Studies of the nutritional quality of commercial 'ready to eat' infant foods in the United Kingdom

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DECLARATION

"I certify this work has not been accepted in substance for any for any degree, and is not concurrently being submitted for any degree, other than that of Doctor of Philosophy, being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised another's work''.

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ABSTRACT

Studies of the nutritional quality of commercial 'ready to eat' infant foods in the United Kingdom

Infancy is a time of rapid physiological (e.g. anthropometric, immunological and neurological) development. Hence, during this period of life nutritional requirements are at their highest in relation to body mass. There is a paucity of data with respect to the nutritional quality of complementary foods manufactured in the UK for infants and young children. The primary objective of this study was to examine the nutritional value of 'ready to feed' complementary infant foods on the UK market in order to ascertain their suitability, relative to dietary guidelines, for the target group.

Quantitative analysis was conducted on eight different products representing four popular commercial brands (meat and vegetable based) currently on sale in the UK for infants aged between 6-12 months. The chemical analyses conducted included Kjeldhal for protein, acid hydrolysis and extraction for fat, phenol sulphuric acid for carbohydrate and AOAC 985.29 for fibre. The results of these studies were referenced to the Recommended Nutrient Intake (RNI) values for 6 to 9 months old children, and a listing of the entire daily intake of nutrients was composed taking into consideration the nutrient and energy intake from milk consumption in order to (1) accurately estimate the daily intake of these nutrients derived from commercial infant food consumption, and (2) ascertain their nutritional suitability relative to dietary guidelines for the 6-9 month age group. The only significant difference found between different product varieties (meat and vegetable-based) was with respect to the protein content ($p = 0.04$) per 100 g of food. The experimentally determined concentrations of macronutrients (g/100 kcal) were compared to the declared values provided by the manufacturers on the product labels and, despite some variations, the values obtained comply with regulatory requirements (Commission Directive 2006/125/EC). The total daily intake of fat (27.0 g/day), based on the menu composed from commercial complementary food, is suggested to exceed the Dietary Reference Values (DRVs) for fat (31%), *if the intake of snacks and desserts are incorporated*. The aforementioned results imply that the formulations of the recipes, based on a standard commercial menu, are of significant importance in relation to the nutritional quality of the diet of infants.

In terms of elemental analysis, the concentrations of up to twenty (essential and non-essential) elements in a selected range of sixteen different products representing meat, poultry, fish and vegetable base varieties were established by ICP-OES and ICP-MS. Six major essential elements, namely: calcium, iron, magnesium, potassium, sodium and zinc were measured by ICP-OES. The concentrations of six essential trace elements (selenium, molybdenum, cobalt, copper, chromium, manganese) and eight non-essential, potentially toxic, elements (arsenic, barium, nickel, cadmium, antimony, lead, mercury, aluminium) in chicken and fish-based varieties were determined by ICP-MS due to the higher sensitivity required.

Based on the results of elemental analysis, there was also some evidence of a lack of attention to micro-nutrient interactions in food. With reference to the guidelines, the RNI values for 6 to 9 month olds, all samples provided less than 20% of RNI values except for potassium (20%). In terms of the risk of exposure to toxicity, the concentration of non-essential elements in ready to feed products analysed were not considered to be of concern.

With regard to the analyses of vitamins, a novel assay for the simultaneous quantitative determination of riboflavin (B_2) and pyridoxine (B_6) has been developed. The method involves a mild hydrolysis step, extraction of the supernatant by centrifugation followed by quantitative analysis using UHPLC. Separation of the two water soluble vitamins achieved is excellent and rapid - within one minute whilst the resultant sample is also LC-MS compatible.

With respect to vitamin B analyses, despite wide individual differences between brands (p = 6.5e-12), no significant differences were observed in the levels of vitamin B_6 between the meat and vegetable-based varieties (p = 0.7) per 100 g of commercial infant food. Vitamin B_2 was not detected in any of the samples, where the detection limit was below 0.07µg/mL. In terms of the RNI of vitamin B_6 for 6 - 9 month old infants, the complementary infant meal products analysed herein provided less than 15% of the RNI values with mean (SD) values of 12.87 (±4.46) % and 13.88 (±4.97) % for the meat- and vegetable-based recipes, respectively. The estimated total daily intake of vitamins B_2 and B_6 from the consumption of commercial complementary food was found to be satisfactory and in accordance with the DRVs. The intake of both vitamin B_2 and B_6 was estimated to be mainly derived from the consumption of formula milk which could be a cause of concern if the quality of an infant's milk diet is compromised by an inadequate or lack of supplemented milk intake. All the foregoing results

suggest that commercial complementary infant foods on the UK market may not contain minimum levels of micronutrients required for labelling declaration of micronutrient content (*Commission Directive 2006/125/EC*).

An attempt, therefore, was made to optimise the formulated version of the meat based infant food as a baseline and measure the post-process retention of its nutrient content after being subjected to different processing condition in terms of a combination of temperature and time. This was achieved by quantitative analysis of the post-process values of the nutrients in the optimised formula using the aforementioned analytical techniques. The results of this study indicates that careful formulation of the recipes, in the context of new product development, is important; the selection of high quality ingredients and the ratios in which they are used have a direct effect on the nutrient content of the final product. It also indicates that a carefully controlled temperature-time combination, pH, pressure and macroscopic conditions of processing (e.g. controlled leaching) are very important in reducing heat loss and improving the nutritional quality of the food product. This provides opportunities and scope for product optimisation, of ready to eat to eat infant foods, in order to improve their nutritional value.

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ABREVIATIONS

Abbreviation	Meaning
ALA	Alpha (α) Linolenic Acid
ANOVA	Analysis Of Variance
ARA	Arachidonic Acid
AOAC	Association of Official Analytical Chemists
BMR	Base Metabolic Rate
BBC	British Broadcasting Corporation
BMD	Bone Mass Density
BRC	British Retail Consortium
CNS	Central Nervous System
CRM	Certified Referenced Material
Codex	Codex Alimentarius Standards
CFU	Colony Forming Units
COMA	Committee on Medical Aspects of Food and Nutrition Policy
DRV	Daily Recommended Value
DVs	Daily Values
DoH	Department of Health
DRI	Dietary Reference Intake
DPASV	Differential Pulse Anodic Stripping Voltammetry
DHA	Docosahexaenoic Acid
ESI-MS	Electrospray Ionization- Mass Spectrometry
EAR	Estimated Average Requirement
EE	Energy Expenditure
EER	Estimated Energy Requirement
EFA	Essential Fatty Acids
EOV	Estimated Optimised Values
FAD	Flavin Adenine Dinucleotide
FDA	Food and Drug Administration
FFM	Fat Free Mass
FMN	Flavin Mononucleotide

FSA	Food Standards Agency
GI	Gastrointestinal
HPLC	High Performance Liquid Chromatography
HPP	High Pressure Processing
HTST	High Temperature/Short Time
HR-ICP-MS	High Resolution Inductivity Coupled Plasma–Mass Spectrometry
INQ	Index of Nutritional Quality
ICP-OES	Inductivity Coupled Plasma –Optical Emission Spectrometry
IOM	Institute of Medicine
LOD	Limit of Detection
LOQ	Limit of Quantification
LA	Linoleic Acid
LC-MS	Liquid Chromatography-Mass Spectrometry
LCPUFA	Long-Chain Polyunsaturated Fatty Acids
LTLT	Low Temperature/Long Time
MRD	Maximum Recovery Diluent Solution
NatCen	National Centre for Social Research
NDNS	National Diet and Nutrition Survey Program
NPD	New Product Development
NADH	Nicotinamide Adenine Dinucleotide
NOFT	Non-Organic Failure to Thrive
ND	Nutrient Density
OFR	Optimised Formulated Recipe
PARNUTS	Particular Nutritional Uses
PPAR	Peroxisome Proliferator-Activated Receptors
PALs	Physical Activity Levels
PPE	Post-Process Evaluations
PPV	Post-Process Values
RNI	Recommended Nutrient Intake
RF	Referenced Values
SACN	Scientific Advisory Committee on Nutrition
SCF	Scientific Committee on Food

TBF	Total Body Fat
TDI	Total Daily Intake
TEE	Total Energy Expenditure
TFA	Trans Fatty Acid
UHPLC	Ultra-High Pressure Liquid Chromatography
UHT	Ultra-High-Temperature
WDXRF	Wavelength-Dispersive X-Ray Fluorescence
WHO	World Health Organisation

Chapter 1

Overview of the thesis

Infancy is a time of rapid physiological (e.g. anthropometric, immunological and mental) development. Hence, during this period of life nutritional requirements are at their highest rate per unit of body mass (Holden & Anita, 2000; Robert & Kraisid, 2001). Phenotypic expression of food preferences in childhood, which may persist into later life, depend on interactions between genetic pre-dispositions and the early life eating environment (Fisk *et al*. 2011; Stang, 2006; Skinner *et al*. 2002).

Observational studies in industrialised countries suggest that there is a strong link between rapid weight gain in infancy and later risk of obesity in childhood and adulthood (Baird *et al*., 2005; Ong & Loos, 2006; Wells & Victora, 2005). National statistical data shows that children typically have a diet high in energy dense food, saturated fat and non-milk extrinsic sugar but low in fibre, fruit, vegetable and oily fish (SACN, 2008). This is evident in the increased sales and consumption of pre-packed foods in the UK, extending to food consumed by infants and young children (Lobstein *et al*. 2004). The modern life style dynamic has, therefore, lead to an increased parental reliance on commercially marketed complementary foods in the UK (White & Hampson 2008; Bolling *et al*,. 2007), which may have potential implications for total energy and fat intake in addition to taste acquisition (DoH, 2006, SACN, 2011). The foregoing factors can negatively impact on the risk of chronic non-communicable diseases (Rudolf 2009).

Currently insufficient attention has been paid to the nutritional quality of ‘ready to feed’ complementary foods and there is a paucity of data in respect of the nutritional quality of “ready to feed” complementary foods marketed in the UK for infants aged between 6-12 months. In relation to the quality of infant formula milk, however, there have been several studies conducted on the composition of fortified formulas in relation to their nutrient quality, bioavailability and the safety levels of toxic elements (Ikem *et al.*, 2001; SCF/CS/NUT/IF/65, 2003; Bass & Chan, 2006; Jestoi *et al.*, 2009).

Under the food labelling regulations in Great Britain [1996 (FLR) (SI 1996 No.1499) provision of the EC nutrition labelling Directive (90/496/EEC)] micronutrient declaration is non-mandatory. According to the Commission Directive 2006/125/EC, the micronutrient content can only be declared when at least 15% of the reference values are supplied per 100 g (or 100 mL) of the product (Food Standard Agency, 2004; the Commission of the European Communities, 2006). The current lack of legislative attention paid to the nutritional quality of infant 'ready to eat' complementary foods (Processed Baby Foods Regulations 1997/2042), suffers from the fact that it comes under the same legislative category as adult 'ready meals'. The requirements concerning the essential nutrient composition of food marketed as 'ready meals' for adults are therefore, generally, not particularly robust, since the nutritional value of these food products are not critical to their target groups. As a result infant ready meals are not as well regulated as- perhaps- they should be.

To date, there has been little discussion relating to the challenges concerning the nutritional composition of such products available in the UK and there have been no controlled studies comparing the dietary intake from the consumption of these foods with reference to the Recommended Nutrient Intake (RNI) in order to ascertain their suitability (DoH, 1991).

Currently, the European Commission has proposed a revised framework for the legislation relating to foodstuffs intended for **par**ticular **nut**ritional uses (PARNUTS) covered by the "Framework Directive on dietetic food" (Directive 2009/39/EC). The Framework aims to remove ambiguity in defining PARNUTS, reduce legislative burden and facilitate innovation and trade in the single market, whilst protecting vulnerable consumers. The proposed regulation will include processed cereal-based foods and baby foods for infants and young children as well as follow on formulas and medical foods. The proposed regulation however, does not introduce any compositional rules for existing PARNUTS foods and will retain the current rules to categorise food established under the current specific legislation. The new regulation is not expected to come into force for the next few years and will "*apply 2 years after the entry to force".*

The focus of the current study is therefore related to the lack of attention paid to the nutritional quality of 'ready to feed' complementary foods marketed for infants of 6 to 9 months of age.

1.1 Research Questions

The issues that this study will address are as follows.

1) What is the macro/micronutrient content of selected commercial infant foods?
2) What is the extent to which commercial infant food meets the nutritional requirements of infants in relation to the current nutritional guidelines?
3) Is it possible to improve the retention of the nutrient content of commercial 'ready to eat' complementary food via re-formulation and by judiciously employing an appropriate thermal processing technique as a part of the production of an infant ready meal

1.2 Aims and objective

This study aims to:

1) ascertain the nutritional suitability of commercial infant foods currently available in the UK market in relation to the relevant dietary guidelines for 6-9 month old infants. This will be achieved by undertaking a quantitative analysis of both the macro- and micronutrient content of a series of commercial 'ready to eat' infant foods.
2) examine potential methods of optimising the nutrient content of 'ready to eat' infant foods marketed in the UK.

The objectives of the studies reported in this thesis are as follows.

1) To quantitatively determine the macro-nutrient content of a series of commercial "ready to-feed" baby meals for infants of 6-9 months old currently available in the UK market. The analytical strategies employed include: (a) derived methods for energy, (b) Kjeldhal for protein, (c) phenol sulphuric acid for carbohydrate and (d) acid

hydrolysis extraction for the analysis of fat content. The results of this work are then evaluated in relation to the dietary guidelines for infants of 6-9 months and are reported in Chapter 3.

2) To quantitatively measure the concentration of up to 20 essential and non-essential elements in the selected range of commercial infant food products in the UK market by Inductivity Coupled Plasma–Optical Emission Spectrometry (ICP-OES) and Inductivity Coupled Plasma–Mass Spectrometry (ICP-MS). The results of this work are also compared to the relevant dietary guidelines and are reported in Chapters 4 and 5.

3) To develop a simultaneous extraction and quantification method for the analysis of vitamins B_2 and B_6 by Ultra High Pressure Liquid Chromatography (UHPLC). The proposed assay will also be specifically developed to be LC-MS (Liquid Chromatography-Mass Spectrometry) compatible and the results are presented in Chapter 6. This novel assay is then employed to evaluate the role of the selected commercial infant food in meeting the vitamin B_2 and B_6 requirements in infants of the target age group based on dietary guidelines.

4) To attempt to optimise the nutritional content of an existing infant food product via the re-formulation of the recipes and examining the effects of the various thermal processing conditions to include: High Temperature Short Time (HTST) and Low Temperature Long Time (LTLT), on the safety and post-process integrity of the optimised formulas, as described in Chapter 7.

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Chapter 2

General introduction

2.1 The impact of nutrition on infant growth and development

As suggested by Karlberg *et al*., (1987) human growth is a dynamic process consisting of three distinct phases described as the infancy-childhood–puberty model (ICP). Based on this model, the regulation of growth differs between each phase and its speed appears to be at its' most rapid rate during the phase of infancy. Growth, during infancy, is a continuation of foetal growth in the pre-natal phase and in the neonatal phase is dependent upon maternal, placental and foetal factors (Barker *et al*., 2002; Collinson *et al*., 2003; Gokhale & Kirschner, 2003, Monaghan, 2008; Cole *et al*., 2009; Wells, 2009).

Normal growth, however, is not a uniform process and other variables may also influence growth such as gender, race and post-natal age (Rupich *et al*,. 1996: Tappy, 2005). On the whole, the process of growth is influenced by the interaction of a range of factors including genotype, intra- and extra-uterine exposure to environmental factors that occur before/during infancy and early childhood e.g. hormonal factors, nutrition, infection and socio-emotional interchanges (Maurage, 2008). Any disruption to the interaction of the foregoing factors, regardless of the cause (e.g. disease, genetic changes or malnutrition) results in an abnormal growth rate (Gibson & Hots, 2000; Melo *et al.*, 2008). As a result, these factors cannot be examined in isolation. Nutrition, however, seems to play a stronger role in the regulation of growth during the infancy phase than later during the childhood phase. Nutrients are likely to have a direct stimulatory effect on e.g. the insulin-like growth factor-1 (IGF-1), bypassing the growth hormone axis, and it is possible that nutrients might also affect growth directly through a stimulation of insulin secretion (Michaelsen & Fariis, 1998). Thus, growth appears to proceed at a normal rate if an infant is adequately nourished. Growth monitoring is an important tool in the early detection of disease in children and, on occasion, may be the initial or only manifestation of an underlying chronic disease (Gokhale & Kirschner, 2003).

The importance of providing an appropriate energy and nutrient intake during early life is emphasized by the so called "thrifty phenotype" hypothesis, which refers to the programming

of cells during pre-natal and early post-natal development. Based on this hypothesis, a "nutritional insult" during foetal life, leads to a permanent metabolic alteration, which predisposes the child to metabolic abnormalities e.g. obesity and diabetes upon exposure to environmental factors such as sedentary lifestyle and/or high energy intake (Tappy, 2005; de Moura & Passos, 2005; Regan , 2006; Rotteveel *et al.*, 2008).

Nutrition therefore, appears to be fundamental in the development of an infant to full potential and the period from birth to two years of age is known as the "critical window" for promotion of growth, health and behavioural development (WHO, 2001). It is therefore important to maintain a balanced nutritional intake during this period as longitudinal studies have consistently shown that this is the peak age for most nutritional related impairments with irreversible consequences on adulthood (Schlenker & Williams, 2003; Taylor *et al.*, 2004, Nomura, 2009).

2.1.1 Nutritional aspects of physiological and biological development

The diet of an infant should be capable of providing adequate energy and nutrients for their growth, building body stores, maintenance of their development and repair followed an infection or illness. One of the useful indicators of healthy growth and development are anthropometric measurements. The sequential measurements of growth or growth velocity are much more meaningful, since they represent growth dynamics over time (Gokhale & Kirschner, 2003). Most healthy neonates should have doubled their birth weight by 5 months and trebled it by the end of their first year. An increase in body length also happens rapidly with an average increase of 25 cm in the first year followed by 12 cm over the next year of life (Gokhale & Kirschner, 2003). Although head circumference is less of an indicator of growth, its measurement during the infancy phase is an important indicator of brain development and possible neurological disorders (Lindley *et al.*, 1999).

Thus physical expansion of the body comprises of an evolution of the body composition in terms of the Total Body Fat (TBF): Fat Free Mass (FFM) ratio (Barclay & Weaver, 2003). This evolution is largely determined by the post-natal accumulation of energy as fat and protein, respectively. In essence, stored energy in adipose tissue decreases with post-natal age possibly due to lower fat: protein ratio in synthesized tissue, which results in an accumulation

of lean body mass (Hulzebus & Sauer, 2007). The increase in lean body mass during infancy is usually accompanied by bone mineralisation, (i.e. an increase in Bone Mass Density (BMD)), and a simultaneous increase in blood volume. The evolution of the foregoing components of the body composition will subsequently result in an increase in the Base Metabolic Rate (BMR) during infancy (Johnstone *et al.*, 2005).

The BMR is the rate at which a person uses energy to maintain the basic functions of the body and for infants and young children it is relatively high due to the dominant contribution of the brain (60-70%) (Shils *et al.*, 2006). The BMR of a term infant ranges between 43 and 60 kcal/kg/day i.e., two to three times greater than adults. The proportionally high BMR, for the size of an infant during early life, necessitates an increase in energy and nutrient intake for maintaining healthy growth.

There are two plausible alternatives for the underlying mechanism associated with the influence of early nutrition on body composition and the risk of disease (Wells, 2007). First, is the early nutritional impact on the epigenetic and hormonal regulation mechanisms (the thrifty gene theory), that is believed to generate an effect on the body composition in term of the "constrained lean mass" and limited metabolic capacity (e.g. impaired pancreatic development) in tolerating a rich diet (Wells, 2007). The second hypothesis relates to the more immediate impact of a high energy diet, at the early stage of development, on body composition (Wells, 2007). The high energy intake appears to divert energy disproportionately to adipose tissue particularly around the abdominal region resulting in an increase in the metabolic load, which is imposed by 'catch up' growth. The risk of disease is predicted to be greatest when there is an extreme disparity between metabolic capacity and load (both slow and fast infant growth) due to inappropriate nutrition during early life (Sesso, 2004; Wells, 2009).

The physical development of a young infant requires the physiological evolution of a functionally maturing gastrointestinal (GI) tract that can digest and absorb nutrients, as well as a renal development function that can cope with an increasing solute load (Wells, 2009). The ability of an infant to feed is usually developed at four to six months of age when the physical activity levels (PALs) also simultaneously increase (Foote & Marriott, 2003). In summary therefore, during the stage of infancy a child is most vulnerable to nutritional

deficiency and insufficiency as a result of low nutritional stores, increased energy and nutrient requirements and immature gastrointestinal/immune systems (Holden & Anita, 2000). It is important, however, that nutrient-energy intake should not result in a disproportional growth rate, as it leads to childhood obesity (Owen *et al*., 2005). A systematic review of absolute size and growth in infancy supports a series of recommendations with regard to appropriate nutritional provision preventing obesity in later life (Baird *et al*., 2005). Martin *et al* (2005) have also established a correlation between birth weight and arterial blood pressure as well as plasma lipid levels. Hence, infant energy requirements should be individualised (Leunissen, 2009).

2.1.2 Motor skills and cognative development: consequences of nutrition

Motor skill development during infancy is pivotal to the ability of an infant to feed. The motor skill development of an infant reflects the ability to control voluntary muscle movements from the central to the peripheral parts of the body (Brown *et al.*, 2008). At around 4-6 months of age an infant develops sufficient neuromuscular coordination to allow for a gradual transition from breast/bottle feeding to an energy-nutrient dense solid diet (Barclay & Weaver, 2003). An infant's ability to achieve the general and neurological stage of development (chewing, swallowing, digestion and excretion), will determine their capability to feed and the level of energy expenditure in any activity (Stevenson, 1991; WHO, 2004).

In addition to motor development, sensory-motor and cognitive development are also essential in respect of the ability of the infant to feed. Research suggests that simultaneous stimulation of the social and emotional growth of the infant are as critical as optimum nutrition for optimal brain development (WHO, 2004).

A stimulating feeding environment, therefore, is important with respect to the ability of an infant to associate mouth sensation with pleasure and exploration through play and emotional connection with the mother. This may explain some later feeding problems in infants who are reluctant to feed as a result of disassociating mouth sensation with pleasure and exploration. Behavioural feeding problems, such as food "fads" in childhood, refusal to chew, vomiting on

demand and limited appetite are usually known to reflect a disturbed mother and child relationship which may ultimately result in growth faltering (Wharton, 1996). Interestingly in a study by Lozoff *et al*. (1998), mothers of anaemic children were rated as being less affectionate, giving them fewer tasks and making fewer attempts to elicit responses (Andreca *et al*., 1997).

A constant dietary supply of nutrients including glucose, amino acids, fatty acids, vitamins and minerals is required for normal brain function including the production of neurotransmitters. Nutritional "insult" during early life can largely cause irreversible effects on both the peripheral nervous system with sensory deficit and the central nervous system (CNS) which can lead to mental retardation and cognitive dysfunction (Nomura, 2009).

The scientific community has shown great interest in the effects of infant malnutrition on cognitive development since the 1960s and is continuing to do so. For instance, a growing number of studies demonstrate an association between iron deficiency anaemia and impaired mental and psychometric development. Other studies demonstrate the important role of iron in relation to cerebral development especially with respect to the myelination process and the effect of iron on the CNS (Andreca *et al*., 1997; Grantham & Cornelius, 2001).These studies have found that anaemic infants (6-month-old) have prolonged central conduction time in auditory brainstem responses (n=5, 29 sec) in comparison with non-anaemic children (n=5, 26 sec) i.e. 3 seconds longer. Furthermore, the central conduction time did not improve with a correction of the anaemia and the difference was even greater 6 and 12 months later (Roncagliolo *et al*., 1998).

Insufficient iron availability during myelination of the nervous tissue, may provide a physiological explanation for certain behavioural changes including a decrease in the child's mental and psychomotor development index as measured by the Bayley infant behaviour ratings (Lozoff, 1997). These infants tend to be more fearful, withdrawn, tense, un-reactive to usual stimuli, more solemn, less involved and unhappy. There are some hypotheses which attribute iron deficiency anaemia to motor and cognitive dysfunction including an alteration of neurotransmitters.

In some animal studies, iron deficiency results in diminishing reversible monoamine oxidase activities, which is responsible for noradrenaline degradation. Moreover, serotonin activity may increase due to deactivation of aldehyde oxidase which catalyses its degradation. Finally iron deficiency can also lead to a reduced function of the dopamine D_2 receptors (Booth & Aukett, 1997). Longitudinal studies consistently indicate that children who are anaemic in infancy continue to have impaired cognition, underachieve at school, and more behavioural problems into middle childhood (Yu *et al*., 1986; Grantham & Cornelius, 2001).

In relation to the role of fatty acids, research has confirmed that long-chain polyunsaturated fatty acids (LCPUFA) are required components of the rapidly growing pre-natal CNS. During late gestation and early post-natal life, the neonate's brain experiences a tremendous increase in growth and cellular proliferation termed the ''brain growth spurt'' (Ryan *et al*., 2010).

In infants numerous studies have found a positive correlation between an adequate blood concentration of docosahexaenoic acid (DHA; 22:6 n-3) and improvements in cognitive, visual and immune function. For the rapidly growing infant, there is a high demand for LCPUFA to form vital cell membrane structures (myelination), thus the requirement of pre-formed substrates such as DHA and arachidonic acid (ARA; 20:4 n-6), as integral neural membrane components, are at a premium (Agostoni *et al*., 2005). Clinical evidence suggests that an ARA: DHA ratio greater than 1 is associated with improved cognitive outcomes (Hoffman *et al*., 2009). The importance of having an appropriate supply of DHA and ARA is important throughout infancy, as both fatty acids continue to accumulate in the brain's grey matter and in visual elements of the retina through at least the first 2 years of life (Ryan *et al*., 2010).

Humans are capable of endogenous synthesis of DHA and ARA from the precursor essential fatty acids α-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6). They should however be considered conditionally essential especially during infancy due to their role in development of neural tissue as assessed by visual function and learning-memory behavioural tasks during early development (Ryan *et al*., 2010). Similarly, they may be considered conditionally essential in long-life health due to their role in prevention of cardiovascular disease.

There are also other micronutrient deficiencies that cause major public health concerns, which can affect brain development and influence its functioning beyond infancy which include deficiencies in zinc (decreased neural replication (Sandstead, 2003)), iodine (reduced intellectual ability), vitamin A (reduced visual preceptors), folate (prevention of neural tube deficit) and vitamin B_{12} (impaired cognitive function) (Benton, 2008).

Evidence also suggests that zinc deficiency during pregnancy and lactation, is associated with deficits in activity and motor development in neonates at six months of age which is proposed to be caused by suppression of gene expression of growth factors and synthesis of nucleic acid and protein (Black, 1998; Bhatnagar & Taneja, 2001; Sandstead, 2003). In a study conducted by Friel *et al*, infants who received a zinc supplement had a greater linear growth over the entire study period than infants in the placebo group. Although there were no differences in the growth velocities in terms of weight or head circumference, infants in the zinc-enhanced supplementation group displayed better scores in motor development (Friel *et al.*, 1993).

On the whole an imbalanced diet, in terms of both inadequate provision of food and inadequate intake, can result in a 'non-organic failure to thrive (NOFT)'. NOFT can be defined as a restrained physiological and mental growth with no underlying medical reason that is caused by nutritional deprivation due to psychological and emotional problems. NOFT is usually manifested by a reduction in linear growth (stunting) and impaired cognitive development during infancy, leading to decreased mental performance during childhood (Skus, 1985; Brown, 2008).

2.1.3 Hormonal systems and nutritional immunology

Hormones and growth factors are responsible for providing the intercellular signals that are responsible for utilisation of nutrients. This can alter the availability of the stored nutrients in times of stress, regulate the use of substrates for growth, and redirect nutrients for cellular repair after infection. Hormones and growth factors that regulate the utilization of nutritional substrates include intestinal, pancreatic, adipose tissue and skeletal muscle hormones as well as hormones of the endocrine glands (Shils *et al.*, 2006)

The post-natal secretion of growth hormones under the circumstances of ample nutrient intake is responsible for enhanced linear growth and the accumulation of lean body mass as well as a decrease in fat mass via their influence on adipocytes to increase lipolysis. It is important to note that nutritional substrates can reciprocally influence the production of hormones and growth factors. For instance in the context of inadequate nutrition, the intermediary sematomedine (IGF-I) which is responsible for linear growth is inhibited although lipolysis and oxidation of fat increases resulting in weight loss and finally muscle wasting (Shils *et al.*, 2006).

Nutrition is also necessary for the development and maintenance of the immune system, and the ability to fight infections. Under-nutrition during gestation, neonatal and weaning can cause impaired immunological development and affect differentiation of the normal immune system; for instance, it can have a direct impact on lymphoid tissues and cells during the "vulnerable period". A study in Gambia has shown that there is a correlation between low birth weight and the rate of survival from infections in infants born during the starvation period relative to those infants born during the harvest period as they had constrained thymus development (Collinson *et al,*. 2003).

In recent years nutritional research and immunologic studies have been extremely valuable in understanding the cellular and molecular mechanisms involved in maintaining healthy growth and prevention of disease. The immaturity of the infant's immune system (e.g. impaired immunoglobulin production, low cytokine production) is the main reason for the exaggerated effect of infection during the early years of life (Robert & Kraisid, 2001). A neonate usually receives circulating antibodies through placental transfer of IgG while developing its own repertoire of immunoglobulins. Human breast milk, however, contains a variety of substances such as immunoglobulins (IgA), cytokines (IL-1β, IL-2, IL-6, IL-8, TNF-α) and trace elements (zinc, copper), which may contribute to an active stimulation of the infant's immune system, interacting with mucosal tissues in the upper parts of the respiratory and alimentary tracts providing an anti-inflammatory effect upon the recipient in infancy (Ustundag *et al*., 2005).

Most of the IgA in breast milk will work in protecting pathogens crossing the oral cavity. When human milk was given to premature new-borns, incidences of infections were reported

to be lower with respect to commercially available formulas (WHO, 2004). The babies who were fed with human milk developed a natural defence against *Bacteroides, Clostridium,* and *Escherichia coli.* Colostrum in particular, provides the infant with passive immunity in the form of *lactobacillus bifidus,* lactoferrin, lysozymes and secretory IgA (Wheeler *et al*., 2007). Lysozyme is present in breast milk in concentrations 5000 times greater than in cow's milk and its activity appears to increase during lactation. Lactoferrin is abundant in human milk but is not present in cow's milk. It affects the absorption of enteric iron, thus preventing pathogenic *E. coli* from obtaining the iron they need for survival. The bifidus factor in human milk promotes the growth of Gram-positive bacilli in the gut flora, particularly *Lactobacillus bifidus*, which discourages the multiplication of pathogens. Babies who are fed on cow's milk formula have Gram-negative (potentially pathogenic) bacilli in their gut flora. IgA protects intestinal epithelium and the mucosal surfaces against entry of pathogenic bacteria (*E. coli, Salmonellae, Shigellae, Streptococci, and Staphylococci*) and enteroviruses (poliovirus and rotaviruses).

The infants' degree of susceptibility to infection is in part due to their acute vulnerability to the immune-depressive effects of hypo-vitaminosis (diseases that are caused by deficiencies in one or more vitamins). Nutrients act as antioxidants and as cofactors at the level of cytokine regulation. The combination of chronic under-nutrition and infection further weakens the immune response, leading to altered immune cell populations and a generalized increase in inflammatory mediators. Inadequate nutrition, therefore, causes debility in children making them more susceptible to infections that can become extensive and serious. For instance, vitamin A, riboflavin, iron, zinc and iodine deficiencies, that are normally associated with protein-energy malnutrition, are known to have a profound influence both on host response to infection and the rate of microbial proliferation (Bhutta, 2006).

Nutritionally acquired immune deficiencies are an immunological consequence of nutritional deficiencies which represent a secondary immunodeficiency. For instance, there is a high incident of TB reported among the children in the Gujarati community in East London which is associated with biochemical deficiency of vitamin D (Ustianowsk *et al.,* 2004). Over-nutrition and obesity also reduce immunity. Leptin is emerging as a cytokine-like immune

regulator that has complex effects in both over-nutrition and in the inflammatory response in malnutrition (Cunningham *et al*., 2005).

Conversely, infection can also induce a metabolic effect on 'nutritional status', which is quite important during infancy as it drives nutrients away from the growth process to immunological responses (Panter *et al.,* 2009). Firstly, in the incident of an infection, the acute phase response may reduce intake and absorption through loss of appetite (Ulijaszek, 2000; Assis *et al*., 2005). Secondly, the acute-phase response may also increase the utilization of nutrients by prioritising the metabolic process to the synthesis of immunoglobulins, proliferation of white blood cells and to tissue repair rather than growth (Michaelsen, 1998). Examples of the foregoing include: HIV and fatty acids, measles and vitamin A (D`Souza *et al.,* 2002; Sudfeld *et al.,* 2010). It has been reported that a substantial number of children who have had measles in the United States, have a low plasma retinol concentration (Arrietta *et al*,. 1992). Other factors aside (e.g. inborn errors of metabolism; immunodeficiency disease), successful infant feeding practice therefore, will result in healthy growth and the prevention of disease.

2.2 Energy requirments and key nutrients

The amount of energy and the nutrient requirement during infancy depends on the degree of the ability of each individual to achieve optimal physical and mental growth. The energy recommendations based on measured energy expenditure rather than reported energy intakes indicate that children 0-24 months have energy needs that are 15-20% lower than previously recommended (Butte *et al*., 2000) Again, this is subject to considerable variation in individual children where the nutrient requirement will be based upon many factors (some of which are outlined above) and include gender, weight, height, age, body stores of nutrient, activity levels and genetics (Thompson, 1998).

In the first six months of life, breast milk can satisfy the nourishment that an infant requires providing that mother is well nourished (Brown *et al*., 2008; Foote & Marriott, 2003). After six months, the utilization of post-natal nutrient stores together with the need for rapid growth

of an infant, creates further demand for energy and nutrients; milk alone becomes inadequate (Barclay *et al*., 2003; Briefel *et al*., 2004; Hulzebos & Sauer, 2007).

At the point that breast milk is no longer adequate, the weaning process commences. At this stage biological systems, mainly gastrointestinal and renal, are rapidly improving and neuromuscular coordination should be sufficiently developed to allow for gradual weaning on to an energy-nutrient dense solid diet (Barclay *et al*., 2003). Complementary semi-solid foods therefore are required to provide *additional* energy and a variety of nutrients to meet these evolving needs (Davies & O'Hare.B, 2004; Foote *et al*., 2003). Thus, milk remains the main source of energy, fat, carbohydrate and protein for nourishing a healthy infant, born at term (Taitz & Wardley, 1989). The amount of energy that is made available from the consumption of different macronutrients varies as follows: a gram of carbohydrate (starch or sugar) provides 16 kJ (3.75 kcal)/g, protein provides 17 kJ (4kcal)/g and fat provides 37 kJ (9 kcal) /g. These are values established by the European Union for the nutritional labelling declaration of foods (90/496/EEC).

The estimated energy needs from complementary foods for infants with an "average" milk intake in both developing and industrialized countries are presented in Table 2.1.

Table 2.1 The energy needs from complementary foods (kcal per day; WHO/UNICEF, 1998).

Age	**6-8 months**	**9-11 months**	**12-23 months**
Developing	200 kcal/day	300	550
Industrialised	130 kcal/day	310	580

The differences in these estimations are because of differences in average milk intake. Energy needs from complementary foods are estimated by subtracting average breast milk energy intake from total energy requirements at each age (Dewey & Brown, 2003). The above guidelines are based on children receiving average amounts of breast milk at each age. If an infant is consuming more or less breast milk than the average, the amount needed from complementary foods will differ accordingly.

An infant's requirement for energy is not only for their growth but also to provide their basic metabolic functions, keeping warm and daily activity.

The amount of energy required for their physiological and biological development is only 20-25% of the total energy expenditure (TEE).

According to the Department of Health (1991), the estimated average requirement (EAR) for energy for each individual is subject to change throughout the growth period due to a large variation in influential factors including: energy expenditure (EE), metabolic rate and energy loss, as demonstrated in the data in Table 2.2.

Table 2.2 Estimated average requirement (EAR) for energy (Department of Health,1991)[a].

Age in Months	*Average weight (kg)*	*Energy requirement kcal/kg per day*
1	4.0*	115
3	5.9	100
6	7.7	95
9	8.9	95
12	9.8	95

*(SCF, 1993)

[a] The figures are assumed to be identical for both females and males when expressed as a function of body weight.

Infants are very efficient in self- regulating their daily intake of energy. Thus, they only need a small amount of energy-dense foods (number of calories per unit of the volume or weight). The provision of energy dense food is essential in feeding an infant, since they have a limited gastric capacity (30-40 g/kg of body weight) and they may not meet their daily energy requirement if they are eating a low-energy diet. When weight faltering has no underlying physical or biological reasons, one of the common diagnoses is inappropriate implementation of healthy- eating concepts in the infant's diet. This is supported in a study by Wharton (1996) which suggests that some mothers are under the misconception that a low fat, high fibre diet (bulky with low energy density) is healthier for their infants, which in essence is inadequate in respect of energy for growth and development.

Therefore, the recommended energy intake from complementary foods varies according to the age of the infant, the volume and fat content of the milk they consume and the frequency at

which they are fed. For 6-9 month old infants, with an average intake of milk, the desirable level of energy density in complementary food ranges between 0.6 kcal/g increasing to 1 kcal/g at 12-23 months (Monte & Giuliani, 2004). It is also worth mentioning that an excessive amount of energy from complementary food can also have negative consequences including a reduction in milk consumption and problems associated with excess body weight and obesity.

