Use of iodised salt in cheese manufacturing to improve iodine status in the UK

by

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Abstract

Iodine is an essential trace mineral. Iodine deficiency during pregnancy can lead to adverse postnatal consequences such as impaired mental development, reduced intelligence scores and impaired motor skills in the offspring of the deficient women (Khazan et al., 2013, Rayman et al, 2008). There is growing evidence in the UK of low dietary iodine intakes and potential iodine deficiency in vulnerable populations (pregnant women and women of child bearing age group) (Rayman and Bath, 2015, Vanderpump et al., 2011) and a paucity of information on the iodine content of food products. In developing countries where iodine deficiency is widespread, salt has successfully been used as a vehicle for iodine fortification, however iodised salt is not widely available in UK supermarkets and there are valid health concerns about promoting salt intake. Salt is an essential component of the cheese production process, and cheese is commonly consumed in the UK. The overall aim of the present study was to explore the use of iodised salt in cheese production to improve the iodine status in the UK. In order to accomplish this, 4 smaller interconnected aims were identified namely development of method and subsequent analysis of iodine content of cheese samples in the UK, examining these results in relation to the legislative policies regarding iodine fortification practised in the country of production of cheese, understanding about the knowledge, attitudes and practices of consumers regarding iodine nutrition and fortification through a survey and then finally manufacturing popularly consumed cheese varieties (soft cheese and cheddar – from the consumer survey) using iodised salt and comparing it with cheese made with non-fortified regular table salt in terms of iodine content and overall sensory acceptability.

A cheese mapping exercise was conducted to gain an overview of cheese varieties available across three major supermarkets in the UK and their country of origin. Selected varieties of cheese were analysed for their iodine content which was subsequently mapped against legislation policies (mandatory or voluntary) for iodine fortification in their country of origin. A questionnaire was designed to explore the knowledge attitude and practices (KAP) of general consumers about the use of iodised salt in food products. After pilot testing and minor modifications, the questionnaire was used to obtain KAP data from general consumers (n=506). To explore the use of iodised salt in cheese production, a collaboration between UCLan and Food technology centre (Anglesey) was established. Two varieties of cheese (cheddar cheese and soft cheese) with two variations (with and without iodised salt) were

developed. Sensory acceptability was assessed by both expert (at Cardiff Metropolitan University) and non-expert consumer (at UCLan) panels. The newly developed cheese samples were analysed for their iodine content to evaluate their potential as a novel vehicle of improving dietary iodine intake.

The iodine content of cheese samples from countries with mandatory legislation ranged from 0.16mg/kg for creamy Danish blue cheese (from Denmark) to 0.76mg/kg for Canadian cheddar. From countries with voluntary legislation, the range in iodine content was much broader, from 0.08 mg/kg (Swiss Le Gruyere) to 0.92 mg/kg (Farmhouse Cheddar). Analysis of milk and salt samples revealed that the iodine content of all the selected salt samples produced in the UK was negligible. Consumer KAP survey results revealed a general lack of awareness about significance of iodine for optimal health with only 11% participants aware that it is 'Extremely important' for maintaining optimal levels of iodine in the body and a high percentage of participants (81%) were not aware of any illness associated with iodine deficiency. 11% of consumers reported seeing brands of iodised salt in supermarkets, indicating low availability in the UK and most (62%) expressed a preference for non-iodised salt. The survey also revealed that 61% of respondents reported consuming cheese more than once a week and that Cheddar cheese was the most popular variety.

The iodine content of the soft and hard cheese made with iodised salt was higher (0.92 mg/kg and 0.77mg/kg respectively) than those made with regular salt (0.76mg/kg and 0.48mg/kg respectively). Sensory evaluation of this cheese by sensory experts and general consumers revealed the use of iodised salt in place of regular salt had no significant impact on the main attributes (colour, appearance, taste, texture, odour and overall quality) or specific characteristics (sweetness, bitterness, aftertaste, creamy odour, saltiness) of the cheese (Chi Square test $p>0.05$).

In conclusion, this research has revealed that on a global scale, mandatory iodine fortification policy may result in more consistency in the iodine content of cheese. In a sample of the UK population, there was a lack of awareness of the importance of iodine for human health. The research demonstrated that replacing non-iodised salt with iodised salt in the production of both soft and hard cheese increased the iodine content of the cheese by 21% and 60%

respectively and had no detectable impact on the sensory attributes, thus is potentially a useful vehicle for iodine fortification in the UK.

