total	100	2,000

(B)

Region	%	N
North East	4.1	83
North West	11.3	226
Yorkshire & Humberside	8.5	170
East Midlands	7.4	149
West Midlands	9.1	183
Greater London	13.7	275
South East/East of England	23.8	475
South West	8.7	173
Wales	4.9	97
Scotland	8.5	169
Total	100	2,000

(C)

Socio-economic grade	%	N
High managerial, administrative or professional	28	560
Intermediate managerial, administrative or professional		
Supervisor, clerical, junior managerial, administrative or professional	28	560
Skilled manual worker	20	400
Semi-skilled or unskilled manual worker or Unemployed	24	480
Total	100	2,000

ii. A table of reference nutrient intakes (RNI)for all age groups for thiamine, riboflavin, folate, vitamin B12 and vitamin C (Department Of Health Committee On The Medical Aspects Of Food Policy, 1991)

Age	Thiamine	Riboflavin	Folate	Vitamin B12	Vitamin C
	Mg/d	Mg/d	µg/day	µg/day	Mg/day
0-3 months	0.2	0.4	50	0.3	25
4-6 months	0.2	0.4	50	0.3	25
7-9 months	0.2	0.4	50	0.4	25
10-12 months	0.3	0.4	50	0.4	25
1-3 years	0.5	0.6	70	0.5	30
4-6 years	0.7	0.8	100	0.8	30
7-10 years	0.7	1.0	150	1.0	30
Males					
11-14 years	0.9	1.2	200	1.2	35
15-18 years	1.1	1.3	200	1.5	40
19-50 years	1.0	1.3	200	1.5	40
50+ years	0.9	1.3	200	1.5	40
Females					
11-14 years	0.7	1.1	200	1.2	35
15-18 years	0.8	1.1	200	1.5	40
19-50 years	0.8	1.1	200	1.5	40
50+ years	0.8	1.1	200	1.5	40
Pregnancy	+0.1**	+0.3	+100	No increase	+10**
Lactation					
0-4 months	+0.2	+0.5	+60	+0.5	+30
4+ months	+0.2	+0.5	+60	+0.5	+30

iii. A table describing the terms used for the literature search strategy in Pubmed, Scopus and Ebscohost, the results yielded from the search and the hand search for consumption of RMs.

Database	Topic	Search Terms	Results	Hand search
Pubmed	Consumption of RMs	("ready-meal"[Title/Abstract] OR "ready-meals"[Title/Abstract] OR "ready-meal"[Title/Abstract] OR "ready-meals"[Title/Abstract] OR "convenience food"[Title/Abstract] OR "ready-to-eat"[Title/Abstract] OR "ready-to-eat"[Title/Abstract] OR "ready-prepared"[Title/Abstract] OR "ready-prepared"[Title/Abstract]) AND "consum*"[Title/Abstract] AND "UK"[Title/Abstract] NOT "cereal"[Title/Abstract]	29	1
Scopus	Consumption of RMs	TITLE-ABS-KEY ((("ready-prepared" OR "ready prepared" OR "ready-to-eat" OR "ready to eat" OR "convenience food" OR "ready-meal" OR "ready meals" OR "ready meal") AND (consum* OR freq* OR "Eating habits") AND "UK" AND NOT (status OR supplementation OR cereal OR cereals OR micro*)))	41	1
EbscoHost	Consumption of RMs	AB ("ready meal" OR "ready meals" OR "ready-meals" OR "convenience food" OR "ready to eat" OR "ready-to-eat" OR "ready prepared" OR "ready-prepared") AND AB ("eating habit" OR consum* OR freq*) AND AB (uk or united kingdom or britain or england or wales or scotland or northern ireland) NOT AB (micro* OR cereal)	46	3

iv. A table of the literature identified (n=5) through the literature and a summary of each article including the aim, setting, participants, dietary assessment methods, question relating to consumption and main finding.

Citation	Aim	Setting	participants	Dietary Assessment Method	Frequency Questions Asked	Main Findings
(Garcia et al., 2014)	To evaluate longitudinally the effectiveness of a cooking programme on self-reported confidence about cooking skills and food consumption patterns in parents of young children.	Deprived communities in Ayrshire and Arran, Scotland.	Parents of nursery age children, 97 % were female and <45 years old	Self-reported questionnaire	How often do you eat ready meals?' 1 = 'never' to 7 = 'more than once a day' for ready meals; from 1 = 'less than once a week' to 7 = 'more than twice a day'	For completers, median baseline consumption of ready meals was 2–4 times/week
(Garcia et al., 2020)	to evaluate the immediate and sustained impacts of the Eat Better Feel Better cooking programme (EBFBCP) developed by the National Health Service Greater Glasgow and Clyde (NHSGGC) Public Health in Scotland on food choices and eating behaviours in families and children.	Greater Glasgow and Clyde, Scotland.	participants (80%) were female, 25–44 years old (51%) and considered socioeconomically deprived (80%).	Self-reported questionnaire	NA	Weekly Frequency / Percent of population Never or <once per week / 34% Once per week / 45% 2-4 times per week / 15% 5-6 times per week / 1% Once per day / 1% Two times per day or more / 1%

Citation	Aim	Setting	participants	Dietary Assessment Method	Frequency Questions Asked	Main Findings
(Mills et al., 2018b)	To identify sociodemographic characteristics associated with frequency of consuming home cooked meals and meals from out of home sources.	Cambridgeshire, UK	Fenland study participants (n=11,326), aged 29 to 64 years at baseline.	Self-reported questionnaire	Fenland General Questionnaire When eating your main meal at home, how often do you usually eat? Ready-made meals/prepared foods Never or rarely 1-2 times per week 3-5 times per week More than 5 times per week	The majority of participants ate ready meals (94.4%) and takeaways (93.7%) only twice per week or less. Lower odds of eating ready meals more than twice per week was associated with household income of over £40,000, compared with less than £20,000 (OR 0.57, 99% CI 0.41 to 0.80).
(Sprake et al., 2017)	This study explored factors associated with body weight gain among British university students who were members of a slimming club	UK Universities	Approximately half of the sample (47.6%) was between 18 and 21 years of age and just under one quarter (22.3%) of respondents were between 22 and 30 years old. The majority of responders had been members of Slimming World for less than a month (46.0%) or between 3-6 months (29.9%) at the point of survey.	Self-reported questionnaire	Frequency of RM consumption over a week	RM consumption was associated with increased weight (44.14, $p<0.001$)
(Pettinger et al., 2006)	To evaluate whether meal patterns and cooking practices in Central England and Mediterranean France conform to popular stereotypes, eating together as a household, preparation of meals, food purchasing patterns, cooking practices and eating out were investigated.	Nottingham, UK	sample comprised 826 subjects in England (58% males, 42% females; mean age 44 years)	Self-reported questionnaire	How often do you consume ready meals, Use by age group	27% of 18-35 year olds consumed ready meals. With the majority (51%) consuming RMs at least once per week.

v. A table describing the terms used for the literature search strategy in Pubmed, Scopus (a), and Ebscohost (b), the results yielded from the search and the hand search for water-soluble vitamin content of RMs.