A summary of the major nutrients involved in infant physiological development are as follows (Monte & Giuliani, 2004; Thompkinson & Kharb, 2007; WHO, 2004).

2.2.1 Macronutrient content of food

Macronutrients are chemical compounds found in food that are required in large quantities by body; they include: proteins, fats and carbohydrates.

2.2.1.1 *Protein*

Dietary protein requirement of an infant per unit of body weight is greater than that of an adult (Brown, 2008), which primarily reflects the infants' additional need to support both maintenance and the high rates of tissue formation (Dupont, 2003). About 87% of protein intake is used for tissue synthesis (Dewey *et al*., 1996). The tissue protein synthesis rate (on a g/kg/d basis), however, is only modestly higher during the first months of life than in older infancy (Young *et al*., 1991). The slower rates of tissue synthesis in later infancy indicate a diminishing anabolic response to nutrient intake as lactation progresses, which are paralleled by a decreased metabolic response to insulin and amino acids (Dupont, 2003). The total-body protein synthesis rate is 4 times greater than actual protein intake at birth and at weaning, which is evidence for extensive reutilization of amino acids in infants.

Protein can also be utilized as a source of energy (although it is not necessarily beneficial to be used in this way); therefore, an adequate energy intake is crucial with respect to linear growth. Inadequate energy intake will result in protein being metabolised as a source of energy rather than being utilised for its primary purpose, i.e., growth. Human milk can

provide most of the protein needed, providing that a modest protein supply is obtained from weaning foods (Lutter & Dewey, 2003).

The protein: energy ratio in the diet of an infant should be within 6-12%, and carbohydrate and fat should be the main source of energy as mentioned earlier in section 2.1.1 (Monte & Giuliani, 2004). The protein dietary reference values for infants are shown in Table 2.3.

Table 2.3 Reference nutrient intake for protein consumption in infancy (DoH, 1991).

Age in months	Protein (g/kg per day)
0-3	2.2
4-6	1.6
7-9	1.6
10-12	1.5

Apart from total protein content, the type and the quality of protein also plays an important role in the infant's diet because of the underdeveloped metabolic activities. In a breast fed infant, low molecular-weight proteins are more efficiently digested and utilised, which reduces the gastric acidity to a lesser degree and results in a lower incident of gastrointestinal infection (Thompkinson & Kharb, 2007). The quality of a protein is a function of its amino acid composition and digestibility (Lonnerdal, 1994; DuPont, 2003; Brown *et al*., 2008). In addition to the 8 essential amino acids designated for adults, a few more are known to be indispensable for infants (Table 2.4). This is due to the fact that the inter-conversion mechanism of the histidine from ribose-5-phosphate, cysteine from methionine, and tyrosine from phenylalanine are not fully developed in infants (Thompkinson & Kharb, 2007).

Table 2.4 Amino acid requirements of an infant.

Types of amino acids	Age of Infants (months)			FAO/WHO 3 to 4 month
	0 to 1	1 to 3	3 to 6	
Histidine				28
Isoleucine	59	43	32	70
Leucine	109	75	54	161
Lysine	116	85	63	103
Methionine + cysteine	64	37	27	58
Phenylalanine + tyrosine	114	88	60	125
Threonine	63	45	34	87
Tryptophan	22	16	11	17
Valine	72	51	38	93

Animal source foods are a good source of high quality protein and they seem to have a special effect on linear growth, either due to increased intake of associated micronutrients (e.g. zinc and calcium) or because of a high content of sulphur containing amino acids (Michaelsen & Friss, 1998). Many plant foods have an "unbalanced" essential amino acid composition. Grains are generally low in lysine whereas legumes tend to be low in sulfur containing amino acids (methionine and cysteine) (WHO, 2004; Iqbal *et al.*, 2006). By combining grains and legumes, an adequate protein quality can, however, be obtained. For example Lutter & Dewey (2003) have proposed that between 0.65- 0.79 g of cereal protein per kilogram body weight per day could provide the amount of lysine needed. Thus the diet of a vegetarian or vegan could have the same quality intake of protein as a person who eats meat, providing that they consume a variety of vegetable proteins in combination. For instance, lysine is the limiting amino acid in wheat protein, tryptophan in maize and cysteine in beef protein (WHO, 2004). At the same time, soya is considered one of the most inclusive vegetable sources of protein (DuPont, 2003). By providing 0.18–0.48 g of bovine milk protein or soy protein per kilogram of body weight per day, the requirements for sulfur containing amino acids can be met (Thompkinson & Kharb, 2007).

A diet of variety, therefore, is more likely to provide a wide range of micronutrients via the complementary combinations of different food compositions.

2.2.1.2 *Fat*

Fat is an important component of an infant diet due to its role in providing essential fatty acids, facilitating the absorption of fat-soluble vitamins, and enhancing dietary energy density and sensory qualities. Lipid supply, particularly of essential fatty acids (EFAs) and LCPUFA, has also been shown to affect neural development and function (Uauy & Hoffman, 1991; Uauy *et al*., 2000). Evidence indicates that specific fatty acids exert their effect by modifying the physical properties of membranes, including membrane-related transport systems, ion channels, enzymatic activity, receptor function and various signal transduction pathways. More recently, specific fatty acids were reported to play a role in determining levels of gene expression for key transcription factors, peroxisome proliferator-activated receptors (PPAR) and retinoic acid receptors, leading to increased interest in better defining the role of these critical nutrients in the regulation of lipid metabolism, energy partitioning, insulin sensitivity, adipocyte development and neural function throughout the human lifespan (Lauritzen *et al*., 2001).

Although there is debate about the optimal amount of fat in the diets of infants and young children, the range of 30-45% of total energy has been suggested for an infant with a medium to high level of milk consumption (Dewey & Brown, 2003; Bier *et al*., 1999). The percentage of energy derived from fat in complementary foods needed in order to prepare a diet with 30%-45% of total energy as fat, for children with an average intake of milk (milk energy of 400 kcal/day), is recommended to be 5% of the non-milk energy at the estimated maximum level of 4.4g/100 g of food (Dewey *et al*., 2004, WHO, 2004).

It is, however, important to take into account the potential effect of added fat on the overall nutrient density of the diet. For example, the addition of one teaspoon of vegetable oil to 100 g of a typical maize based recipe, would increase the energy density from 0.28 to 0.73 kcal/g, but would reduce the proportion of energy derived from protein from 8.9% to 3.3%, and iron density from 0.5 to 0.2 mg/100 kcal (WHO/UNICEF, 1998). These effects could exacerbate the micronutrient deficiency in vulnerable populations unless other measures (such as fortification or supplementation) are taken to ensure adequate micronutrient intake. For instance, intestinal absorption of calcium is influenced by how calcium is bound to fats

(medium chain fatty acids) and proteins (casein). This issue is discussed in more detail in section 2.3.2.2.

In addition, a high level of fat intake, and in particular saturated fatty acids, has been associated with raised blood cholesterol levels (Lawlor *et al*., 2006), which is one of the risk factors for coronary heart disease. Diets rich in saturated fatty acids are associated with the development of insulin resistance and dyslipidemia (abnormal blood fat levels) as part of the 'Metabolic Syndrome' (a cluster of risk factors for cardiovascular disease) (Mozzaffarian *et al*., 2007).

The Government's advisory committee, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) has recommended that no more than 35% of food energy intake should come from fat and no more than 11% of energy intake should come from saturated fatty acids. These figures are intended as population averages (goals for the population to attain on average), and not as targets for individuals. The successful removal of hydrogenated oils in recent years from many of the manufacturing practices in the UK, however, is a prime example of a response to the concerns raised in relation to the negative implications of high *trans* fatty acid intake, which has resulted in a reduction in the population's consumption of *trans* fatty acids. In agreement with the recommendation for the reduction of the population's dietary fat intake, it has been suggested that this guideline also be applied to infants and young children. There are however concerns that, since fat is the major sources of energy for infants as well as the only source for essential fatty acids (EFAs), such diets may limit growth.

Until recently limited data were available relating to infants and young children's growth on a "low fat" diet. According to a study conducted in Finland (Videon & Manning, 2003) there was no significant difference observed between infants on a low fat diet and those in a control group with respect to their growth. Interestingly, although the intervention group had lower energy and fat intake than the control group, the mean fat intake of both groups was close to 30% of the total energy intake, but the intervention group proved to have a lower serum cholesterol concentration at 3 years.

The total diet should provide infants with at least 3–4.5% of energy intake (EI) from LA and 0.4-0.6% EI from ALA to meet DHA and ARA requirements. Very high intakes of EFA confer no advantage and are associated with potential health risks. Intake of LA and other n-6 fatty acids should be limited to <10% EI and intake of total polyunsaturated fatty acids should be limited to <15% EI (FAO, 2008). The proposed values can be justified by emerging information on the effect of excess n-6 PUFA on eicosanoid related functions and the implications for oxidative stress and chronic inflammation. Unfortunately the introduction of food sources of LCPUFA, such as liver and fish are currently being delayed due to concerns about allergies (FAO, 2008).

2.2.1.3 *Carbohydrate and fibre*

The term "carbohydrate" describes a family of compounds synthesized from monosaccharide building blocks, ranging from simple sugars, or mono- and disaccharides, through sugar alcohols, oligosaccharides and dextrin, to the more complex starch and non-starch polysaccharides (Table 2.5).

Human breast milk contains both digestible (predominantly lactose) and indigestible (oligosaccharides) carbohydrates. It is well documented that lactose is assimilated from the digestive tract more slowly than other carbohydrates. The change of lactose into glucose and galactose when feeding breast milk proceeds so slowly that some lactose remains intact almost up to the last section of the digestive tract (Engfer *et al.*, 2000). This is of considerable importance in breastfeeding as it leads to the production of lactic acid in the large intestine under the influence of 'bifidus' bacteria, making the intestinal environment acidic (pH = 5.50 to 6.00). The acid medium is also conducive to the growth of lactose fermenting rather than putrefactive bacteria, thus decreasing the likelihood of infection. This effect is more notable in breast-fed infants. Moreover, the extra lactose present in breast milk stimulates the synthesis of B-groups vitamins and increases the absorption of calcium and iron (Thompkinson & Karb, 2007).

Carbohydrates are the major source of energy and dietary fibre. Glucose is the most abundant endogenous monosaccharide vital for *de novo* synthesis of fatty acids and a number of amino acids (Kalhan & Kilic, 1999).

The minimum level of carbohydrate (Table 2.5) intake must be enough to allow for healthy growth and development; ideally it should minimise the protein carbon use for glycogenesis and should also avoid ketosis or hypoglycaemia (Bier *et al*,. 1999).

Table 2.5 The major dietary carbohydrates (FAO/WHO, 1997).

Class	Sub-Group	Components
Sugars (1-2)	Monosaccharaides Disaccharides Polyols (sugar alcohols)	Glucose, galactose, fructose Sucrose, lactose, trehalose Sorbitol, mannitol
Oligosaccharides (3-9)	Malto-oligosaccharides Other oligosaccharides (RO)*	Maltodextrins Raffinose, stachyose, fructo-oligosaccharides
Polysaccharides (>9)	Starch/ RS**	Amylose, amylopectin, modified starches
	Non-starch polysaccharides	Cellulose, hemicellulose, pectin, hydrocolloids

*Resistant oligosaccharide
** Resistant starch

In addition to providing easily available energy for oxidative metabolism, carbohydrate-containing foods are vehicles for the delivery of important micronutrients and phytochemicals. Dietary carbohydrate is important for maintain glycaemic homeostasis and for gastrointestinal integrity and function. An optimum weaning diet should consist of at least 57% carbohydrate obtained from a variety of food sources (Lutter & Dewey, 2003; WHO, 2004). The upper limit of an infant's carbohydrate intake is constrained by the minimum need for other macronutrients, which were described earlier. Thus, carbohydrate intake is calculated after subtracting the energy contribution from fat (31%) and protein (12%). The starting point for 12% energy value from protein and 31% fat is based on the recommendation for infants with average to high level of milk consumption as a main source of nutrient (WHO, 2004).

Fibre in the diet of infants, however, is not recommended due to their limited gastric capacity and the effect of fibre on nutrient density and early satiation (WHO, 2004).

2.2.2 Micronutrients and their interactions

Micronutrients, as opposed to macronutrients (protein, carbohydrate and fat), are nutrients required by the body in minute quantities to ensure healthy growth as well as maintenance and repair through the entire life cycle.

- ***Vitamins*** are classically defined as essential organic nutrients, most of which are not synthesised by the body, or only in insufficient amounts, and are mainly obtained via food. There are thirteen vitamins known today with specific functions in the body: four are fat soluble (vitamins A, K, D, and E) and nine are water soluble, i.e., vitamin B complex (B_1, B_2, B_6, and B_{12}), biotin, vitamin C, folic acid, niacin and pantothenic acid (Preedy *et al*., 2008).

- ***Dietary Minerals*** are inorganic compounds including essential and essential trace elements. Essential elements are those that occur in the body in mg/kg/day quantities. They include calcium, magnesium, sodium, potassium, iron, zinc as well as chloride and fluoride ions. Essential trace elements, however, are required in mg/day quantities, and include copper, manganese, cobalt, chromium, selenium, molybdenum, nickel and iodine (Mann & Trustwell, 2007).

Providing an appropriate diet for a growing infant is critical in terms of healthy growth and development, as low intake or reduced bioavailability of nutrients may lead to deficiencies and cause body function impairment (Gokhale & Kirschner, 2003; Hulzebos & Sauer, 2007). For example, vitamins and minerals have a crucial influence on the interaction between genetics and biological factors (Gibson & Hotz, 2000; Melo *et al*., 2008). They are involved in many important functions in the body, e.g. bone mineralisation, enzymatic reactions, secretion of hormones, as well as protection of cells and lipids in biological membranes (Schlender & William, 2003; Taylor, Gallagher, & McCullough, 2004).

Examples of the role and function of some of the major vitamins and minerals in the body and recommended intake in relation to growth and development during infancy are presented in Tables 2.6 and 2.7, respectively.

Table 2.6 Examples of the major vitamins and their function in the body (DoH, 1991).

Nutrient	RNI* / Day	Functions	Deficiency
Vitamin A (μg)	350	Development of normal vision, healthy skin and mucous lining of body organ, resistance to infection	Xerophthalmia, night blindness and susceptibility to infection
Thiamin (mg)	0.2	Co-enzyme in metabolism of carbohydrates, normal function of the nervous system and other excitable tissues such as skeletal muscles and the heart	Beri-beri, polyneuritis, Wernicke-Korsakoff syndrome
Riboflavin (mg)	0.4	Co-enzyme functions in numerous oxidation and reduction reactions, release of energy, transport and metabolism of iron, normal structure and function of mucous membranes and the skin	Lesions of the mucocutaneous surface of the mouth (angular stomatitis), dermatitis
Niacin (mg)	4	Precursor to the coenzymes NAD **and NADP*** in carbohydrate metabolism, normal function of the skin and mucous membranes and for normal functioning of the nervous system	Pellagra, dermatitis, Inflammation of mucus membranes, diarrhea and vomiting. High intake (1g/day) results in hepatotoxicity (Powers, 1997)
Pyridoxine (mg)	0.3	Major role in metabolism of amino acids, breakdown of glycogen, maintenance of normal blood homocysteine levels together with folate and B_{12}	Failure to thrive, weakness, microcytic anemia, sleepiness, dermatitis peripheral neuropathy, cheilosis (Mann & Trustwell, 2007)
Folate (μg)	50	Healthy red blood cells, normal cell division, normal structure of the nervous system specifically in the development of the neural tubes in the embryo	Megaloblastic anaemia, growth falteration, low white cell & platelet, delayed maturation of central nerve system, susceptibility to infection
Vitamin B_{12} (μg)	0.4	Aid maturation of red blood cell and metabolism of folate, cofactor for enzymes involving normal function of the nervous system and energy production	Megaloblastic anemia, peripheral neurological damage. lethargy, irritability
Vitamin C (mg)	24	Prevention of scurvy and for the optimal functioning of the immune system	Weakness, fatigue, inflamed and bleeding gums, impaired wound healing and bruising

*UK's recommended nutrient intake for an infant 6-9 months of age (DoH, 1991).
** Nicotinamide adenine dinucleotide
***Nicotinamide adenine dinucleotide phosphat

Table 2.7 Examples of the major essential and trace elements and their function in the body (DoH, 1991).

Nutrients	RNI/Day	Functions	Deficiency
Calcium (mg)	525	Healthy bone and teeth, regulation of muscles contraction and nerve conductivity, blood clotting, enzyme activation and hormone secretion	Rickets, irritability, jitteriness, tremors and convulsions in new-born babies
Magnesium (mg)	75	Involve in glycolysis, replication of DNA and synthesis of RNA regulatory effect on Ca and k level , parathyroid hormones secretion (PTH), vitamin D metabolism and subsequently bone function, normal neuro-muscular function, steady heart rate	Muscle spasm and weakness, sleep disorders, irritability, poor nail growth, neuromuscular excitability
Iron (mg)	7.8	Major part of hemoglobin and role in cognitive development	Anemia, pale skin, tiredness, motor and mental problems
Zinc (µg)	5	Growth and immune function, site specific antioxidant, synthesis and activation of enzymes and proteins such as insulin, vitamin A, nucleic acid.	Growth retardation, immune deficiency, loss of appetite night blindness
Copper (mg)	0.3	Catalyst in mobilization of iron, connective tissue synthesis	Skeletal demineralization, decreased skin tone, decreased in plasma iron
Selenium (µg)	10	Antioxidant, maintenance of healthy immune system, interaction with heavy metals	Asthma, vulnerability to infection

* UK recommended nutrient intake for an infant 6-9 months of age (DoH, 1991).

To meet the mineral and vitamin requirements of infants, a variety of micronutrient-rich complementary foods should be offered as their nutritional bio-availability is directly influenced by the interaction between specific or groups of nutrients (Lonnerdal, 2005). For example consumption of foods of animal origin rich in iron, as well as fruit and vegetables rich in vitamin C and A is highly recommended. Vitamin C rich foods are important not only as a source of vitamin C itself, but to enhance non-haem iron absorption.

Calcium and vitamin D are another example of an interaction between a vital nutrient for linear growth and bone health in an infant diet. The accumulation of calcium or bone mineralisation is positive during the first 18 months of life, with the highest rate of accretion taking place during the first year (Bass & Chan, 2006). Many nutritionists believe that early bone mass accumulation is important in the prevention of adult osteoporosis (Bass & Chan,

2006; Cole *et al*., 2009). Vitamin D is one of the major factors affecting intestinal calcium absorption and it is required for the secretion of parathyroid hormone (PTH). Infant vitamin D stores are usually adequate unless there is insufficient maternal dietary intake or lack of exposure to sunlight. Children with vitamin D deficiency develop rickets and they often show bowed limbs (Crocombe *et al*., 2004; Cooper *et al*., 2008).

The B vitamins are also essential for child growth and development, and those listed (riboflavin, vitamin B_6 and folate) are often limited in the diets of young children (Dewey & Brown, 2003).

The nutritional composition of complementary foods, however, will differ widely due to socio-economic factors. For instance, the diets of infants of certain ethnic backgrounds as well as vegetarian infants are particularly poor in vitamin B_{12}, iron and zinc, as well as being high in carbohydrates and phytic acid (Davis & O'Hare, 2004). In most developing countries, complementary foods do not provide sufficient iron, zinc and vitamin B_6 (WHO, 2004). Even in the U.S., iron and zinc were identified (WHO, 2004) as problem nutrients in the first year of life, despite the availability of iron-fortified products.

Currently, nutritional iron deficiency continues to be the most commonly diagnosed nutritional disorder of early childhood worldwide, and Britain is no exception (Yeung, 1998; Aggett *et al*,. 2002). The evidence is that iron deficiency is directly linked to bad weaning practices especially amongst ethnic minorities and socially disadvantaged inner city families in the UK (Daly & Booth, 1998; Kimmones *et al*., 2005; Koster, 2009). A large survey carried out in 1992 has suggested that the median iron intake of full term babies 6–12 months of age are below the RNI and the mean zinc intake for this age group is only 90% of the RNI for zinc (Foote & Marriott, 2003). It is worth mentioning that a high intake of cheese and milk powder in the diet of young children in Britain, has been identified as one of the factors contributing to low iron bioavailability (Aggett *et al*., 2002; Singh *et al*., 2006). With iron being a vital component of haemoglobin and an integral part of various enzymes, poor weight gain and increased vulnerability to infection and impaired intellectual development are all worrying clinical consequences of iron deficiency. It is, however, important to note that

excessive iron and zinc intake may be detrimental in respect of other minerals, for example they can counter the effect on absorption of copper (Aggett *et al*., 2002; Singh *et al*., 2006).
In general, zinc and iron are known as problematic micronutrients in terms of their bioavailability as a result of poor utilisation as well as the effect of inhibitors (calcium, phytate and tannin) (Wellinghausen, 2001, Dewey & Brown, 2003, de Costa *et al.*, 2008).
The concept of bio-availability, natural inhibitors and enhancers is further discussed in section 2.3.2.2.

2.2.2.1 *Recomended Nutrient Intake*

There are different sources available for daily requirements of micronutrients for infants and young children (Table 2.8). The dietary requirements frequently used for infants and young children are the Recommended Nutrient Intake (RNI) from the Dietary Reference Values (DRVs) recommended by the Department of Health (DoH, 1991). RNIs are based on the Estimated Average Requirements (EAR) + 2 (SD) and reflect the average daily intake of a nutrient sufficient to meet the needs of almost all members (97.5%) of a healthy population. Intakes above this amount are thought to almost certainly be adequate. Values set may vary according to age, gender and physiological state (e.g. breastfed or formula fed).

DRVs are based on the amount of nutrients given to a wholly breast fed infant. There are, however, a few limitations associated with how these values have been set including uncertainty concerning the actual intake of infant foods, changes in composition of milk during the course of lactation between women, variability in the volume of milk supply, and because of difficulties in measuring these factors as well as ethical issues (Aggett *et al.*, 1997). Another major limitation is the variation in efficacy of nutrient absorption in breast fed infants which is higher than for formula fed infants. Examples of such differences are energy, protein and iron requirements between the two feeding regimes. Breast fed infants absorb 50% of the iron in milk whilst formula fed infants absorb only 10% of the iron in the formula milk. The higher rate of weight gain in formula-fed infants may be related to the higher protein and energy content of formula than of human milk (Dupont, 2003). In addition, estimates of nutrient requirements usually match the amounts required to correct or avoid deficiency and therefore will supply the nutrients at a marginally adequate level.

Table 2.8 Comparison of recommended nutrient intake of infants [WHO 2002, RNI (DoH, 1991) and DRI by Institute of Medicine (IOM)].

Nutrients	Recommended nutrient intake								
	6-8 month			9-11 month			12-23 month		
	WHO 2002	RNI DoH	DRI[b] IOM[c]	WHO 2002	RNI DoH	DRI[b] IOM[c]	WHO 2002	RNI DoH	DRI IOM[c]
Protein (g/day)	NA	12.7	NA	NA	13.7	NA	NA	14.9	NA
Vitamin A (RE µg/day)	400	350	500	400	350	500	400	400	300
Folate (µg/day)	80	50	80	80	50	80	160	70	150
Niacin (mg/day)	4	4	4	4	5	4	6	8	6
Riboflavin(mg/day)	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.6	0.5
Thiamine(mg/day)	0.3	0.2	0.3	0.3	0.3	0.3	0.5	0.5	0.5
Vitamin B_6 (mg/day)	0.3	0.3	0.3	0.3	0.4	0.3	0.5	0.7	0.5
Vitamin B_{12} (µg/day)	0.5	0.4	0.5	0.5	0.4	0.5	0.9	0.5	0.9
Vitamin C (mg/day)	30	25	50	30	25	50	30	30	15
Vitamin D (µg/day)	5	7	5	5	7	5	5	7	5[b]
Vitamin K (µg/day)	10	NA	2.5	10	NA	2.5	15	NA	30[b]
Calcium (mg/day)	400	525	270	400	525	270	500	350	500[b]
Chloride(mg/day)	NA	500	NA	NA	500	NA	NA	800	NA
Copper (mg/day)	NA	0.3	0.2	NA	0.3	0.2	NA	0.4	0.3
Fluoride (µg/day)	NA	60	0.5	Na	60	0.5	NA	70	0.7
Iodine (µg/day)	90	NA	130	90	NA	130	90	NA	90
Iron (mg/day)[d]	9.3	7.8	11	9.3	7.8	11	5.8	6.9	7[b]
Magnesium (mg/day)	54	75	75	54	80	75	60	85	80
Manganese (mg/day)	NA	NA	0.6	NA	NA	0.6	NA	NA	1.2[b]
Phosphorus (mg/day)	NA	400	275	NA	400	275	NA	270	460
Potassium (mg/day)	NA	700	NA	NA	700	NA	NA	800	NA
Selenium (µg/day)	10	10	20	10	10	20	17	15	20
Sodium (mg/day)	NA	320	NA	NA	350	NA	NA	500	NA
Zinc (mg/day)	4.1[e]	5	3	4.1[e]	5	3	4.1[e]	5	3

[a] Department of Health 1991.
[b] Based on Adequate Intake (AI) estimates, apart from Zinc
[c] Dietary reference Intake by Institute of Medicine, National academy Press, Washington D.C. (IOM)
[d] assuming medium bioavailability (10%)
[e] assuming moderate bioavailability (30%)

2.3 Current infant practices in the UK

According to a report by the World Health Organisation (WHO), a review of feeding guidelines promoted by various national and international organizations has shown that there are inconsistencies in the specific recommendations for feeding infants and young children (WHO, 2004). Some of the feeding guidelines are based more on tradition and speculation than on scientific evidence, or are far more prescriptive than is necessary regarding issues such as the order of foods introduced and the amounts of specific food to be given.

Based on the above discussions, in this section, an outline of common practices of early feeding in Britain is presented. In addition, issues around the timely introduction of complementary food, the characteristics of appropriate complementary food content, and the amount and frequency of the feed are considered.

2.3.1 First six months

It is common knowledge that the best option for feeding a normal infant during the first six months of life is a mother's milk (Taitz *et al*., 1989). Breast milk is a unique combination of nutrients which is perfectly adapted to the immature gastrointestinal, renal and metabolic systems of an infant (Table 2.9).

Table 2.9 Breast milk composition[a].

Constituent (per liter) [b]	Early Milk	Mature Milk	Constituent (per liter)	Early Milk	Mature Milk
Energy		653-704	**Water soluble vitamins**		
Carbohydrate			**Vitamin C (mg)**		100
Lactose	20-30	67	**Thiamine (mg)**	20	200
Glucose	0.2-1.0	0.2-0.3	**Riboflavin (mg)**		400-600
Oligosaccharides	22-24	12-14	**Niacin (mg)**	0.5	1.8-6.0
Total nitrogen	3	1.9	**Vitamin B_6 (mg)**		0.09-0.31
Non-protein nitrogen	0.5	0.45	**Folate (mg)**		80-140
Protein nitrogen (g)	2.5	1.45	**Vitamin B_{12} (mg)**		0.5-1.0
Total protein	16	9	**Pantothenic acid(mg)**		2.0-2.5
Casein	3.8	5.7	**Biotin (mg)**		5-9
β-Casein	2.6	4.4	**Fat-soluble vitamins**		
κ-casein	1.2	1.3	**Vitamin A(mg)**	2	0.3-0.6
α-Lactalbumin	3.62	3.26	**Carotenoids (mg)**	2	0.2-0.6
Lactoferrin	3.53	1.94	**Vitamin K (mg)**	2-5	2-3
Serum albumin	0.39	0.41	**Vitamin D (mg)**		0.33
Serum IgA	2	1	**Vitamin E (mg)**	8-12	3-8
IgM	0.12	0.2	**Macro-minerals**		
IgG	0.34	0.05	**Calcium**	250	200-250
Phospholipids	1.1	0.6-0.8	**Magnesium**	30-35	30-35
Total lipid	2	3.5	**Phosphorus (mg)**	120-160	120-140
Triglycerides	97-98	97-98	**Sodium(mg)**	300-400	120-250
Cholesterol[c]	0.7-1.3	0.4-0.5	**Potassium (mg)**	600-700	400-500

Constituent (per litre) [b]	Early Milk	Mature Milk	Constituent (per litre)	Early Milk	Mature Milk
Fatty acids	88	88	**Chloride (mg)**	600-800	400-450
Total saturated	43.44	44-45	**Micro-minerals**		
C12:0		5	**Iron (mg)**	0.5-1.0	0.3-0.9
C14:0		6	**Zinc (mg)**	08-10	1-3
C16:0		20	**Copper (mg)**	0.5-0.8	0.2-0.4
C18:0		8	**Manganese (mg)**	5-6	3
Monounsaturated		40	**Selenium (mg)**	40	7-33
C18:1 ω-9	32	31	**Iodine (mg)**		150
Polyunsaturated	13	14-15	**Fluoride (mg)**		4-15
total ω-3	1.5	1.5			
C18:3 ω-6	0.7	0.9			
C22:5 ω-3	0.2	0.1			
C22:6 ω-3	0.5	0.2			
Total ω-6	11.6	13.06			
C18:2 ω-6	8.9	11.3			
C20:4 ω-6	0.7	0.5			
C22:4 ω-6	0.2	0.1			

[a]From: Picciano, M.F. (2001) *Paediatric Clinic of North America*, *48*, 263-4.

[b] All values are expressed as per litre of milk with the exception of lipids, which are expressed as a percentage on the basis of either milk volume or weight of total lipids.

[c] The cholesterol content of human milk ranges from 100-200 mg/L in most samples of human milk after day 21 of lactation.

The benefits of breast milk are thought to possibly persist throughout the individual's lifespan (Fall *et al.*, 1997; Wright *et al*., 2001). An extensive body of scientific evidence supports the consensus that failure to breast feed increases the risk of illness in both the mother and infant (SACN, 2011).

- Risk to the infant: formula fed babies are more likely to develop problems with their gastrointestinal and respiratory systems as well as conditions such as urinary tract infections (Horta *et al*., 2007; Ip *et al.,* 2007). In addition blood pressure, total cholesterol (Owen *et al*., 2002), the prevalence of obesity, type-2 diabetes (Owen *et al.*, 2006) and childhood cancer (Martin *et al*., 2005) are lower amongst breast fed children than those who are fed formula (Horta *et al.,* 2007). To date, breast-feeding is strongly recommended by midwives and physicians. Some positive constituents in breast milk have been described for the prevention of allergic disorders or pathogen related diseases in neonates. For instance, the content of oligosaccharides in the milk of healthy mothers seems to reduce the risk of allergic disorders through prebiotic effects on the intestinal milieu (Pali *et al*, 2009).

- Risk to the mother: aside from the effect on the infants, mothers who have not breast fed could be at greater risk of breast and ovarian cancer in later life and they are less likely to return to their pre-pregnancy weight (World Cancer Research Fund, 2007).

The current UK recommendation relating to infant feeding is exclusive breast feeding for the first six months of life (DoH, 2008). The key finding from latest infant feeding survey in the UK suggests that initial breastfeeding rate has increased from 76% in 2005 to 81% in 2010. The incidence of breastfeeding has continued to follow the same pattern of variations as in 2005, according to the socio-demographic characteristic of mothers such as maternal age and educational attainment. The duration of breast feeding across the UK illustrates that the greatest increase is seen in older mothers from higher socio-economic backgrounds who typically have a higher educational attainment (Infant Feeding Survey 2005: Infant Feeding Survey 2010).

Maternal milk however, has some limitations in terms of maternal nutritional status, maternal nutritional intake and period of gestation. For instance, breast milk becomes an insufficient source of iron and zinc during the course of lactation and as the infants body stores become depleted (Hurley & Lonnerdal, 1988; Shashiraj, 2006). In the case of limited exposure to sunlight, maternal milk cannot compensate for the vitamin D deficiency either (Balasubramanian *et al.,* 2008). It also appears not to provide a sufficient amount of vitamin K and fluoride required for optimum health.

The level of ARA in human milk however, is relatively constant while the level of DHA is more variable and depends on maternal diet and lifestyle (Yuhas *et al*., 2006; Agostoni *et al*., 2003). In a Canadian study of human breast milk, trans fatty acids (TFAs) averaged 7.2% of the total fatty acids at the expense of essential fatty acids due to excess consumption of partially hydrogenated vegetable oils. Gibson *et al*. (1997) reported a dose-dependent response between maternal DHA consumption and DHA levels in human milk, although human milk DHA levels above 0.8 % FA did little to increase the plasma or red blood cell DHA content of the infants studied.

2.3.1.1 *Human milk model for infant formula milk*

Human milk is the ‘‘gold standard’’ model for development of infant formula, both in composition and in physiologic effects in relation to composition. The formulation of infant formula has evolved during the past decades and the technological advancement for the production of infant formula has come a long way in the manufacture of a variety of infant formulae for the dietary management of infants. For example, emphasis has been laid on the manufacturers to materialise the formulation in terms of the compositional and biochemical characteristics similar to human milk (Table 2.10).

Table 2.10 Compositional characteristics of human milk (HM), conventional infant formula (CIF), and maternalised infant formula (MIF) on dry weight basis.

Nutrient	HM	CIF	MIF
Casein (%)	3.67	17.8	4.96
Whey (%)	5.5	4.45	7.44
Total Protein (%)	9.17	22.25	12.4
Lipid (%)	31.67	19.57	26.24
Carbohydrates	62.6	52.32	59.91
Ca (mg/100g)	249.9	1014.3	998.11
P (mg/100g)	124.96	786.8	495.42
K (mg/100g)	423.6	592.2	875.62
Na (mg/100g)	134.1	262.5	239.68
Fe (mg/100g)	1.21	5	5.01
Cu (µg/100g)	0.33	Na	0.51
Mn ((µg/100g)	0.38	Na	0.51
Zn (mg/100g)	3.06	Na	3.01

Human breast milk promotes the development of a microbial flora in the colon, which is largely predominated by lactobacilli and bifidobacteria (up to 90% of the total flora) (Harmsen *et al*., 2000). In recent years two approaches have been proposed to achieve an intestinal flora more similar to that of breastfed infants: the addition to formulae of non-digestible carbohydrates that may act as prebiotics, such as oligo-fructosyl-saccharose and oligo-galactosyl-lactose, and the addition of bacterial cultures such as specific strains of *lactobacilli* and *bifidobacteria* considered as probiotics to formulae. A committee of Swedish scientists convened by the Swedish Nutrition Foundation reviewed and evaluated human studies on the health effects of probiotics and prebiotics (Andersson *et al*., 2001). The group concluded that certain probiotic bacteria might improve symptoms of lactose intolerance, acute rotavirus induced diarrhoea, and possibly also other forms of infectious diarrhoea. The application of *Lactobacillus rhamnosus* to infants and young children (age 2 to 16 months) allergic to cow milk protein and of *Lactobacillus GG* or *Bifidobacterium lactis* Bb12 added to a hydrolysed formula to previously breast-fed infants suffering from atopic dermatitis improve the clinical symptoms (Isolauri *et al*., 2000). Follow-on formulae with added bacteria regarded as probiotics have been introduced since about 2003.

2.3.2 Introduction of semi-solid food

As outlined above, in the first four to six months the infant's requirements can be totally satisfied by breast milk (Brown *et al*., 2008; Foote *et al*., 2003). After the first six months of life, the utilization of post-natal nutrient stores together with the need for rapid growth of an infant, creates further demand for energy and nutrients; milk alone becomes inadequate (Barclay *et al*., 2003; Briefel *et al*. 2004; Hulzebos & Sauer, 2007).

At this point, complementary food is required to boost energy and nutrient intake (Barclay *et al*., 2003; Briefel *et al*., 2004; Hulzebos *et al*., 2007). Complementary food can therefore be reasonably described as 'transitional'; bridging the gap between a diet solely composed of milk to that which is mostly made up of solid, more dense food (Barclay *et al*., 2003). This transitional period is thus appropriately referred to as "the weaning period" (Fig 2.1).

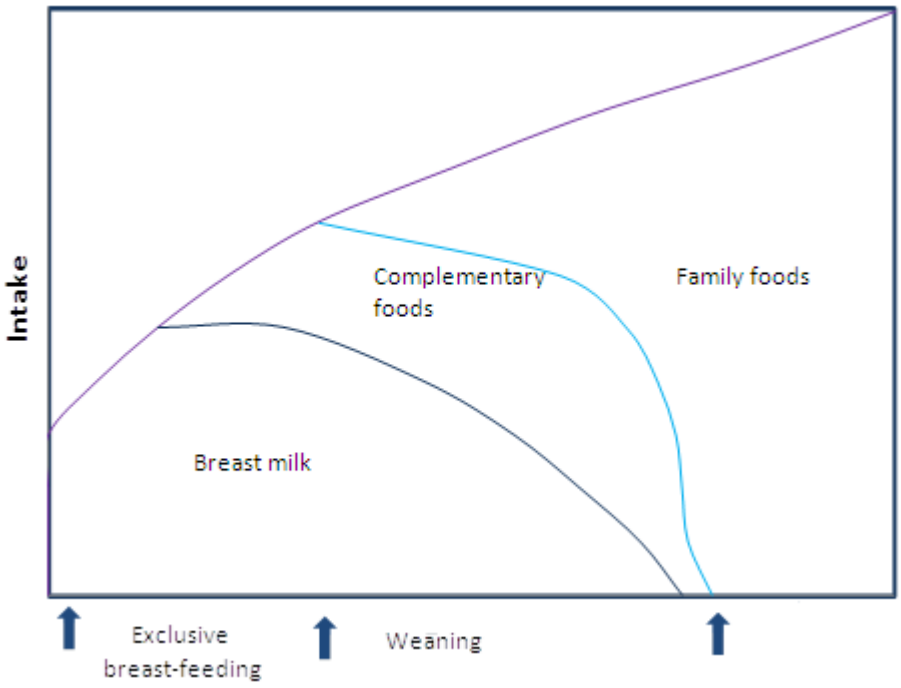

Fig.2.1 Contribution of different food sources to the energy intake of growing infant

After adapting to extra-uterine life and the establishment of milk feeding, weaning becomes the next challenging experience of infancy and early childhood. There are many debates usually embedded in issues associated with the weaning process which include: the appropriate age for introduction of first semi-solid; quality and safety of the food

composition; quantity and frequency of feeds; the feeding environment and the bonding between the carer and infant (Davies & O'Hare, 2004).