(A)

Database	Topic	Search Terms	Results	Hand search
Pubmed	Ready meals and Micronutrient content	("ready meal"[TIAB] OR "ready meals"[TIAB] OR "ready-meal"[TIAB]OR "ready-meals"[TIAB] OR "convenience food"[TIAB] OR "ready to eat"[TIAB] OR "ready-to-eat"[TIAB] OR "ready prepared"[TIAB] OR "ready-prepared"[TIAB] OR "meals on wheels"[TIAB] OR "meals-on-wheels"[TIAB]) AND (thiamin[TIAB] OR Thiamine[TIAB] OR "vitamin b1"[TIAB] OR riboflavin[TIAB] OR "vitamin b2"[TIAB] OR "vitamin b9"[TIAB] OR Folate[TIAB] OR "folic acid"[TIAB] OR "vitamin b12"[TIAB] OR cobalamin[TIAB] OR "vitamin C"[TIAB] OR "Ascorbic acid"[TIAB] OR "l-ascorbic acid"[TIAB]) NOT cereal	61	2
Scopus	Ready meals and Water-soluble vitamin content	TITLE-ABS-KEY (("community meal" OR "meals-on-wheels" OR "meals on wheels" OR "ready-prepared" OR "ready prepared" OR "ready-to-eat" OR "ready to eat" OR "convenience food" OR "ready-meal" OR "ready meals" OR "ready meal") AND ("l-ascorbic acid" OR "ascorbic acid" OR "vitamin c" OR cobalamin OR "vitamin b12" OR folate OR "folic acid" OR "vitamin b9" OR "vitamin b2" OR riboflavin OR "vitamin b1" OR thiamin) AND NOT (status OR supplementation OR cereal OR cereals))	153	9

(B)

Search ID	Search Terms	Result	Hand search
S3	S1 AND S2	36	1
S2	thiamine OR thiamin OR "vitamin b1" OR riboflavin OR "vitamin b2" OR "vitamin b9" OR "folic acid" OR folate OR "vitamin b12" OR cobalamin OR "vitamin C" OR "Ascorbic acid" OR "l-ascorbic acid"	19,598	-
S1	"ready meal" OR "ready meals" OR "ready-meals" OR "convenience food" OR "ready to eat" OR "ready-to-eat" OR "ready prepared" OR "ready-prepared" OR "meals on wheels" OR "community meals" OR "community meal"	519	

vi. A table of the literature identified (n=15) through the literature and a summary of each article including the aim, country, food analysed, vitamins analysed, Methods used and main findings

Citation	Aim	Country	Food analysed	Vitamin Analysed and method used	Main finding
(Bognar, 1980)	To assess the nutritive value of chilled ready meals, and to evaluate the changes in vitamin content after storage and reheating	Germany	Beef Goulash, Sauerbraten, roast pork, pork chops, boiled meatballs with caper sauce, Hamburgers with sauce, chicken leg, fish fillet covered in breadcrumbs.	Thiamine, Riboflavin, AA – Methods not specified	AA varied between 0.7 and 38mg/100g depending on the meal. Industrially prepared chilled meals contained 30-90%less AA compared to fresh meals. Addition of ascorbic acid mitigated AA loss during sterilisation. Thiamine contents was between 0.001 and 1.137mg/100g. There was little fluctuations upon heating in chilled vs. freshly prepared. Riboflavin content was 0.002-4.379mg/100g. There was little difference in riboflavin content in meals. There is a positive correlation between length on time chilled, storage length and AA retention. No change for thiamine or riboflavin. AA decreases 23-49% upon reheating – little change in riboflavin and thiamine
(Agte et al., 2002)	study of different categories of food items in the traditionally cooked form grouped according to their consumption for the levels of five important vitamins, namely ascorbic acid, folic acid, beta carotene, thiamine and riboflavin	India	Sago, Panicum m., peanut curry, potato with peanut, sago, amaranth pancake, sweet amaranth cake, peanut ladu, sweet potato with ground nut	AA - Reduction of 2,6 dichlorophenol indophenol Thiamine -fluorometrically using excitation (364 nm) and emission (435 nm) Riboflavin: fluorometrically with excitation (436 nm) and emission (510 nm) Folic acid: Spectrophotometrically using the complex formation with N-1 naphthyl ethylene diamine dihydrochloride at 550 nm	There was trace amounts of vitamin C, <0.05mg/100g thiamine, <0.02mg/100g riboflavin, and <5µg/100g of folic acid overall in the foods tested.

Citation	Aim	Country	Food analysed	Vitamin Analysed and method used	Main finding
(O'Leary et al., 2000)	The effects on quality over a short time period of 2 week and a longer time period of 32 week.	Ireland	RM components: instant mashed potato; and steamed broccoli	AA - reduction of 2,6 dichlorophenol indophenol	The vitamin C content of mashed potato underwent Freeze-chill, freeze and freshly prepared decreased over 32-week storage; most lost after 4 weeks. Freezing of broccoli retained more vitamin C than freeze chill, or chilling, and larger losses occurred after being cooked.
(Redmond et al., 2002)	Examine the effect of different freezing conditions during the freezing stage of the freeze-chilling process on the quality of recon-situated mashed potato.	Ireland	Mashed potato from ready meal	AA – reduction of 2,6-dichloroindophenol titrimetric method	Average vitamin C content was 3.8 mg/100 g which was lower than the vitamin C content of the freshly made reconstituted mashed potato (approximately 12 mg/100 g). This loss in vitamin C content was due to the chilling stage of the freeze- chilling process.
(Redmond et al., 2004)	freeze-chilling of mashed potatoes (three cultivars)	Ireland	Mashed potato	AA – Reduction of 2,6-dichloroindophenol titrimetric method	Vitamin C content of the non-supplemented mashed potato was approximately 2mg/100g in the fresh and in the processed products. Chill and freeze-chilling treatments had a dramatic reducing effect on the vitamin C content ($P<0.001$) of the potato with added vitamin C.
(Fajardo et al., 2017)	To analyse the current availability of chilled ready-to-eat meals from the Spanish market and provide new data on its folate content by a validated microbiological assay	Spain	Various dishes*	Folate: Microbiological assay with *Lactobacillus casei* ssp. rhamnosus	Chilled ready-to-eat meals may provide a significant amount of the vitamin needs. Total folate content in the analysed chilled ready-to-eat products was found to vary from 4.6 to 103.8 µg/100 g fresh weight.

Citation	Aim	Country	Food analysed	Vitamin Analysed and method used	Main finding
Johansson et al, 2008	Quantification of folate concentrations in 10 selected industrially processed vegetable-based RMs which had been precooked and then frozen	Sweden	GoGreece, GoIndia, GoMorocco,	Folate: HPLC determination with fluorescence and UV detection	For GoMorocco and GoGreece, microwave reheating resulted in a significantly ($P<0.05$) lower retention compared to the thawed ready meal. GoMorocco showed true vitamin retention of only 50% after reheating in the oven. The ready meal with the lowest amount of antioxidant-rich vegetables showed the smallest folate retention.
(Hammink, 1978)	To assess the consumer preference and vitamin content of frozen ready meals supplies in hospitals	The Netherlands	Pork, pork liver, minced pork meat, minced beef meat, beef, chicken, cod, sauce/gravy, potatoes (mashed or boiled), endive, red cabbage, French beans, spinach, peas, potato/carrot/onion medley	Thiamine, Riboflavin, Vitamin B12, AA – Methods not specified	Sulphites in the potatoes led to low thiamine content. Gravies do have a B-vitamin content. AA was the most susceptible to cooking. They do not contribute to vitamin B12 intake. Frozen ready meals had the least amount of vitamin C compared to model cooking and institutionally prepared.
(Faulks, 1991)	Retention or losses of the vitamins in foods regenerated from cook-chill or frozen by microwave or conventional ovens	UK	Brussel sprouts, peas, roast potatoes, baby bakers, chips, potato cakes, vegetable lasagne, carrots (whole and sliced)	Thiamine, Riboflavin, Folate, AA- methods not specified.	Samples with the greatest losses of AA per portion were small baked potatoes, roast potato (microwave) at 60% and 55% respectively and potato cakes, peas (conventional) at 67% and 58% respectively. No values were given for thiamine. Riboflavin values in foods were about half that of the food composition tables. Lasagne had the highest source of riboflavin before cooking but lost the most during cooking. Folate losses were 10-40%, however the largest losses were in peas, which was around 90%.

Citation	Aim	Country	Foods Analysed	Vitamin analysed and method used	Main finding
(Gatherer, 1971)	Investigate the effects of conventional cooking and end-cook freezing on the ascorbic acid content of ready meal components	UK	Conventionally cooked or re-heated from frozen meal; peas, carrots, brussels sprouts, cabbage, mashed potato, fried cod, minced meat, roast lamb, baked beans, rice pudding	AA - reduction of 2,6 dichlorophenol indophenol Thiamine - Derivatisation to thiochrome and fluorometric determination	The retention of AA from the beginning of the round to the end was 45-100%, and 0-52% for conventionally cook and end-cooked frozen meals, respectively for. The largest losses were in minced meat that was conventionally cooked; 100% loss. However, there was better thiamine retention overall in end-cooked frozen meals.
(Patterson and Stewart, 2003)	The effect of irradiation (1, 2 or 3 kGy) of thiamine and AA content of meals with the addition of reheating	UK	Roast port gravy and mixed vegetables	AA - HPLC with UV detection at 245nm Thiamine– derivatisation to thiochrome and fluorescence detection using HPLC	13% loss of thiamine in non-irradiated meals A further 11% loss with low dose irradiation, and further 10% with high dose irradiation. Further 10% loss on reheating according to manufactures instructions. There was between 51-54% loss upon irradiation compared to 24% in non-irradiated samples. Reheating caused a further 83% reduction of Total AA
(De Ritter et al., 1974)	Determine the vitamin content of ready meal dinners and pot pies	USA	Chicken Pie, Fried chicken dinner, Turkey dinner, beef dinner, Salisbury steak, fish dinner, fried shrimp dinner, macaroni and cheese, beef pie	Thiamine: Thiochrome Riboflavin: Permanganate oxidation followed by fluorimetry Vitamin B12: Microbiological assay AA: Microfluorometric method	Beef pot pie had the highest thiamine content (0.22mg/meal), with beef dinner being the lowest (0.02mg/meal). Up to 80% vitamin loss on preparation. Only Salisbury steak dinner, turkey dinner and fried shrimp dinner met recommendation of 0.2mg/meal. Chicken pie had the highest riboflavin content 0.8mg/meal. Heat did not affect content. Fish dinner and. Friend shrimp dinner did not meet the 0.2mg/meal recommendation. Turkey dinner, beef dinner, Salisbury steak dinner provided 1.28-2µg/meal. Losses on heating on average 4%. Fried chicken dinner, Turkey dinner and fried shrimp dinner were less than 1.1µg/meal recommendation. Turkey dinner was highest in vitamin C content (8.9mg/meal) due to cranberry sauce. Heat caused up to 85% loss of vitamin C. Chicken pie provided no Vitamin C. Overall meals only provided up to 15% of 60mg daily intake.

Citation	Aim	Country	Foods Analysed	Vitamin Analysed and Method used	Main Findings
(Hoppner et al., 1973)	Calculate the folate levels in frozen RMs, measure the contribution of individual meal components, determine the effect of reheating on folate content.	USA	Frozen ready meals: Chinese-style, Swiss Steak, Loin of pork, Ham, Salisbury steak, Turkey, Fried chicken, Beef, Shrimp and chips, Fish and Chips, Macaroni and cheese, Macaroni and beef, Spaghetti and meatballs, shrimp.	Folate (Free Folic acid [FFA] and Total folate [TFA]): Microbiological assay with *Lactobacillus casei*	44% of meals contained less than 3µg/100g of FFA and 10µg/100g TFA. 36% between 7-10µg/100g FFA and 10-15µg/100g TFA, 20% >7µg/100g and >15µg/100g TFA. Most folate provided by vegetables followed by beef and potatoes. Fried potatoes had higher folate content compared to other cooking methods. Reheating reduces FFA by around 22%, TFA not changed. Meals that met the guidelines had a higher folate concentration
(Salunkhe et al., 1979)	Investigate the effect of long-term storage and temperature on the vitamin content of Ready-to-eat meals	USA	Ham and chicken loaf, beef steak, beef stew, frankfurters, fruitcake, pineapple, and chocolate covered brownies	Ascorbic Acid (AA) - titrated with 2,5-dichlorophenol-indophenol Thiamine– thiochrome method Riboflavin – fluorometric method	Storage at 4.4ºC or 54 months did not decrease thiamine, riboflavin and niacin content for ham and chicken loaf, beef steak, cheese spread. However, storage at 21oC decreased for all vitamins. Thiamine decreased in beef stew when kept at 4.4oC for 54 months.

*Cantonese fried rice, Lasagne with vegetables, Seitan, Broccoli pie, Vegetable pie, Chickpeas cooked (cocido), Chickpeas with spinach, Chickpeas with tripe (callos), Hummus, Lentil stew, Lentils with chorizo, Peas with ham, Couscous salad with chicken, Tabbouleh, Pumpkin, Vegetables mix, Zucchini, Artichokes sautéed, Artichokes with ham, Green beans sautéed, Omelette with spinach, Ratatouille, Vegetables sautéed, Mushrooms with garlic, Algae and eggplant, Broccoli, Cheese and broccoli, Seitan, tofu and vegetables, Soy and carrot, Soy and vegetables mix, Tofu and algae, Tofu, cheese and spinach, Tofu and mushrooms, Tofu and spinach, Tofu, spinach and cheeses

vii. RNI AND LRNI for amount of thiamine, riboflavin, folate, vitamin B12 and Vitamin C in different age groups according to the recommendation for MOW (Caroline Walker Trust, 2004)

		Age Group (years)				
		1.5-3	4-10	11-18	19-64	65+
Thiamine (mg/d)	RNI	0.5	0.7	1.3	1.3	1.3
	30%	0.15	0.21	0.39	0.39	0.39
Riboflavin (mg/d)	RNI	0.6	1	1.3	1.3	1.3
	30%	0.18	0.3	0.39	0.39	0.39
Vitamin B12 (µg/d)	RNI	70	150	200	200	200
	30%	21	45	60	60	60
Folate (µg/d)	RNI	50	100	200	200	200
	30%	15	30	60	60	60
Vitamin C (mg/d)	RNI	30	30	40	40	40
	30%	9	9	12	12	12

viii. The distribution of ready meal portion size according to NDNS age group. Descriptive statistical analysis (SD, mean, min, max, 95% CI, Shapiro Wilks test) and Chi-square statistical test.