The importance of accurately addressing these issues is well understood in many studies that identify that early nutritional imbalance is linked to impairments in intellectual performance, work capacity, reproductive outcomes and overall health during adolescence and adulthood (Hulzebos *et al*., 2007). Addressing these issues, therefore, present opportunities to promote and protect child health.

As mentioned earlier in this chapter, an infant's neurological stage of development will determine their ability to begin to feed on semi-solids. It is therefore important to undertake a careful assessment of the specific infant's growth and development before initiating the weaning process.

From a nutritional perspective, the main disadvantages associated with the early introduction of semi-solid food relates to the risk of compromised milk consumption. More recently, there has been some indication that the early introduction of complementary food is associated with atopic disease and type-1 diabetes mellitus (SACN, 2011). Based on the WHO report in 2001 on the complementary feeding of infants both in the industrialised and developing world, infants who are exclusively breastfed up to six months are less affected by diarrhoea and do not have growth deficits (WHO, 2001; Kramer *et al*., 2004; Monte, 2004). The late introduction of complementary food beyond six months is also not advisable as it could lead to feeding problems in respect of limited appetite, restricted food preferences and consequently micronutrient deficiencies.

2.3.2.1 *The characteristics of an "appropriate" diet*

The optimal characteristics of processed complementary foods include: adequate energy density, appropriate macronutrients, energy ratios, suitably low renal solute load, appropriate viscosity for age, desired sensory properties, resistance to microbiological contamination, simple preparation and low cost (Monte & Giugliani, 2004; WHO, 2004).

The following sections will examine the challenges faced by infant feeding, the characteristics of an appropriate diet and nutritional quality of the final food composition in relation to processing.

2.3.2.1.1 *Meal frequency and energy density*

The accepted amount and frequency of complementary food required during the weaning process varies according to individual requirements, the volume of milk consumed and the composition of complementary food. The current recommendation regarding the frequency of meals are theoretically calculated based on the estimated non-milk energy requirement of an infant, their gastric capacity (30 g/kg of body weight) and energy density of at least 0.6-1.5 kcal/g. Thus, the WHO currently recommends two to three meals a day of complementary foods for breastfed infants between 6 and 8 months of life and three to four meals a day for those between 9 and 24 months, with additional nutritious snacks (pieces of fruit or bread) once or twice a day at 12 months (WHO, 2001).

It should be emphasised that the consumption of complementary food should not result in a displacement of energy intake from milk, and according to the Codex Alimentarius Standards (Codex STAN 074-1981, REV.1-2006) for complementary food, they are not meant to substitute milk but to complement (Clark & Shrimpton, 2000).

2.3.2.1.2 *Food consistency and taste acquisition*

Improper food consistency compromises the appropriate intake of nutrients by the infant and it may lead to food refusal and disinclination to chew **(**Monte & Giugliani, 2004**).** It is common for infants to reject new foods, but this should not be interpreted as a permanent aversion to that food. The recommendation is that new foods be introduced gradually, and infants need to be exposed to a new food eight to ten times until they accept it (WHO, 2004). Infants who have been breast fed are more willing to accept new foods, because via the breast milk, they have already been exposed to different flavours, via the maternal diet. In essence, infants are introduced to the family eating habits from the moment of birth (probably during the intrauterine life too). The findings of a study by Mennella *et al*, suggests that infants are able to distinguish between different flavours from early on. It also suggests that *re-*

introduction to a particular taste or variety of food may be easier after the first refusal, as the infant is already familiar with the taste. This helps especially with the consumption of fruit and vegetables, which are thought to be poor amongst infant and difficult to pursue beyond toddlerhood (Mennnella *et al.*, 2007). There is some evidence that an interactive feeding environment also plays a positive role with respect to improved ingestion and, subsequently, the infant's nutritional status and development.

2.3.2.1.3 *Macronutrient composition*

In formulating a complementary food, feeding frequency, energy density and the energy intake from milk should be considered (Dewey & Brown, 2003). As suggested by Lutter and Dewey (2003), the concept used to calculate the recommended macronutrient composition of complementary foods for an infant with medium to high intake of milk (600 mL/day; 373.5 kcal/d), is based on the calculated gastric capacity of 30 g/kg body weight/day and a minimum energy density of foods of 0.6 kcal/g (Dewey & Brown, 2003; WHO, 2004). This approach uses the recommended protein energy: total energy of 12% as the target starting point (Monte & Giugliani, 2004). Next the fat content is calculated to ensure the food contains at least 31% of its energy as fat for an infant. The remaining energy requirement, not provided by fat or protein, is then used as the basis for calculating the carbohydrate content. The data in Table 2.11 illustrates the above calculations which yield macronutrient composition of 12 % protein, 31 % fat and 57 % carbohydrate.

Table 2.11 Calculation of macronutrient content of a desired complementary food for 6-9 months old infant [a]

Without Fortified Breakfast Porridge (BP)	
Gastric capacity [b]	249 g
Portion size (g/meal)[c]	83
Breakfast energy	-
Number of meals/d	3
Energy density(kcal/d) If desired energy density of food is 0.6- 1.5 kcal/g[*]	149 –373.5 kcal (249g)
Protein (kcal/d) [**] If % of protein energy: total energy is 12 %	18-45 kcal
Protein content (g/meal) [**]	1.5 – 4 g
Fat (kcal/d)	45- 116 kcal
Fat content (g/meal) [***]	1.6– 4.2 g
Carbohydrate (kcal/d)[****]	86 - 212.5 kcal
Carbohydrate (g/meal)	7.6 – 18.8 g

[a] Weight about 8.3 kg
[b] The gastric capacity of an infant aged 6-9 months old (30g/kg body weight/day)
[c] The portion size is calculated based on the gastric capacity of an infant aged 6-9 months old (30g/kg body weight/day) divided by 3 to make up for breakfast, lunch and dinner.
[*](Monte & Giugliani, 2004)
[**] Protein-energy ratio of 12% (DoH, 1991; WHO, 2004)
[***] Desired percentage energy from fat 31% (DoH, 1991; WHO, 2004)
[****]57% remaining energy to be provided by carbohydrate (Lutter & Dewey, 2003)

2.3.2.1.4 *Nutrient density*

As mentioned previously and as a result of the rapid rate of growth and metabolic rate taking place during the first two years of life, the nutrient needs per unit body weight of infants and young children is very high. Given the relatively small amounts of foods that are consumed at 6-24 months, the nutrient density (ND) (amount of each nutrient per 100 kcal of food) of the di*et al*so needs to be very high. In essence, this means food that provides substantial amounts of micronutrients (especially iron, zinc, calcium, vitamin A, vitamin C and folates), with relatively fewer calories (Monte & Giugliani, 2004).

Nutrient density is calculated by the following formula;

ND = (amount of energy (kcal) from one particular nutrient (g) in food/total energy derived from food) x 100

The evaluation of the Index of Nutrient Quality (INQ) in food is another good indicator of nutrient density and it relates to the amount of a nutrient per 1000 kcal in comparison to the recommended intake of that particular nutrient (Lee & Nieman, 2003). A food with an INQ substantially greater than '1' is generally considered to be a good source for that specific nutrient in excess of calories. Lipids are an exception to the foregoing premise; in excess they are known to be detrimental.

$$INQ = \frac{\text{Amount of nutrient (g) per 1000 kcal of sample food}}{\text{* Allowance of nutrient (g) in 1000 kcal of food}}$$

*The allowance of nutrient in 1000 kcal = (RNI for each age group / estimated energy requirement for that age group) x 1000

The micronutrient density of complementary food is of particular importance, due to the nutrient need per unit body weight and the limitations associated with ingestion, digestion and storage of nutrients during infancy. It is estimated that the complementary food target for a nine month old infant should provide about 97% of the intake of iron, 86% for zinc, 76% for magnesium, 73% for sodium and 72% for calcium (Bhutta, 2000; Dewey, 2001; Kimmons *et al*., 2005; Nobel & Emmet, 2006).

2.3.2.2 *Bio-availability*

Appropriate semi-solid foods should include a low content of anti-nutrients (e.g. phytate and polyphenols) as only a portion of ingested nutrients are biologically available (Barclay *et al*., 2003). The bioavailability of nutrients is most influenced by the presence of a dietary component that interferes with digestion and inhibits absorption.

For example, non-haem iron is affected by the many compounds in the meals which impact on solubility, oxidation state (ferric), and amounts of 'free iron' and also alter the iron

available for uptake by specific transporters on the surface of enterocytes in the upper intestine (Roughead *et al*., 2005).

The natural inhibitors of iron include: tannin, non-starch polysaccharides, phytic acid, phosphates, oxalates and polyphenols. The promoters for non-haem iron in the diet include vitamin C and animal protein as vitamin C keeps iron in its ferric state (Singh *et al.*, 2006). On the other hand, there are also concerns that high dietary intake of calcium may also interfere with iron absorption in food although one study reported an inhibitory effect on haem iron only (Roughead *et al*., 2005). Thane *et al* (2000), in a study of the risk factors associated with poor iron status in British toddlers, identified an over-dependence on milk and cream consumption as one of the risk factors associated with a high prevalence of poor iron status.

Another example of nutrient interaction in food is the counter-effect of iron and zinc on the absorption of copper where amino acids, in particular histidine, will act as an enhancer (Rosado, 2003). Conversely, copper is believed to have a regulatory effect on the homeostasis of iron via participation in ferroxidase activity as a catalyst. This suggests that anaemia due to copper deficiency is not caused by iron limitation but rather impairment in iron utilization (Prohaska, 2011)

In relation to the bioavailability of calcium, there have been a number of studies on formula milk in relation to their Ca^{2+} content, its bioavailability and inter-relationship with other nutrients which affect its absorption and retention in the body (Bass *et al.*, 2006). These studies have shown that calcium bioavailability is slightly increased by carbohydrates such as lactose; however, fibrous non-digestible carbohydrates (e.g. guar gum and locust bean gum) are known to decrease the availability of calcium. Green leafy vegetables are also a good source of calcium, but in those foods that are also high in oxalates (such as spinach), the bioavailability of calcium becomes poor (WHO, 2004).

While casein has been reported to improve (accelerate) the passive absorption of calcium, phytate from soy protein in soy based formulas was found to bind with Ca^{2+} and inhibit its absorption. Thus, soya based formulas have been associated with inadequate growth and bone

mineralisation in term and pre-term infants. The rate of calcium absorption is also influenced by the type of fat that that interacts (binds) to calcium ions. Calcium absorption increases when medium chain triacylglycerols are used as the fat source, whereas long chain fatty acids will form fatty acid calcium soaps that reduce absorption (Bass *et al*., 2006). Neville *et al*., (1991) and Koo *et al*., (2003) reported that infants fed on formulas containing vegetable palm oil, which is different to the palmitate in human milk, developed reduced total body bone mineral content compared with infants fed on formula free from palm oil.

As outlined above, much attention has been placed on the nutritional quality and bioavailability of nutrients in formula milk. Conversely, very little data is available on the nutritional quality and bioavailability of infant complementary foods. The exceptions are a few studies on the influence of phytic acid on trace element and mineral bioavailability with specific focus on iron and zinc (Yeung, 1998; Bhutta, 2000; Thane *et al.*, 2000; Beard, 2006; Nobel & Emmett, 2006). Based on these limited studies, it is evident that phytic acid prevalent in vegetable protein sources is a potent inhibitor of iron but its influence on bioavailability of zinc is thought to be modest and maybe more important in children recovering from infection (Hurrell, 2003). A high concentration of calcium in a diet composed of a high level of dairy products is also believed to aggravate the inhibitory effect of phytic acid on zinc absorption. These findings could be a cause for concern in the formulation of products with a high level of calcium, phytic acid and non-starch polysaccharides, due to their inhibitory effects on iron and zinc absorption.

2.3.2.3 *Food Allergy*

Food allergy involves immunologically mediated reactions (IgE) and sensitisation to a food allergen (specific protein), whereas food intolerance occurs in the absence of an enzyme (e.g. lactase) required for digestion or could be a non-IgE mediated immune response to a substance in food.

Infants who experience the development of allergy may already have an altered immune response at birth. However, increasing studies demonstrate that the relationship is slightly more complicated (Sohi & warner, 2008). Recent studies and meta-analyses could not

confirm the protective effect of an *allergen-poor* diet on the part of the mother during pregnancy and lactation. Likewise, *breast feeding* or the *timing of the introduction of solid food* into the infant's diet might not significantly influence the development of atopy, allergy, or asthma in the infant's life (Sohi & warner, 2008). For instance, low dose exposure to an allergen in the second trimester may induce foetal sensitisation while high dose exposure may, instead, induce tolerance. When considering ovalbumin sensitisation as an indicator of subsequent development of egg allergy, it has been demonstrated that a bell-shaped curve of risk exists for allergic sensitisation. Very low and very high dose exposure to allergen will protect, but exposure in the middle range will promote sensitisation (Vance *et al*., 2004).

Epidemiological evidence, however, demonstrates that the quality of maternal diet e.g. reduced antioxidant intake is associated with increased allergic sensitisation (Butland *et al*., 1999). In a study by Shaheen et al. the low levels of selenium in the umbilical cord blood was found to be associated with persistent childhood wheezing and low levels of iron with both wheezing and eczema (Shaheen *et al*., 2004)

It has also been shown that supplementation with anti-inflammatory n-3 polyunsaturated fatty acids do modify neonatal allergen-specific immune responses as infants whose mothers received supplementation had a significantly reduced severity of their eczema (Dunstan *et al*., 2003). On the other hand, it is not only the absolute content but also the ratio of n-6/n-3 PUFAs which might influence the development of either tolerance or sensitization to food. In a study using a rat model a ratio of 9:1 (n-6/n-3) in the mothers' diet prevented tolerance induction in neonatal rats, which otherwise could have been achieved if the diet was n-3 PUFA biased (Korotkova *et al*., 2004).

With respect to the weaning diet, potential allergic reactions related to the consumption of certain high-protein foods during infancy have been a concern in some industrialized countries. Although, genetic and an immunological pre-disposition are the most important determinants of allergic disease, it is highly advisable for infants with a family history of atopic diseases to have a delayed weaning process (beyond that recommended) with a more selective choice of diet (Ziger, 2003). The American Academy of Paediatrics recommends that infants with a strong family history of allergy should not receive cow's milk until one

year of age, eggs until two years, and peanuts, nuts, fish and shellfish until three years of age (AAP, 2004).

2.3.3 Challenges associated with infant feeding in the UK

Parents need to ensure that the diets of their infants and young children benefit from a wide range of different food groups in order to provide for the right nutritional amount of proteins, fat, carbohydrate, fibre, mineral, vitamins & water (Thompson, 1998); Gokhale & Kirschner, 2003; Wells, 2007). There are however, a few feeding challenges that are claimed to threaten the availability of an adequate nutritional intake including (Thompson, 1998):

1) social; low income, lack of knowledge, poor stimulation and a working mother,
2) dietary; early weaning, inappropriate weaning diet, irregularity of meals, vegetarianism, prolonged food refusal & faddiness, high intake of sweet and excessive fluid intake,
3) cultural; ethnicity, and
4) special needs and clinical challenges.

Amongst the above, social and dietary factors in relation to income and lack of knowledge are the issues of relevance in the UK (DoH, 2008; SACN, 2011). To illustrate this point, the major causes of iron deficiency during infancy identified by the Department of Health in 1994 included early weaning and introduction of cow's milk, prolonged milk drinking, delayed weaning, poor intake of vitamin C, consumption of solid food close to milk intake and a general lack of awareness. All of these issues can be alleviated via a better understanding of the weaning process.

There is evidence to suggest that mothers usually have little or no knowledge about the nutritional requirements of their infants and young children (Barclay *et al*., 2003; Briefel *et al.*, 2004; Davies & O'Hare, 2004; Bolling *et al*., 2007). There has recently been considerable media attention devoted to breast feeding and the quality of the diet of school-aged children; however, not as much attention has been placed on the "transitional'' diet for weanlings (Barclay & Weaver, 2003; Davies & O'Hare, 2004). This lack of knowledge is reflected in the

selection of a limited diet in terms of its diversity and nutrient content. Davis & O'Hare (2004), have reported on the outcome of a survey which indicates many mothers were introducing their babies to a low fat, high fibre diet as a step towards preventing their children becoming overweight and obese.

A national survey in the UK has reported that some toddlers and young children are at risk of inadequate energy, vitamin A, iron and zinc intake (Barclay *et al*., 2003). Iron deficiency anaemia alone affects 1 in 8 toddlers in the UK (Holden *et al*., 2000). In contrast, excess body weight and obesity, are growing problems effecting an increasing number of children, due to the imbalance of dietary intake and energy expenditure worldwide (Hulzebos *et al*., 2007). In the UK alone, excess energy intake and decreased physical activity has resulted in 20% of pre-school age children being overweight and at least 10% being clinically obese (Barclay & Weaver, 2003).

2.4 Role of commercial ready to feed infant foods

Over recent decades, changes in life style and to the food economy have contributed to a shift in dietary patterns. This has led to increased consumption of energy-dense diets high in fat, particularly saturated fat, and low in unrefined carbohydrates (Lobstein *et al*. 2004). This is evident in the increased sales and consumption of pre-packed foods in the UK, extending to infant and young children's food (Lobstein *et al*. 2004). The modern life style dynamic has, therefore, lead to an increased parental reliance on commercially marketed complementary foods in the UK (White & Hampson, 2008), which may have potential implications for total energy and fat intake in addition to taste acquisition. The foregoing factors can negatively impact on the risk of chronic non-communicable diseases (Rudolf, 2009).

2.4.1 Parental reliance

Commercially prepared complementary foods are an important part of the diet of many infants and toddlers (Michaelsen & Fariis, 1998; Briefel *et al*., 2004: Melo, *et al*., 2008).
Given the nature of a modern life style many parents in the UK rely on these foods for their young children because they are convenient and perceived to be time-saving (DoH, 2000; Foote *et al*., 2003; Briefel *et al*., 2004; Davies & O'Hare, 2004;Weaver, 2008).

It is estimated that, in the UK alone, 50% to 60% of parents choose commercially prepared baby foods for feeding their infants and young children at some stage (White & Hampson, 2008). It is, therefore, important that commercially prepared foods contain sufficient amounts of energy and nutrients to fulfil the requirements needed for growth and development at this critical period of dietary transition (Barclay *et al*., 2003; Holden *et al*., 2000; Hulzebos *et al*., 2007; Taitz *et al*., 1989; Wells, 2007). The aforementioned trend enhances the importance of clear labelling relating to food safety and nutritional information.

2.4.2 Current debates on safety and quality of foods

Currently, there are a range of food products on the market designed for infants, which are available for parents to purchase. The extent to which these products meet the nutritional requirement of infants is an area of uncertainty (Michaelsen & Friis, 1998; DoH, 2000; Thiel, 2000; Cheesa & Kathryn, 2003; Briefel *et al.*, 2004; Davies & O'Hare, 2004; Mennella *et al.*, 2007; Melo *et al.*, 2008). This issue of uncertainty relates to factors such as (1) nutrient stability, (2) bioavailability, (3) composition of the finished product, (4) processing conditions and (5) storage.

The lack of a clear understanding of the nutritional quality of these food products is further hindered by the current lack of robust regulations relating to the nutritional quality of these food compositions. It is important to note that there are two ends to the nutritional imbalance spectrum which are associated with 1) low intake resulting in deficiencies and 2) high intake resulting in potential toxicity (Goldhaber, 2002). Most essential trace elements present in infant formula have received very little attention in relation to their safe intake. For example, in the case of eight out of the eleven essential elements, whose levels are regulated in formula, the data required for a science-based risk assessment of infant exposure is currently lacking (Codex Alimentarius Commission, 2007).

The implication is that a number of important nutrients may be limited or excessive in some brands thus affecting their nutritional quality and suitability for infants. For example a Norwegian study carried out on commercially fortified complementary foods suggested that

the menu of industrially prepared foods contained almost 2.5 times more potassium than the recommended daily intake for the 6-12 months age group, which is 800 mg per day (Melo *et al*., 2008).

Complementary foods have only recently become an area of public focus due to the recent occurrence of several food crisis e.g. melamine in infant formula (Qiao *et al*., 2010) and the recall of baby rusks from the market due to an excess amount of fat (BBC, 2009).

In response to these concerns, the Food Standard Agency (FSA) and the Department of Health have commissioned a dietary survey of infants in September 2009 [National Center for Social Research (NatCen); report for 2012]. This survey aims to collect data on the food and nutrient intake of children from the age of 4 to 18 months, as well as weaning practices among parents. As this survey progresses, the data from the survey will be used to measure whether parents are taking notice of the Government's advice on breastfeeding and weaning. It will also underpin the Department of Health's work on promoting healthy diets and protecting consumer's rights.

2.4.3 Regulation and legislative requirements with respect to nutritional labelling of ready-to-feed infant foods

Food labelling remains a major issue in the United Kingdom and across the European Union. According to UK Food Standards Agency, there is anecdotal evidence that nutritional labelling of foods is not uniform and is a subject of on-going interest for the FSA in the UK (White & Hampson, 2008).

To date, increased emphasis has been given to improving the preparation methods for infant formula milk and official guidelines have been declared in order to ensure the safety of baby food products (Commission Directive 2006/125/EC). In contrast, insufficient attention has been paid to the nutritional quality of ‘ready to feed’ complementary foods intended for infants during the period of weaning. The current nutritional information labelling formats for ‘ready to eat’ complementary food (Processed Baby Foods Regulations 1997/2042) have primarily been due to legislative requirements rather than being designed specifically to

ensure that the nutritional requirements of the consumer are being met or as an aid to parents being able to make an informed choice at the point of purchase. For example, certain data has not yet been required to be included in relation to nutritional information content such as the mandatory classification of fat and carbohydrates. This recommendation has already been adopted by the US Food and Drug Administration (FDA) in relation to the nutrition fact label; i.e. mandatory declaration of saturated fat and dietary cholesterol as well as *trans* fats by the Nutrition Facts Panel has been included since January 1st, 2006 (68 FR 41434). The foregoing is in addition to the guide to Daily Values (DVs) of energy and macronutrients. In Europe the inclusion of such information has been controversial and much disputed both within the European Committee and by the stakeholders involved in the technical field (Commission Directive 90/496/EEC on Nutrition Labelling for Foodstuffs).

According to the FSA (White & Hampson, 2008) there is, currently, no coherent and complete analytical nutritional data available for ready to feed complementary foods. The McCance & Widdowson's composition of foods integrated data set (FSA, 2002) contains only limited data regarding the micronutrient composition (none in the case of essential fatty acid) content of commercial infant foods and most of the data included is for composite samples rather than specific products or brands (White & Hampson, 2008).

The lack of attention in respect of complementary foods for infants and young children is also highlighted in the National Obesity Action Plan (Lobstein *et al.*, 2004). This plan deals with a number of areas such as improved breast feeding, clear and consistent food labelling, production of more nutritious and lower energy food for children, healthier school meals and, finally, the plan also outlines criteria for advertising (Lobstein *et al.*, 2004). There is, however, no mention about the weaning diet or products intended for infants during the progressive adaptation to ordinary foods. This is not the only plan where there is a nutritional gap. The National Diet and Nutrition Survey Program (NDNS), which is conducted every five years on behalf of the Department of Health, also excludes nutritional information relating to the solid component of the diet for children less than 18 months of age (DoH, 2008).

Labelling may not only be beneficial to the consumer (education and informed choice) but could put the manufacturer at a competitive advantage, particularly if the nutrient quality of their product is found to be good. It is also important to inform future product development

and marketing if there are commercial advantages (or disadvantages) to consider in relation to complete 'nutrient disclosure' (Cheftel, 2005).

2.5 Identification of the gap in relation to the nutritional quality

It is clearly evident that, currently, insufficient attention has been paid to the nutritional quality of 'ready to feed' complementary foods intended for infants during the period of weaning. Manufacturers of infant foods in the UK do not make any reference to the micronutrient composition of their products on the labels.

Under the food labelling regulations in Great Britain *[1996 (FLR) (SI 1996 No.1499) provision of the EC nutrition labelling Directive (90/496/EEC)]* micronutrient declaration is non-mandatory. According to Commission Directive 2006/125/EC, micronutrients content can only be declared when at least 15% of the reference values are supplied per 100 g or 100 mL of product and where appropriate per specified quantity of product as proposed for consumption (Food Standard Agency, 2004; The Commission of the European Communities, 2006).

Moreover, there has been little discussion relating to the challenges concerning nutritional composition of such products in the UK and there have been no controlled studies comparing the dietary intake from commercial 'ready to eat' complementary food with reference to the Recommended Nutrient Intake (RNI) (DoH, 1991) in order to ascertain their suitability.

2.6 Challenges associated with the processing of commercial food

In general, adequate food safety and shelf-life or the alteration of the sensory characteristics of food cannot be achieved unless multiple methods of processing are applied. Food processing has inevitable consequences in relation to the nutritional value of foods depending on the molecular structure/physico-chemical properties of the nutrients and the severity of the processing. For example, the nutritional value of protein is influenced by heat treatment and the presence of iron, vitamin C and lactose in the food composition. Previous studies have shown that heat treatment (122 to 132^{o}C for 5 to 8 min) applied in manufacturing of ready-to- feed liquid infant formula reduced the apparent and true digestibility of protein

(74% to 76%: 88% to 90%) compared to powdered formula (79% to 83%; 93% to 97%) and that the true digestibility of lysine, methionine and cysteine in liquid products were 5% to 13% lower than the powdered form (Thompkinson & Kharb, 2007). It is, therefore, necessary to examine the nutritional value of food formulations to establish whether infants are receiving the correct level of non-milk related nutrients during this critical period of their development.

2.6.1 Biological and chemical safety of food

Microbiological safety and maximum permissible levels of toxic elements in food is vital to the manufacture of safe food intended for infants and young children due to their immature immune system, and exacerbated level of high intake relative to their body size. The presence of potential hazards in food is usually attributed to a) naturally occurring sources such as raw materials or b) contamination during processing of food throughout the supply chain.

The Food Safety Act (1990) in combination with the General Food Law (No. Regulation (EC) 178/2002), are currently being enforced to ensure the safe production, processing, storage, distribution and sale of various ready-to-eat foods at the point of purchase. The British Retail Consortium Standards e.g. BRC Global Standard for Food Safety Issue 5 is an example of the Total Quality Management system implemented by food companies in compliance with the foregoing safety regulations.

The guidelines for the microbiological quality of various ready-to-eat foods including ready meals are presented in Table 2.12.

Table 2.12 Guidelines for the microbiological quality of various ready -to-eat foods (Public Health Laboratories, 2000).

Food Category *	Criterion	Microbiological Quality (CFU per gram unless stated)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable/ Potentially hazardous**
	Aerobic colony count 30°C/48 h				
1		$<10^3$	10^3-$<10^4$	$\geq10^4$	N/A
2		$<10^4$	10^4-$<10^5$	$\geq10^5$	N/A
3		$<10^5$	10^5-$<10^6$	$\geq10^6$	N/A
4		$<10^6$	10^6-$<10^7$	$\geq10^7$	N/A
5		N/A	N/A	N/A	N/A
	Indicator organism				
1-5	*Enterobacteriaceae*¶	<100	100-$<10^4$	$\geq10^4$	N/A
1-5	*E. coli (total)*	<20	20-<100	≥100	N/A
1-5	*Listeria spp(total)*	<20	20-<100	≥100	N/A
1-5	*Salmonella spp*	Not detected in 25 g			Not detected in 25 g
	Pathogen				
1-5	*Campylobacter spp*	Not detected in 25 g			Not detected in 25 g
1-5	*E. coli 0157* & other *VTEC*†	Not detected in 25 g			Not detected in 25 g
1-5	*V. Cholerae*	Not detected in 25 g			Not detected in 25 g
1-5	*V. parahamolyticus*‡	<20	20-<100	100-$<10^3$	$<10^3$
1-5	*L. monocytogenes*	<20***	20-<100	N/A	≥100
1-5	*S. aureus*	<20	20-<100	100-$<10^4$	$\geq10^4$
1-5	*C. prefringes*	<20	20-<100	100-$<10^4$	$\geq10^4$
1-5	*Cereus and other pathogenic Bacillus spp.*	$<10^3$	10^3-$<10^4$	10^4-$<10^5$	$\geq10^5$

*Categories 1-5 are based solely on expected aerobic colony count, according to the type of food and processing undertaken.
** Prosecution solely based on high colony count /or indicator organisms (Colony Forming Unit (CFU) per gram unless stated).
¶ not applicable to fresh fruit, vegetable and salads.
† Verocytotoxin producing *E. Coli*.
‡ Relevant to seafood only.
*** Not detected in 25 g for certain long-shelf life products under refrigeration.

2.6.1.1 *Current methods of processing: nutritional consequences*

As far as nutritional quality is concerned, regardless of the mode of preparation (homemade or industrial), there are a number of areas of concern. These include lack of knowledge of child nutritional requirements, traditional recipe development and natural inhibitors, low nutrient density and high energy content, poor nutrient retention, poor diversity of ingredients in relation to cultural differences and cost (Michaelsen & Friis, 1998; Davies & O'Hare, 2004).

The demand by consumers for high-quality foods having 'fresh' or natural characteristics with an extended shelf-life has led to the application of combined processing techniques in order to maximise the post-process integrity and quality of the food, as first developed by Leistner and others, into the so-called 'hurdle' concept (Leistner, 2000; Fellow 2008).

In combined processes, an understanding of the interaction between temperature, water activity (a_w), pH, chemical preservation and modified atmosphere packaging is used to design a series of hurdles to ensure the safety of processed foods.

The data in Figure 2.2, illustrates the influence of water activity as one of the major hurdles on enzyme activities and microbiological growth in reduced moisture products.

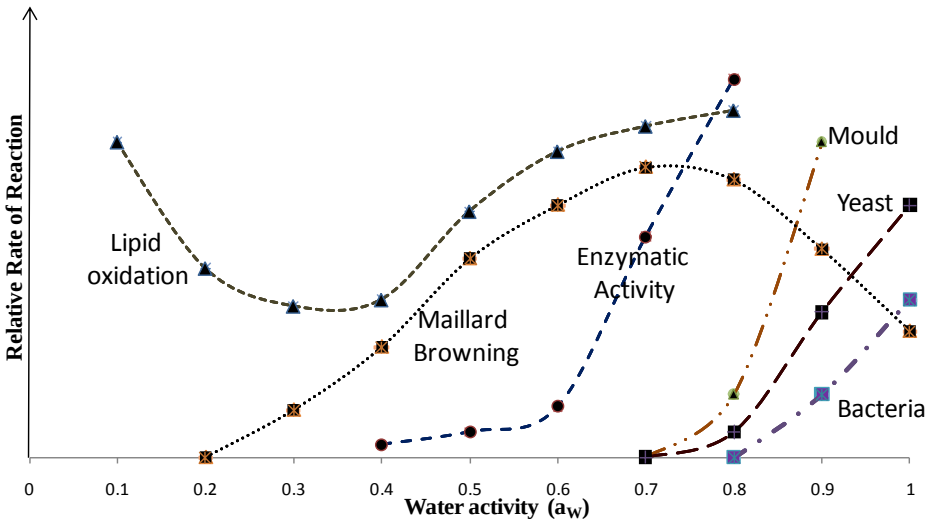

Fig. 2.2. Influence of water activity on the chemistry and microbiology of food (Slade & Levine, 1991).

By combining Hurdle technology (Fellow, 2008) and Hazard Analysis Critical Control Point (HACCP) (Mortimore, 2001) in process design, the intensity of preservation techniques are kept comparatively low to minimise the loss of product quality, while overall there is a high impact on controlling microbial growth. For example, in the case of fruit juice the

combination of minimal processing and high pH (< 4.5) can result in the destruction of most pathogenic organisms, reduction of spoilage organisms and enzyme inactivation e.g. polyphenol oxidase and pectinases, via pasteurisation (Fellow, 2008)

Thermal processes also enhance the digestibility of protein and complex carbohydrates and improve the bioavailability of indigenous micronutrients such as vitamin B_6, folic acid, niacin and carotenoids via destruction of antagonistic ligands e.g. phytate - zinc antagonism (Shills *et al*., 1996).

A summary of the applications, conditions as well as advantages and disadvantages of common thermal processes applied to food are presented in Table 2.13.

Table 2.13 Application, conditions and advantages/disadvantages of common thermal processes applied to food (Shills *et al*., 2006).

Treatment	Temperature	Application	Advantages	Disadvantages
Blanching	75-95^{o}C for 1-10 min	Fruit and vegetables for canning and freezing	Inhibition of damaging enzymes, air removal (canning)	Loss of water soluble vitamins and minerals
Pasteurisation	LTLT* : 63^{o}C-30 min HTST**: 72^{o}C-10s	Milk	Killing of pathogens Lipase, reduced spoilage organism	Refrigeration required, loss of volatile compounds
UHT ***	135^{o}C<10 s	Milk, juices, deserts	Shelf stable, good flavour	Nutrient losses damages texture
Canning	115-125^{o}C for 10-120 min	Fruit, vegetable, canned food	Shelf stable	Nutrient losses, damages texture
Baking	175-260^{o}C for 4-30 min	Bread and cookies	Flavour development and preservation	Loss of vitamin from browning reaction
Extrusion	115-200 ^{o}C for 10-90 s	Expanded snacks	Rapid processing, texture	High cost

*LTLT: low temperature long time, **HTST: high temperature short time, ***UHT: ultrahigh temperature

2.6.1.2 *Blanching*

Blanching is a pre-treatment process that is mainly used for vegetables for the inactivation of enzyme reactions. Water soluble vitamins such as vitamin C (10-15%) and thiamine (9-60%) tend to be lost due to leaching during water blanching, thus steam blanching is preferred. Blanching has also proved effective in the reduction of surface contaminant micro-organisms, hence facilitating the subsequent preservation operation (Breidt *et al*., 2000).

2.6.1.3 *Pasteurisation*

Pasteurisation is a moderate thermal process during which food is treated to mild temperatures below 100^{o}C. Pasteurisation eliminates vegetative organisms; however, temperature controlled storage is necessary for the inhibition of spores. There are some new techniques that have broadened the definition of pasteurisation to any treatment that reduces the number of heat resistant organisms to a level that is not likely to present a public health risk under normal condition of distribution and storage (Sugarman, 2004). Details of the techniques are provided in section 2.6.1.8.

The High Temperature Short Time regimen (HTST) offers a considerably higher retention of nutrients and a tenfold increase in bacterial elimination by a 10^{o}C increase in the processing temperature (Fig 2.3).

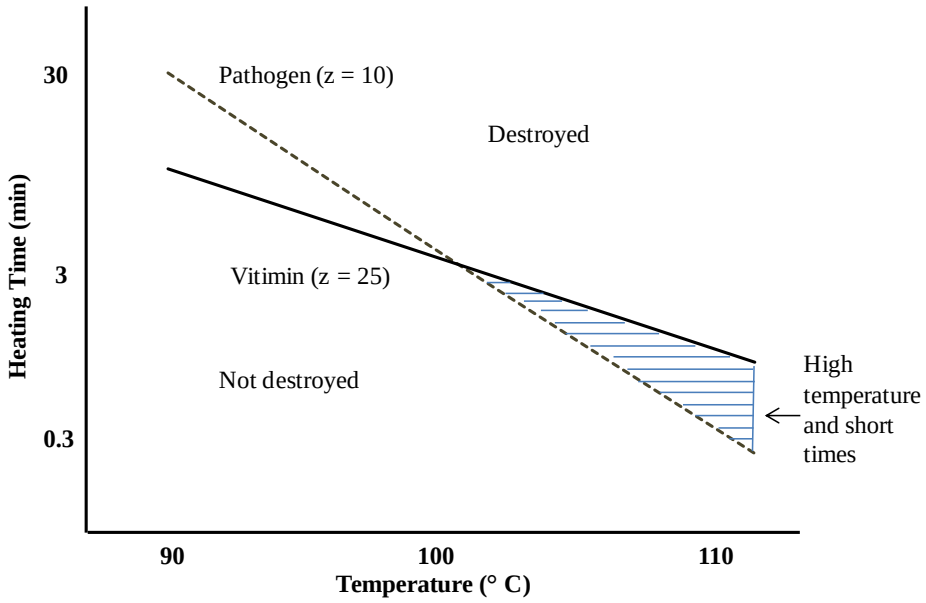

Fig 2.3. The temperature/time relationship for pasteurisation (the shaded area is the commercial HTST region). The Z-value is the number of degrees Celsius required to reduce the decimal reduction time (D-value) of the microorganism by tenfold. D-value is the time (min) required to destroy 90% of the organisms (to reduce their number by a factor of 10) (Fellow, 2009)

Figure 2.3 (Fellow, 2009) illustrates the relative changes in time/temperature profiles for the destruction of microorganisms. Above and to the right of each line the microorganisms or quality factors would be destroyed, whereas below and to the left of each line, the microorganisms or compositional integrity would not be destroyed. Due to the differences in Z values it is apparent that at higher temperature- short time combination (shaded area), pathogens can be destroyed while vitamins can be maintained. The same holds true for other quality factors such as colour and flavour components. Thus in milk processing the high temperature-short time (HTST) process combination (72°C/16 sec) is favoured compared to a lower temperature longer time process since it results in a slightly lower loss of vitamins and better sensory quality of the milk. A Z value of 10°C is typical for a spore forming bacterium. Heat induced chemical changes have much larger Z values than microorganisms, as shown in Table 2.14.

Table 2.14 Heat resistance of vitamins and chemicals in relation to heat resistance of microorganisms (Holdsworth, 1992).