Age Group	N	Mean (g)	SD	Min	Max	95% CI		Shapiro-Wilks	X2	Sig
						Lower	Upper			
1.5-3	96	144.63	73.94	14.40	360.00	129.65	159.61	0.007	288.10	<0.001
4-10	182	193.68	1.0.47	10.00	500.00	178.25	209.11	<0.001		
11-18	276	298.69	127.85	6.60	600.00	283.54	313.84	<0.001		
19-64	542	335.53	123.34	2.70	800.00	325.12	345.93	<0.001		
65+	209	326.44	112.36	50.00	750.00	311.11	341.76	<0.001		

ix. A table of descriptive statistics of the frequency of Cuisine types consumed by age group. Chi-Squared statistical analysis and significance values.

		Cuisine type						X2	Sig.
		British	Italian	Chinese	Indian	Mexican	Other		
Age group	1.5-3	29	50	3	2	0	1	109.8	<0.001
	4-10	52	90	6	18	3	0		
	11-18	45	125	4	55	14	0		
	19-64	133	169	21	104	20	10		
	65+	81	54	13	19	3	5		

x. A table to show the Percentage of thiamine, riboflavin, Vitamin B12 and folate intake provided by ready meals by NDNS age group, descriptive statistics (n, mean, SD, min, max, percentiles, 95% CI) and Kruskall-Wallis test significance

.

Vitamin (% from ready meals)	Age Group	N	Mean	SD	Min	Max	Percentile			95% CI		Sig
							25th	50th Median	75th	Lower	Upper	
Thiamine	1.5-3	87	6.62	7.67	0.13	31.97	1.30	3.46	9.11	4.99	8.26	<0.001
	4-10	169	5.79	5.83	0.11	37.94	1.65	3.86	8.04	4.90	6.67	
	11-18	243	8.66	8.36	0.12	50.86	2.50	6.16	12.40	7.60	9.71	
	19-64	457	8.56	8.71	0.01	47.84	2.55	5.41	12.06	7.76	9.36	
	65+	175	10.56	10.55	0.07	55.52	3.06	6.45	12.38	8.99	12.14	
Riboflavin	1.5-3	87	4.27	3.79	0.35	14.96	1.47	3.08	6.20	3.47	5.08	<0.001
	4-10	169	4.46	3.28	0.40	15.70	2.16	3.23	6.29	3.96	4.95	
	11-18	243	7.15	5.87	0.24	41.03	3.57	5.80	8.79	6.41	7.89	
	19-64	457	7.52	6.26	0.04	49.93	3.32	5.98	10.07	6.94	8.09	
	65+	175	7.77	6.80	0.06	49.11	3.81	6.06	10.83	6.75	8.78	
Folate	1.5-3	87	3.56	2.86	0.38	14.98	2.52	6.89	11.57	2.95	4.17	<0.001
	4-10	169	3.42	2.43	0.28	12.51	3.87	6.41	11.11	3.05	3.78	
	11-18	243	5.53	4.09	0.52	30.16	4.90	9.28	17.00	5.01	6.04	
	19-64	457	6.35	5.41	0.11	36.47	4.73	9.50	17.28	5.85	6.85	
	65+	175	6.39	5.17	0.32	29.93	4.32	12.56	20.88	5.62	7.17	
Vitamin B12	1.5-3	87	9.61	10.21	0.00	43.81	1.55	2.76	4.83	7.43	11.79	<0.001
	4-10	169	9.04	9.01	0.00	56.01	1.76	2.77	4.38	7.67	10.41	
	11-18	243	12.62	12.45	0.00	92.31	2.78	4.38	7.07	11.04	14.19	
	19-64	457	13.21	12.73	0.00	69.96	2.80	4.61	7.98	12.03	14.38	
	65+	175	14.84	13.56	0.00	74.47	2.92	4.75	8.32	12.81	16.87	
Vitamin C	1.5-3	87	1.36	1.94	0.00	9.19	0.00	0.51	1.98	0.95	1.78	<0.001
	4-10	169	1.81	3.71	0.00	30.57	0.03	0.64	1.85	1.24	2.37	
	11-18	243	3.59	6.22	0.00	52.23	0.45	1.43	3.64	2.80	4.37	
	19-64	457	5.68	10.24	0.00	81.70	0.32	1.69	6.87	4.74	6.62	
	65+	175	5.53	9.09	0.00	4.87	0.00	1.59	5.94	4.17	6.89	

xi. A table to show the average concentration of thiamine, riboflavin, vitamin B12, folate and vitamin C in ready meals consumed by age group, descriptive statistics (n, mean, SD, min, max, percentiles, 95% CI) and Kruskall-Wallis test significance.

	Age group	N	Mean	SD	Min	Max	Percentile			95% CI		P-Value
							25th	50th	75th	Lower	Upper	
Thiamine (mg/meal portion)	1.5-3	96	0.212	0.239	0.0036	0.99	0.040	0.105	0.317	0.164	0.261	<0.001
	4-10	182	0.283	0.302	0.0050	1.32	0.069	0.170	0.419	0.239	0.328	
	11-18	276	0.435	0.426	0.0064	1.98	0.129	0.280	0.600	0.385	0.486	
	19-64	542	0.396	0.380	0.0022	2.15	0.150	0.241	0.525	0.364	0.428	
	65+	209	0.487	0.505	0.0215	2.48	0.160	0.288	0.600	0.418	0.556	
Riboflavin (mg/portion)	1.5-3	96	0.175	0.133	0.0143	0.83	0.092	0.126	0.240	0.148	0.202	<0.001
	4-10	182	0.233	0.165	0.0140	0.84	0.124	0.180	0.300	0.209	0.258	
	11-18	276	0.327	0.220	0.0059	2.60	0.194	0.300	0.399	0.301	0.353	
	19-64	542	0.358	0.230	0.0014	1.68	0.214	0.320	0.440	0.339	0.378	
	65+	209	0.407	0.520	0.0370	5.20	0.218	0.325	0.440	0.336	0.478	
Folate (µg/portion)	1.5-3	96	16.072	10.032	1.5840	50.40	9.700	14.250	20.970	14.040	18.105	<0.001
	4-10	182	21.829	12.862	2.1000	67.50	12.969	17.800	28.500	19.948	23.710	
	11-18	276	35.740	19.780	2.6510	121.50	20.719	32.000	45.000	33.395	38.083	
	19-64	542	44.592	30.955	0.9450	326.40	28.000	39.050	56.000	41.980	47.204	
	65+	209	45.744	31.396	6.4000	192.00	28.000	40.000	55.500	41.462	50.026	
Vitamin B12 (µg/portion)	1.5-3	96	1.147	1.326	0.0000	9.00	0.483	0.800	1.303	0.878	1.416	<0.001
	4-10	182	1.360	1.618	0.0000	9.00	0.600	0.927	1.500	1.123	1.597	
	11-18	276	1.646	1.483	0.0000	13.80	0.800	1.355	2.000	1.470	1.821	
	19-64	542	2.062	2.464	0.0000	18.00	0.900	1.500	2.400	1.854	2.270	
	65+	209	2.403	2.337	0.0000	13.50	1.063	1.823	3.105	2.084	2.722	
Vitamin C (mg/portion)	1.5-3	96	2.388	3.650	0.0000	27.20	0.168	1.139	3.336	1.648	3.127	<0.001
	4-10	182	4.376	8.354	0.0000	64.00	0.000	1.500	5.000	3.154	5.598	
	11-18	276	6.766	10.359	0.0000	64.00	0.932	2.650	8.175	5.538	7.993	
	19-64	542	11.081	18.865	0.0000	159.12	1.175	3.600	11.700	9.489	12.672	
	65+	209	11.996	20.213	0.0000	93.60	0.000	3.000	13.130	9.240	14.752	