Reactions	Z –value (°C)
Bacteria	5-10
Enzymes	30-40
Vitamins	20-25
Pigments	40-70

Nutrient retention during pasteurisation is best achieved under acidic conditions as most nutrients are heat stable at low pH < 4.5 (Burger & Walters, 1973).

The exclusion of oxygen during processing and storage is another factor which helps to protect nutrients including folic acid, vitamins B_{12} and C, which are vulnerable to oxidation (Burger & Walters, 1973; Fellow, 2009). Low temperature/long time (LTLT) processing in combination with vacuum processing is one of the examples applied in processing of heat sensitive liquids and concentrates such as juices.

2.6.1.4 *Ultra-high-temperature (UHT)/aseptic process*

UHT is a useful process for sterilisation of products intended for storage at ambient temperatures where refrigeration is not viable. Examples of UHT include aseptic packed infant vegetable and fruit purees with a texture, flavour and nutritional quality that is far superior to those of retorted products (traditional sterilisation-canning) (Fellow, 2009).
Aseptic *pouches* are rapidly replacing the previous canned products in the infant food industry due to their improved organoleptic and nutritional quality from which they are manufactured. These pouches are heat-sealable and due to the flexibility of the polymeric laminates, and a greater surface: volume ratio, the food processing time is greatly reduced (Shills *et al*., 2006).

2.6.1.5 *Extrusion*

In extrusion processing, the combination of high temperature (140-180^{o}C) and shear (high pressure: 60-80 bar) provides several benefits in developing food for infants including improved digestibility of starch and protein and the effective removal of anti-nutrients (Shills *et al*., 2006) The additional advantage of extrusion is that the short residential time minimises

the damages to vitamins. The current application of extrusion in the infant food industry includes colour shaped pastas, snacks and fruit bars.

2.6.1.6 *Dehydration*

There are many applications for dehydration techniques including air drying (65-70^{o}C), spray-drying and freeze-drying in the infant food industry. These include the production of infant formula powders (spray drying), fruit and vegetable-based breakfast cereals and meals, and dried crispy fruits (freeze drying). There are a number of nutrients such as lysine, vitamin A and C which are lost during the dehydration process although the retention of thiamine is improved by 95%. In soya based products nutritional quality is improved because the trypsin inhibitor activity is reduced. In the UK, however, the fortification of cereal based products intended for infants is permitted; hence the processing losses can be compensated by controlled fortification. Losses during storage are very much dependent on water activity (a_w) can be reduced by removal of oxygen and exclusion of light and reduction of storage temperature (Fellow, 2009). Moreover, dehydrated products offer less convenience compare to extrude or UHT packed products as a result of rehydration being required.

2.6.1.7 *Microwaving*

In microwave processing, the electromagnetic waves that are applied to food induce a vibration in any molecule that has a dipole moment and will ultimately generate heat (Shills *et al.*, 2006).

Microwaving has a greater impact in home cooking. Industrially microwaves are often used for defrosting (Shills *et al*., 2007). In feeding practices in the UK, microwave reheating of baby food and formula milks is commonly applied.

From a review by Gerster (1989), on the effect of microwaves on vitamin stability, it is concluded that when heat sensitive vitamins such as C, B_1 and B_2 are used as markers, the retention during blanching, cooking, reheating and thawing in the microwave is comparable to the retention obtained with conventional methods of heating. There are no published studies,

however on the effect of microwave reheating on the non-endogenous vitamins and minerals as well as the EFAs in fortified infant foods.

2.6.1.8 *New approches in processing*

There are new approaches used in processing of food including ohmic heating and high pressure processing. It is, however, worth noting that there are many obstacles for introducing the new processing technologies as the food industry is unwilling to replace the traditional methods due to regulations, demands on facilities and public reluctance toward new technology. For example in Europe, products that are obtained via minimal processing are classed as novel foods and are regulated following the "Novel Food Legislation" (Sun-Waterhouse, 2011).

2.6.1.8.1 *Ohmic heating (OH)*

During ohmic processing, the heat is internally generated using an alternating current through a material that provides electrical resistance (Kirinch *et al*., 2010). More specifically OH is a HTST method that can heat 80% of a solid from room temperature to 129^{o}C in 90 seconds. In OH over-processing can be avoided as simultaneous heating of both the liquid and solid food phase is achieved (Zell *et al*., 2010; Jacob *et al*., 2010).

The application of OH in the food industry is limited due to the lack of temperature monitoring and knowledge of the critical processing factors affecting heating using OH. Thus commercialisation of the techniques is hindered and further studies are needed on food safety and retention time (Shills *et al*., 2006; Kirinch *et al*., 2010).

2.6.1.8.2 *High pressure processing (HPP)*

In HPP hydrostatic pressure is employed to pasteurise food non-thermally, hence the techniques offers significant advantage in terms of having a minimal effect on the colour, flavour, taste and vitamin retention especially in heat- and chill-sensitive products (Houska *et al*,. 2006). The application of HPP, however, in combination with temperature has shown to be more effective in the destruction of bacterial spores depending on the pressure and strains

of the organism. Few studies are currently available on the effect of HPP on anti-nutrient stability.

2.6.2 Assesment of nutritional quality: optimisation and food design

To assess and improve the nutritional quality of composite food, it is important to consider the effect of the different stages of processing on the nutritional constituents of the food.

As outlined above, thermal treatment in particular leads to significant changes in the structure of food constituents e.g. hydrolysis of polymeric carbohydrates, hydrolysis and coagulation of protein (Sun *et al*., 2008). As a result, essential nutrients present in the raw materials are exposed to different processing conditions that can reduce the nutritional and sensory values of the final product (Kaur *et al*., 2007). The extent of any change depends on many factors e.g. the kind of ingredients, cooking method, equipment, temperature and time. The damaging pathways pointed out for thermal treatment concerning nutritional quality include heat degradation of nutrients, oxidation of fat soluble vitamins and lipids and leaching of water-soluble vitamins and minerals (Thiel, 2000; Morris *et al*., 2004). Most of the research that has been carried out, to date, with respect to the effect of processing on foods has been undertaken by examining a particular ingredient in isolation e.g. the influence of dehydration on the composition of e.g. spinach rather than spinach as part of a "meal" (Kaur *et al*., 2007).

Very few studies have been undertaken to determine the effect of varying conditions of thermal processing on the nutritional quality of the final food composite (Holdsworth 2004; Morris *et al*., 2004; Meade *et al*., 2005). Recent research shows that different constituents in food may have a protective effect on vulnerable nutrients and thereby promote the organoleptic properties of food, e.g., the retention of the naturally occurring antioxidants in fish (Aubourg, 2001; Kaur *et al*., 2007; Sun *et al*., 2008). Such studies (Aubourg, 2001; Kaur *et al*., 2007) show that when accurate time and temperature conditions are employed during thermal treatments, the retention of most of the nutrients remain at an acceptable level, providing that high quality raw materials are used in the recipe.

While consumer interest lies in natural, healthy and nutritious food products, there is scope and opportunity for optimisation and product development. Ingredient selection for a food formulation is one of the critical aspects. In formulating an optimised product, target population, safety, nutrient content and molecular interactions, food processing functionality and legislative requirements as well as consumer acceptance needs to be considered. While improving food formulation is an effective approach, the use of encapsulation in design and fortification is an emerging approach in development of functional foods (Song *et al*., 2006; Mozafari *et al*., 2008; Given, 2009).

2.7 References

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Chapter 3

Commercial "ready to-feed" infant foods in the UK: macro-nutrient content and composition

3.1 Introduction

Early infant feeding provides nutrients for optimal growth and development. Breast milk provides sufficient amounts of energy and essential macro- and micro-nutrients for rapid growth and development at least for the first six months of the infant life, in addition to being perfectly adapted to the immature gastrointestinal, renal and metabolic systems of an infant (Brown *et al*. 2008; Foote *et al*. 2003; Wells, 2009). An extensive body of scientific evidence supports the consensus that failure to breast feed increases the risk of illness in both the mother and infant (SACN, 2011).

The current UK recommendation relating to infant feeding is exclusive breast feeding for the first six months of an infant life (DoH 2008). The key finding from the latest infant feeding survey in the UK suggests that the initial breastfeeding rate has increased from 76% in 2005 to 81% in 2010. The incidence of breastfeeding has continued to follow the same pattern of variation as in 2005, according to the socio-demographic characteristics of mothers such as maternal age and educational attainment. The duration of breast feeding across the UK illustrates that the greatest increase is found in older mothers from higher socio-economic backgrounds who, typically, have a higher educational attainment (Infant Feeding Survey 2005, 2010).

After the first six months of life, the utilization of post-natal nutrient stores together with the need for rapid growth of an infant, creates further demand for energy and nutrients; milk alone becomes inadequate (Barclay *et al*., 2003; Briefel *et al*., 2004; Hulzebos & Sauer, 2007). This is the beginning of the weaning period, when complementary food (i.e. food that is additional rather than a substitute to mother's or formula milk) is required to boost energy and nutrient intake (Briefel *et al*., 2004; Hulzebos *et al*., 2007). It is therefore important that complementary foods contain the right amount of energy, protein , fat , minerals and vitamins

to fulfil the requirements needed for growth and development at this critical period of dietary transition (Barclay *et al*., 2003; Wells, 2007).

Over recent decades, the modern life style dynamic has lead to an increased parental reliance on commercially marketed complementary foods in the UK (White & Hampson, 2008), which may have potential implications for total energy and fat intake in addition to taste acquisition (Lobstein *et al*. 2004). The result of early nutritional imbalances is already evident as, currently in the UK, one in five children starts school overweight (DoH, 2006); data suggests that children who are overweight at an early age are likely to continue to be overweight in later life (DoH, 2006). This in turn, increases the risk of developing chronic diseases such as type 2 diabetes, heart disease and a variety of other co-morbidities in early adulthood (Lobstein *et al*., 2004; Rudolf, 2009). On the other hand, a national survey in the UK has reported that some toddlers and young children are at risk of inadequate energy, vitamin A, iron and zinc intake (Barclay *et al*., 2003). Iron deficiency anaemia alone is estimated to affect 1 in 8 toddlers in the UK (Holden *et al*., 2000). The foregoing factors can negatively impact on the risk of chronic non-communicable diseases (Rudolf, 2009; Wells, 2009) and therefore enhances the importance of robust regulatory systems both in the areas of food safety and nutritional quality of the commercially prepared food products relevant to their target age group.

To-date, insufficient attention has been paid to the nutritional quality of 'ready to feed' complementary foods intended for infants during the period of weaning. Increased emphasis has been given to improving preparation methods for infant formula milk and also official guidelines have been declared in order to ensure the safety of baby food products (Commission Directive 2006/125/EC). The current nutritional information labelling formats for 'ready to eat' complementary food (Processed Baby Foods Regulations 1997/2042) is, in general, a duplicate of the legislative requirements for manufacturing 'ready meals' rather than being designed specifically to ensure the nutritional requirements of their vulnerable target group. For example, certain data has not yet been required to be included on the nutritional information content such as mandatory classification of fat and carbohydrates or declaration of micronutrient content. This recommendation has already been adopted by the US Food and Drug Administration (FDA) in relation to the nutrition fact label; i.e. mandatory declaration of saturated fat and dietary cholesterol as well as *trans* fats by the Nutrition Facts

Panel has been included in the USA since January 1st, 2006 (68 FR 41434). In Europe the inclusion of such information has been controversial and much disputed both within the European Community and by the stakeholders involved in the technical field (Commission Directive 90/496/EEC on Nutrition Labelling for Foodstuffs).

The lack of attention in respect of complementary foods for infants and young children is also highlighted in the National Obesity Action Plan (Lobstein *et al*., 2004). This plan deals with a number of areas such as improved breast feeding, clear and consistent food labelling, production of more nutritious and lower energy food for children, healthier school meals and, finally, the plan also outlines criteria for advertising (Lobstein *et al.*, 2004). There is, however, no mention about the weaning diet or products intended for infants during the progressive adaptation to ordinary foods. This is not the only plan where there is a nutritional gap. The National Diet and Nutrition Survey Program (NDNS), which is conducted every five years on behalf of the Department of Health, also excludes nutritional information relating to the solid component of the diet for children less than 18 months of age (DoH, 2008).

According to the FSA (White & Hampson, 2008) there is, currently, no coherent and complete analytical nutritional data available for ready to feed complementary foods. The McCance & Widdowson's composition of foods integrated data set (FSA, 2002) contains only limited data regarding the nutrient composition of commercial infant foods and most of the data included is for composite samples rather than specific products or brands (White & Hampson, 2008).

Complementary foods have become an area of public focus due to the recent occurrence of several food crisis e.g. melamine in infant formula (Qiao *et al*., 2010) and the recall of baby rusks from the market due to an excess amount of fat (BBC, 2009). To date, there has been little discussion relating to the challenges concerning nutritional composition of such products in the UK and there have been no controlled studies comparing the dietary intake from this food with reference to the Recommended Nutrient Intake (RNI) in order to ascertain their suitability (DoH, 1991).

The focus of the current study is concerned with the lack of quantitative data available with respect to the nutritional quality of 'ready to feed' complementary foods marketed for infants of 6 to 9 months of age in the UK. This study addresses several important issues; 1) the macronutrient content of a series of commercially prepared meat and vegetable-based meals; 2) the discrepancy between the label claims and the actual macronutrient content of the infant foods; 3) a hypothetical, non-numerical format of labelling information is proposed and 4) the role of these foods in meeting the dietary requirements of infants. The essential and trace element content of the same range of infant food products have already been reported (Zand *et al.*, 2011).

One of the main limitations of this study is that it is unlikely to represent the actual amount of food that is ingested and retained by an infant; neither does it take into account any wastage of food. A further limitation with this analytical approach is that it fails to take into account any contribution derived from breast milk, snacks or homemade food.

3.2 Material and Methods

3.2.1 Sample collection and preparation

Eight samples of "ready to feed" infant meals, representing four brands of two different varieties of meat and vegetable based products, were purchased from retail outlets in the UK between November 2010 and March 2011. The sampling plan was intended to reflect the main products and brands within the major categories of commercial infant foods on sale, taking into account available market share data. Two categories of commercial infant foods were sampled: (i) meat based and (ii) vegetable based from the same lot/batch for each individual product. These products were semi-pureed and packed in glass jars. Exact corresponding recipes were not available for all the brands. The sample jars (in triplicate) were stored unopened at room temperature, similar to their distribution and market environment. The main ingredients of the baby food samples and their characteristics are presented in Table 3.1. The samples were first homogenised using a blender (Multi-quick, Braun 300) prior to any analysis. All samples were analysed in triplicate and the mean value reported for energy, protein, fat, carbohydrate and fibre.

Table 3.1 Infant complementary food sample characteristics.

	BRAND Code	PRODUCT NAME(n=8)	INGREDIENTS	NUTRITIONAL INFORMATION per 100g
Meat based (6-9 Months)	A	Cottage pie	Organic vegetables (64%) (potato (22%), carrots, tomatoes, onions), water, organic cooked rice, organic beef (8%), organic sunflower oil, organic herbs [parsley, oregano],organic vegetable stock [salt, organic rice flour, organic vegetable (carrots, onions, celeriac),organic yeast extract, organic vegetable oil, organic spices]	**Energy** 278kJ/66kcal, **Protein** 2.8g, **Carbohydrate** 8.7g of which sugars 1.9, **Fat** 2.3g, of which saturates 0.6g , **Fibre** 1.6g , **Sodium** 0.05g
	B	Cottage pie	Vegetables (45%), (carrots (22%), potatoes (14%), onions (7%), peas(4%)) water, whole milk, beef (9%), corn flour, unsalted butter, natural flavouring, yeast extract, herbs, iron sulphate	**Energy** 253kJ/60kcal , **Protein** 2.8g, **Carbohydrate** 7.5g of which sugars 1.8g, **Fat** 2.1g of which saturates 1.2g , **Fibre** 1.3g, **Sodium** 0.8g , **Iron** 1.3mg
	C	Cottage pie	Baby grade vegetables (56%) (potato, green beans, carrot, peas), cooking water, beef (10%), white beans, wheat starch (gluten free),yeast extract	**Energy** 279kJ/66kcal , **Protein** 3.8g, **Carbohydrate** 7.6g, of which sugars 0.7g, **Fat** 2.3g of which saturates 0.9g, Fibre 2.3g, **Sodium** 0.07g
	D	Pasta & Lamb	Cooking water, potato, halal lamb (10%), pasta(10%), carrot, tomato, onion, rice flour, sunflower oil, herbs	**Energy** 272kJ/64 kcal , **Protein** 3.1g ,**Carbohydrate** 8.5g, **Fat** 2.1g , **Sodium** mg < 50
Vegetable based (6-9 Months)	A1	Creamy vegetable pasta	Organic vegetables (50%) (carrot, sweet corn, cauliflower, fennel), organic wholemeal spaghetti(durum wheat) (18%), organic whole milk, water, organic cream (4%),organic sunflower oil, organic spice (pepper), rice, white beans, tapioca starch, oregano (0.5%), yeast extract	**Energy** 275kJ/65kcal , **Protein** 2.0g, **Carbohydrate** 8.5g of which sugars 2.8g, **Fat** 2.6g of which saturates 1.2g, **Fibre** 1.9g , **Sodium** Trace
	B1	Cheesy tomato pasta star	Tomatoes (20%), pasta (18%) (water, drum wheat semolina), vegetarian cheddar cheese (8%), corn flour, natural flavouring (contain celery, celeriac), yeast extract, iron sulphite	**Energy** 283 kJ/67kcal, **Protein** 2.9g, **Carbohydrate** 8.5 g of which sugars 0.7g, **Fat** 2.4,of which saturates 1.8g, **Fibre** 0.3g, **Sodium** 0.1g **Iron** 0.9mg
	C1	Vegetable lasagne	Baby grade vegetables (64%) (tomatoes, aubergine (9%), courgette (9%), green paper, onion), pasta (18%) (drum wheat, egg albumin), full cream milk, cream (9%), cheese (3%), tapioca starch, basil, nutmeg	**Energy** 266kJ/63 cal, **Protein** 2.9g , **Carbohydrate** 8.9g, of which sugars 1.8 g, **Fat** 1.8g of which saturates 1.0 g, Fibre 0.7 g, **Sodium** 0.05g
	D1	Garden vegetable	Vegetable (67%) (carrots, potato, peas, cauliflower), skimmed-milk, Sweet corn, cooking water, sunflower oil	**Energy** 247kJ/58kcal , **Protein** 2.2g , **Carbohydrate** 8.2g, of which sugars ,**Fat** 1.9 g , **Sodium** < 50 mg

3.2.2 Quantitative analysis of macronutrient content in the food samples

All the methods employed in this study are those approved by the FSA as well as UK Trading Standards and currently in use by public analysts (Kent Scientific Laboratories) (White & Hampson, 2008).

3.2.2.1 *Protein*

The technique used in this study for protein quantification is based on colorimetric analysis of Kjeldhal nitrogen (indophenol method; Mantoura & Woodward, 1983). Three independent replicates of 0.5 g (wet weight), of food samples, were weighed using a four-figure analytical balance. The replicates were transferred into 25 x 300 mm boiling tubes containing 0.2 g copper sulphate pentahydrate and anti-bumping crystals, following the addition of 10 mL concentrated sulphuric acid. The samples were then digested using a Keldotherm thermal heating block held at 400^{o}C for between 180-240 minutes until the digests turned green in colour. The temperature of the heating block was monitored using a 500^{o}C mercury thermometer (Fisher Scientific, Loughborough, UK). The level of concentrated sulphuric acid in each tube was periodically topped up to the 10 mL mark. After digestion, samples were allowed to cool to room temperature (approx. 20^{o}C). Each digest was then transferred into 250 mL volumetric flasks and diluted with ultra pure water to act as stock. All water used was de-ionized to 0.05 μS cm^{-1} using a Purite reverse osmosis system (RO 200 fitted with a HP 700 cartridge) attached to a polishing cartridge (StillPlus HP, fitted with a N340 and C340 cartridge). A further 1:50 dilution of the stock solution was prepared using 1% (v/v) sulphuric acid solution, ready for analysis. A stock solution of nitrogen (1000 μg/L) was prepared by dissolving 2.3596(5) g of ammonium sulfate $(NH_4)_2SO_4$ in 500 mL of 1% (v/v) sulphuric acid. The ammonium sulphate had previously been heated in an oven for 10 hours at 110^{o}C and cooled in a desiccator before weighing. All solutions were de-gassed in an ultrasonic bath before use. The stock solution was further diluted (1:100) to make a 10 ppm working standard, required for the preparation of a calibration curve (r^2= 0.9989) at different concentrations (min. 0.5 ppm – max. 4.0 ppm) ; 2.0 mL of each of the digests and standards were measured directly into 4 mL plastic macro-cuvettes. This was followed by the consecutive addition of 1 mL of both *solution one* (50 g L^{-1} sodium hydroxide and 4 g L^{-1} sodium dichloroisocyanurate dissolved in water) and *solution two* (240 g L^{-1} sodium

salicylate, 1.4 g L^{-1} sodium nitroprusside and 130g L^{-1} trisodium citrate dissolved in water). Prior to the measurements, the cuvettes were covered with aluminium foil and stored at room temperature in the dark for 20 minutes. The absorbance of each replicate was measured at 650 nm (UV-160A, UV-Visible Recording Spectrophotometer, Shimadzu, Japan). The level of organic nitrogen liberated by sodium hydroxide was obtained by multiplying the nitrogen factor by 6.25 (AOAC, 1990).

3.2.2.2 *Fat*

Acid hydrolysis followed by solvent extraction was used for the determination of the fat content of the food samples (adapted from the AOAC Official method 922.06). Three independent replicates of 2.0 g (wet weight), of food samples, were weighed using a four-figure analytical balance. The replicates were transferred into labelled Pyrex 100 mL conical flasks, followed by the addition of 2 mL ethanol and 10 mL concentrated hydrochloric acid (37%). The samples were hydrolysed for 40 minutes using a water bath maintained at 70 - 80^{o}C. The conical flasks were frequently shaken to prevent the formation of aggregates. On completion of the digestion process, 10 mL of absolute ethanol was added to each sample and the mixtures were left to cool at room temperature. After cooling, 25 mL of diethyl ether was added to each flask, which were stoppered and shaken for 1 minute. To each sample, 25 mL of petroleum ether (40 to 60^{o}C) was then added and the flasks were shaken for a further minute. At this point a dark greenish colour was attained and the flasks were left to stand until the upper layer was completely clear of the dark greenish colour. A Pasteur pipette was used to siphon off the upper layer containing ethereal-fat into weighed beakers previously dried to constant weight. The extraction procedure was repeated by the addition of 15 mL of both diethyl and petroleum ether to the remaining mixtures in the conical flasks. The re-extracted ether-fat layer was then added to the corresponding beaker. The beakers were placed in a water bath at 40 - 50^{o}C until the solvents in the mixture evaporated. Samples were then dried in an air-oven for 90 minutes at 100^{o}C and left to cool for another 30 minutes in desiccators prior to weighing (AOAC, 1990; Pomeranz & Melon, 1994). The fat content in the samples was calculated as follows:

Fat content (%) = (fat content (g) / weight of sample before extraction) x 100

3.2.2.3 *Carbohydrate*

Total carbohydrate was measured using the phenol-sulphuric acid method during which, carbohydrates are first hydrolysed to simple sugars by using diluted hydrochloric acid. In hot acidic medium glucose is dehydrated to 5-(hydroxymethyl)-2-furaldehyde (hydroxymethylfurfural). The hydroxymethylfurfural form a green coloured product with phenol which exhibits an absorption maximum at 490 nm (Pomeranz & Melon, 1994; Masuko *et al*., 2005; Mecozzi, 2002). Three independent replicates of 100 mg of sample were weighed and transferred into 25 x 300 mm boiling tubes followed by the addition of 5 mL of 2.5 N HCl. The samples were then hydrolysed by placing the tubes in a boiling water bath for three hours prior to being cooled to room temperature. After cooling, the samples were neutralised with solid sodium carbonate until the complete cessation of effervescence. The sample volume was then made up to 100 mL prior to being centrifuged. After centrifugation, 0.2 mL of the sample solution was transferred to test tubes and the volume was made up to 1 mL using pure water. To each tube, 1 mL of phenol solution (5%; v/v) was then added followed by 5 mL of 96% sulphuric acid and the tubes were shaken constantly for 10 minutes. Finally, the tubes were placed in a water bath for another 20 minutes at 25-30 ^{o}C prior to measuring the absorbance (optical density) at 490 nm. A glucose stock solution was prepared by dissolving 100 mg of glucose in 100 mL of water (1000 ppm). The stock solution was further diluted (1:10) to make a 100 ppm working standard required for the preparation of a calibration curve (r^2= 0.9992) at different concentrations (min. 20 – max. 100 mg/mL).

3.2.2.4 *Energy*

The energy density of the complementary food samples was determined by multiplying the protein and carbohydrate content by 4 kcal (17 kJ) and the fat content by 9 kcal (37 kJ). These are values established by the European Union for the nutritional labelling declaration of foods.

3.2.2.5 *Fibre*

The percentage of total fibre was analysed by enzymatic-gravimetric method AOAC 985.29 (AOAC, 1990).

3.2.3 Quality assurance

The accuracy of the above methods was verified by analysing Certified Referenced Materials (CRM 8418: Wheat Gluten, Wheatex 2240) and the concentration for each of the samples were typically within the certified range or ± 5% of the certified value. Blank samples of ultrapure water and reagents were also prepared using the same procedures as for the samples. All blank levels obtained were subtracted appropriately.

3.2.4 Proposed nutritional profiling of the products based on the FSA "Front of pack traffic light signpost labelling" guidelines (FSA, 2007).

A nutritional profiling model, using data from the traffic light signpost labelling on the product label is hypothetically proposed as a tool to categorise the food samples analysed in this study based on their nutrient content. The colour coding of the nutritional criteria, is based on EC N^{o} 1924/2006 and recommendations by the Committee on Medical Aspect of Food and Nutrition Policy (COMA) and the Scientific Advisory Committee on Nutrition (SACN) for fat, sugar and salt using 25% of the recommended intake level per 100 g. Based on the traffic light colour approach to nutritional signpost labelling, criteria are defined as the green/amber (low/medium) and amber/red (medium/high) boundaries for the key nutrients (fat, saturated fat, sugars and salt).

One of the main benefits of the non-numerical labelling format of food products is to help consumers to make judgements on the nutritional information presented on the food labels.

3.2.5 Approaches used for estimation of total daily dietary intake from a diet based on commercially prepared complementary foods

In order to estimate the total daily dietary intake of an infant from the consumption of manufactured infant foods and formulas, it was necessary to adopt the following approaches

in combination. In the first approach an example menu based on the manufacturer's feeding recommendations was composed, which included the ready to feed type foods, such as jars of semi-solid meals and readymade formulas. The second approach was then introduced to estimate the gastric capacity (30 g/kg body weight/day) based on data from the World Health Organization (WHO) expert Consultation on Complementary Feeding (Dewey & Brown, 2003). The average weight for infants of different age ranges have been taken from the report by the Committee of Medical Aspects of Food and Nutrition Policy (COMA): Weaning and The Weaning Diet (1994). The aforementioned methods have been used by the Scientific Committee on Food (SCF) for assessing the maximum level of residue of pesticides in foods. The estimated amount for milk consumption, however, was set at 600 mL, as advised by COMA, for infants up to 12 months old.

Based on this approach the daily recommended intake of energy and macronutrients by an infant aged between 6-9 months, is calculated considering the medium recommended daily intake of milk (600 mL/day). As a starting point, the energy provided from recommended total protein intake of the target population (13.7 g/d) is calculated (DoH, 1991). Next the fat content is calculated to ensure that the diet contains at least 31% of energy as fat for an infant who consumes 600 mL of milk per day. The remaining energy requirement not provided by fat or protein is then used as the basis for calculating the carbohydrate content of the diet (Lutter & Dewey, 2003). Hence, the daily dietary energy and macronutrient requirements for the diet of an infant 6-9 months old are mainly calculated based on the DRVs for protein and fat, yielding 6 % (13.7 g) and 31 % (27.3 g) of the Estimated Energy Requirement (EER) from protein and fat, respectively (DoH, 1991; WHO, 2004). The remaining 63% of the EER needs are provided by 131.7 g of carbohydrate.

3.2.6 Statistical analysis

The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0. The minimum, maximum, mean (standard error of mean) and standard deviation of the data were compared using a 1-sided unpaired *t*-test at $p = 0.05$ level of significance (with 95 % confidence interval) to examine mean differences between meat and vegetable-based varieties.

3.3 Results

3.3.1 Macronutrient content and discrepancy of the labels

3.3.1.1 *Protein*

The results of the protein analyses (Table 3.2) indicate that there is a significant difference (p= 0.02) between the meat and vegetable based foods with respect to protein content. The mean protein content of meat and vegetable based recipes were 3.2 (± 0.37) and 2.3 (±0.56) g/100 g, respectively. Since the consumption of nutrient dense food (those foods that provide substantial amounts of nutrients with relatively few calories) is critical during infancy, the nutrient density [amount of nutrient per 100 kcal of food (ND)] for both meat and vegetable based food samples in relation to protein content was also calculated; the data is presented in Table 3.2. With respect to the protein density, all the products were found to be a good source of protein with an ND of > 12 % as recommended by the nutritional guidelines for composition of complementary food (DoH, 1980; WHO, 2004).

Table 3.2. Comparison between the experimental value of protein content (g/100 g) of meat and vegetable-based samples and the declared values on the labels.

Brand (n=8)	Experimental (Mean)	SD±	Declared	% Variation *	ND**	%RNI ¶
A	2.7	0.02	2.8	4	16.4	19.7
B	3.2	0.01	2.8	+14	21.3	23.4
C	3.6	0.02	3.8	5	21.8	26.3
D	3.3	0.01	3.1	+6	20.6	24.1
Mean	3.2	0.37	-	-	-	23.4 (±2.7)
A1	1.8	0.03	2.0	10	11.1	13.1
B1	3.0	0.01	2.9	+3	17.9	21.9
C1	2.5	0.02	2.9	14	15.9	18.2
D1	1.9	0.03	2.2	14	13.1	13.9
Mean	2.3	0.56	-	-	-	16.8 ± 4.09
CRM †	1.18	0.02	1.25	95.4	-	-

*Percentage difference between the experimental values *vs.* declared.
**Nutrient Density (ND) = (percentage of energy (kcal) derived from a particular nutrient per 100 kcal of food; Lee & Nieman, 2003).
¶The % of a day intake from 100 g of the food towards the Recommended Nutrient Intake (DoH, 1991).
† Certified Reference Material (CRM).

The meat-based recipes were found to provide, on average, 23.4 (±2.7) % of the RNI of protein for infants aged 6-9 months old (DoH, 1991), which is 6.6 % i.e. higher than the percentage of RNI provided by the vegetable based recipes at 16.8 ± 4.09 %. It is also important to note that the protein in vegetable based varieties could also be of less "biological significance" due to the lack of particular amino acids in vegetable sources if the recipes are not carefully designed. This could be an issue, especially where the vegetarian diet is concerned and requires further investigation (Iqbal *et al*., 2006).

In order to ascertain the transparency of the labels, the experimentally determined protein concentrations of different food samples were also compared to the protein content declared by the manufacturers on the product labels. The results show a greater variation, on average 10-14 %, between the experimental and declared values of vegetable based varieties. Despite the discrepancies between the experimental and declared values on the labels, the average protein content (gram/100 kcal) of the food samples in both varieties was found to be in line with legislative requirements (Commission Directive 2006/125/EC) at a minimum of 3g/100 kcal of food. These observations confirm that, the protein content of the commercial infant foods is within the regulatory requirements established for complementary food.

3.3.1.2 *Fat*

The mean fat content of meat and vegetable based recipes (Table 3.3) were found to be 2.1 (± 0.02) and 2.5 (± 0.2) g per 100 g, respectively and there was no significant difference (p = 0.3) between meat and vegetable based recipes regarding the fat content. The fat density in both meat and vegetable based food samples was calculated and all the products were found to have relatively high percentage of fat specifically in two of the vegetarian brands with a fat density higher than the recommended 31% for nutritional composition of complementary food as demonstrated in the data presented in Table 3.3.

Table 3.3 Comparison between the experimental value of fat content (g/100 g) of meat and vegetable-based samples and the declared values on the labels.

Brand (n=8)	Experimental (Mean)	SD±	Declared	% Variation *	ND**
A	1.9	0.02	2.3	17	25.9
B	2.0	0.02	2.1	5	30.0
C	2.4	0.02	2.3	+4	32.7
D	2.2	0.01	2.1	+5	30.9
Mean	2.1	0.02	-	-	-
A1	3.5	0.03	2.6	+35	48.5
B1	3.0	0.01	2.4	+25	40.3
C1	1.4	0.02	1.8	22	20.0
D1	1.8	0.03	1.9	8	27.2
Mean	2.5	0.2	-	-	
CRM †	1.4	0.02	1.5	95	-

*Percentage difference between the experimental values *vs.* declared.
**Nutrient Density (ND) = (percentage of energy (kcal) derived from a particular nutrient per 100 kcal of food; Lee & Nieman, 2003).
† Certified reference material (CRM).

The experimentally determined fat concentrations of different food samples were also compared to the fat content declared by the manufacturers on the product labels to check the transparency of the labels. The data (Table 3.3) shows greater variations between the experimental and declared values of fat content in vegetable-based (min.8% - max.35%) recipes than meat-based (min. 4 % - max. 17%). The average fat content (gram per 100 kcal) of the food samples in meat (3.5g/100 kcal) and vegetable (4.1 g/kcal) varieties were, however, found to be compliant with the maximum permitted level established by legislative bodies (Commission Directive 2006/125/EC) at a maximum of 4.1 g/100 kcal of food despite the aforementioned variations. These results, however, reveal that the fat content in the vegetarian products is almost at the maximum regulatory requirements established for complementary food.

The surprisingly high level of fat in some vegetable-based recipes was further investigated and is due to the inclusion of cream, cheese and whole milk powder, declared by the manufactures in almost all the vegetarian options available for purchase (Table 3.1).

3.3.1.3 *Carbohydrates*

The results of the carbohydrate analysis are presented in Table 3.4. The mean carbohydrate content of meat and vegetable based recipes were found to be 8.1 (±0.02) and 7.4 (± 0.02) g per 100 g, respectively. No significant difference in carbohydrate content (p = 0.2) was found between the meat and vegetable based recipes. The carbohydrate density for both meat and vegetable based food samples was also calculated on the basis of the experimentally determined values given in Table 3.4. The ND with respect to carbohydrate for all the products was satisfactory and below the recommended 57 % for nutritional composition of complementary food intended for infants with medium/high intake of milk (DoH, 1980; WHO, 2004).

Table 3.4 Comparison between the experimental value of carbohydrate content (g/100 g) of meat and vegetable-based samples and the declared values on the labels.

Brand (n=8)	Experimental (Mean)	SD±	Declared	% Variation *	ND**
A	7.8	0.02	7.8	-	47.3
B	7.9	0.02	8.7	26	42.7
C	8.5	0.02	7.6	12	40.6
D	8.0	0.01	8.5	2	54.4
Mean	8.1	0.02		-	-
A1	7.9	0.03	8.5	7	48.5
B1	8.9	0.01	8.5	4	52.1
C1	7.2	0.01	8.9	19	45.9
D1	5.7	0.03	8.2	31	39.3
Mean	7.4	0.02		-	-
CRM †	10.2	0.02	10.3	98.8	-

*Percentage difference between the experimental values *vs.* declared.
**Nutrient Density (ND) = (percentage of energy (kcal) derived from a particular nutrient per 100 kcal of food; Lee & Nieman, 2003).
†Certified reference material (CRM).

The experimentally determined carbohydrate concentrations of different food samples were also compared to the carbohydrate content declared by the manufacturers on the product labels to ascertain the transparency of the labelling information. The percentage variation between the experimental and declared values of carbohydrate in the meat and vegetable based products (Table 3.4) show the same variation in both vegetable and meat based recipes. It is, however, difficult to validate the legislative compliance of the samples in relation to the

carbohydrate content, since there is no legislative requirement defined for the carbohydrate content (gram per 100 kcal) of food (Commission Directive 2006/125/EC).

3.3.1.4 *Energy*

Data for the energy levels of the food samples tested are presented in Table 3.5. The average energy density (kcal/g) of the food samples was found to be at the recommended level of 0.6 kcal/g (Dewey & Brown 2003; Monte & Giugliani, 2004). There was no significant difference (p = 0.9) found between the meat and vegetable based varieties, regarding the calorific values.

Table 3.5 Energy content of the food samples based on the nutrient values (g/100 g) obtained in this investigation and proposed nutritional profiling.

Brand (n=8)	Protein*	Fat*	Carbohydrate*	Energy kJ/kcal	ED** (kcal/g)	Sodium [a]	Fibre*
A	2.7	1.9	7.8 (1.9 sugar) [a]	247 / 59.1	0.59	0.05	1.5
B	3.2	2.0	6.4 (1.8 sugar)	236 / 56.4	0.56	0.8	1.8
C	3.6	2.4	6.7 (0.7 sugar)	263 / 62.8	0.62	0.07	1.1
D	3.3	2.2	8.7	284 / 67.8	0.67	<50 mg	0.9
Mean	3.2	2.1	7.4	257 / 61.5	0.58		1.3
SD±	0.37	0.2	1.1	4.9	0.01		0.4
95%CI	2.8-3.6	1.9-2.3	6.4-8.4	56.7-66.4	0.57-0.59	0.9-1.7	
A1	1.8	3.5	7.9 (2.8 sugar)	294 / 70.3	0.70	Trace	1.6
B2	3.0	3.0	8.9 (0.7 sugar)	312 / 74.6	0.75	0.1	0.5
C3	2.5	1.4	7.2 (1.8 sugar)	215 / 51.4	0.51	0.05	0.8
D4	1.9	2.0	5.7	353 / 48.4	0.45	<50 mg	1.0
Mean	2.0	2.5	7.4	256 / 61.2	0.61		1.0
SD±	0.56	0.95	1.35	13.2	0.13		0.5
95% CI	1.75-2.85	1.54-3.41	6.11-8.78	48.24-74.11	0.48-0.74		0.5-1.4
P value †	0.02	0.3	0.2	0.9	-		0.3

*Based on the analytical results obtained in this study (g/100g)

**Energy density= Amount of energy per gram of food (kcal/g)

	Low	Medium	High
Fat	≤3 g/100g	>3 g/100g	>20 g/100g
Sugar	≤5 g/100g	>5 g/100g	>12.5 g/100g
Salt	≤ 0.30 g/100g	>0.30 g/100g	>1.50 g/100g

[a] The information on the sugar and sodium content are obtained from the label, the data have not been available for those not declared.