xii. A table to show the vitamin content of ready meals compared to 30% the RNI recommendations for thiamine, riboflavin, folate, vitamin B12 and vitamin C, descriptive statistics (mean difference, 95% confidence intervals) and one-sample test significance.

	Age group	Mean Difference	CI		-Value
Thiamine (mg/meal portion)	1.5-3	0.062	0.014	0.111	<0.05
	4-10	0.073	0.029	0.118	<0.05
	11-18	0.045	-0.005	0.096	0.079
	19-64	0.005	-0.026	0.038	0.726
	65+	0.12	0.052	0.19	<0.005
Riboflavin (mg/portion)	1.5-3	-0.01	-0.032	0.022	0.711
	4-10	-0.066	-0.066	-0.090	<0.001
	11-18	-0.0.62	-0.089	-0.037	<0.001
	19-64	-0.031	-0.051	-0.012	<0.001
	65+	0.017	-0.054	0.088	0.630
Folate (μg/portion)	1.5-3	6.829	4.943	8.710	<0.001
	4-10	-8.171	-10.05	-6.290	<0.001
	11-18	-24.261	-26.604	-21.917	<0.001
	19-64	-15.408	-18.020	-12.795	<0.001
	65+	-14.265	-18.537	-9.975	<0.001
Vitamin B12 (μg/portion)	1.5-3	-19.85	-20.12	-19.58	<0.001
	4-10	-43.64	-43.87	-43.40	<0.001
	11-18	-58.35	-58.53	-58.18	<0.001
	19-64	-57.94	-58.15	-57.73	<0.001
	65+	-57.60	-57.92	-57.28	<0.001
Vitamin C (mg/portion)	1.5-3	-6.61	-7.35	-5.87	<0.001
	4-10	-4.62	-5.85	-3.40	<0.001
	11-18	-5.23	-6.46	-4.01	<0.001
	19-64	-0.92	-2.51	0.672	0.257
	65+	-0.00	-2.76	2.75	0.998

xiii. A table of the sample information and preparation for the ready meals tested (Wiltshire Farm Foods, ASDA, ALDI, Tesco's Finest and Tesco's) including the component weights (g), date purchased (DD/MM/YY), cost (£) and microwave cooking method.

Provider	Range	N	Weight on packaging (g)	Average weight before cooking (g)	Average weight after cooking (g)	Mean sausage weight (g)	Mean Mashed Potato Weight (g)	Mean Gravy Weight (g)	Mean Peas Weight (g)	Date Purchased	Cost (£)	Cooking method
Wiltshire Farm Foods	Own Brand	2	380	417	384	95	105	56	126	10.07.2018	3.15	Microwave 900W, 2.5 min., stand 1 min., 2 min.
ASDA	Own Brand	2	450	439.5	430.5	116	231	71	Not applicable	31.01.19	2.20	Microwave 900W, 5.5 min.
ALDI	Own Brand	2	450	446	426.5	101.5	227	90.5	Not applicable	04.02.19	1.89	Microwave 900W, 5 min., stand 1 min., 2.5 min.
Tesco's	Tesco's Value Range	2	450	449.5	439.5	90.5	225.5	111.5	Not applicable	04.02.19	2.50	Microwave 900W, 5 min.
	Tesco's Finest	2	500	494	457.5	158	183	103.5	Not applicable	04.02.19	3.50	Microwave 900W, 5 min.

xiv. A table of exact Kruskal-Wallis analysis and significance values for the comparison of total vitamin content of thiamine, riboflavin, folate and vitamin C in ready meals between five different sausage and mash ready meals from four different providers

		Test statistic
Folate	Chi-Square	8.291
	Df	4
	Exact Sig.	.010
Riboflavin	Chi-Square	7.964
	Df	4
	Exact Sig.	.022
Thiamine	Chi-Square	7.636
	Df	4
	Exact Sig.	.034
Vitamin C	Chi-Square	8.400
	Df	4
	Exact Sig.	0.005

xv. A table of the comparison of riboflavin, folate and thiamine content in Wiltshire Farm Foods, ASDA, ALDI, Tesco's Finest and Tesco's sausage and mashed potato ready meals compared to 30% of thiamine and riboflavin, and 50% of the folate and vitamin C UK reference nutrient intakes for 1.5-3 years, 4-10 years, 11-18 years, 19-64 years and 65+ years.

			NDNS Age Group				
			1.5-3 years	4-10 years	11-18 years	19-64 years	65+ years
Thiamine	30% UK RNI (mg/meal portion)		0.15	0.21	0.39	0.39	0.39
	Wiltshire Farm Foods	Mean±SD (mg)	1.32±0.26				
	ASDA	Mean±SD (mg)	2.87±0.49				
	ALDI	Mean±SD (mg)	3.33±0.56				
	Tesco's Finest	Mean±SD (mg)	1.94±0.37				
	Tesco's	Mean±SD	1.62±0.172				
	Sign test sig. (exact)		0.001 (1 tailed), 0.002 (2 tailed)				
Riboflavin	30% UK RNI (mg/meal portion)		0.18	0.30	0.39	0.39	0.39
	Wiltshire Farm	Mean±SD (mg)	0.035±0.002				
	ASDA	Mean±SD (mg)	0.04±0.01				
	ALDI	Mean±SD (mg)	0.062±0.01				
	Tesco's Finest	Mean±SD (mg)	0.09±0.01				
	Tesco's	Mean±SD (mg)	0.07±0.02				
	Sign test sig. (exact)		0.001 (1 tailed), 0.002 (2 tailed)				
Folate	30% UK RNI (µg/meal portion)		15.00	30.00	60.00	60.00	60.00
	Wiltshire Farm	Mean±SD (µg)	7.94±0.39				
	ASDA	Mean±SD (µg)	9.64±0.16				
	ALDI	Mean±SD (µg)	6.63±1.05				
	Tesco's Finest	Mean±SD (µg)	11.97±1.24				
	Tesco's	Mean±SD (µg)	8.22±1.03				
	Sign test sig. (exact)		0.001 (1 tailed), 0.002 (2 tailed)				

			NDNS Age Group				
			1.5-3 years	4-10 years	11-18 years	19-64 years	65+ years
Vitamin C	50% UK RNI (mg/meal portion)		15	20	20	20	20
			1.5-3	4-10 years	11-18	19-64 years	
	Wiltshire Farm Foods	Mean±SD (mg)	0.84±0.18				
	ASDA	Mean±SD (mg)	0.21±0.05				
	ALDI	Mean±SD (mg)	0.28±0.07				
	Tesco's Finest	Mean±SD (mg)	0.55±0.03				
	Tesco's	Mean±SD (mg)	1.03+0.02				
	Sign test sig. (exact)		0.001 (1 tailed), 0.001 (2 tailed)				

xvi. Ingredients used in RMs Tested as of May 2021.

Wiltshire Farm Foods

Water, Peas, Potato, Cooked Pork (10%), WHEAT Flour (With Calcium, Iron, Niacin, thiamine), Vegetable Oils (Rapeseed, Sunflower), Onion, Cornflour, Butter (MILK), Salt, Dextrose, WHEAT Starch, WHEAT GLUTEN, Dried MILK, Yeast Extract, Tomato Puree, Beef Collagen Casing, Caramelised Sugar, WHEAT Protein, Natural Flavourings, Pepper, Concentrated Carrot Juice, Onion Powder, Chilli Powder, Concentrated Onion Juice, Mushroom Extract, Sugar, Dark Brown Sugar, Tomato Concentrate, Clove Extract, Mace Extract, Nutmeg Extract, Pepper Extract.

Aldi

Potato (45%), Cooked Pork Sausages (20%) [British Pork, WHEAT Flour (WHEAT Flour, Calcium Carbonate, Iron, Niacin, Thiamin), Water, Salt, Potato Starch, Dextrose, Rubbed Sage, Spices, Stabiliser: Diphosphates; Flavouring, Raising Agent: Ammonium Carbonates; Pork Rind, Ground Bay Leaf], Water, Onion (9%), Whole MILK, Butter (MILK), Cornflour, Rapeseed OIL, Double Cream (0.5%) (MILK), Beef Stock (Water, Beef Extract, Salt, Yeast Extract, Sugar, Beef Fat, Onion Concentrate, Carrot, Onion, Tomato Purée), Tomato Purée, WHEAT Flour (WHEAT Flour, Calcium Carbonate, Iron, Niacin, Thiamin), Worcester Sauce (Water, SPIRIT Vinegar, Sugar, Tamarind Extract, Garlic, Onion, Chilli, Clove, Concentrated Lemon Juice, Ginger), Pork Gelatine, Salt, Demerara Sugar, Garlic Purée, BARLEY Malt Vinegar, Colour: Plain Caramel; Black Pepper, White Pepper. Pork Sausages Filled into Beef Collagen Casings.

Tesco's finest

Mashed Potato [Potato, Skimmed Milk, Butter (Milk), Single Cream (Milk), Whole Milk, Salt, Sea Salt, White Pepper], Cumberland Sausage (27%) [Pork, Water, Rice Flour, Salt, Black Pepper, Sage, White Pepper, Mace, Preservative (Sodium Metabisulphite), Dextrose, Vegetable Fibre, Stabilisers (Tetrasodium Diphosphate, Disodium Diphosphate), Parsley, Caramelised Sugar, Colour (Paprika Extract)], Onion, Water, Tomato Purée, Chicken Extract, Cornflour, Gelling Agent (Pectin), Black Treacle, Sugar, Salt, Rapeseed Oil, Garlic Purée, Caramelised Sugar, Black Mustard Seeds, Sage, Thyme, Spirit Vinegar, Onion Concentrate, Black Peppercorns. Filled into natural casings.

Tesco's own brand

Mashed Potato [Potato, Skimmed Milk, Butter (Milk), Salt, White Pepper], Onion Gravy [Water, Onion, Cornflour, Tomato Purée, Chicken Extract, Rapeseed Oil, Caramelised Sugar, Muscovado Sugar, Wheat Flour, Vegetable Juices (Carrot, Mushroom, Onion), Garlic Purée, Sugar, Salt, Thyme, White Pepper], Pork Sausages (17%) [Pork, Wheat Flour, Water, Salt, Rapeseed Oil, Dextrose, Stabiliser (Sodium Triphosphate), White Pepper, Parsley, Mace, Yeast Extract, Ginger, Sage, Nutmeg]. Wheat Flour contains: Wheat Flour, Calcium Carbonate, Iron, Niacin, Thiamin. Sausages filled into natural casings.

Asda

Potatoes (45%), Water, Pork Sausage in Beef Protein Casing (20%) [Pork (70%), Fortified Wheat Flour [Wheat Flour, Calcium Carbonate, Iron, Niacin (B3), Thiamin (B1)], Water, Salt, Potato Starch, Dextrose, Dried Herbs, Spices, Stabiliser (Diphosphates), Flavouring, Rapeseed Oil, Sausage Casing [Beef Protein, Cellulose]], Onions, Red Wine, Unsalted Butter (Milk), Cornflour, Single Cream (Milk), Garlic Purée, Worcester Sauce [Water, Spirit Vinegar, Sugar, Tamarind Extract, Onion Powder, Garlic Powder, Spices, Concentrated Lemon Juice], Sugar, Salt, Tomato Paste, Beef Extract, Rapeseed Oil, Colour (Plain Caramel), Beef Powder, Spices, Caramelised Sugar Syrup

xvii. A table to show the descriptive statistics of the thiamine (mg/meal component portion) content of sausage, mashed potato, gravy and mean, standard deviation, interquartile rage (IQR), minimum and maximum values for Wiltshire Farm Foods (n=6), ASDA (n=6), Tesco's Finest (n=6) and Tesco's (n=6).

		n	Mean	Standard deviation	median	IQR	Min	Max
Sausage	Wiltshire Farm Foods	6	0.51	0.11	0.50	0.19	0.37	0.66
	ASDA	6	0.67	0.24	0.64	0.49	0.41	0.96
	ALDI	6	0.45	0.12	0.43	0.25	0.30	0.60
	Tesco's Finest	6	0.52	0.05	0.51	0.08	0.47	0.60
	Tesco's	6	0.80	0.39	0.74	0.74	0.42	1.31
Mash	Wiltshire Farm Foods	6	1.27	0.41	1.26	0.76	0.82	1.73
	ASDA	6	0.16	0.06	0.15	0.12	0.10	0.25
	ALDI	6	0.31	0.04	0.31	0.06	0.25	0.37
	Tesco's Finest	6	1.26	0.82	1.18	1.52	0.47	2.19
	Tesco's	6	0.77	0.32	0.76	0.61	0.44	1.10
Gravy	Wiltshire Farm Foods	6	1.12	0.81	0.72	1.49	0.44	2.36
	ASDA	6	0.34	0.04	0.33	0.06	0.28	0.41
	ALDI	6	0.17	0.05	0.16	0.11	0.12	0.24
	Tesco's Finest	6	1.76	1.10	1.58	2.07	0.62	3.15
	Tesco's	6	0.13	0.05	0.13	0.08	0.08	0.20

xviii. A table to show the descriptive statistics of the riboflavin (mg/meal component portion) content of sausage, mashed potato, gravy and mean, standard deviation, interquartile rage (IQR), minimum and maximum values for Wiltshire Farm Foods (n=6), ASDA (n=6), Tesco's Finest (n=6) and Tesco's (n=6).