† To examine mean differences (p = 0.05) between meat and vegetable-based varieties.

3.3.1.5 *Fibre*

The mean fibre content of meat and vegetable based recipes were found to be 1.3 (± 0.4) and 1.0 (± 0.5) g per 100 g, respectively (Table 3.5). No significant difference in fibre content ($p = 0.3$) was found between the meat and vegetable based recipes.

3.3.2 Nutritional profiling of the products based on the FSA "Front of pack traffic light signpost Labelling" guidelines (FSA, 2007)

The data in Table 3.5 was also categorised based on a hypothetical nutrient profiling model (FSA 2007) and products were found to be within the green (low) boundaries for most of the nutrients in each category. The level of fat in two of the vegetarian based meals, however, was in the amber/medium zone. Currently, the non-numerical labelling information of commercial infant foods is not required and is not practiced. From the consumer point of view, it may be useful to present such information as a quick and "easy to understand" rule in making an informed choice.

3.3.3 Estimation of daily dietary intake from sample menu composed of commercially prepared infant foods

In order to determine the total daily intake of an infant from consumption of complementary food menus, a sample menu (Table 3.6) was composed, as advised by the complementary food manufacturers (Heinz, 2010).

Table 3.6 Total daily intake of nutrients by an infant age 6-9 months old[¶] (based on gastric capacity of an eight months old infant and the standard feeding regime composed of commercially prepared infant food products).

Meals		Infant formula		Breakfast porridge *		Lunch (meat-based)			Dinner (vegetable-based)			Total Daily Intake [e] (a + b+ c +d)	**DRVs
Amount		**100 mL**	**600 mL [a]**	**100g**	**(83g/2[*]) [b]**	**100g**		**83 g [c]**	**100g**		**83 g [d]**		
Macronutrient						**Mean**	**SD**		**Mean**	**SD**			
Energy	kcal	68	407.4	394.5	163.7	61.3	4.9	50.9	61.3	13.2	50.9	672.9	795
Protein	g	1.8	10.8	13.2	5.5	3.2	0.4	2.7	2.3	0.6	1.9	20.8	13.7
Fat	g	3.5	21	5.3	2.2	2.1	0.2	1.7	2.5	1.0	2.1	27.0	27.3
Carbohydrate	g	7.3	43.8	73.5	30.5	7.4	1.1	6.1	7.4	1.3	6.1	86.6	131.7

[¶] Weight about 8.3 kg.

* prepared using warm water (50:50) as advised by the manufacturer.

** DRVs are based on recommendation for protein and fat, yielding 6 % (13.7g) and 31 % (27.3g) of the EER (795 kcal/d) from protein and fat, respectively (DOH, 1991; WHO 2004).

[a] Recommended volume of milk intake for a 6-9 months old infant .

[b , c & d] The portion size is calculated based on the gastric capacity of an infant aged 6-9 months old (30g/kg body weight/day) divided by 3 to make up for breakfast, lunch and dinner.

[e] TDI is calculated by the sum of milk and non- milk intake to compare with DRV.

Table 3.6, provides an illustration of the daily dietary macronutrient requirements of an infant aged 6-9 months old and compares it to the DRVs (DoH, 1991; Lutter & Dewey, 2003; WHO, 2004). Based on this comparison the average daily intake of protein (20.8 g) is suggested to be in excess of the DRVs (13.7 g/d) and for carbohydrates (86.6 g) below the recommendations (131.7g/d).

The limitation with this approach is the exclusion of snacks and desserts which in effect could, if incorporated in the menu, make up for the shortfall of calories and carbohydrates. Based on the World Health Organization (WHO) expert Consultation on Complementary Feeding (Dewey & Brown, 2003) , the percentage EER provided from milk (407.2 kcal from 600 mL/day) can be estimated to be approximately 50 %, and the remaining 50 % is expected to be provided by complementary food at e.g. breakfast, lunch and dinner. A typical 200 g jar of infant food will usually provide ~16% of the EER of an infant. Two jars a day will therefore provide 32% which is just short of the total energy requirements when milk at 600 mL per day is factored in. Between the energy provided from milk (50 %) and 2 jars of infant food at 32 % the total energy in the food ingested is 82 %. The short fall would need to be made up from breakfast and snacks during the day. A menu based on the complementary jar foods should, therefore, be within the recommended limits providing on average 32% of the EER.

On the other hand, the borderline intake of fat (27.0 g) based on this menu could also be a cause for concern as the fat intake will be in excess of the guidelines if the contribution made from intake of snacks and desserts are also incorporated.

3.4 Discussion

Based on the finding of this study, the vegetarian products do not provide a lower fat option in comparison to meat-based varieties. This is despite a report on parent's perception of the vegetable based product as being a low fat choice, where they limit their options to only vegetarian products in an attempt to prevent their children becoming overweight or obese in the future (Davis & O'Hare, 2004). The foregoing trend in reduction of dietary fat intake, however, could be a reflection of the recommendations for the reduction of the population's

dietary fat intake by the Commission of the European Communities (1989/1990). These data are supported by Davis and O'Hara (2004), as they suggest parents in the UK tend to limit the dietary fat intake of infants and children. The issue of a high energy and low fat diet is not exclusive to commercial foods. Boom *et al.* (1997) have identified the same findings, with regard to the fat content of homemade meals in the UK, in a comparison conducted on nutritional composition of home-prepared baby meals in Spain and England. In the aforementioned study, Spanish homemade food was shown to have a low energy density and high fat/salt content. In contrast English homemade baby food had higher energy density, lower protein and fat content and wider variation in micronutrient content. There are, however, concerns that, since fat is the major source of energy for infants as well as the only source for EFAs and fat soluble vitamins, such diets may limit growth (Garrow *et al.,* 2000; Shils *et al.,* 2006; Ells *et al*., 2008, Siri-Tarino *et al*., 2010). According to a study in Finland (Lapinleimu *et al*., 1995), no significant difference was found between infants on a low fat diet and those in a control group with respect to their growth. Interestingly, although the intervention group had lower energy and fat intake than the control group, the mean fat intake of both groups was close to 30% of the total energy intake, but the intervention group proved to have a lower serum cholesterol concentration at 3 years (Lapinleimu *et al*., 1995).

Although there are debates over the optimal amount of fat in the diet of infants and young children (Garrow *et al.,* 2000; Shils *et al.,* 2006; Ells *et al*. 2008, Siri-Tarino *et al*., 2010), the current recommendations suggest a range of 31-45 % of the energy from fat, based on the low or average amount of energy intake from milk (WHO, 2004). Since the current UK recommended intake of daily milk at 600 mL/d provides an average energy of 396 kcal/d (energy density of 0.66 kcal/g), 31% of energy from fat (27.3 g fat per day) seems reasonable if it contributes to adequate intake of essential fatty acids and less saturated fat intake (< 8% EI) (DoH, 1991). The borderline daily intake of fat (27.0 g) based on the menu in Table 6 could, therefore, be a cause for concern as the fat intake will be in excess of the guidelines if the contribution made from intake of snacks and desserts are also incorporated.

This particular aspect of the results which relates to the excess daily intake of fat based on the commercial feeding menus should be investigated further. Furthermore, although the quantification of different classes of lipid in terms of fatty acid content is not required, under

current legislation, the inclusion of such information could be helpful with respect of consumer's right to healthy choice in full knowledge of the facts.

3.5 Conclusions

The primary aim of this study was to examine the macronutrient content of complementary infant foods currently for sale in the UK market. The study was undertaken in order to ascertain the suitability of the complimentary products in relation to current dietary guidelines for infants aged between 6 - 9 months old. The following approach was adopted to address the aims of the study. Quantitative analysis was conducted on eight different food products representing four popular commercial brands (meat and vegetable based). The analytical strategies included: (a) derived methods for energy, (b) Kjeldhal for protein, (c) phenol sulphuric acid for carbohydrate and (d) acid hydrolysis extraction for the analysis of fat content. The results of the quantitative analysis of macro-nutrients (protein, fat, carbohydrate) were compared with the information provided on the labels in order to ascertain the transparency of the labelling and the level of compliance of the products with the legislative requirements. The selected infant food products were hypothetically categorised on the basis of a traffic light based nutrient profiling model (FSA, 2007), examples of which are already in practise in helping consumers make an informed choice with respect to the nutritional quality of food for children and adults. Finally, the role of commercially prepared infant food products in meeting the dietary requirement of their relevant target group with reference to the RNI based on a sample menu, as advised by the complementary food manufacturers (Heinz, 2010), was ascertained.

The results of the analysis indicate that despite the variations seen in the above comparisons, the concentration of macronutrients (g/100 kcal) in complementary food were still satisfactory and within the regulatory requirements (Commission Directive 2006/125/EC). The total daily intake of fat (27.0 g/day) based on the menu composed in this study from commercial complementary food are suggested to exceed the DRV for fat (27.3 g/day), if the intake of snacks and desserts are incorporated in the menu. The results of this current study suggest that the formulation of infant recipes are of significant importance in relation to the nutritional quality of an infant's diet in which the consumption of nutrient dense food (those foods that provide substantial amounts of vitamins and minerals with relatively few calories) is critical.

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Chapter 4

Essential and trace-essential elements content of commercial infant foods in the UK

4.1 Introduction

Infancy is a time of rapid physical, biological, immunological, mental, growth and development (Holden & Anita, 2000; Robert & Kraisid, 2001). Providing an appropriate diet for a growing infant is critical in terms of healthy growth and development, as low intake or reduced bio-availability of nutrients may lead to deficiencies and cause body function impairment (Gokhale & Kirschner, 2003; Holden *et al*., 2000; Hulzebos *et al*., 2007; Robert & Kraisid, 2001; Taitz *et al*., 1989). For example trace elements and minerals have a crucial influence on the interaction between genetics and physiological factors (Gibson & Hots, 2000; Melo *et al.*, 2008). They are involved in many important functions in the body, e.g. bone mineralization, enzymatic reactions, secretion of hormones, as well as protection of cells and lipids in biological membranes (Schlenker & Williams, 2003; Taylor *et al.*, 2004).

Nutritional imbalances at an early stage of life can influence health in later life and have also been linked to developmental delay, increased risk of ischemic heart disease, hypertension, diabetes and obesity in adult life ((Barclay *et al*., 2003) Monte & Giuliani, 2004, (Hulzebos *et al*., 2007).

In the first four to six months, an infant's requirements can be totally satisfied by milk regardless of whether the infant is being breast or formula fed (Brown *et al*., 2008; Foote *et al*., 2003). After this period, complementary food (i.e. food that is additional rather than a substitute to mother's or formula milk) is required to boost energy and nutrient intake (Barclay *et al*., 2003; Briefel *et al*., 2004; Hulzebos *et al*., 2007). Recently, there have been several studies of infant diets both in developing and industrialized countries (Bhutta, 2000; Kimmons *et al*., 2005; Nobel & Emmet, 2006). It is estimated that the complementary food targeted for a nine month old infant should provide about 75-100% of the intake of iron and zinc (Melo, *et al.*, 2008).

Currently, commercially prepared complementary foods have become an important part of the diet of many infants and toddlers (Briefel *et al*., 2004; Melo *et al*., 2008; Michaelsen & Friis, 1998) and this includes sufficient quantities of minerals and vitamins to fulfil the requirements of their target group. In the UK many parents are increasingly relying on commercially marketed, ready to eat, infant foods (FSA, 2004). Despite the growing trend, the type, nature and extent to which these products meet nutrient needs of infants are subject to much debate and are an area of uncertainty. These issues relate to factors such as (1) nutrient stability, (2) bio-availability, (3) composition of the finished products, (4) processing conditions and (5) storage. Under the current legislation [1996 (FLR) (SI 1996 No.1499) provision of the EC Nutrition Labelling Directive (90/496/EEC); Commission Directive 2006/125/EC], micronutrient declaration is not mandatory. Currently, the nutritional information declared on the labels of these products does not contain adequate data regarding the mineral and vitamin content (Table 1). The foregoing comments imply that a number of important nutrients may be limited or excessive in some brands thus affecting their nutritional quality and suitability for infants. It is, therefore, necessary to examine the nutritive value of the food composition to establish whether infants are receiving the correct level of nutrients (non-milk related) during this critical period of development.

The primary objective of this study was to determine the level of eight major minerals and trace elements: calcium, copper, magnesium, iron, zinc, potassium, sodium and selenium in eight different complementary infant food products in order to (1) ascertain their suitability relative to dietary guidelines for the 6 to 9 month age group, and (2) to estimate the daily intake of these elements from commercial infant food consumption. A number of techniques have been proposed in the literature for the determination of the elemental content of e.g. milk and infant formulas; these include High Resolution Inductivity Coupled Plasma–Mass Spectrometry (HR-ICP-MS), Differential Pulse Anodic Stripping Voltammetry (DPASV) and Wavelength-dispersive X-Ray Fluorescence Analysis (WDXRF) (Melo, *et al.*, 2008*;* Jannat, *et al.*,2009; Pashkova , 2009). The current study, however, was conducted using ICP-OES and ICP-MS.

4.2 Materials and methods

4.2.1 Sample Collection

Eight different of baby food samples were obtained from leading supermarkets in UK between November-December 2009. The samples represented four popular brands available on the market, including an organic and a Halal range. All brands were represented by two different product categories: (i) meat based and (ii) vegetable based; these were semi pureed and packed in glass jars. Exact corresponding recipes were not available for all the brands. The main ingredient of the baby food samples and their characteristics are presented in Table 4.1. Three independent replicates of all samples were analysed originating from the same batch. The sample jars were stored unopened at room temperature similar to their distribution and market environment.

Table 4.1 Infant complementary foods sample characteristics.

	BRAND Code	PRODUCT NAME(n=8)	INGREDIENTS	NUTRITIONAL INFORMATION per 100g
Meat based (6-9 Months)	A	Cottage pie	Organic vegetables (64%) (potato(22%), carrots, tomatoes, onions), water, organic cooked rice, organic beef (8%), organic sunflower oil, organic herbs[parsley, oregano],organic vegetable stock[salt, organic rice flour, organic vegetable(carrots, onions, celeriac),organic yeast extract, organic vegetable oil, organic spices] chicken (10%),corn flour, corn oil.	**Energy** 278kJ/66kcal, **Protein** 2.8g, **Carbohydrate** 8.7g of which sugars 1.9, **Fat** 2.3g, of which saturates 0.6g, **Fibre** 1.6g, **Sodium** 0.05g
	B	Cottage pie	Vegetables (45%), (carrots (22%), potatoes (14%), onions(7%), peas(4%)) water, whole milk, beef (9%), corn flour, unsalted butter, natural flavouring, yeast extract, herbs, iron sulphate.	**Energy** 253kJ/60 kcal, **Protein** 2.8g , **Carbohydrate** 7.5 g of which sugars 1.8g, **Fat** 2.1g of which saturates 1.2g , **Fibre** 1.3g, **Sodium** 0.8g, **Iron** 1.3mg
	C	Cottage pie	Baby grade vegetables (56%) (potato, green beans, carrot, peas), cooking water, beef (10%), white beans, wheat starch (gluten free),yeast extract	**Energy** 279kJ/66kcal , **Protein** 3.8g, **Carbohydrate** 7.6g, of which sugars 0.7g, **Fat** 2.3g of which saturates 0.9g, Fibre 2.3g, **Sodium** 0.07g
	D	Pasta & Lamb	Cooking water, potato, halal lamb (10%), pasta(10%), carrot, tomato, onion, rice flour, sunflower oil, herbs	**Energy** 272kJ/64 kcal , **Protein** 3.1g, **Carbohydrate** 8.5g , **Fat** 2.1g, **Sodium** mg < 50
Vegetable based (6-9 Months)	A1	Creamy vegetable pasta	Organic vegetables (50%) (carrot, sweet corn, cauliflower, fennel), organic wholemeal spaghetti(durum wheat) (18%), organic whole milk, water, organic cream (4%),organic sunflower oil, organic spice (pepper), rice, white beans, tapioca starch, oregano (0.5%), yeast extract	**Energy** 275kJ/65kcal , **Protein** 2.0g, **Carbohydrate** 8.5g of which sugars 2.8g, **Fat** 2.6g of which saturates 1.2g, **Fibre** 1.9g, **Sodium** Trace
	B1	Cheesy tomato pasta star	Tomatoes (20%), pasta (18%) (water, durum wheat semolina), vegetarian cheddar cheese (8 %), corn flour, natural flavouring (contain celery, celeriac), yeast extract, iron sulphite.	**Energy** 283 kJ/67kcal, **Protein** 2.9g, **Carbohydrate** 8.5 g of which sugars 0.7g, **Fat** 2.4,of which saturates 1.8g, **Fibre** 0.3g, **Sodium** 0.1g **Iron** 0.9mg
	C1	Vegetable lasagne	Baby grade vegetables (64%) (tomatoes, aubergine (9%), courgette (9%), green pepper, onion), pasta (18%) (durum wheat, egg albumin), full cream milk, cream (9%), cheese (3%), tapioca starch, basil, nutmeg.	**Energy** 266kJ/63 kcal, **Protein** 2.9g , **Carbohydrate** 8.9g, of which sugars 1.8 g, **Fat** 1.8g of which saturates 1.0 g, Fibre 0.7 g, **Sodium** 0.05g
	D1	Garden vegetable	Vegetable (67%) (carrots, potato, peas, cauliflower), skimmed-milk, sweet corn, cooking water, sunflower oil	**Energy** 247kJ/58kcal , **Protein** 2.2g , **Carbohydrate** 8.2g, of which sugars, **Fat** 1.9 g , **Sodium** < 50 mg

4.2.2 Sample Digestion and ICP-OES/MS

A microwave accelerated reaction system (CEM MARS 5® with XP-1500 vessels), equipped with the standard temperature and pressure control systems, was used to digest all the samples. Each baby food sample was mixed and homogenised using a domestic blender (Multi-quick, Braun 300) and three independent replicates of 0.5 g (wet weight) were weighed prior to adding 5.0 mL of concentrated nitric acid (70 % trace analysis grade, Fisher Scientific) and 0.5 mL of hydrogen peroxide (30 % trace analysis grade, VWR International). The samples were then heated for 20 min using microwave digestion; the operating conditions are shown in Table 4.2.

Table 4.2 CEM MARS 5 ®, (XP-1500 vessels) microwave digestion conditions [a].

Microwave conditions	**Nitric acid digestion of semi- solid samples**
Sample	0.5 g
Nitric acid (HNO_3)	5 mL
Hydrogen peroxide (H_2O_2)	0.5 mL
Pressure [b]	Max 400 psi
Power	1200 W- 100%
Temperature [c]	Step 1: ramp to 190 ^oC over 20 min Step 2: hold at 190 ^oC for a further 5 min; allow to cool to room temperature over 1 hr

[a] Microwave condition and digestion procedures were adapted and modified based on the CEM operation Manual (674007 version).
[b & c] The electronic temperature and pressure sensors (EST & ESP -1500) were used to control and monitor the conditions inside the vessels to avoid exothermic reaction and over pressurization of the digestion vessels.

The digested samples were quantitatively analysed using an Inductivity Coupled Plasma –Optical Emission Spectrometer (ICP-OES, Perkin Elma Optima 4300 DV), for which the operating conditions are shown in Table 4.3.

Table 4.3 Instrument operating parameters applied for determination of elements by ICP-OES.

Parameter	Value
View mode	Axial
View distance	15
Plasma gas flow	15 L/min
Auxiliary gas flow:	0.2 L/min
Source equilibration time	15 s
Pump flow rate	1.50 mL/min
Detector	Segmented array change coupled device
Power	1300 watts
Nebulizer	0.80 min
Sample aspiration rate	1.50 mL/min
Read	Peak area
Number of replicates	3
Background correction	2 –point
Read delay	60 s
Rinse delay	30 s

Selenium was analysed by Inductivity Coupled Plasma – Mass Spectrometry (Thermo Scientific, X Series 2 ICP-MS). The ICP-MS operation parameters are summarised in Table 4.5.

Table 4.5 Thermo Scientific, X Series 2 ICP-MS operating parameters.

Parameter	Value
Plasma flow	18
Auxiliary flow	0.5
Nebulizer flow	0.9
RF Power	1400 kW
Stabilization time	60 s
Sampling depth	120 mm
Operating mode	Kinetic energy discrimination
Collision cell gas	8%H in He, 3.5mL min^{-1}
Measurement mode	Peak jumping
Dwell time	100 ms
Point per peak	1
Sweeps	75
Nebulizer	Quartz micro-concentric (0.4 mL /min)
Number of replicates	3

A collision cell operated in kinetic energy discrimination mode was used to minimise polyatomic-and argon-based interferences on the first row transition metals and selenium. Method parameters were checked on a daily basis using an in-house optimisation programme.

4.2.3 Standards

Seven multi-element calibration solutions were prepared at different concentration levels (250 – 5000 µg/L) from 1000 mg/L single element ICP grade standards (Inorganic Ventures) using high purity nitric acid (70 % trace analysis grade, Fisher Scientific) matched to the samples matrix.

A calibration curve, at seven different concentrations (min. 50 ppb –max. 1 ppm), was made using these multi-element standards (r^2= 0.9999). A further three point calibration curve at higher concentrations levels (5 ppm, 10 ppm and 25 ppm) was required to perform the analysis of calcium, magnesium, potassium and sodium due to the high concentration level of these elements in the samples. To check for instrumental drift, one of the multi-element standards was analysed for every 15 samples.

4.2.4 Quality assurance

The accuracy of the method was verified by analysing the Certified Referenced Material (CRM 8418: Wheat Gluten) and the concentration for each of the samples were typically within the certified range or ± 10% of the certified value, demonstrating the validity of the above methods (Table 4.6). The blank samples of ultrapure water and reagents were also prepared using the same procedures as for the samples. All blank levels obtained were subtracted appropriately.

Table 4.6 Results for Certified Referenced Material (Wheat Gluten 8418).

Element (mg/kg)	Measured	Certified		% Recovery
Calcium	383	369	± 35	104
Iron	58.9	54.3	±6.8	108
Zinc	56.5	53.8	±3.7	105
Magnesium	512	510	±47	100
Copper	5.83	5.94	±0.72	98
Potassium	431	472	±61	91
Sodium	1277	1420	±0.011	90
Selenium	2.90	2.58	± 0.19	112

4.2.5 Statistical methods

The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0. The minimum, maximum, mean (standard error of mean) and standard deviation of the data were compared using a 2-sided paired *t*-test at 5% level of significance (95 % CI).

4.3 Results and discussion

4.3.1 Results and discussion

The concentration of calcium, iron, magnesium, zinc, copper, potassium, sodium and selenium in eight different infant food products each representing a different variety of four popular brands (including the halal and organic range), targeted for infants aged 6 to 9 month (stage 2 of weaning), were determined by analysis using ICP-OES and ICP-MS. The products were divided into two groups of meat and vegetable base.

The results obtained, using ICP-OES and ICP–MS, are presented as per 100 g of the food samples in Table 4.7, which shows a considerable variability between samples with respect to mineral content.

Table 4.7 Mineral content of four popular brands of infant complementary in the United Kingdom (mg per 100 g)

	Meat-based				Vegetable-based				
Mineral	**Mean**	**SD**	**% RNI**	**INQ*** **	**Mean**	**SD**	**% RNI**	**INQ**	***p*-value**
Calcium	17.4	9.4	3	0.4	56.4	19.7	11	1.3	0.01
Iron	0.8	0.4	10	1.2	0.5	0.4	6	0.8	0.46
Zinc	0.54	0.14	11	1.3	0.34	0.10	7	0.8	0.07
Magnesium	12.0	2.7	16	1.9	12.7	3.4	17	2.0	0.79
Copper	0.05	0.02	18	2.1	0.02	-	-	-	0.02
Potassium	140	34	20	2.5	126	33	18	2.2	0.63
Sodium	48.3	21.3	15	1.9	47.3	32.6	15	1.8	0.96
Selenium*	** <0.24	-	-	-	<0.24	-	-	-	-

***µg/100g**

** below the limit of quantification

*** Index of Nutritional Quality **(**INQ) = amount of nutrient (g) in 1000 kcal of sample food / Allowance of nutrient (g) in 1000 kcal of food [a]

[a] Allowance of nutrient in 1000 kcal = (RNI for each age group / estimated energy requirement for that age group)*1000

Vegetable-based recipes contained significantly more calcium than meat-based varieties (*p=0.01*) but significantly less copper (*p=0.02*). The relatively high levels of calcium in the vegetable based recipes may be attributable to the inclusion of cheese and/or cream as one or more of the major ingredients.

Despite wide individual variations within varieties, no significant differences were observed in iron, zinc, magnesium, potassium and sodium contents between meat and vegetable-based products. With reference to RNI values for 6 to 9 month olds, all samples provided less than 20% of RNI values except potassium (20%) with mean (SD) values of 14.37 (±7.6) % and 10.61 (±7.91) % for meat- and vegetable-based recipes, respectively. Selenium in general was not detected in any of the samples where the detection limit for selenium was 0.24µg/L.

Since the consumption of nutrient dense food (those foods that provide substantial amounts of vitamins and minerals with relatively few calories) as opposed to energy-dense food (also called "empty calorie" food) is most critical during infancy, the Index of Nutritional Quality (INQ) for both meat and vegetable based food samples in relation to their mineral content was also calculated and the data presented in Table 4.7. The evaluation of INQ in food is a good indicator for the nutrient content of food and relates to the amount of a nutrient per 1000 kcal of food in comparison to the recommended intake of that particular nutrient (Lee & Nieman, 2003). A food with an INQ substantially greater than '1' is generally considered a good source for that specific nutrient. From the data, it is apparent that vegetable based food products are generally of a poorer quality in relation to iron and zinc, than meat based, whilst containing high level of calcium. It is worth mentioning that a high intake of cheese and milk powder in the diet of young children in Britain, has been identified as one of the factors contributing to low iron bioavailability (Agett *et al*., 2002; Singh *et al*., 2006). Based on the INQ levels meat based complementary food varieties do appear to be a good source for most of the essential and trace elements apart from calcium and selenium (Table 4.7).

While these results are insightful, it is important to examine the entire daily intake when studying the nutrient quality of the complementary food. In Table 4.8, therefore, as suggested

by Davis and O'Hare, a standard feeding regime has been composed to calculate the total daily intake of an infant, fed on commercial infant food (Davis & O'Hare, 2004).

Table 4.8 Total daily intake of nutrients by an infant age 6-9 months old a, based on gastric capacity of an eight months old infant and the standard feeding regime composed of commercially prepared infant food products.

Meals	Infant formula		Lunch (meat-based)			Dinner (vegetable-based)			Total Daily Intake [e]	RNI
Amount	100 mL	600 mL [b]	100g		124.5 g [c]	100g		124.5g [d]		
Mineral (mg)			Mean	SD		Mean	SD			
Calcium	50	300	17.4	9.4	21.6	56.4	19.7	70.2	391.8	525
Iron	1.2	7.2	0.8	0.4	0.9	0.5	0.4	0.6	8.7	7.8
Zinc	0.8	4.8	0.54	0.14	0.67	0.34	0.10	0.42	5.89	5
Magnesium	6.4	38.4	12.0	2.7	14.8	12.7	3.4	15.7	68.9	75
Copper	0.03	0.18	0.05	0.02	0.06	ND	-	-	0.24	0.3
Potassium	70	420	140	34	174	126	33	157	751	700
Sodium	16	96	48.3	21.3	60.0	47.3	32.6	58.8	215	320
Selenium*	1.4	8.4	ND	-	-	ND	-	-	8.4	10

[a] Average weight about 8.3 kg.
[b] Recommended volume of milk intake for a 6-9 months old infant .
[c&d] The portion size is calculated based on the gastric capacity of an infant aged 6-9 months old (30g/kg of body weight) divided by 2 to make up for lunch and dinner. (30 g x 8.3 kg = 249g/2).
[e] Daily intake is simply calculated by the sum of milk and none milk intake to compare with RNI.
* µg/100g.
ND= not detected; below the limit of quantification.

The calculations used in Table 4.8 are based on the World Health Organization (WHO) expert Consultation on Complementary Feeding (Dewey & Brown, 2003). In WHO's consultation, the same principle used in the formulation of fortified complementary foods suggested by Lutter and Dewey, is used (Lutter & Dewey, 2003). They consider a number of factors for the proposed fortification levels of nutrients including: age range; daily ration size; recommended nutrient requirements and contribution of human milk to these requirements (Dewey & Brown, 2003). In the data in Table 4.8 the daily intake from the milk contribution, as well as the gastric capacity of an average eight months old infant (30 g per kg of body weight) were taken into account to ascertain the nutritional value of these products in relation to the recommended daily intake (RNI) (Dewey, 2003). The gastric capacity of an eight month old infant, with an average weight of approximately 8.3 kg, is estimated to be 249 g per day which, ideally, should be divided by three to make up breakfast, lunch and dinner. In the above regime however, the entire daily intake only comprises the intake from infant's formula milk and semi-puree ready meals in the form of lunch and dinner, omitting the intake from cereal based breakfast porridges due to their powdered nature. The gastric capacity, therefore, is divided by 2 to compensate for the exclusion of breakfast devoting portion sizes of 124.5 g for each lunch and dinner at meal times. Based on the data in Table 4.8, the average value of daily calcium intake from consumption of commercial complementary food is suggested to be lower (391.8 mg/day) than the RNI (525 mg/day). In the case of the other elements such as iron, zinc and potassium, however, the data presented suggests an excessive daily intake at 8.75, 5.89 and 751.8 mg/day, respectively.

Since zinc and iron are problematic in terms of bioavailability and due to poor utilization as well as the effect of inhibitors (calcium, phytate and tannin) (Dewey & Brown, 2003), their higher concentration level, as demonstrated by the data in Table 4.8 may be of little concern in terms of food safety (Agett *et al*., 2002; Singh *et al*., 2006). A large survey carried out in 1992 has suggested that the median iron intake of full term babies 6-12 months of age are below the RNI and the mean zinc intake for this age group is only 90% of the RNI for zinc (Foote & Marriott, 2003). It is, however, important to note that excessive iron and zinc intake may be detrimental in respect of other minerals, for example the counter effect on copper (Agett *et al*., 2002; Singh *et al*., 2006). Nevertheless, potassium level remains alarmingly

high as similarly indicated by the Norwegian study where it was also suggested that the menu of commercial complementary food contained a high level of potassium; almost 2.5 times more potassium than the recommended daily intake for the 6-12 months age group (Melo *et al.*, 2008). This may be due to the replacement of salt with a palatable low-sodium alternative such as potassium chloride in the ingredient by manufacturers of seasoning and natural flavours (e.g. tomato extract), in order to maintain the flavour and functionality of food during processing as a response to the urge to reduce the sodium content of ready meals, which should be investigated further.

In Table 4.9, the data is further analysed to evaluate the nutritive value of each individual variety (meat and vegetable based) in respect of the mineral and trace elements content of an ideal complementary food based on the infants' estimated daily non-milk requirements.

Table 4.9 The nutrient quality of an ideal complementary food based on the estimated daily none milk requirements compared to the samples analysed.

Meals	Infant formula		RNI [b]	Daily none milk intake requirements C = b-a	Nutritive value of samples	
Amount	100 mL	600 mL[a]		Gastric Capacity 249 g [*]	per 249 g	
Mineral (mg)					Meat based	Veg based
Calcium	50	300	525	225	43.3	140.4
Iron	1.2	7.2	7.8	0.6	2.0	1.2
Zinc	0.8	4.8	5	0.2	1.34	0.85
Magnesium	6.4	38.4	75	36.6	31.6	31.6
Copper	0.03	0.18	0.3	0.12	0.12	ND
Potassium	70	420	700	280	349	315
Sodium	16	96	320	224	120	118
Selenium*	1.4	8.4	10	1.6	ND**	ND

[a] Amount of nutrients provided by daily milk intake based on the nutritional information declared on the label.
[b] Recommended Nutrient intake for a 6-9 months old infant.
[c] Daily none milk requirement is simply calculated by subtracting the milk intake from advised requirements.
[*] Daily amount of complementary food intake based on the gastric capacity of a 6-9 months old infant.
* µg/100g.
** ND = not detected/below the limit of quantification.

Unfortunately there is no universal data available for the required nutritional constitution of complementary foods. The basic concept used to calculate nutrient requirements from complementary foods is simple. The starting point for these calculations is current knowledge of the recommended energy and nutrient levels in infants. By subtracting the amount of energy and nutrient provided by milk from these theoretical requirements, the amount of energy and nutrient required from complementary food can be calculated. The data in Table 4.9 indicates the adequate level of magnesium in both varieties, i.e. almost at the required levels. The vegetable based products, however, had higher calcium content. The level of iron, zinc and potassium content, in both varieties of food, are relatively high.

Comparing the different individual brands the halal brand was found to be relatively poor in respect of the level of most elements (Table 4.10).

Table 4.10 Mean level of 8 minerals and trace elements in complementary infant food samples from the UK.

Element [a]	**Calcium**		**Iron**		**Zinc**		**Magnesium**		**Copper**		**Potassium**		**Sodium**		**Selenium**	
Brand [b]	Meat	Veg*	Meat	Veg	Meat	Veg	Meat	Veg	Meat	Veg	Meat	Veg	Meat	Veg	Meat	Veg
A (organic)	13.7	36.2	0.6	0.3	0.46	0.41	11.2	15.8	0.03	ND	151	109	44.7	16.9	ND**	ND
B	29.9	75.5	1.4	1.2	0.55	0.43	12.1	7.0	0.03	ND	142	81	71.3	102	ND	ND
C	19.7	71.0	0.7	0.3	0.75	0.32	16.1	13.7	0.04	ND	181	155	61.9	30.5	ND	ND
D (halal)	7.7	43.3	0.3	0.2	0.39	0.18	8.6	14.0	0.10	0.02	86	160	15.4	39.7	ND	ND

[a] All values are presented in mg/100 g apart from zinc and selenium in µg/100g.

[b] Eight products representing four brands including the organic and halal range in two varieties of meat and vegetable based*.

**ND = not detected, below the limit of quantification <0.24 µg/100g.

From the same Table, it also became apparent that the high level of iron, as indicated in the previous analyses, is only due to the high concentration of iron in one particular brand (brand B). This could be explained by the ingredient information given in Table 4.1, where the products are declared to be fortified with ferrous sulphate. Fortification with this compound may be cheap but there are large numbers of inhibitors and promoters that influence its bioavailability and its high solubility causes the undesirable sensory changes, especially colour.

Finally, the average energy density (kcal/g), based on the information declared on the labels, was also calculated and it was found to be at the desirable level of 0.6kcal/g for both meat and vegetable based varieties (Monte & Giuliani, 2004). A comparison between the desired energy density for complementary food and the energy density of the analysed food products (based on the labels) is shown in Table 4.11.

Table 4.11 The energy density of an ideal complementary food based on the estimated daily none milk energy requirements compare to the samples analysed.

Meals		Infant formula		EER[a]	Daily none milk energy requirements [b]	Desired energy density [c]	Energy density of the samples (249g)	
Amount		**100 mL**	**600 mL** [a]	**kcal/day**	**Gastric capacity 249 g**	**kcal/g**	**kcal/g**	
							Meat based	**Veg based**
Energy	(kcal)	67	402	795	393	1.6	1.6	1.6

[a]Estimated Energy Requirements for a 6-9 months old child declared as the average for boys and girls (DoH ,1991).
[b] Daily none energy requirement = EER- Daily intake from formula.
[c] Desired Energy density level is the amount of daily none milk energy requirement/ration size of the complementary food=393 kcal/249 g.

In general the value of some mineral and trace elements in complementary food were found to be inadequate in meeting the recommended intakes. The frequency of the feed as a solution to improved uptake however must be addressed very carefully. Increasing frequency has potential implications for total energy and fat intake and taste acquisition which can impact negatively on the risk of chronic non-communicable disease that are currently causing major health concerns for society particularly in the UK e.g. obesity. It may also result in a displacement of the milk intake, whilst complementary food must not be a substitute for milk according to the Codex Alimentarius Standards for canned baby food (Clark & Shrimpton, 2000).

4.4 Conclusions

The results from the analyses reported herein indicate a considerable variability between samples with respect to mineral content which are due to the proportion of different ingredients used in the composition of the foods. With reference to the guidelines, the RNI values for 6 to 9 month olds, all samples provided less than 20% of RNI values except for potassium (20%). The index of nutritional quality for vegetarian food products with respect to iron and zinc seems to be relatively poor. Meat based products are shown to be a good source for most of the essential and trace elements in question apart from calcium and selenium. Furthermore, when the entire daily intake from the consumption of these complementary food products in addition to the daily milk intake is compared to RNI values in the proposed menu, it is apparent that the recommended daily intake were not met, except for iron, zinc and potassium. In light of the aforementioned comments, it can therefore be concluded that commercial complementary infant foods on the UK market may not contain the minimum levels of minerals required for labelling declaration of micronutrient content (*Commission Directive 2006/125/EC*). This may be one of the reasons as to why manufacturers of complementary 'ready to eat' infant meals do not declare the micronutrient contents of their products, as it is difficult to satisfy the minimum required level due to adverse affects of the production process throughout the supply chain. This may provide opportunities and scope for both product and process optimizations to improve the nutritive value.

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Chapter 5

Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK

5.1 Introduction

Infant growth and development critically depend on the provision of an appropriate diet (Gokhale *et al*., 2003; Holden *et al*., 2000; Hulzebos *et al*., 2007; Robert *et al*., 2001; Taitz *et al*., 1989). Essential elements, for instance, are crucial for interaction between genetic and physiological factors and if a dietary deficiency of these elements exists it will lead to physiological and structural abnormalities which are preventable and may be reversed by administration of the element (Gibson & Hots, 2000; Melo *et al*., 2008). There are, however, two aspects to the nutritional imbalance spectrum which are associated with 1) low intake resulting in deficiencies and 2) high intake resulting in potential toxicity (Goldhaber, 2002).

Essential elements are those that occur in the body in mg/kg/day quantities (Mann & Trustwell, 2007). The essential elements analysed in this study include calcium, iron, magnesium, potassium, sodium and zinc. Trace elements, however, are required in µg/kg/day quantities and include selenium, molybdenum, cobalt, copper, chromium and manganese (Mann & Trustwell, 2007). There are also non-essential elements (e.g., arsenic, barium, nickel, cadmium, antimony, lead, mercury, aluminium) that are food contaminants with cumulative properties, and are thus considered ''potentially'' dangerous (toxic) for the consumer. The presence of non-essential elements in food is usually attributed to a) naturally occurring sources such as raw materials or b) contamination during processing of food throughout the supply chain. It should be noted that infants have a relatively higher absorption (intake/body size) and less effective excretion of several elements compared to adults (Koletzko *et al*., 2005). The contamination of food by these non-essential elements must, therefore, be kept at an absolute minimum - ideally they should not be present - especially if the food is intended for consumption by infants. In the study by Ljung *et al*, (2011) the arsenic concentration of rice-based infant powdered food was found to be in excess of what is considered to be safe. The situation is further hindered by the fact that the data required for

science based risk assessment of infant exposure to non-essential elements is currently lacking (Codex Alimentarious Commission, 2007).

Currently there is a paucity of data regarding the nutritional value of commercial infant foods in the UK. Under the current legislation [1996 (FLR) (SI 1996 No.1499) provision of the EC Nutrition Labelling Directive (90/496/EEC); Commission Directive 2006/125/EC], micronutrient declaration is not mandatory. Hence, the nutritional information declared on the labels of these products does not contain adequate data regarding the mineral and vitamin content (Table 5.1) (FSA, 2004). The foregoing comments imply that a number of important nutrients may be limited or excessive in some brands of food, thus affecting their nutritional quality and suitability for infants (Bhutta, 2000; Melo, *et al.*, 2008; Kimmons *et al*., 2005; Nobel & Emmet, 2006). It is, therefore, necessary to examine the nutritive value of infant foods in order to establish whether infants are receiving the correct level of (non-milk related) nutrients during this critical period of development (Briefel *et al.*, 2004; Brown *et al.*, (Barclay *et al*., 2003; Briefel *et al*., 2004; Hulzebos *et al*., 2007).

The primary objective of this study was to determine the level of twenty elements in eight different ready to feed infant food products in order to (1) evaluate the adequacy of intake at the deficiency end of the spectrum with reference to DRVs, and (2) to assess the risk of exposure at the toxicity end of the spectrum relevant to safety guidelines. The current study was conducted using ICP-OES and ICP-MS.

5.2 Materials and methods

5.2.1 Sample collection

Eight different of baby food samples were obtained from leading supermarkets in the UK between February/March 2011. The samples represented four popular brands available on the market, including an organic and a Halal range. All brands were represented by two different product categories: (i) chicken based and (ii) fish based; these were semi-pureed and packed in glass jars. Exact corresponding recipes were not available for all the brands. The declared ingredients of the baby food samples and their characteristics are presented in Table 5.1. Three

independent replicates of all samples, from the same batch, were analysed. The sample jars were stored unopened at room temperature, similar to their distribution and market environment.

Table 5.1 Infant complementary foods sample characteristics.

	BRAND Code	PRODUCT NAME(n=8)	INGREDIENTS	NUTRITIONAL INFORMATION
Chicken based (6-9 Months)	A	Tasty sweet corn tomato & chicken risotto	Organic vegetable (47%) [tomato(23%), carrots, sweet corn (6%), red pepper, onion], water, organic cooked rice (21%), organic chicken (8%), organic herbs and spices [parsley, paprika, rosemary, pepper], organic rapeseed oil (0.9%), organic vegetable stock [salt, organic rice flour, organic vegetable stock (carrots, onions, celeriac), organic yeast extract, organic vegetable oil, organic spices], antioxidants [ascorbic acid, tocopherol rich-extract].	**Energy** 286 kJ/68 kcal, **Protein** 2.8 g **Carbohydrate** 9.4 g of which sugars 1.7 g, **Fat** 2.1 g, of which saturates 0.4 g, **Fibre** 1.2g, **Sodium** 0.05 g.
	B	Fruity chicken with rice	Vegetables (32%, carrots, potatoes, onions), water, apple juice from concentrate (17%), rice (16%), chicken (8%), apricot (3%), corn flour, vegetable oil, natural flavouring, iron sulphate.	**Energy** 274 kJ/65 kcal, **Protein** 2.4 g, **Carbohydrate** 10.0 g of which sugars 3.4g, **Fat** 1.5 g, of which saturates 0.2 g **Fibre** 1.0 g, **Sodium** 0.06 g , **Iron** 1.4 mg.
	C	Autumn orchard chicken	Baby grad vegetables (43%) (tomato, carrot, sweet corn, courgette, cauliflower), rice (19%), cooking water, chicken (11%), apple (7%),apple juice from concentrate, wheat starch (gluten free), corn flour, corn oil, parsley.	**Energy** 307 kJ/73 kcal, **Protein** 3.1 g, **Carbohydrate** 10.6 g, of which sugars 2.9 g, **Fat** 2.0g of which saturates 0.4 g, Fibre 1.2 g, **Sodium** 0.05 g.
	D	Organic vegetable & chicken hot pot	Organic potato (37%), water (25%), organic parsnip (12%), organic carrot (11%), organic chicken (8%), organic onion (7%), organic thyme (<1%).	**Energy** 185 kJ/44 kcal , **Protein** 2.4 g ,**Carbohydrate** 7.4 g , of which sugars 0.7 g **Fat** 0.5 g , of which saturates 0.2 g, **Sodium** trace.
Fish based (6-9 Months)	A1	Scrummy tuna penne	Baby-grade vegetables (39%) (tomato, courgette, carrot, peas, onion), pasta (25%)(durum wheat flour, egg albumen), cooking water, dolphin-friendly tuna(11%), wheat starch, corn flour, /corn oil, oregano.	**Energy** 296 kJ/70 kcal , **Protein** 4.6 g, **Carbohydrate** 8.9 g of which sugars 0.8 g, **Fat** 1.8 g of which saturates 0.3 g, **Fibre** 0.9 g, **Sodium** 0.05 g.
	B1	Fabulously filling fish pie with mash	Organic whole milk (29%), organic broccoli (14%), organic onion (14%), organic peas (14%), organic potato (14%), organic salmon (11%), organic unsalted butter (4%), organic parsley (<1%),	**Energy** 403 kJ/97 kcal, **Protein** 5.2 g, **Carbohydrate** 6.2 g of which sugars 2.6 g, **Fat** 5.6 g, of which saturates 2.9 g, **Fibre** 1.9 g, **Sodium** < 0.1g.
	C1	Super scrummy salmon risotto with sprinkle of cheese	Organic vegetables stock (50%) (water and organic vegetable: leeks, carrots and parsley), organic salmon (12%), organic carrots (9%), organic onions (8%), organic peas (8%), organic cheese (6%), organic rice (5%), organic unsalted butter (2%).	**Energy** 266 kJ/63 cal, **Protein** 2.9 g , **Carbohydrate** 8.9 g, of which sugars 1.8 g, **Fat** 1.8 g of which saturates 1.0 g, Fibre 0.7 g, **Sodium** 0.05 g.
	D1	Spinach with salmon and parsnip	Organic vegetable (57%) (organic parsnip 32%, organic spinach 13%, organic leek 7%, organic celeriac root 5%), water, organic salmon 12%, organic quinoa flakes 4%, organic dill, organic ground ginger.	**Energy** 289 kJ/69 kcal, **Protein** 2.8 g, **Carbohydrate** 6.8 g, of which 1.0 g sugars, **Fat** 2.8 g, of which saturates 0.4 g, **Fibre** 1.2 g, **Sodium** trace.

5.2.2 Sample Digestion and ICP-OES/MS

A microwave accelerated reaction system (CEM MARS 5® with XP-1500 vessels), equipped with standard temperature and pressure control systems, was used to digest all the samples. Each baby food sample was mixed and homogenised using a domestic blender (Multi-quick, Braun 300) and three independent replicates of 0.5 g (wet weight) were weighed prior to adding 5.0 mL of concentrated nitric acid (70 % trace analysis grade, Fisher Scientific), 1.7 mL of hydrochloric acid (36% analytical grade, Fisher Scientific) and 0.5 mL of hydrogen peroxide (30 % trace analysis grade, VWR International). The samples were then heated for 20 min using microwave digestion; the operating conditions for which are shown in Table 5.2.

Table 5.2 CEM MARS 5 ®, (XP-1500 vessels) microwave digestion conditions [a].

Microwave conditions	**Nitric acid digestion of semi-solid samples**
Sample	0.5 g
Nitric acid (HNO_3)	5 mL
Hydrochloric acid (HCl)	1.7 mL
Hydrogen peroxide (H_2O_2)	0.5 mL
Pressure [b]	Max 400 psi
Power	1200 W- 100%
Temperature[c]	Step 1: ramp to 190 ^oC over 20 min Step 2: hold at 190 ^oC for a further 5 min; allow to cool to room temperature over 1 hr

[a] Microwave condition and digestion procedures were adapted and modified based on the CEM operation Manual (674007 version).

[b & c] The electronic temperature and pressure sensors (EST & ESP -1500) were used to control and monitor the conditions inside the vessels to avoid exothermic reaction and over pressurization of the digestion vessels.

The digested samples were quantitatively analysed for calcium, iron, magnesium, manganese, sodium, potassium and zinc using an Inductivity Coupled Plasma–Optical Emission Spectrometer (ICP-OES, Perkin Elma Optima 4300 DV), for which the operating conditions are shown in Table 5.3.

Table 5.3 Instrument operating parameters applied for determination of elements by ICP-OES.

Parameter	Value
View mode	Axial
View distance	15
Plasma gas flow	15 L/min
Auxiliary gas flow	0.2 L/min
Nebulizer	0.80 L//min
Pump flow rate	1.50 mL/min
Detector	Segmented array change coupled device
Power	1300 watts
Sample aspiration rate	1.50 mL/min
Read	Peak area
Number of replicates	3
Background correction	2 –point
Read delay	30s
Wash delay	60 s

Selenium, chromium, molybdenum, antinomy, barium, cobalt, copper, nickel, cadmium, arsenic, aluminium and lead were analysed by Inductivity Coupled Plasma – Mass Spectrometry (Thermo Scientific, X Series 2 ICP-MS). The operational parameters for the ICP-MS are summarised in Table 5.4.

Table 5.4 Operating parameters for the ICP-MS instrument (Thermo Scientific, X Series 2).

Parameter	Value
Plasma flow	18
Auxiliary flow	0.5
Nebulizer flow	0.9
RF Power	1400 kW
Stabilization time	60 s
Sampling depth	120 mm
Operating mode	Kinetic energy discrimination
Collision cell gas	8%H in He, 3.5mL min^{-1}
Measurement mode	Peak jumping
Dwell time	100 ms
Point per peak	1
Sweeps	75
Nebulizer	Quartz micro-concentric (0.4 mL /min)
Number of replicates	3

A collision cell operated in kinetic energy discrimination mode was used to minimise polyatomic-and argon-based interferences of the first row transition metals and selenium. Both samples and standards were spiked with rhodium at a concentration level of 10 µg/L (0.01 ppm).Method parameters were checked on a daily basis using an in-house optimisation programme.

5.2.3 Standards

Seven multi-element calibration solutions were prepared at different concentration levels (50 – 4000 µg/L) from 1000 mg/L single element ICP grade standards (Inorganic Ventures) using high purity nitric acid (70 % trace analysis grade, Fisher Scientific) matched to the sample matrix.

A calibration curve, at six different concentrations (min. 10 ppb –max. 800 ppb), was obtained using these multi-element standards (r^2= 0.9999). To check for instrumental drift, one of the multi-element standards was analysed for every 15 samples.

5.2.4 Quality assurance

The accuracy of the analysis was verified by analysing the Certified Referenced Materials (NCS ZC73008:wheat and NCS ZC73009: rice) and the concentration for each of the samples were typically within the certified range or ± 10% of the certified value, demonstrating the validity of the above methods, as presented in Table 5.5. Blank samples of ultrapure water and reagents were also prepared using the same procedures as for the samples. All blank levels obtained were subtracted appropriately.

Table 5.5 Results of elemental analyses for certified reference materials (NCS ZC73008/NCS ZC73009).

Element (mg/Kg)	Measured	Certified		% Recovery
Essential				
Calcium*	0.031	0.034	± 0.002	91
Iron	15.3	18.5	±3.1	83
Magnesium	40	41	±6	97
Potassium	131	140	±6	95
Sodium	16.9	17	±5	99.6
Zinc	10.2	11.6	±0.7	105
Trace				
Selenium	0.050	0.053	± 0.007	95.5
Molybdenum	0.48	0.48	± 0.05	98
Cobalt	0.009	0.01	-	91
Copper	2.6	2.7	±0.2	96
Chromium	0.082	0.096	±0.014	85
Manganese	16.1	17	± 1	98
Toxic				
Arsenic	0.023	0.031	±0.005	75
Barium	2.2	2.4	± 0.3	94
Nickel	0.24	0.27	±0.02	92
Cadmium **	12.5	18	± 4	69
Antimony	0.0057	0.006	-	94
Aluminium	0.0081	0.0104	± 0.0019	78
Mercury	1.08	1.6		68
Lead	0.052	0.065	± 0.024	80

*g/100g

**µg/kg

5.2.5 Limit of detection (LOD) and quantification (LOQ)

The LOD is most commonly determined using a multiple of the standard deviation derived from 10 measurements of the preparation blank. The LOD in this study for all the relevant elements was calculated using the instructions provided by the instrument manufacturers, quoting 3 x standard deviation of the blank. A reagent blank was therefore analysed ten consecutive times, with a routine rinsing procedure between each run. The LODs and LOQs, defined as 10 x standard deviation of the blank, are presented in Table 5.6.

Table 5.6 ICP-OES and ICP-MS detection limits for the elements examined.

Element	SD±	LOD*	LOQ**
ICP-OES [a]			
Ca	0.0052	0.015	5.2
Fe	0.0005	0.0015	0.5
Mg	0.0001	0.0003	0.1
K	0.0012	0.0036	1.2
Na	0.0011	0.0033	1.1
Zn	0.0005	0.0015	0.5
ICP-MS [b]			
Cu	0.12	0.36	120
Se	0.022	0.066	22
Mo	0.023	0.069	23
Co	0.005	0.0015	5
Cr	0.026	0.078	26
Mn	0.0481	0.1443	48.1
As	0.01	0.03	10
Ba	0.053	0.159	53
Ni	0.080	0.24	80
Cd	0.020	0.060	20
Sb	0.006	0.018	6
Al	0.332	0.996	332
Hg	0.010	0.03	10
Pb	0.014	0.042	14

*LOD = 3 x SD of ten consecutive blanks
** LOQ = (10 x SD of ten consecutive blanks)*100 solid conversion
[a] mg/Kg
[b] µg/Kg

5.3 Results and discussion

The concentration of twenty elements in eight different infant food products each representing a different variety of four popular brands, targeted for infants aged 6 to 9 month (stage 2 of weaning), were determined by analyses using ICP-OES and ICP-MS. The products were divided into two groups of chicken and fish base. Two of the chicken based food products were organic. The results obtained, using ICP-OES and ICP–MS, are presented as per 100 g of the food samples in Figure 5.1 (I) and Figure 1(II), which shows a considerable variability between samples with respect to essential and trace element content, respectively.

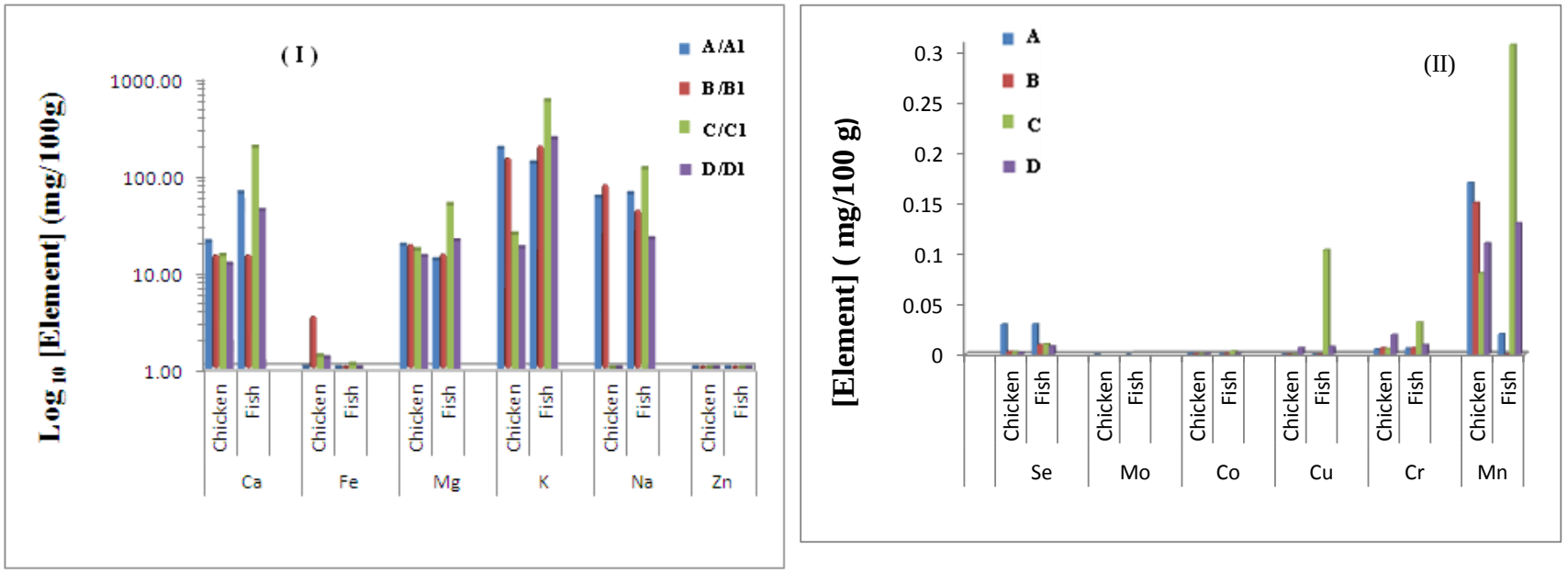

Fig.5.1 Comparison between elemental content of vegetable and fish based infant food products: (I) essential and (II) trace elements.

The data in Table 5.7 shows the concentration of all the investigated elements in the analysed samples, which varied markedly between the brands. By comparing the different individual brands, the organic chicken brands (A and D) were found to contain higher concentrations of essential and trace elements, especially in respect of potassium at 185.7 and 231.57 mg/100 g, respectively. Brand D was also the only chicken based product containing copper (0.006 µg/100 g). From the data in Table 5.7, it is also apparent that the high level of iron (1.09 mg/100 g), potassium (579 mg/100 g), chromium (0.103 µg/100 g) and magnesium (48.91 mg/100) in fish based recipes, is attributed to one particular brand (C1) compared to the other brands.

Table 5.7 Concentration of essential and toxic elements in 'ready to-feed' chicken and fish-based infant foods intended for consumption from 6 months of age (mean ±SD).

Element *	A[a]	B	C	D[a]	A1	B1	C1	D1
Essential (mg/100g)								
Ca	20.22 ±0.27	14.57 ± 0.05	14.58 ±0.02	11.78 ±0.09	64.70 ±0.03	14.60 ±0.05	191.48 ± 0.16	42.61 ±0.07
Fe	1.01 ±0.05	3.38 ± 0.01	1.34	1.27	0.88 ±0.005	0.48 ±0	1.09	0.52
Mg	18.68 ±0.05	18.59 ±0.05	15.81 ±0.02	15 ±0.6	13.14 ±0.005	14.84 ±0.002	48.91 ±0.125	20.73 ±0.09
K	185.7 ±0.05	146.3 ±1.4	162.1 ±0.07	231.6 ±1.2	131.6 ±0.8	192.9 ±1.65	579.0 ±1.5	235.3 ±0.07
Na	58.08 ±0.51	77.85 ±0.35	26.34 ±2.81	17.55 ±0.08	63.26 ± 0.57	42.43 ±2.3	115.30 ±0.9	21.78 ± 0.29
Zn	0.20 ±0.002	0.16 ±0.010	0.23 ±0.002	0.19 ±0.018	0.29 ±0.0235	0.14 ±0.008	< 0.05**	0.29 ±0.027
Trace (µg/100g)								
Se	29 ±0.045	< 2.2**	< 2.2	5	29 ±0.045	9±0.005	10±6.5E-05	8±0.005
Cu	<12**	<12	<12	<12	<12	<12	103 ± 0.003	<12
Cr	5	6	5	190	5	5	31	9
Mn	170 ±0.05	150±0	80	110	20	<4.8	310 ± 0.01	130
Toxic (µg/100g)								
Ba	6 ±0.0002	11 ± 0.0118	<5.3	18 ±0.0099	<5.3	<5.3	29 ±0.0139	8 ±0.0008
Ni	8 ±0.001	9	8	14 ±0.001	<8	<8	41 ±0.001	11 ±0.001
Cd	< 2 **	< 2	< 2	< 2	< 2	< 2	2.2 ±0.0003	<2

[a] Organic.

* Mo < 2.3, Co <0.5, Sb <0.6, Pb <1.4 , As <1, Hg <1 and Al< 33.2 µg/100g were all below the limit of quantification for all brands.

** Not detected; below the limit of quantification.

The concentration of most of the toxic elements including arsenic, molybdenum, cobalt, lead, mercury, aluminium and antimony were below the limit of detection (Table 5.7). The only trace and non-essential elements found in the samples were manganese, chromium, nickel and cadmium at very low levels insufficient to present health hazards.

With reference to RNI values for 6-9 month olds (Table 5.8), chicken based samples provided less than 15 % of RNI values with mean (SD) values of 9.6 (±9.3) %. Conversely, fish based recipes provided 2-fold the percentage of RNI provided by the chicken based meals with mean (SD) values of 17 (13.4) %.

Table 5.8 Concentration of selected* essential and trace element content of four popular brands of infant complementary in the United Kingdom (mg per 100 g).

	Chicken-based			Fish-based		
Mineral	**Mean**	**SD**	**% RNI**	**Mean**	**SD**	**% RNI**
Calcium	15.31	3.51	3	78.35	78.18	14
Iron	1.75	1.10	22	0.74	0.29	9
Zinc	0.20	0.03	4	0.18	0.14	10
Magnesium	17.07	2.07	23	24.14	16.66	32
Potassium	93.48	85.32	13	285.04	200.48	41
Sodium	33.98	40.06	11	60.69	40.15	19

*Those with defined DRVs detected in the samples.

While these results are insightful, it is important to examine the total daily intake when studying the nutrient quality of the complementary food.

Therefore, an example menu based on the manufacturer's feeding recommendations was composed (Table 5.9), which included the ready to feed type foods, such as jars of semi-solid meals and ready-made formulas. In the data in Table 5.9 the daily intake from the milk contribution, as well as the gastric capacity of an average eight months old infant were taken into account to ascertain the Total Daily Intake (TDI) of elements in these products in relation to the recommended daily intake (RNI) (Dewey, 2003). The calculations of gastric capacity (30 g/kg body weight/day), used in Table 5.9, are based on the World Health Organization

(WHO) expert Consultation on Complementary Feeding (Dewey & Brown, 2003). The gastric capacity, therefore, is divided by 2 to compensate for the exclusion of breakfast, devoting portion sizes of 124.5 g for lunch and dinner at meal times.

The average weight for infants of different age ranges have been taken from the report by the Committee of Medical Aspect of food and nutrition policy (COMA): Weaning and The Weaning Diet (1994). The aforementioned methods have been used by the Scientific Committee on Food (SCF) for assessing the maximum levels of pesticide residues in food. The estimated amount for milk consumption however, was set at 600 mL, as advised by COMA, for infants up to 12 months old. The gastric capacity of an eight month old infant, with an average weight of approximately 8.3 kg, is estimated to be 249 g per day which, ideally, should be divided by three to make up breakfast, lunch and dinner. In the above regime however, the TDI only comprises the intake from infant formula milk and semi-puree ready meals in the form of lunch and dinner, omitting the intake from cereal based breakfast porridges due to their powdered nature. One of the main limitations with this menu is that it is unlikely to represent the actual amount of food that is ingested and retained by the infant; neither does it take into account any wastage of food. The other problem with this approach is that it fails to take into account any contribution from breast milk, snacks or homemade food.

Table 5.9 Total daily intake of selected essential and trace elements* by an infant age 6-9 months old [a], based on gastric capacity of an eight month old infant and a standard feeding regime composed of commercially prepared infant food products.

Meals	Infant formula		Lunch (chicken-based)			Dinner (Fish-based)			Total Daily Intake [e]	RNI
Amount	100 mL	600 mL [b]	100g		124.5 g [c]	100g		124.5g [d]		
Mineral (mg)			Mean	SD		Mean	SD			
Calcium	50	300	15.31	3.51	19.06	78.35	78.18	97.55	416.61	525
Iron	1.2	7.2	1.75	1.1	2.18	0.74	0.29	0.92	10.30	7.8
Zinc	0.8	4.8	0.2	0.03	0.25	0.18	0.14	0.22	5.27	5
Magnesium	6.4	38.4	17.07	2.07	21.25	24.14	16.66	30.05	89.71	75
Potassium	70	420	93.48	85.32	116.38	285.04	200.48	354.87	891.26	700
Sodium	16	96	33.98	40.06	42.31	60.69	40.15	75.56	213.86	320

[a] Average weight about 8.3 kg.
[b] Recommended volume of milk intake for a 6-9 months old infant.
[c&d] The portion size is calculated based on the gastric capacity of an infant aged 6-9 months old (30 g/kg of body weight) divided by 2 to make up for lunch and dinner (30 g x 8.3 kg = 249 g/2).
[e] Total daily intake is calculated by the sum of milk and non- milk intake to compare with RNI.
[*]Those with defined DRVs detected in the samples.

Based on the data in Table 5.9, the average value of daily calcium intake from consumption of commercial complementary food is suggested to be lower (416.61 mg/day) than the RNI (525 mg/day). In the case of the other elements such as iron, zinc, magnesium and potassium, however, the data presented suggests an excessive daily intake at 10.30, 5.27, 89.71 and 891.26 mg/day, respectively.

Since zinc and iron are problematic in terms of bioavailability and due to poor utilization as well as the effect of inhibitors (calcium, phytate and tannin) (Dewey & Brown, 2003), their higher concentration level, as demonstrated by the data in Table 5.9, may be of little concern in terms of food safety (Agett *et al*., 2002; Singh *et al*., 2006). A large survey carried out in 1992 has suggested that the median iron intake of full term babies 6-12 months of age are below the RNI and the mean zinc intake for this age group is only 90% of the RNI for zinc (Foote & Marriott, 2003). It is, however, important to note that excessive iron and zinc intake may be detrimental in respect of other minerals; for example the counter effect on copper (Agett *et al*., 2002; Singh *et al*., 2006). Nevertheless, potassium levels remain alarmingly high as similarly indicated by the Norwegian study where it was also suggested that the menu of commercial complementary food contained a high level of potassium; almost 2.5 times more potassium than the recommended daily intake for the 6-12 months age group (Melo *et al.*, 2008). This may be due to the replacement of salt with a palatable low-sodium alternative such as potassium chloride in the ingredients by manufacturers of seasoning and natural flavours (e.g. tomato extract), in order to maintain the flavour and functionality of food during processing as a response to the urge to reduce the sodium content of ready meals. The high level of magnesium may also be due to the application of fertiliser on vegetable farms and the agricultural industry. The data in Table 5.9 are supported by the data in Tables 5.6 and 5.8 which indicate a high concentration of potassium and magnesium in the organic range and fish based recipes, which should be investigated further.

With regard to the assessment of the daily risk exposure associated with consumption of commercially prepared ready to feed infant foods, a previously proposed standard menu (Table 5.9) was replicated to provisionally evaluate the total daily intake (TDI) of manganese, chromium, nickel and cadmium (Table 5.10).

The Food Standard Agency survey (FSA, 2006) was used as a basis for calculating the mean elemental concentration (Table 5.10) of infant formula milk powder (µg/kg). The preparation guidelines provided on the labels were then used to calculate the daily amount of milk powder required to prepare the equivalent of 600 mL of milk [7 scoops (4.5g) /feed x 5 times/day].

Table 5.10 Estimated dietary exposure of infants to selected trace and non-essential elements in commercially prepared infant foods (µg /kg bw/day).

Meals	Infant formula *		Lunch (chicken-based)		Dinner (Fish-based)		Total daily exposure [e] (µg /kg bw/Day)	Safety guideline (µg /kg bw/Day)
Amount	µg/kg[a]	157.5g[b]	100g	124.5 g[c]	100g	124.5g [d]		
Mineral			Mean		Mean			
Manganese	78	12.28	127.5	158.7	115	143.5	35	16 (Doh,1991)
Chromium	22	3.4	51.5	64.1	12.5	15.5	9	1 (Doh,1991)
Nickel	35	5.5	9.75	12.1	26	32.4	5.6	5 (WHO,1996)
Cadmium	2.0	0.3	nd**	-	nd	-	0.03	1 (WHO,2001)

* Element concentration in dry matter excluding water.
** not detected ;below the limit of quantification
[a] Mean element concentration of dried infant formula milk powder (µg/kg) (FSA,2002).
[b] Preparation guidelines provided by manufacturer on the product label [7 scoops (4.5g) /feed x 5 times/day] - excluding water.
[c&d] The portion size is calculated based on the gastric capacity of an infant aged 6-9 months old (30g/kg of body weight) divided by 2 to make up for lunch.
[e] Total daily intake is calculated by the sum of milk and none milk intake to compare with RNI (b+c+d).

Based on the data in Table 5.10, there is a degree of daily exposure to intake of manganese, chromium, nickel and cadmium associated with consumption of commercially prepared ready to feed infant foods. Interestingly, the TDI of manganese, chromium and nickel appears to be in excess of the safe intake. The safe intake of chromium for infants and young children set by the Department of Health is 1 µg/kg bw/day (DoH, 1994). Since trivalent chromium is classified as essential and is the most abundant form found in the majority of foods (unlike the hexavalent form) it is appropriate to compare the total chromium levels with the recommended safe intakes (DoH, 1994). However, since the TDI is likely to be an overestimation, due to the use of maximum portion size, which was mentioned earlier as one of the limitation with the set menu, the overall dietary exposure to neither chromium nor manganese is considered to be of concern. Similarly, taking into account the worse scenario consumption, the exceeded WHO guidelines for nickel (5 mg/kg bw/day) is considered not to be significant. Ingestion of nickel may exacerbate contact dermatitis/eczema in pre-sensitised individuals and since infants are less susceptible to pre-sensitization to nickel (SACN, 2003), within limits, overall dietary exposure is not considered to be of concern.

5.4 Conclusions

In general the levels of some essential and trace elements in complementary food were found to be inadequate in meeting the recommended intakes and in some cases e.g. magnesium and potassium in excess of the recommended intake, which warrant further investigations. For most of the essential and trace elements, inadequate daily intake may lead to nutritional deficiencies. However, consideration of nutritional deficiencies is not within the remit of this study. The frequency of the feed as a solution to improved uptake of the nutrients must be addressed very carefully. Increasing frequency of food intake has potential implications for total energy and fat intake and taste acquisition which can impact negatively on the risk of chronic non-communicable disease that are currently causing major health concerns for society particularly in the UK e.g. obesity. It may also result in a displacement of the milk intake, whilst complementary food must not be a substitute for milk according to the Codex Alimentarius Standards for canned baby food (Clark & Shrimpton 2000). In terms of non-essential elements, the concentration of toxic elements in food samples analysed in this study, were found not to present any hazards to health.

In light of the aforementioned comments, it can be concluded that commercial complementary infant foods on the UK market may not contain the minimum levels of minerals required for labelling declaration of micronutrient content (*Commission Directive 2006/125/EC*). This may be one of the reasons as to why manufacturers of complementary 'ready to eat' infant meals do not declare the micronutrient contents of their products, as it is difficult to satisfy the minimum required levels due to adverse effects of the production process throughout the supply chain. This may provide opportunities and scope for both product and process optimizations to improve the nutritive value.

5.5 References

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Chapter 6

Simultaneous extraction and quantification of vitamin B_2 and B_6 by UHPLC/LC-MS in UK commercial infant meal food products

6.1 Introduction

Providing an appropriate diet for a growing infant is critical in terms of growth and development. The consequences of inappropriate early infant feeding include energy and micronutrient deficiency which may cause body function impairment and a slower growth rate (Schlenker & Williams, 2003; Taylor *et al.*, 2004). For example vitamin B_2 (riboflavin) is claimed to be abundant in milk and the precursor for the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Many enzymes that catalyze oxidation-reduction reactions require FAD or FMN as a prosthetic group. Furthermore, the ability of FMN to couple two-electron transfers with one electron transfers is important in mitochondria, where the transfer of reducing power from nicotinamide adenine dinucleotide (NADH) to O_2 includes a number of single electron steps. Vitamin B_6 (pyrodixine) is the precursor of pyridoxal phosphate which is the prosthetic group for many enzymes that catalyze a variety of reactions involving amino acids (including isomerization, decarboxylation, and side-chain elimination or replacement reactions), glycogen releasing enzymes, the maturation of blood cells and in the absorption of iron (Bessey *et al.*, 1957; Ronnenberg, 2008).

Currently, commercially prepared complementary foods play an increasing role in the diets of infants (Briefel *et al*., 2004; Melo *et al*., 2008; Michaelsen *et al*., 1998). To-date, little attention has been paid to the nutritional quality of 'ready to feed' complementary foods intended for infants during the period of weaning. The lack of a clear understanding of the nutritional quality of these food products is further hindered by the current lack of robust regulations relating to the nutritional quality of these compositions. Under current legislation *[1996 (FLR) (SI 1996 No.1499) provision of the EC nutrition labelling Directive (90/496/EEC)*; Commission Directive 2006/125/EC], micronutrient declaration is not mandatory. At present the nutritional information declared on the labels of these products does not contain sufficient data regarding the mineral and vitamin content (Table 3.1). The

implication being that a number of important nutrients may be limited in some brands thus affecting their nutritional quality and hence suitability for infants.

Food processing has inevitable consequences in relation to the nutritional values of foods depending on the molecular structure/physico-chemical properties of the nutrient and the severity of the processing. The vitamin B group, in particular, is susceptible to degradation when food is processed or stored. Vitamin B_2, for example, is unstable to UV light and once milk has been exposed to sunlight for 4 hours, up to 70% of the B_2 is lost. Vitamin B_6 can also be lost/degraded in highly processed foods e.g. by heating (DoH, 1991). In 1953, a minor epidemic of convulsions in infants in the US was traced to an infant formula which had undergone a severe heating during processing and lost much of its B_6 (Bessey *et al.,* 1957). It is, therefore, necessary to examine the nutritional value of food formulations to establish whether infants are receiving the correct level of non-milk related nutrients during this critical period of their development.

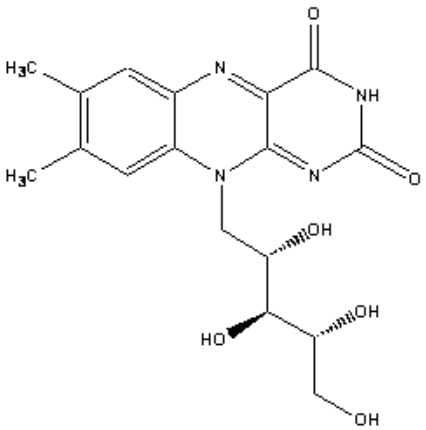
A number of techniques have been proposed in the literature for the simultaneous determination of water soluble vitamins in pharmaceutical products, biological samples and dietary supplements. These include spectrophotometric, spectrofluorometric, and electrochemical, methods as well as capillary zone electrophoresis and HPLC (Aslam *et al*., 2008; Blake, 2007; Chatzimichalakis *et al.,* 2004; Karatapanies *et al.,* 2008; Li *et al*., 2000; Lebiedzinksa *et al*., 2007; Marszall *et al.,* 2009; Ndaw *et al.,* 2000; Zefra-Gomez *et al.,* 2006). Difficulties arise, however, when an attempt is made to determine the different types of endogenous vitamins in food (Blake, 2007; Zefra Gomez-2006). While vitamin analysis of pharmaceutical samples is, relatively speaking, more straight forward (as they contain much higher levels of vitamins) an extraction technique e.g. hydrolysis or solid phase extraction is necessary prior to HPLC analysis in order to remove interfering components from matrices such as biological and food samples (Chatzimichalakis *et al.,* 2004; Lebiedzinksa *et al*., 2007; Ndaw *et al.,* 2000).