		n	Mean	Standard deviation	median	IQR	Min	Max
Sausage	Wiltshire Farm Foods	6	0.01	0.00	0.01	0.00	0.00	0.01
	ASDA	6	0.02	0.01	0.02	0.02	0.01	0.03
	ALDI	6	0.02	0.01	0	0.01	0.01	0.02
	Tesco's Finest	6	0.04	0.01	0.03	0.01	0.03	0.05
	Tesco's	6	0.02	0.01	0.02	0.01	0.01	0.02
Mash	Wiltshire Farm Foods	6	0.01	0.00	0.01	0.01	0.01	0.00
	ASDA	6	0.01	0.00	0.01	0.00	0.01	0.00
	ALDI	6	0.02	0.00	0.02	0.00	0.02	0.01
	Tesco's Finest	6	0.03	0.01	0.03	0.02	0.04	0.02
	Tesco's	6	0.01	0.00	0.01	0.00	0.01	0.01
Gravy	Wiltshire Farm Foods	6	0.02	0.01	0.02	0.01	0.01	0.02
	ASDA	6	0.02	0.01	0.02	0.02	0.01	0.03
	ALDI	6	0.03	0.01	0.03	0.02	0.02	0.04
	Tesco's Finest	6	0.03	0.01	0.03	0.01	0.02	0.04
	Tesco's	6	0.05	0.02	0.05	0.04	0.03	0.07

xix. A table to show the descriptive statistics of the folate (µg/meal component portion) content of sausage, mashed potato, gravy and mean, standard deviation, interquartile rage (IQR), minimum and maximum values for Wiltshire Farm Foods (n=6), ASDA (n=6), Tesco's Finest (n=6) and Tesco's (n=6).

		n	Mean	Standard deviation	median	IQR	Min	Max
Sausage	Wiltshire Farm Foods	6	1.22	0.41	1.16	0.69	0.83	1.81
	ASDA	6	2.09	0.42	2.05	0.70	1.55	2.66
	ALDI	6	1.65	0.22	1.68	0.20	1.24	1.91
	Tesco's Finest	6	1.90	0.14	1.91	0.27	1.75	2.08
	Tesco's	6	1.08	0.21	1.07	0.40	0.86	1.30
Mash	Wiltshire Farm Foods	6	1.53	0.14	1.51	0.28	1.38	1.69
	ASDA	6	7.21	1.71	7.04	3.06	5.41	9.60
	ALDI	6	4.32	1.12	3.99	1.68	3.13	6.29
	Tesco's Finest	6	8.74	1.18	8.70	2.17	7.59	9.97
	Tesco's	6	5.78	0.57	5.80	1.03	5.17	6.32
Gravy	Wiltshire Farm Foods	6	0.71	0.14	0.70	0.27	0.52	0.89
	ASDA	6	0.35	0.18	0.44	0.22	0.00	0.47
	ALDI	6	0.65	0.01	0.65	0.02	0.63	0.66
	Tesco's Finest	6	1.33	0.08	1.31	0.15	1.25	1.43
	Tesco's	6	1.36	0.03	1.35	0.05	1.34	1.41

xx. A table to show the descriptive statistics of the vitamin C (mg/meal component portion) content of sausage, mashed potato, gravy and mean, standard deviation, interquartile rage (IQR), minimum and maximum values for Wiltshire Farm Foods (n=2), ASDA (n=2), Tesco's Finest (n=2) and Tesco's (n=2).

		n	Mean	Standard deviation	median	IQR	Min	Max
Sausage	Wiltshire Farm Foods	2	015.	0.01	0.15		0.15	0.16
	ASDA	2	0.18	0.06	0.18		0.13	0.22
	ALDI	2	0.00	0.00	0.00		0.00	0.00
	Tesco's Finest	2	0.42	0.14	0.42		0.32	0.52
	Tesco's	2	0.70	0.02	0.70		0.69	0.71
Mash	Wiltshire Farm Foods	2	0.20	0.00	0.20		0.20	0.20
	ASDA	2	0.00	0.00	0.00		0.00	0.00
	ALDI	2	0.23	0.05	0.23		0.20	0.26
	Tesco's Finest	2	0.09	0.12	0.09		0.00	0.17
	Tesco's	2	0.33	0.06	0.33		0.29	0.38
Gravy	Wiltshire Farm Foods	2	0.05	0.07	0.05		0.00	0.11
	ASDA	2	0.04	0.05	0.04		0.00	0.08
	ALDI	2	0.05	0.07	0.05		0.00	0.10
	Tesco's Finest	2	0.05	0.07	0.05		0.00	0.09
	Tesco's	2	0.00	0.00	0.00		0.00	0.00

xxi. A table of test statistics and significance values comparing mean folate, riboflavin, thiamine and vitamin C content of sausage, mashed potato and gravy components across ready meal providers in sausage and mash ready meals.

	Folate		**Riboflavin**		**Thiamine**		**Vitamin C**	
	F	Sig.	F	Sig.	F	Sig.	X2	Sig. exact
Sausage	12.406	P<0.001	15.114	P<0.001	2.442	0.073	8.341	<0.05
Mashed potato	38.812	P<0.001	31.295	P<0.001	8.571	P<0.001	8.161	<0.05
Gravy	6.664	0.001	6.664	0.001	8.092	P<0.001	1.731	1

xxii. A table of descriptive statistics for riboflavin content (mg/meal portion), folate (µg/meal portion), thiamine (mg/meal portion), price (£), meat content in meal (%) and mashed potato content in meal (%) across the ready meals tested.

		Statistic	Std. Error
Riboflavin (mg/meal portion)	Mean	.0611	.00741
	Median	.0582	
	Std. Deviation	.02343	
	Range	.06	
	Interquartile Range	.05	
Folate (µg/meal portion)	Mean	8.8812	.64021
	Median	8.5845	
	Std. Deviation	2.02451	
	Range	6.96	
	Interquartile Range	2.63	
Thiamine (mg/meal portion)	Mean	2.2160	.27065
	Median	1.9694	
	Variance	.732	
	Std. Deviation	.85586	
	Range	2.59	
	Interquartile Range	1.50	
Vitamin C (mg/meal portion)	Mean	0.5843	0.10987
	Median	0.5508	
	Variance	0.121	
	Std. Deviation	0.3475	
	Range	0.93	
	Interquartile Range	0.73	
Price (£)	Mean	2.6680	.20459
	Median	2.5000	
	Std. Deviation	.64696	
	Range	1.61	
	Interquartile Range	1.19	
Meat in meal (%)	Mean	26.8382	2.13604
	Median	23.9336	
	Std. Deviation	6.75475	
	Range	14.50	
	Interquartile Range	13.66	
Mashed Potatoes in meal (%)	Mean	47.3311	2.03078
	Median	50.8009	
	Std. Deviation	6.42190	
	Range	14.85	
	Interquartile Range	11.94	

xxiii. A table of spearman's rank correlations and significance values for the associations between riboflavin content (mg/meal portion), folate (µg/meal portion), thiamine (mg/meal portion), price (£), meat content in meal (%) and mashed potato content in meal (%)

Variable 1	Variable 2	Spearman's Correlation	Sig. (2-tailed)
Riboflavin (mg/meal portion)	Folate (µg/meal portion)	0.285	0.425
	Thiamine (mg/meal portion)	0.224	0.533
	Price (£)	0.197	0.586
	Meat in meal (%)	-0.615	0.058
	Mashed potato in meal (%)	-0.049	0.893
	Vitamin C (mg/meal portion)	0.127	0.726
Folate (µg/meal portion)	Thiamine (mg/meal portion)	-0.079	0.829
	Price (£)	0.64	<0.05
	Meat in meal (%)	-0.345	0.329
	Mashed potato in meal (%)	0.098	0.787
	Vitamin C (mg/meal portion)	-0.067	0.855
Thiamine (mg/meal portion)	Price (£)	-0.689	<0.05
	Meat in meal (%)	0.566	0.088
	Mashed potato in meal (%)	-0.788	<0.01
	Vitamin C (mg/meal portion)	-0.842	<0.01
Price (£)	Meat in meal (%)	-0.8	<0.01
	Mashed potato in meal (%)	0.8	<0.01
	Vitamin C (mg/meal portion)	0.517	0.126
Meat in meal (%)	Mashed potato in meal (%)	-0.700	<0.05
	Vitamin C (mg/meal portion)	-0.665	<0.05
Vitamin C (mg/ meal portion)	Mashed potato in meal (%)	0.665	<0.05

xxiv. Descriptive statistics of the weight reduction after reheating and hot-holding for 0, 1, 2 and 3 hours, and the temperature of the meals after heating and after hot-holding.

Time (T)	Mean Meal Weight Before Reheating (G)	Mean Meal Weight After Reheating (G)	Average Change in Weight After Reheating (%)	Weight of Meal After Hot-Holding (G)	Average Change in Weight After Heating and Hot-Holding (%)	Average Temperature of Meal After Reheating (°C)	Average Temperature of Meal After Hot Holding (°C)
0	377.5	355	5.964			75	
1	373.5	355.5	4.821	349	1.809	72.5	88.5
2	374	362	3.225	345	4.746	76.5	82.5
3	371.5	358.5	3.500	321.5	10.340	71.5	90

xxv. Descriptive statistics of thiamine (mg/portion), riboflavin (mg/portion), folate (μg/portion), and vitamin C (mg/portion) in sausage and mash ready meal components (sausage, mashed potato, peas and gravy).

		N	Mean	SD	Min	Max	Percentile			X2	Sig.
							25th	50th	75th		(exa
Thiami	Sausa	8	0.3988	0.34279	0.04	1.04	0.1091	0.3326	0.6686	4.1	0.24
	Mash	8	0.3860	0.25509	0.19	0.98	0.2091	0.3317	0.4169	3.0	0.39
	Peas	8	0.5102	0.63738	0.04	1.76	0.0899	0.2350	1.0245	517	0.16
	Grav	8	0.2331	0.15032	0.09	0.53	0.1236	0.1774	.3430	0.5	0.95
Ribofla	Sausa	8	0.00867	0.005737	0.001	0.021	0.00546	0.00777	0.01025	4.5	0.26
	Mash	8	0.00908	0.003614	0.003	0.013	0.00576	0.01036	0.01172	3.8	0.36
	Peas	8	0.01466	0.003988	0.007	0.022	0.01259	0.01481	0.01596	6.1	0.03
	Grav	8	0.01650	0.005917	0.008	0.025	0.01181	0.01551	0.02227	2.0	0.68
Folate	Sausa	8	1.2679	0.37596	0.86	1.98	0.9180	1.2041	1.5201	3.5	0.41
	Mash	8	1.1163	0.34159	0.72	1.65	0.7588	1.1400	1.3841	6.6	0.10
	Peas	8	8.0455	3.21112	4.42	13.37	4.8419	8.0394	10.5457	4.1	0.32
	Grav	8	0.5883	0.21900	0.22	0.83	0.4425	0.5790	.8169	2.5	0.60
Vitami	Sausa	8	0.0763	0.05795	0.00	0.16	0.0172	0.0712	0.1339	5.7	0.08
	Mash	8	0.1313	0.08949	0.00	0.23	0.0285	0.1531	0.2034	2.2	0.65
	Peas	8	0.2413	0.19358	0.09	0.58	0.1129	0.1560	0.4342	5.5	0.11
	Grav	8	0.0451	0.04200	0.00	0.11	0.0000	0.0522	0.0775	2.9	0.54

xxvi. Descriptive statistics for the retention of vitamins in sausage and mashed potato ready meal components (sausage, mashed potato, peas and gravy)

		N	T=0		T=1		T=2		T=3	
			mg/portion	Retention (%)	mg/portion	Retention (%)	Mg/portion	Retention (%)	Mg/portion	Retention (%)
Thiamin	Sausag	8	0.737	100	0.667	90.5	0.448	60.8	0.998	135.5
	Mash	8	0.418	100	0.315	75.3	0.109	26.0	0.653	156.2
	Peas	8	0.107	100	0.190	177.3	0.149	138.5	0.184	171.2
	Gravy	8	0.333	100	0.372	111.8	0.227	68.2	0.205	61.8
Riboflavi	Sausag	8	0.007061	100	0.006948	98.4	0.016026	227.0	0.004648	65.8
	Mash	8	0.010455	100	0.004918	47.0	0.012405	118.7	0.00858	82.1
	Peas	8	0.01433	100	0.01354	94.5	0.020795	145.1	0.018445	128.7
	Gravy	8	0.01458	100	0.010148	69.6	0.014918	102.3	0.019023	130.5
Folate	Sausag	8	0.001215	100	0.001290	106.1	0.001598	131.5	0.000966	79.5
	Mash	8	0.001528	100	0.001254	82.1	0.000952	62.3	0.000729	47.7
	Peas	8	0.000708	100	0.000611	86.3	0.000639	90.2	0.000393	55.5
	Gravy	8	0.004490	100	0.008046	179.2	0.009532	212.3	0.010112	225.2
Vitamin	Sausag	8	0.155	100	0.035	22.6	0.085	54.8	0.035	22.6
	Mash	8	0.2	100	0.115	57.5	0.12	60.0	0.09	45.0
	Peas	8	0.055	100	0	0.0	0.06	109.1	0.065	118.2
	Gravy	8	0.55	100	0.14	25.5	0.165	30.0	0.115	20.9

xxvii. Statistics analysis of the total thiamine (mg/meal), riboflavin (mg/meal), folate (mg/meal) and vitamin C (mg/meal) of the sausage and mashed potato ready meals

	N	Mean (mg/meal)	SD	Min	Max	Percentile			Sig. (exact)
						25th	50th Median	75th	
Thiamine	8	1.53	1.01	0.52	3.56	0.91	1.03	2.21	0.881
Riboflavin	8	0.05	0.01	0.03	0.06	0.04	0.05	0.06	0.418
Folate	8	11.02	3.11	7.67	16.90	8.30	10.63	12.88	0.244
Vitamin C	8	0.50	0.32	0.11	1.04	0.25	0.44	0.68	0.212