Currently there is no uniform standard method for the extraction and determination of water soluble vitamins in composite food. A limited number of methods for the determination of vitamins in single food ingredients have been reported in the literature. With respect to the vitamin B group, evidence from the literature suggests that hydrolysis using mineral acid followed

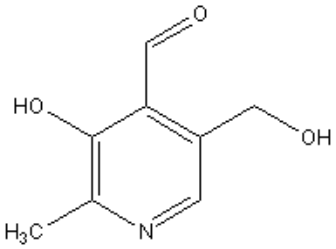
by enzymatic digestion and addition of buffer solutions is effective (Zefra-Gomez *et al.*, 2006). The drawbacks of the foregoing include the time required for analysis, high solvent consumption and the severity of the procedures used for sample digestion, especially when low concentrations of vitamins need to be quantitated e.g. in the case of processed foods (Blake, 2007).

The primary objective of this study was to develop an assay for the simultaneous determination of vitamins B_2 and B_6 (Fig. 6.1) in eight different complementary infant meal products in order to: (1) estimate the daily intake of these elements from commercial infant food consumption and (2) ascertain their nutritional suitability relative to dietary guidelines for the 6-9 month age group.

In the present work a novel, simple and accurate method for the simultaneous determination of vitamins B_2 and B_6 is proposed. The method involves a mild hydrolysis and an extraction of the supernatant by centrifugation followed by quantitative analysis using UHPLC. The separation achieved is excellent and rapid - within one minute - whilst the retention time reported in the literature for the separation of these compounds by HPLC varies between 6 to 35 min (Blake, 2007; Nollet *et al.*, 2000). The proposed assay has also been specifically developed to be LC-MS compatible which will enable further validation of the chromatographic data in addition to identification of other water soluble organic species.



Vitamin B_2



Vitamin B_6

Fig. 6.1 Chemical structures of the water soluble vitamins B_2 and B_6.

6.2 Materials and methods

6.2.1 Chemicals

Riboflavin (vitamin B_2) was obtained from ChromaDex (A1044 B) and pyridoxine hydrochloride (vitamin B_6) from ACR S Organics (99%). The reference material (BCR® - 121), containing both vitamins, was purchased from the Institute for Reference Materials and Measurements for Certified Reference Materials (Geel, Belgium). HPLC-grade acetonitrile (99.99%) and glacial acetic acid (99.7%) were purchased from Fisher (UK). All other reagents used were of analytical grade.

6.2.2 Sample collection and preparation

Eight different baby food samples were obtained from leading supermarkets in the UK between November and December 2010 to allow for randomness. The samples represented four popular brands available on the market, including an organic and a Halal range. All brands were represented by two different product categories: (i) meat based and (ii) vegetable based; these were semi-pureed and packed in glass jars. Exact corresponding recipes were not available for all the brands. The main ingredient of the baby food samples and their characteristics are presented in Table 3.1. Three independent replicates of all samples were analyzed from the same batch. The sample jars were stored unopened at room temperature, similar to their distribution and market environment.

The samples were treated according to the methods described by Aslam *et al*. (2008) and Luo *et al*. (2006), with some modifications. Each of the food samples were mixed and homogenised using a domestic blender (Multi-quick, Braun 300) and three independent replicates of 10 g (wet weight) were weighed and spiked with 1 mL of a standard solution (250 µL/mL) prior to being transferred into 50 mL volumetric flasks. Samples were then diluted with 25 mL of solution A (1% acetic acid) and incubated in a water bath at 70^{o}C for 40 min. After cooling to room temperature, the volumes were made up to 50 mL with solution A. The solutions obtained were shaken and centrifuged at 8000 rpm for 10 min at 5^{o}C. The supernatants were filtered through filter paper and passed through a 0.45 µm membrane filter prior to injection.

6.2.3 HPLC analysis

Samples were first quantitatively analysed using an HPLC system (1200, Agilent Tech, UK) equipped with a quaternary pump and diode array detector (1200, DAD). For the simultaneous separation of vitamins B_2 and B_6, a C_{18} Ultrasphere Behcam (4.6 mm x 25 cm, 5 µm particle size) column was used at 22^{o}C.

6.2.3.1 *HPLC solvent composition*

The mobile phase of the HPLC system, delivered at a flow rate of 1 mL/min, consisted of solution A [water: acetic acid (99:1; v/v)] and solution B [acetonitrile: acetic acid (99:1; v/v)].

6.2.3.2 *HPLC gradient system*

A mobile phase gradient was used starting at an A: B composition of 90:10 (v/v), respectively. This composition was changed linearly to reach 90% of solvent B after 9 min with a stop time at 12 min. A two minute equilibration time was used between injections. The injection volume was 20 µL. The chromatographic conditions were optimised for best peak shapes, column efficiency, chromatographic analysis time, selectivity and resolution. The optimum intensity for vitamin B_2 and B_6 were obtained at wavelengths (λ) of 273 and 280 nm, respectively.

6.2.4 UHPLC analysis

The foregoing chromatographic conditions were further optimised using an Ultra High Pressure Liquid Chromatographic system (UHPLC 1290 Infinity, Agilent Tech, UK) equipped with a binary pump and diode array detector (1290, DAD). The simultaneous detection and quantification of vitamins B_2 and B_6 by UHPLC was achieved using a rapid resolution high definition C_{18} column (2.1 x 50 mm, 1.8 µm particle size; RRHD Eclipse Plus), coupled with an in-line filter (5067-1551, Agilent Tech, UK) at 39^{o}C.

6.2.4.1 *UHPLC solvent composition*

The mobile phase of the UHPLC system, delivered at a flow rate of 0.5 mL/min, consisted of solution A [water: acetic acid (99:1; v/v)] and solution B [acetonitrile: acetic acid (99:1; v/v)].

6.2.4.2 *UHPLC gradient system*

A mobile phase gradient was used, starting at an A: B composition of 90:10 (v/v), respectively.

This composition was changed linearly to reach 90% of solvent B after 3 min with a stop time at 3.30 min. A two minute equilibration time was used between injections. The injection volume was 20 µL. The chromatographic conditions were optimised for peak shapes, column efficiency, chromatographic analysis time, selectivity and resolution. The optimum intensity for vitamin B_2 and B_6 were obtained at wavelengths (λ) of 273 and 280 nm, respectively.

6.2.5 LC-MS analysis

The proposed assay was specifically developed to be LC-MS compatible; therefore the analysis by LC-MS was further trialled. The LC-MS system used consisted of an Agilent 1200 Tech (Japan) HPLC system equipped with a quaternary pump and a C_{18} Ultrasphere Behcam (4.6 mm x 25 cm, 5 µm particle size) column coupled to a ThermoQuest (Finingan AQA, UK) mass spectrometer.

The chromatographic conditions for the LC system remained the same as the HPLC system already described. The electrospray ionization- mass spectrometry (ESI-MS) was operated in positive ion electrospray mode. Nitrogen was used as both desolvation gas (at a flow rate of 350 L/h) and cone gas (at a flow rate 50 L/h). Desolvation temperature was set at 350^{o}C and the ionization source temperature was 105^{o}C. The capillary and cone voltages were set at 4000 and 60 V, respectively.

6.2.6 Linearity and LOD

Six standard solutions of vitamin B_2 and B_6 were prepared at different concentrations (2.5 -50 µg/mL) following dilution of stock solutions (50 µg/mL) using solution A to match the samples matrix. Two linear calibration curves, at six different concentrations (min. 2.5- max. 50 ppm), were defined by the best-fit line equations (Table 6.1).

Table 6.1 UHPLC limit of detection and quantification of vitamins B_2 and B_6.

Vitamin		**Y= *m*x +b**		**LOD (µg/mL)**	**LOQ (µg/mL)**
B_2	Pyridoxine HCl	Y=1.180x+0.050	(n=7, r^2=0.9997)	0.01	0.06
B_6	Riboflavin	Y=1.013x+0.073	(n=7, r^2=0.9993)	0.02	0.07

m = slope, b = intercept and r^2 = correlation coefficient

The lower limit of detection (LOD) was determined by using a signal-to-noise ratio (S/N) ascertained from the relationship:

$$LOD = 3.3(s_b/m)$$

The value of s_b is the standard deviation of the intercept, whilst *m* is the slope of the response near the lower limit of detection. This determination was also performed for LOQ, except that a S/N of 10:1 was used as a cut-off point at 0.07 and 0.06 (µg/mL) for vitamins B_6 and B_2, respectively:

$$LOQ= 10 (s_b/m)$$

To check for instrumental drift, one of the standards was analysed for every 15 samples.

6.2.7 Quality assurance

The accuracy of the method was verified by analysing the certified reference material (BCR®-121) as well as the use of internal standards. The concentrations for each of the samples were typically within the certified range (± 10% of the certified value), demonstrating the validity of the above methods. Data for percentage recoveries for both CRM and spiked samples are shown in Table 6.2.

Table 6.2 Vitamin B_2 and B_6 content of certified reference material (BCR®-121).

Vitamin (mg/kg)	Measured	Certified value	% Recovery	%RSE
B_2*	13.01±1.50	12.5	108	0.025
B_6	4.69 ± 0.05	4.1 ± 1.02	113	0.045

**Spiked*

Blank samples of ultrapure water and reagents were also prepared using the same procedures as for the food samples and no obvious interferences in relation to the determination of vitamins B_2 and B_6 were observed from the blank chromatograms.

6.2.8 Statistical methods

The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0. The minimum, maximum, mean (standard error of mean) and standard deviation of the data were compared using a 2-sided paired *t*-test at $p = 0.05$ level of significance (with 95 % confidence interval) to examine mean differences between products and within meat and vegetable-based varieties. The data was further subjected to ANOVA at $p = 0.05$ to examine differences in variation between products and replicated (n = 3) measurements.

6.3 Results and discussion

A simultaneous method for extraction and quantification of B_2 and B_6 in eight different infant food products, each representing a different variety of four popular brands (including the halal and organic range), targeted for infants aged 6-9 month (stage 2 of weaning), was developed. The products were divided into two groups of meat and vegetable base.

6.3.1 HPLC gradient elution

The separation of the vitamin B_2 and B_6 using the HPLC system was relatively fast compared to the average reported time in the literature varying between 6 to 35 min (Blake, 2007; Nollet *et al.*, 2000). The retention time (t_R) for vitamin B_2 ($\lambda = 273$ nm) in this method was 5.50 min, whilst the t_R for vitamin B_6 ($\lambda = 280$ nm) was 2.96 min (Fig. 6.2).

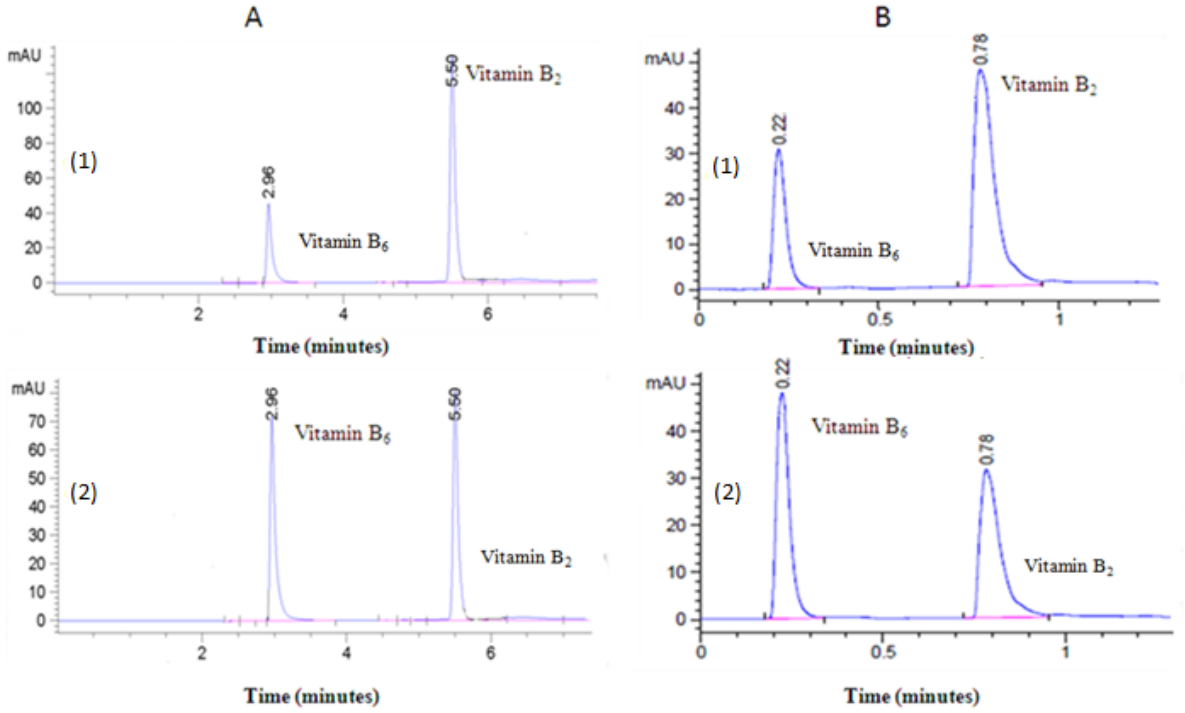


Fig. 6.2 A: HPLC results (1200, Agilent Tech.), quaternary pump, C_{18}, Ultrasphere Behcam (4.6 mm x 25 cm, 5 µm particle size) column, diode array detector, flow rate = 1 mL/min. Mobile phase: A is water: acetic acid (99:1; v/v); B is acetonitrile: acetic acid (99:1; v/v). Gradient A: B (90:10; v/v) to A:B 10:90 (v/v) over 9 min. B_2 (1) at λ = 273 nm (t_R = 5.50 min), B_6 (2) at λ = 280 nm (t_R = 2.96 min). B: UPLC results (1290 infinity, Agilent Tech.), RRHD Eclipse Plus, C_{18} (2.1 x 50 mm, 1.8 µm particle size) column, binary pump, 1290 diode array detector, flow rate = 0.5 mL/min. Mobile phase: A is water: acetic acid (99:1; v/v); B is acetonitrile: acetic acid (99:1; v/v). Gradient A:B 90:10 (v/v) to A:B 10:90 (v/v) over 9 min. B_2 (1) at λ = 273 nm, (t_R = 0.78 min), B_6 (2) at λ = 280 nm (t_R = 0.22 min).

6.3.2 UHPLC gradient elution

The separation achieved by UHPLC was excellent and rapid, within one minute, whilst the total run time was only 3.30 minutes. In comparison to the HPLC, the t_R value for vitamin B_2 at λ = 273 nm using UHPLC was reduced to 0.78 min from 5.50 min , whilst the t_R for vitamin B_6 at λ = 280 nm was reduced from 2.96 min to 0.22 min (Fig. 6.2).

6.3.3 LC-MS compatibility

The proposed assay has also been specifically developed to be LC-MS compatible, as illustrated in Figs. 6.3 and 6.4. This will enable further validation of the chromatographic data in addition to identification of other water soluble organic species.

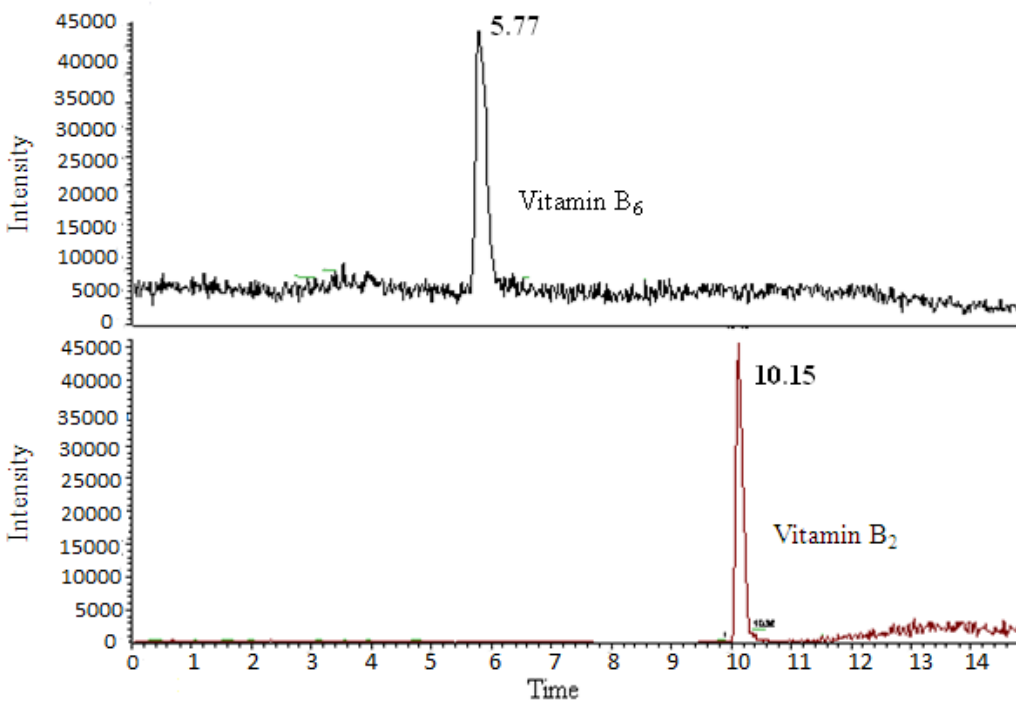


Fig. 6.3 LC-MS chromatogram; LC (1200, Agilent Tech, Japan) quaternary pump, C_{18} Ultrasphere Behcam (4.6 mm x 25 cm, 5 µm particle size) column; flow rate = 1 mL/min, A is water:acetic acid (99:1; v/v); B is acetonitrile:acetic acid (99:1; v/v). Gradient A:B = 90:10 (v/v) to A:B = 10:90 (v/v) over 9 min. B_6 at λ = 273 nm, (t_R = 5.77 min), B_2 at λ = 280 nm (t_R = 10.13 min).

The obtained ESI-MS spectra showed the protonated molecular ions at m/z values of 377 and 168 representing vitamin B_2 and B_6, respectively (Fig. 6.4 (A) and (B)).

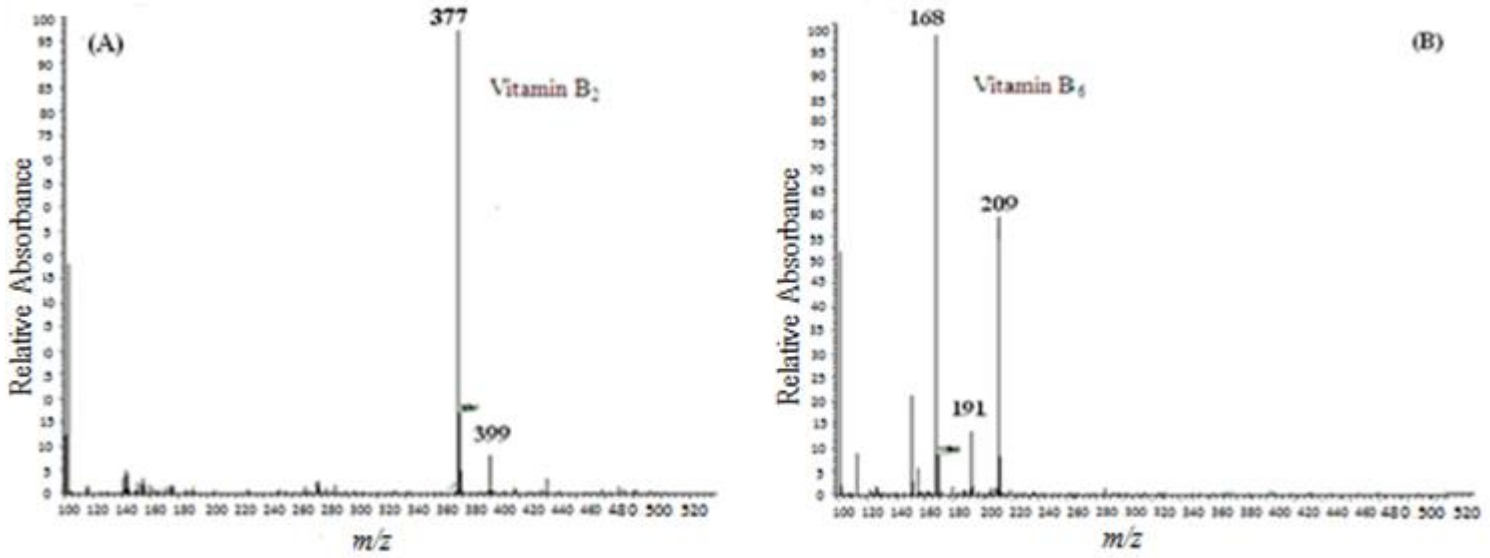


Fig. 6.4 ESI-MS chromatogram (ThermoQuest Finingan AQA, UK) operated in positive ion electro-spray mode, cone voltage 60 (V), desolvation gas flow rate 50 L/h and 350^{o}C desolvations temperature. (A) B_2 m/z 377, ESI-MS spectrum shows the protonated molecular ion at m/z 377 with the adducted ion MNa^+ at m/z 399. (B) B_6 m/z 168, ESI-MS spectrum shows the protonated molecular ion at m/z 168. The adducted ions MNa^+ at m/z 191 and (M^+CH3CN) H^+ at m/z 209 are also observed.

The ESI-MS spectra in Fig.6.4 (A) show the protonated molecular ion at an m/z of 377 with the adducted ion MNa^+ at an m/z of 399. The adducted ions MNa^+ were also observed at *m/z* 191 and $(M+CH_3CN)H^+$ at m/z 209, Fig. 6.4 (B).

6.3.4 Determination of B_2 and B_6 in infant food products

Quantification of the vitamins in the current study was finally conducted using UHPLC for which the chromatographic conditions were optimized for peak shapes, column efficiency, chromatographic analysis time, selectivity and resolution. The results obtained are presented as per 100 g of the food samples (Fig.6.5), which shows considerable differences between products with respect to vitamin B_6 content.

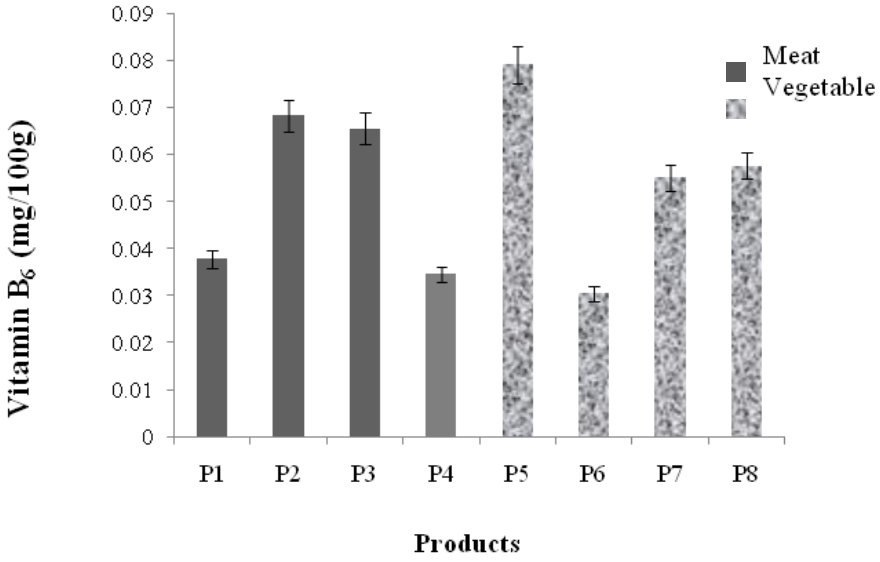


Fig. 6.5 Comparison of vitamin B_6 content of meat and vegetable-based products.

The results of the vitamin B_6 content in the selected infant food products (mg/100 g) were further subjected to both single factor with replication and two factor ANOVA without replication analyses. The calculated F value after ANOVA between products showed a significant difference between the products. The calculated *p*-value (p = 6.5e-12) was significantly lower than the critical *p*-value at 0.05, as shown in Table 6.3.

Table 6.3 Single factor ANOVA with replication for B6 content (mg/100 g) of meat and vegetable based products.

Source of Variation	*df*	*F*	*p-value*	*F crit*
Between products	7	97.79	6.25E-12	2.65
Within groups	2	0.018	0.981	3.46

The calculated F-value for the ANOVA within groups (between the replicates), showed no significant difference with the calculated *p*-value (0.98), as also illustrated in the data in Table 6.3, which indicates the consistency of the measurements.

Despite wide individual variations between each brand, no significant differences (Table 6.4) were observed in the vitamin B_2 and B_6 content between the meat and vegetable-based varieties ($p = 0.7$).

Table 6.4 Comparison between vitamin B_6 content (mg/100 g) of meat and vegetable based varieties of four poplar brands in the UK.

	Meat-based				**Vegetable-based**				
Vitamin (mg/100g)	**Mean**	**SD**	**% RNI**	**INQ**	**Mean**	**SD**	**% RNI**	**INQ**	***p*-value**
B_2*	ND				ND				
B_6	0.0515	0.0179	12.8796	1.5999	0.0556	0.0199	13.8886	1.7252	0.7730
95% CI	0.03-0.06				0.03-0.07				

* <LOQ (0.06µg/mL), ND = not detected

With reference to RNI values for 6-9 month olds, all samples provided less than 20 % of RNI values with mean (SD) values of 12.87 (±4.46) % and 13.88 (4.97) % for meat- and vegetable-based recipes, respectively. Vitamin B_2 was not detected in any of the samples where the detection limit for vitamin B_2 was 0.07µg/mL. In addition to leaching and degradation via the heating process, the lack of this particular nutrient at detectable levels could be explained by the sensitivity of vitamin B_2 to light exposure during storage of the product in glass jars.

Since the consumption of nutrient dense food (those foods that provide substantial amounts of vitamins and minerals with relatively few calories) as opposed to energy-dense food (also called "empty calorie" food) is critical during infancy, the Index of Nutritional Quality (INQ) for both meat and vegetable based food samples in relation to their vitamin content was also calculated (Table 6.4). Evaluation of INQ in food is a good indicator of the nutrient content of food and relates to the amount of a nutrient per 1000 kcal of food in comparison to the recommended intake of that particular nutrient (Lee & Nieman, 2003). A food with an INQ substantially greater than '1' is generally considered to be a good source for that specific nutrient. From the data, it is apparent (as expected) that vegetable based food products are a good general source of vitamin B_6.

While these results are insightful, it is important to examine the entire nutrient daily intake when studying the nutrient quality of complementary food. In order to estimate the total daily dietary intake of an infant from the consumption of manufactured infant foods and formulas, an example menu based on manufacturer's feeding recommendations was composed (Table 6.5).

Table 6.5 Total daily intake of vitamin B_2 and B_6 by an infant age 6-9 months old [a], based on gastric capacity of an eight months old infant and the standard feeding regime composed of commercially prepared infant food products.

Meals	Infant formula		Lunch (meat-based)			Dinner (vegetable-based)			Total Daily Intake [e (b+ c+ d)]	RNI*
Amount of vitamin	100 mL	600 mL[b]	100 g		124.5 g[c]	100 g		124.5 g [d]		
			Mean	SD		Mean	SD			
B_2 mg	0.15	0.9	ND*			ND			0.9	0.4
B_6 mg	0.04	0.24	0.0515	0.0179	0.0641	0.0556	0.0199	0.0692	0.3733	0.4

[a] Average weight about 8.3 kg.
[b] Recommended volume of milk intake for a 6-9 months old infant.
[c&d] The portion size is calculated based on the gastric capacity of an infant aged 6-9 months old (30 g/kg of body weight) divided by 2, to make up for lunch and dinner (30 x8.3/2 ≡ 249 g/2).
[e] Daily intake is calculated by the sum of milk (b) and none milk intake (c +d) to compare with the RNI*
*ND= not detected, below the limit of quantification.

This included the ready to eat type foods, such as jars of semi-solid meals and readymade formulas. The volume of ingested meal based on the gastric capacity of an infant (30 g/kg body weight/day) was estimated in accordance with the Committee on Medical Aspects of Food and Nutrition Policy (COMA) Weaning and the Weaning Diet Report (1994). The aforementioned methods have been used by the Scientific Committee on Food (SCF) for assessing the maximum level of residue of pesticides in food. The estimated amount for milk consumption however, was set at 600 mL, as advised by COMA, for infants up to 12 months. It is important to note that one of the main limitations of such an approach is that it is unlikely to represent the actual amount of consumption that is ingested and retained by the infant; neither does it take into account any wastage. The other problem with this approach is that it fails to take into account of any contribution from breast milk, snacks or homemade food.

From the data in Table 6.5, it can be concluded that the total daily intake of vitamin B_6 from the consumption of commercially complementary food is satisfactory and is in accordance with the DRVs value. The intake of vitamin B_6 is, however, mainly attributable to the consumption of formula milk which could be a cause of concern if an infant's diet is based on un-supplemented milk (Bessey *et al.*, 1957; Heiskanen *et al*., 1996). On the other hand, if the RNI for a 6 months old infant is recommended to be 8 µg /g protein of the food (DoH, 1991), then the amount of vitamin B_6 in complementary food with an average protein content of 3 g/100 g as presented in the labels of the products in Table 1 should be 24µg /100 g of the products. From the results of this study, the mean B_6 content was estimated to be 50 µg /100 g of the product. This amount is in excess of the recommendations, especially as the intake from snacks and breast milk is not included and as already mentioned is a limitation associated with such an approach.

The intake of vitamin B_2 (mg/day) only from consumption of formula milk however, already seems to be in excess of the RNI at 0.4 mg/day. The absorption of B_2 is believed to be saturated at levels above 25 mg and the excess amount is excreted primarily in the urine. In terms of vitamin deficiencies however, the same concern exists if the intake of formula or other food supplements is compromised (Heiskanen *et al*., 1996).

6.4 Conclusions

A fast and robust UHPLC method for simultaneous extraction and quantification of vitamin B_2 and B_6 in processed infant food has been developed. The separation achieved is excellent and rapid (within one minute) and the resultant sample is also LC-MS compatible, which enables further validation of the chromatographic data in addition to the identification of other water soluble organic species. The estimated total daily intake of vitamins B_2 and B_6 from the consumption of commercial complementary food was found to be satisfactory and in accordance with DRVs values. The intake of both vitamin B_2 and B_6, however, was revealed to be mainly attributed to the consumption of infant formula milk. This could be a cause of concern if the quality of an infant's milk diet is compromised by inadequate amounts or lack of supplemented milk intake. These results suggest that commercial complementary infant foods on the UK market may not contain minimum levels of vitamins required for the labeling declaration of micronutrient content (*Commission Directive 2006/125/EC*). This provides opportunities and scope for product optimization to improve their nutritive value.

6.5 References

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Chapter 7

Product Optimisation

7.1 Introduction

The work reported in this chapter is concerned with the impact of processing on the nutritional quality of newly formulated infant food products. The processing is considered in relation to the various heat treatments applied to the products. The microbiological safety of the new products has also been examined and the inter-relationship between the different heating regimes and the microbiological content is also presented. This investigation will also explore potential methods for new product development (NPD) with a specific focus on nutritional optimisation via recipe design and the application of an appropriate thermal process in order to maximise the retention of nutrients and minimise any adverse microbiological content.

In the development of infant formula milk, the formulation is usually based on human milk composition after allowing for differences in bioavailability (Dupont, 2003). In designing complementary food, however, the intake of nutrients from the consumption of milk defines the nutritional baseline and it has been previously suggested by Dewey that an age specific micronutrient content and ration size should be specified (Dewey, 2003).

The strategy proposed for the nutrient composition of a newly optimised formulated recipe (OFR) in this development therefore, is based on the methods applied in formulation of fortified complementary food by the World Health Organisation (WHO), as described in section 2.3.2.1.3 (Lutter & Dewy, 2003; WHO, 2004).

The proposed composition for the OFR considers a number of factors such as age range, daily ration size, recommended nutrient requirement, contribution of human milk to these requirements, macronutrient interactions, expected losses and product safety. It also aims to fulfil the labelling requirement (Commission Directive 2006/125/EC) for the declaration of micronutrient to be at least 15% of the Referenced Values (%RV).

7.2 Materials and methods

7.2.1 Selection of ingredients

For the purpose of optimisation, the choice of ingredients for the new OFR was deliberately restricted to those typically used e.g. lamb or beef for the development of meat-based varieties. The products currently marketed in the UK, were used to provide a baseline for the post process evaluations (PPE) of the OFR. The nutritional content of the meat-based varieties has already been analysed quantitatively and the list of ingredients is presented in Chapter 3, Table 3.1.

7.2.2 Design of the optimised formulated recpie (OFR)

A food composition table was compiled using the McCance & Widdowson's composition of foods integrated data set (FSA, 2002). The optimum ratios of the ingredients were calculated using this table to develop the OFR, and are presented in Table 7.1. The compositional calculations were based on the approach described in chapter 2 section 2.4.2.1.3 (Table 2.5) and were designed to provide an average energy of 0.6 kcal per gram of the OFR. The protein (3.6 g/100g) and fat (2.3 g /100g) content were calculated to provide 12 and 31% of the energy content of the OFR, respectively (DoH, 1991; WHO, 2004). The remaining 57% of the energy is expected to be contributed by the carbohydrate content (10.5 g/100g).

Table 7.1 Estimation of the optimum ratios of the ingredients in development of the OFR.

Food	Weight (g)	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Ca (mg)	Cu (mg)	Fe (mg)	Mg (mg)	K (mg)	Na (mg)	*Zn* (mg)	Se (µg)	B2 (mg)	Vitamin B6 (mg)
%RV		0.6 kcal / g	>3	<4	ND	26.89	17.4	70.6	54.7	50.0	8.3	18.2	13.0	16.6	35.2
Food codes/ RV and Recommendations		49.7-124.5	1.5-4	1.6-4.2	7.8-18.8	400	0.4	6	40	700	320	4	10	0.8	0.7
13-009	22.000	16.434	0.462	0.044	3.784	1.100	0.018	0.088	3.740	79.200	1.540	0.066	0.220	0.004	0.097
13-446	20.000	6.945	0.120	0.060	1.580	5.000	0.004	0.060	0.600	34.000	5.000	0.020	0.200	0.002	0.028
13-438	5.000	4.174	0.345	0.075	0.565	1.050	0.003	0.140	1.700	16.500	0.050	0.055	0.000	0.001	0.006
13-460	12.000	2.055	0.084	0.036	0.372	0.840	0.001	0.060	0.840	30.000	1.080	0.012	0.000	0.001	0.017
13-304	6.000	2.174	0.072	0.012	0.474	1.500	0.003	0.018	0.240	9.600	0.180	0.012	0.000	0.080	0.064
water	20.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11-435	1.000	3.513	0.000	0.007	0.920	0.150	0.001	1.400	0.070	0.610	0.520	0.003	0.000	0.000	0.000
18-040	6.000	10.440	1.314	0.576	0.000	0.600	0.004	0.090	1.140	17.400	5.400	0.264	0.420	0.009	0.025
17-045	0.500	4.496	0.000	0.500	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13-845	*2.000*	3.612	0.316	0.140	0.290	21.600	0.003	0.830	2.400	81.800	3.600	0.076	0.100	0.006	0.000
13-842	*3.500*	11.281	0.385	0.361	1.733	55.300	0.033	1.540	9.450	58.450	0.525	0.154	0.000	0.000	0.000
12-032	2.000	9.793	0.526	0.526	0.788	20.400	0.000	0.008	1.680	25.400	8.800	0.064	0.360	0.028	0.010
Totals	100.000	74.916	3.624	2.336	10.506	107.540	0.070	4.235	21.860	`352.96	26.695	0.726	1.300	0.132	0.247

7.2.3 Thermal Processing

The different methods of thermal processing and their impact on the retention of the nutritional quality of processed foods are discussed in chapter 2, section 2.6.1.2. The thermal methods employed in the process design adopted in this investigation include both direct (conduction) and in-direct (conventional) methods of heating (Fellow, 2009) and are presented in Figure 7.1. Maintaining a processing temperature at a constant level (<100 ^{o}C) has been reported to be an effective way of minimising nutrient loss as a result of heating (Morris *et al.*, 2004; Sugarman, 2004).

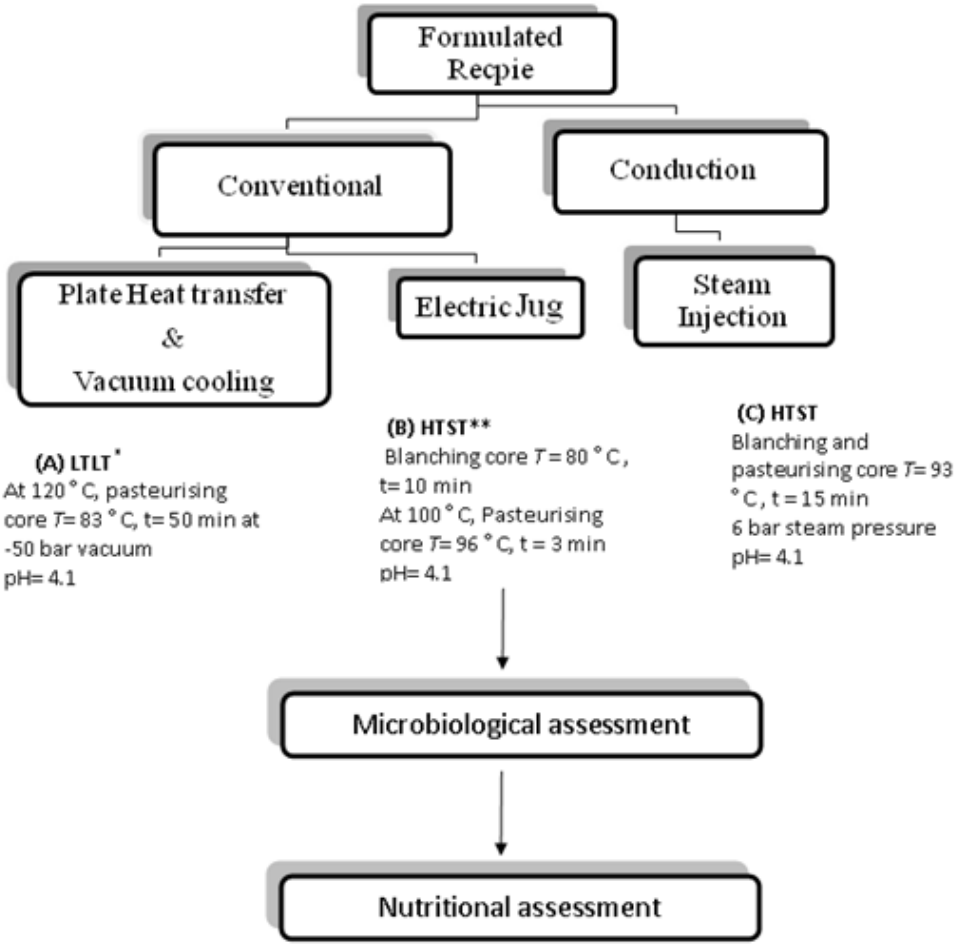

Fig. 7.1 Process design and the related parameters, (A) *LTLT-low temperature/long time, (B) & (C) **HTST-high temperature/short time (Fellow, 2009).

7.2.4 Post process analysis of the optimised recipe

7.2.4.1 *Primary shelf-life assessment*

Ensuring food safety is a high priority in any type of new product development (DoH, 2003). The heat treatments employed in the above processing steps (typically below 100^{o}C), require temperature controlled storage (< 8^{o}C) of all of the OFRs. A primary shelf life assessment of the OFRs was carried out on both pre and post-process formulas on the day of processing (day 0) and 72 hours later (day 3). A post-process shelf life assessment of all OFRs following different processing conditions, were also carried out for the duration of a week under refrigeration (< 8^{o}C). Further isolation and identification of the different bacteria

present in the OFRs was undertaken by the Gram Staining method. This work was carried out at a third party laboratory (NRI) by the author of the thesis for validation purposes.

7.2.4.1.1 *Microbiological analysis*

Standard plate count (reference) was the method of choice for an evaluation of the impact of the three processing methods in order, primarily, to assess the effectiveness of the heating process on the microbiological safety of the new formulas. 30g of the food samples were diluted in 270 mL of a maximum recovery diluent solution (MRD CM 0733, OXOID, UK) and were digested for 1 min using a Stomacher (Seward 400 Stomacher, Lab Blender Medical UAC, UK). A series of serial dilutions 10^2, 10^3 –> 10^6 were prepared by transferring 1 mL of the previous dilution to 9 mL of MRD and then mixing the resultant media for few seconds using a Rota mixer (Vortex, UK). Labelled sterile Petri dishes were inoculated by 1mL of a representative aliquot from each media in duplicate for each of the processing methods. Plates were then covered with 15 mL of the 30^{o}C plate count agar (Nutrient Agar, OXOID CM0003, UK) following the pour plate procedures and left for 10 min for the gel to set. Finally, the inverted plates were incubated (LMS Cooled Incubator, UK) for 48 hours at 37^{o}C and were examined for evidence of growth. The colonies were counted using a magnifying colony counter (Gallenkamb, UK) and the colony forming units per gram (CFU/g) were calculated using the following formula:

$$\text{CFU/ g} = \text{CFU/plate} \times \text{dilution factor} \times 1/\text{aliquot}$$

7.2.4.2 *Post-processing nutrtional assessment*

7.2.4.2.1 *Macronutrient analysis*

The post process concentration of the macronutrient content of the OFR were quantitatively determined by applying the same methods of analysis employed for the analysis of macronutrient content of selected infant foods in the market in Chapter 3, section 3.2.

7.2.4.2.2 *Micronutrient analysis*

The micronutrient content including essential elements and vitamins were also analysed by the same methods described in section 4.2 and 6.2 of Chapter 4 and 6, respectively.

7.3 Results and discussion

7.3.1 Shelf-life assessment

The results of the primary shelf-life assessment of both pre and post-processed OFRs on the day of processing (day 0) and 72 hours later (day 3) are presented in Fig 7.2.

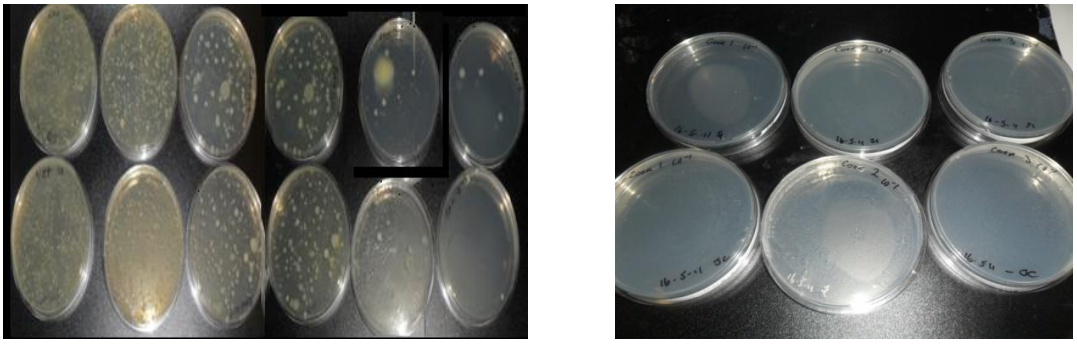

Fig 7.2 (I) Pre-process (10^6 fold dilution) . (II) Post-process (10 fold dilution).

The colony forming units of the bacterial organisms per gram (CFU/g) of the formula for different processing conditions (A, B and C) are presented in Table 7.2.

The complete inhibition of micro-organism activity on day 0 of the sampling shows that all the different processing parameters (A, B and C) and the prerequisite hygiene protocols had been effective.

Table 7.2 Standard plate count (CFU/g) of the pre- and post-process OFRs.

Day/process	**Pre-Process** †	**(A)***	**(B)***	**(C)***
Day 0 (CFU)‡	87000	0	0	0
Day 3 (CFU)	-	20	15	15

† Pre-processed OFR.
*Different processing condition.
‡ Colony forming units.

The results of a further bacterial assessment carried out at the third party laboratory (NRI) for validation purposes, are presented in Table 7.3.

Table 7.3 Microbiological assessment of the refrigerated processed formulas stored for one week.

Sample	Test	Result	Units *
(A)	Escherichia coli	<10	CFU/g
	Salmonella sp.	Not Detected	/25 g
	Listeria sp.	Not Detected	/25g
(B)	Escherichia coli	<10	CFU/g
	Salmonella sp.	Not Detected	/25 g
	Listeria sp.	Not Detected	/25g
(C)	Escherichia coli	<10	CFU/g
	Salmonella sp.	Not Detected	/25 g
	Listeria sp.	Not Detected	/25g

*Colony forming units per gram (CFU/g)

According to the guidelines for the microbiological quality of various ready to eat foods (Public Health Laboratories, 2000) presented in Table 2.6 Chapter 2, section 2.7.1, the effectiveness of the thermal processes employed in this work are shown to be satisfactory as *E. c*oli is less than < 20 cfu/g and both *Salmonella* and *Listeria* were not detected in any of the OFRs.

7.3.2 Comparison between estimated optimised values (EOV) of nutrients in the OFR with the nutrient content of the meat based varieties of commercial infant food analysed in this study.

The EOV for the macro- and micro-nutrient content of the OFR are presented in Tables 7.4 and 7.5, respectively.

Table 7.4 EOVs of the macronutrients content of the OFR in relation to the recommended and legislative requirements.

Nutrient (100g)		EOV	Market [b]		Recommendation *	Legislative Requirement**
			Mean (SD)	95% CI		
Energy	**kcal**	74.9	61.5 ±4.9	56.7-6.4	**49.7-124.5**	**100 kcal**
Protein	**g**	3.6	3.2 ±0.4	2.8-3.6	1.5-4	>3
Fat	**g**	2.3	2.1 ±0.2	1.9-2.3	1.6-4.2	<4
CHD	**g**	10.5	7.4 ±1.1	6.4-8.4	7.8-18.8	nd

[a] Estimated optimised values, pre-process values of macronutrients content of the OFR.
[b] Selected range of commercial infant foods analysed in this study.
*Recommended value for macronutrient content of complementary food (WHO, 2003).
** (Commission Directive 2006/125/EC).

It is evident that the macronutrient content of the OFR for protein, fat and carbohydrate are all within the recommended range (WHO, 2003) and legislative requirements (Commission Directive 2006/125/EC). With regards to the current infant ready meals market, it is apparent that the macronutrient content of the OFR compared to the range of complementary foods currently on the market (95% CI), is at the higher range of the spectrum. The high EOVs were inevitable due to the challenge associated with fulfilling labelling requirements (Commission Directive 2006/125/EC) for a declaration of the micronutrient content to be at least 15% of the referenced values. The EOVs are, however, still within the legislative requirements but it should be noted that this does result in a high total daily intake of fat as previously discussed in Chapter 3, section 3.6.7. It is also important to note that these values are the pre-process concentrations of macronutrient and some losses are expected due to processing (Morris *et al*., 2004; Fellow, 2009).With regards to micronutrients, the concentration of the selected trace elements and vitamins were intended to achieve 15% of the RVs. The EOVs of the micronutrient content of the OFR and the %RV required for labelling declaration of micronutrients met by EOVs are presented in Table 7.5.

Table 7.5 EOVs of the micronutrients content of the OFR and the %RV met by the EOVs as required for labelling.

Nutrient (100g)		EOV [a]	Market [b]		RNI[†]	RV[‡]	%RV[¶]
			Mean (±SD)	% RNI			
Ca	**(mg)**	107.5	17.4 (± 9.4)	3	525	400	27
Cu	**(mg)**	0.1	0.05 (±0.02)	18	0.3	0.4	17
Fe	**(mg)**	4.2	0.8 (±0.4)	10	7.8	6	70
Mg	**(mg)**	21.9	12 (±2.7)	16	75	40	55
K	**(mg)**	353.0	140 (±34)	20	700	700	50
Na	**(mg)**	26.7	48.3 (±21.3)	15	320	320	8
Zn	**(mg)**	0.7	0.54 (±0.14)	11	5	4	18
Se	**(µg)**	1.3	ND*	-	10	10	13
Vitamin B_2	**(mg)**	0.1	ND	-	0.4	0.8	17
Vitamin B_6	**(mg)**	0.2	0.05 (±0.01)	13	0.4	0.7	35

[a] EOVs, pre-process values of macronutrients content of the OFR.[b] The micronutrient content of selected range of ready to feed commercial complementary foods in the UK analysed in this study.
[†] Recommended Nutrient Intake (DoH, 1991).
[‡] Reference values for micronutrient labelling of commercial infant foods in the UK (Commission Directive 2006/125/EC).
[¶] %RV met by the EOV of the micronutrients in the OFR.
*Not detected.

The data in Table 7.5 shows that the concentration of the selected micronutrients in the OFR were designed to meet 15% of the RV required for labelling purposes (Commission Directive 2006/125/EC) except in the inappropriate cases such as sodium.

A comparison between the market and the EOV, discussed above, is shown in Figure 7.3.

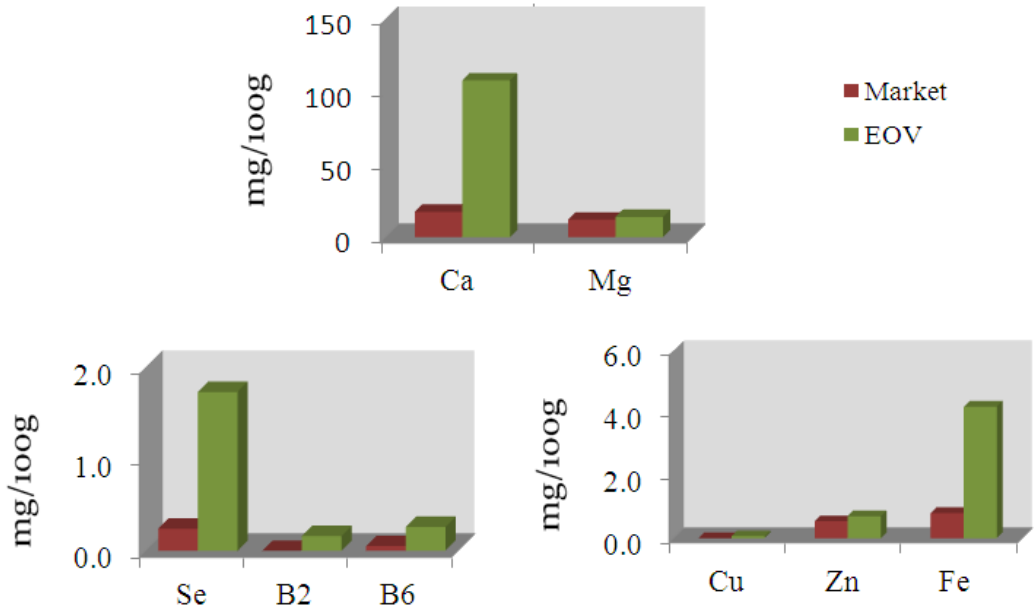

Fig. 7.3 Comparison between the EOVs of the selected nutrients in the OFR and the nutrient content of the average complementary food in the market

One of the major drawbacks of this approach however, is the reliance on nutritional data bases as they are not always up to date and they do not take any account of the season or the cultivar differences (Greenfield & Southgate, 2003). This problem is not so difficult to address in a large scale production situation as a supplier quality assurance system ensures a consistency in the supply chain.

7.3.3 Post-process evaluation of nutrient retention in the OFR *vs* EOV

As is shown in Table 7.6, the PPVs of macronutrients in the OFR under all three processing conditions (A, B and C) are below the EOV, apart from the PPV of the carbohydrate content determined under process condition (A).

The PPVs of fat in particular is of great interest when comparing with the 95% CI of the current market products. They were found to be contributing to a lower TDI of fat based on the proposed menu which is contrary to the marketed products presented in the same table. The levels of PPVs for all macronutrients are also within the legislative requirements (Commission Directive 2006/125/EC).

With regards to the selection of the least damaging heat processing conditions, maximum nutrient retention was found under condition (A) when compared to that obtained under conditions (B) and (C) which may be due to a concentrating effect of the vacuum and a reduction in water activity (a_w). Vacuum conditions are also known to reduce oxidation and as a result, fat-soluble vitamins, flavours and aromas are expected to be better preserved under condition (A) (Burger & Walters, 1973; Fellow, 2009). This processing condition also helps with respect to the integrity of the ingredients and a reduced loss of weight (Shills, 2006). Reduced a_w, under vacuum conditions (A), also increases the effectiveness of the process in eliminating the microbial activity (Holdsworth, 2004).

Table 7.6 Post-process evaluation of macronutrient content of the OFR *vs* EOVs

Nutrient (100g)		EOV [a]	Post Process Values [b] (Mean ±SD)			Recommendation*	Legislative requirement**	Market Mean (SD)
			A	B	C			
Energy	**kcal**	74.9	73	52	66	**49.7-124.5**	**100 kcal**	**56.7-6.4**
Protein	**g**	3.6	2.8	2.1	2.6	1.5-4	>3	2.8-3.6
Fat	**g**	2.3	1.9	1.5	1.8	1.6-4.2	<4	1.9-2.3
CHD	**g**	10.5	11.3	7.5	9.8	7.8-18.8	nd	6.4-8.47
Moisture	**g**		83.4	88.3	85.1	-	-	

[a] Estimated OVs, pre-process values of macronutrients content of the OFR.
[b] Post-process values of macronutrient content of the OFR.
*Recommended value for macronutrient content of complementary food (WHO, 2003).

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The influence of the concentrating effect of vacuum processing is also evident in the PPVs of the micronutrients, in particular with respect to the essential and trace elements, as demonstrated in Table 7.6. It is however important to note that macronutrients and vitamins are more likely to be affected by processing than minerals (Morris *et al*., 2004). In general maintaining the process temperature at a constant point (<100^{o}C) allows a longer time of processing without any significant detrimental effects on the nutrient content.

Table 7.6 PPVs of the micronutrient content of the OFR and the %RV met for labelling purposes.

Nutrient (mg/100g)	EOV [a]	Post Process [b]			P value	% RV*
		A	B	C		
Ca	107.5	232.0	161.7	193.6	4.24E-11	40
Cu	0.1	0.09	0.05	0.05	0.1	12.5
Fe	4.2	4.4	3.4	2.8	0.08	56
Mg	21.9	19.36	14.09	15.63	3.65E-08	35
K	353.0	137.05	85.34	102.3	1.17E-06	12
Na	26.7	11.78	5.02	9.01	1.77E-07	1.5
Zn	0.7	0.92	0.65	0.60	3.41-05	16.25
Vitamin B_2	0.1	0.05	0.02	0.02	0.2	2.5
Vitamin B_6	0.2	0.08	0.64	0.05	1.19E-07	91

[a] EOVs, pre-process values of micronutrients content of the OFR.
[b] PPVs of micronutrient content of the OFR processed under different conditions.
* % RV met by the PPVs of the micronutrient content of the OFR.

Vitamin B_2 is sensitive to light and is more heat-labile at alkaline conditions (Fellow, 2009; Shills *et al*., 2006). The pH of the food composition and the potential leaching of nutrients during the processing have a higher impact on the retention of vitamin B_2 than does the temperature-time combinations of the processing treatment itself. The outcome of the statistical comparisons of nutrient composition of the three processing conditions confirms the above, where there is no significant difference (p = 0.2) found between the retention of vitamin B_2 under the three different processing temperature-time combinations. Vitamin B_6 is also heat stable at acidic conditions and therefore is most preserved under the HTST condition (B) which has the shortest time of processing (t =13 min) and also has a reduced level of leaching.

Although conditions (B) and (C) benefit from a relatively short processing time (t), the steam injection utilised in system C was expected to have the least heat loss as a result of the rapidity of the introduction of the steam (Fellow, 2009). The diluting effect of the condensing vapour, resulting from the introduction of steam, however, has resulted in the nutritional content of the final composition to be lower following this processing condition (C) than that typically obtained from processing conditions (A) and (B). This is an important point in terms of satisfying the legislative requirements in relation to labelling. The consistency of the composition of the OFR after processing under condition (B) was found to have a better consistency as well as being a more cost-effective processing option. With regards to the % RV, PPVs in condition (B) were therefore chosen to evaluate the retention of nutrients in relation to micronutrient declaration, presented in Table 7.7

Table 7.7 Comparison of the pre and post-process retention of micronutrient content of the OFR under condition (B) in relation to %RV.

Nutrient (mg/100g)	EOV [a]	%RV* (estimated)	PPVs [b]	%RV** (post-process)	RV
Ca	107.5	27	161.7	40	400
Cu	0.1	17	0.05	12.5	0.4
Fe	4.2	70	3.4	56	6
Mg	21.9	55	14.09	35	40
K	353.0	50	85.34	12	700
Na	26.7	8	5.02	1.5	320
Zn	0.7	18	0.65	16.25	4
Vitamin B2	0.1	17	0.64	2.5	0.8
Vitamin B6	0.2	35	0.7	91	0.7

[a] EOVs, pre-process values of micronutrients content of the OFR.
[b] PPVs of micronutrient content of the OFR
[*]% RV required for labelling of the micronutrient met by the EOV in OFR.
**%RV required for labelling met by the PPVs of the micronutrient in OFR.

The PPVs for calcium, iron, magnesium, zinc and vitamin B_6 prove satisfactory in relation to the % RV as they are all in excess of the required 15%. The high post process value of calcium could be due to the use of tap water in the recipe and should be investigated further.

Although, the retention of copper and vitamin B_2 is lower compared to the estimated %RV, the PPVs of all the selected nutrients in the optimised version (OFR) has been greatly improved

compared to the selected range of commercial infant food analysed in this study. A comparison between the level of selected nutrients in the commercial meat-based infant food with the pre- and post- processed values of the optimised version (OFR) are presented in Figure 7.4.

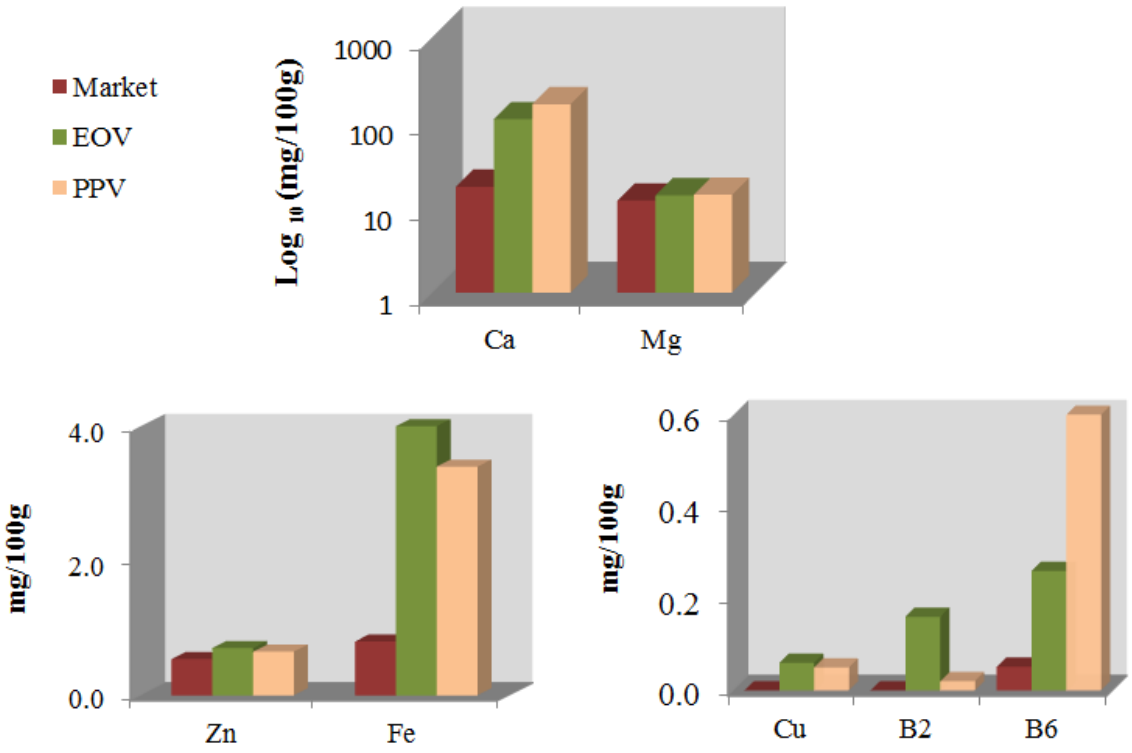

Fig.7.4 Comparison between the levels of selected nutrients in the commercial meat-based infant food, EOVs and PPVs in the optimised OFR.

7.4 Conclusions

From the above investigation, it is evident that the formulation of the recipes in the context of new product development is an important stage and the selection of high quality ingredients and the ratios in which they are used have a direct effect on the nutrient content of the final composition.

The post process evaluation of the formulated recipes was also as necessary in order to assess adverse effects of processing on the nutritional quality of the final product.

The results of the above evaluation indicate that a carefully controlled temperature–time combination, pH, pressure and overall conditions of processing e.g. controlled leaching are very important in reducing heat loss and in improving the nutritional quality of the food product. The above results warrant further investigation of the development of a range of new formulas under different processing parameters with respect to the optimal retention of the nutrients.

7.5 References

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Chapter 8: General discussion

The work reported in this thesis is concerned with the nutritional quality of commercially prepared infant ready meals available for purchase in the UK. In general there is a paucity of data with respect to the nutritional quality of "ready to feed" complementary foods marketed in the UK for infants aged between 6-12 months. This situation seems to be further hindered by the current legislation (Commission Directive 2006/125/EC) which is generally not robust enough considering the nutritional requirements of its intended target group. Currently, the European commission has proposed a revised legislation (Directive 2009/39/EC) on foodstuffs intended for "**Par**ticular **Nut**ritional use (PARNUTS)", with the aim of removing the ambiguity around variable food labelling and to also reduce the overall legislative burden. It does not, however introduce any compositional rules for existing PARNUTS foods, Thus far this new legislation is yet to be approved and it will only apply 2 years after its initial introduction (DoH, 2011).

This current study has focused on the apparent lack of attention to the nutritional quality of 'ready to feed' complementary foods marketed for infants and is the first primary quantitative experimental evaluation of the macro- and micronutrient content of *ready to feed* infant foods in the UK.

As mentioned in the literature review, the impact of early life nutrition on growth and its role in the prevention of later life chronic disease has been reported in many epidemiological and experimental studies (Chapter 1 and 2). Although the associated evidence is variable in quality, both experimental and observational studies give cause for concern about the later life consequences of compromised or excessive nutrient supply during early growth and development.

It is estimated that in the UK alone, 50 to 60% of parents choose commercially prepared baby foods for feeding their infants and young children at some stage (Food Standard Agency, 2004), which may have potential implications for the total daily intake of energy and fat in addition to an infant's development of taste acquisition.

To date, there has been no controlled study comparing the total daily dietary intake from the consumption of these types of food with reference to the RNI and therefore any attempt to ascertain the suitability of commercially prepared infant ready meals (DoH, 1991) is problematic. Although the RNI may have limitations in the context of optimum growth and development (Section 2.2.2.1), the % RNI was chosen as a reasonable indicative factor in measuring the nutritional quality of food in the context of an infant requirements for healthy growth and development.

In order to achieve the above goal, a standard feeding regime was developed and composed to calculate the total daily dietary intake of an infant who is fed on commercial infant food. The menus were based on the WHO expert Consultation on Complementary Feeding (Dewey & Brown, 2003) guidelines. The aforementioned approach has a precedence as it has been used by the SCF e.g. for assessing the maximum level of residue of pesticides in food (EC, 1998). It is important to note, however, that one of the main limitations found with this approach is that it is unlikely to represent the actual amount of consumption that is ingested and retained by the infant as a result of variable feeding regimes. The other problem with this approach is that it fails to take into account any nutritional contribution from breast milk, snacks or homemade food.

In terms of the chemical analysis of the infant ready meals, a number of techniques have been proposed in the literature for the quantitative experimental determination of different nutrients in food. Due to the enquiring nature of this investigation in terms of regulation and legislation relevant to food, it was decided to employ analytical experimental methods which were approved by the FSA and Trading Standards. All the methods outlined in Chapters 3, 4, 5 and 6 are therefore, approved and currently in use by public analysts (White & Hamptone, 2008; Kent Scientific Laboratories).

The key findings of each of the macro- and micronutrient analysis of the selected ready to feed complementary infant food products in the UK (Chapters 3, 4, 5, and 6) are highlighted and reviewed in context of the recommend nutritional requirements of an infant and are presented below.

8.1 Key declerations from the macronutrient analysis (Chapter 3)

- Experimentally determined concentrations of macronutrients (g/100 kcal) are within the regulatory requirements (Commission Directive 2006/125/EC).

The results of the analysis indicate that despite the variations observed between the declared values provided by the manufacturers on the product labels and the obtained values, the concentration of macronutrients (g/100 kcal) in complementary food were still satisfactory and within the regulatory requirements (Commission Directive 2006/125/EC). Although the quantification of different classes of lipid and carbohydrates are not currently a part of the regulatory requirements, the inclusion of such information could be helpful with respect to the consumer's right to choose a product in full knowledge of the nutritional facts. The declaration of the "group two" nutritional information is already being implemented in the 'Nutrition Fact Label' by the FDA and should be at least explored as a potential labelling requirement.

- Total daily dietary intake of fat from the consumption of commercial complementary food may be in excess of the recommended guidelines if the intake of dessert and snacks are incorporated.

The total daily intake of fat (27.0 g/day) - based on a menu composed from commercial complementary food – is suggested to exceed the DRV of fat (27.1 g/day), if the intake of snacks and desserts are incorporated. Although there are debates over the optimal amount of fat in the diet of infants and young children (Garrow *et al* 2000; Shils *et al* 2006; Ells *et al*. 2008, Siri-Tarino *et al*. 2010), the current recommendations suggest a range of 31-45 % of total energy should be derived from fat, based on a low or average amount of energy intake from milk (WHO 2004). Since the current UK recommended intake of daily milk at 600 mL/d provides an average energy of 396 kcal/d (energy density of 0.66 kcal/g), 31% of energy from fat (27.3 g fat per day) seems reasonable if it contributes to adequate intake of essential fatty acids and less saturated fat intake (< 8% EI) (DoH, 1991). The contribution from the intake of snacks and desserts can distort the results which relate to the excess daily intake of fat based on the commercial feeding menus and therefore the precise food intake would need to be taken into account when evaluating a child's total macronutrient consumption.

8.2 Highlights of the essential and trace elements analysis (Chapter 4)

- Evidence of a lack of attention to micro-nutrient interactions in food.

The bioavailability of nutrients is mostly influenced by the presence of dietary components that interfere with digestion and therefore potentially inhibit the absorption of nutrients (Section, 2.3.2.2). The result of the quantitative analysis of the elements iron, calcium and zinc suggests an excessive daily intake. Iron and zinc are known to be problematic nutrients in terms of their bioavailability. In addition it is important to note that excessive iron and zinc intake may be detrimental with respect to the absorption of other minerals, for example their counter effect on the absorption and hence bioavailability of copper (Agett *et al*., 2002; Singh *et al*., 2006). On the other hand, calcium is known as an iron inhibitor and high intake of cheese and milk powder in the diet of young children in Britain, has already been identified as one of the factors contributing to low iron bioavailability (Agett *et al*., 2002; Singh *et al*., 2006).

- Evidence of an inadequate intake of some elements from an example menu with reference to the RNI.

The entire daily intake of nutrients from the consumption of ready to feed complementary food products including the daily milk intake were compared with the RNI values in order to ascertain their nutritional adequacy in meeting the requirements of their target group. The results of the comparison suggest that the recommended daily intake were, in some cases, not met and in others were in excess compared to the RNI. These findings are supported by similar studies reported in Chapter 4, section 4.4.1.

Increasing the frequency of feeding as a solution to addressing the issue of inadequate nutrient intake has potential implications for the total energy, fat intake and taste acquisition which can impact negatively on the risk of chronic non-communicable disease that are currently causing major health concerns for society particularly in the UK e.g. obesity. It may also result in a displacement of the milk intake which is potentially alarming as complementary food must not be used as a substitute for milk according to the Codex Alimentarius Standards for canned baby food (Clark & Shrimpton 2000).

8.3 Highlights of the analysis of vitamins: B_2 and B_6 (Chapter 5)

- Development of a simultaneous extraction and quantification method for the analysis of Vitamins B_2 and B_6

Currently there is no uniform standard method for the extraction and determination of water soluble vitamins in composite food. A limited number of methods for the determination of vitamins in single food ingredients have been reported in the literature (Zefra-Gomez *et al.,* 2006). The drawbacks of the foregoing include the time required for analysis, high solvent consumption and the severity of the procedures used for sample digestion, especially when low concentrations of endogenous vitamins need to be quantified e.g. in the case of processed foods (Blake, 2007). The approach adopted to overcome these issues involved an optimization of the analytical parameters for the rapid separation of vitamins B_2 and B_6 by UHPLC. The resolution achieved by this technique is excellent and rapid - within one minute and is therefore significantly superior to the retention time currently reported in the literature for the separation of these compounds by HPLC which vary between 6 to 35 min (Blake, 2007; Nollet *et al.,* 2000). Further work has examined the LC-MS compatibility of the above approach. An assay has also been specifically developed to be LC-MS compatible which will enable further validation of the chromatographic data to be made in addition to identification of other water soluble organic species.

- Evidence of inadequate intake of vitamins B_2 and B_6 with reference to the RNI if consumption of formula milk is compromised

The estimated total daily intake of vitamins B_2 and B_6 from the consumption of commercial complementary food was found to be satisfactory and was in accordance with DRVs values. The intake of both vitamins B_2 and B_6 was however, found to be mainly attributed to the consumption of infant formula milk. This could be a cause of concern if the quality of an infant's milk diet is compromised by inadequate amounts or a lack of supplemented milk intake.

The aforementioned highlights the significance of the formulation of the complimentary food in relation to the nutritional quality of the infants' total diet. It also suggests that commercial complementary infant foods in the UK market may not contain the minimum levels of vitamins required for the labelling declaration of micronutrient content (*Commission*

Directive 2006/125/EC). As a result an attempt was made to examine the nutrient retention through a combination of recipe optimization and the judicious application of different thermal processes (Chapter 7), the key finding of which are described below.

8.4 Key message on formulation of new products

A preliminary investigation of the optimisation of potential new recipe formulas coupled with a detailed examination of the effects of various thermal processing methods on the safety and post-process integrity of the nutrients indicates that improving the nutrient retention, via a combination of recipe optimization and the application of appropriate processing conditions is not impossible and is an area ripe for further development.

The results of the above investigation indicate that optimising temperature-time combination, controlling pH, environmental pressure and the overall conditions of the processing e.g. controlling the oxidation and leaching of water soluble components are effective in reducing heat loss and improving the nutritional quality of the composition

The present findings, however, do not suggest a relationship between formulation design and nutrient bio-availability and therefore more investigation is required with regards to nutrient interaction and the design of recipes. This provides scope and opportunity for the development of a new range of infant food products with optimised nutritional values.

8.5 References

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Chapter 9

Overall Conclusions and Future work

In this study, the importance of complementary food in relation to the growth and development of an infant as well as its role in health during adulthood has been well established (Chapter 2). It has also been demonstrated that the infant feeding regimes in the UK have not been immune to the shift in the dietary patterns which are taking place as a result of rapid changes in life style. Parents are increasingly relying on commercial infant food products as a convenient alternative to homemade food. In the absence of robust regulation, a number of shortfalls in relation to the safety and quality of these products have already been highlighted in both the public media and scientific literature and these issues are now at the heart of public health concerns e.g. childhood obesity.

The current investigation was designed to identify the nutritional gaps associated with the current infant food market in the UK. The work included quantitative analysis of both the macro and micro nutrient content of a representative range of infant food products (Chapters 3-6). All the analytical techniques are those employed by third party public analysts and in the absence of standardised method; e.g. analysis of vitamin B group, new methods were developed and validated (Chapter 5). The outcome of the quantitative analysis lead to a number of gaps in the current infant food market in relation to nutritional requirements of their target groups being highlighted (Chapter 8).

The next phase of the work was an attempt to improve and address the "identified gaps" via an optimisation of the recipes and post-process evaluation of the nutritional quality of the optimised formulas which proved satisfactory. A summary of the highlights and key findings of this work is provided in Chapter 8.

Although the formulation and optimisation work proved successful, there is scope for further investigation in relation to the following areas.

9.1 Future work in relation to the current infant food market

a) Complete the assessment of the investigated foods in terms of fatty acid profiles and analysis of fat soluble vitamins as well as other B vitamins.
b) Investigate the effect of re-heating of the food products especially using the domestic microwave with respect to the nutritional quality of the food composition. This is important as domestic microwaves are commonly utilised for the heating and preparation of infant food products.
c) Nutritional assessment of the infant food products stored under different conditions during the given shelf-life by the manufacturer.

9.2 Future work in relation to new product development

a) Additional investigations are required on the association between the type of ingredients used in the new formulations and the processing parameters with respect to the optimal retention of the nutrients.
b) Further studies are required with specific focus on the bio-availability of the various key nutrients in the new food formulations.
c) In future the use of high pressure processing (UHT) could be an option for sterilisation and could provide a solution to the requirement of controlled cold storage. Such an approach might be especially helpful where export markets might include areas of limited electricity supply.
d) An investigation into the introduction of a wider range of ingredients for the development of products with specific health benefit is an emerging topic and could be integrated into new future food formulations. This work will need ethical approval for clinical trials. The employment of a wider range of varieties of ingredients will also help to overcome the limitation associated with season and cultivar.
e) Further studies on the effect of different variables on the nutritional quality e.g. types of packaging and storage conditions will need to be undertaken.
f) Further work could be undertaken to examine the issue of palatability and the organoleptic properties of infant food product.

ADDENDUM

Conferences/Seminars

Nutrition Society Summer Meeting, 4-6 July 2011, 70th Anniversary Meeting: From plough through practice to policy, Determination of mineral content of commercial infant foods in the United Kingdom (University of Reading)

Nutrition Society Summer Meeting, 28 June-1 July 2010, Nutrition and health: cell to community. Commercial infant foods in the UK: macro-nutrient content and composition (Harriot Watt University)

School of Science Seminars, 25 May 2011."A quantitative evaluation of the nutritional quality of commercial infant foods in the UK. Is there a gap in the market?" UoG, Medway Campus.

School of Science Seminars, 23 November 2009. "Studies of nutritional quality of commercial 'ready to eat' infant foods in the UK". UoG, Medway Campus.

Online Publications

Nazanin Zand*, Francis B. Zotor, Babur Chowdhry, John Tetteh, Dave Wray and Paul Amuna (2010). Determination of mineral content of commercial infant foods in the United Kingdom. *Proceedings of the Nutrition Society*, 69, E485 doi:10.1017/S0029665110003484

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Research Publications/Manuscripts

1. Nazanin Zand*, Babur Z. Chowdhry, Francis B. Zotor, David S Wray, Paul Amuna, Frank S. Pullen (2011). Essential and trace element content of commercial infant foods in the UK. *Food Chemistry*, 28, 123-128.

2. Nazanin Zand*, Babur Z. Chowdhry, Lucie V. Pollard, Frank S. Pullen, M. J. Snowden and Francis B. Zotor (2011). Commercial infant foods in the UK: macro-nutrient content and composition. *Journal of Maternal and Child Nutrition* (In Press, ID MCN-08-11-OA-0520).

3. Nazanin Zand*, Babur Z. Chowdhry, Frank S. Pullen, John Tetteh and M. J. Snowden (2011). Simultaneous extraction and quantification of vitamin B_2 and B_6 by UHPLC/LC-MS in UK commercial infant meal food products. *Food Chemistry* (Under Review: FOODCHEM-D-11-01433).

4. Nazanin Zand*, Babur Z. Chowdhry, David S Wray, Frank S. Pullen, M. J. Snowden (2011). Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK, *Food Chemistry* (Submitted).