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Abbreviations

AFSSA – Agence Française de Sécurité Sanitaire des Aliments (French food safety authority)

APC – Aerobic Plate Count

BCB – British Cheese Board

BDA – British Dietetics Association

CRM – Certified reference material

DASH – Dietary Approaches to Stop Hypertension

E – Sensory experts

EFSA – European Food Safety Authority

FCN - Federal Commission for Nutrition

FSA – Food Safety Standards

FTC – Food Technology Centre

HPA – Health Protection Agency

ICCIDD – International Council for Control of Iodine Deficiency Disorders

ICP-MS – Inductively coupled plasma mass spectrometry

ICP-OES – Inductively coupled plasma optical emission spectrometry

IDD – Iodine deficiency disorders

IGN – Iodine Global Network

IIH – Iodine induced hyperthyroidism

MRC – Medical Research Council

NE – Non-expert consumers

NHS – National Health Service

PTM – Proficiency test material

RNI - Recommended Nutrient Intake

SACN – Scientific Advisory Committee on Nutrition

TA – Titrable Acidity

TVC – Total Viable Count

UIC - Urinary iodine concentration

UNICEF - The United Nations International Children's Emergency Fund

USI – Universal Salt Iodisation

WHO – World Health Organisation

Chapter 1 : Introduction

1.1 Significance of Iodine for human health

Iodine is one of the essential minerals required for normal health and wellbeing. Iodine is predominantly required for synthesis of thyroid hormones (throxine –T_4 and triiodothyronine –T_3) which are critical for growth and organ development during gestation especially for optimal brain function. The foetal thyroid does not develop until 13-15 weeks of gestation and so the required neurological development of the growing foetus is dependent entirely on the maternal iodine levels. Thus, iodine deficiency during pregnancy can have adverse postnatal consequences such as impaired mental development, reduced intelligence scores and impaired motor skills in the offspring of the deficient women (Khazan et al., 2013, Rayman et al, 2008, Haddow et al., 1999).

1.2 Physiological roles of iodine in the body

Iodine, through its role in production of thyroid hormones, plays an essential role in human metabolism. Thyroid hormones (T_3 and T_4), iodinated forms of tyrosine, are key regulators of cellular oxidation and through their binding to nuclear receptors on DNA can have significant impact on various metabolic activities including calorigenesis, thermoregulation and protein synthesis. Moreover, these hormones promote nitrogen retention, glycogenolysis, intestinal absorption of glucose and galactose, as well as lipolysis, and the uptake of glucose by adipocytes (Kapil, 2007). Literature suggests that a reserve of about 60 µg of iodine within the thyroid gland is required to meet the physiological demand for thyroid hormones. If dietary intake of iodine is insufficient to meet the physiological demand, production of these key hormones is compromised, thereby adversely affecting functioning of vital organs including the kidney, liver, brain and heart.

Iodine is an essential micronutrient, and the daily reference intakes have been clearly identified as seen in table 1.1

Table 1. 1 Daily reference intake for different life stages

Life stage	Iodine required per day (mcg)
Adults	150
Pregnant women	200 (British Dietetic Association (BDA), 2016, proposed by The European Food Safety Authority) 250 (Bouga et al., 2018, WHO, 2007)
Breastfeeding women	200 (BDA, 2016)
Lactating women	250 (International Council for Control of Iodine Deficiency Disorders (ICCIDD), 2007)
Children < 2 years	90

Source: British Dietetic Association, (2016), ICCIDD, (2007)

1.2.1 Iodine requirements during pregnancy

Optimal iodine status is required during the entire course of pregnancy. The iodine requirements during pregnancy are increased to 250 µg/day compared with 150 µg/day for non-pregnant women (Scientific Advisory Committee on Nutrition (SACN), 2014., WHO, 2007).

During pregnancy, additional iodine is required to facilitate 50% increase in thyroid hormone production required to meet the demands of the mother as well as the growing foetus. In addition, there is an increase in renal excretion of iodine and so additional dietary iodine is required to compensate for this increased urinary loss. Finally, the foetal thyroid function begins only around mid-gestation (13-15 weeks) and so until this time the foetus is entirely dependent on maternal thyroid hormone obtained from placental transfer (SACN, 2014., Bath and Rayman, 2013, Zimmermann, 2009, Delange, 2007). Explaining the scientific mechanisms that lead to increase in iodine requirements, recently, the SACN (2014) highlighted the alternations in the thyroid function and thereby changes encountered in the iodine metabolism in the body during pregnancy. According to this, it has been postulated that in early gestation there is increase in maternal thyroid hormone production along with increase in glomerular filtration rate leading to decrease in circulating plasma iodine concentration. This iodine deficient state further could lead to renal retention of iodine to cope with the increased requirements. Further, the fall in plasma iodine concentration is also attributed to expansion of plasma volume and transfer of maternal thyroid hormone to the foetus. However, the authors have indicated that more work is required to confirm these changes during pregnancy. (SACN, 2014., Zimmermann, 2009b., Glinoer, 2001).

1.2.2 Iodine requirements during lactation and infancy

WHO/ICCIDD/UNICEF have recommended an intake of 250 µg/day of iodine for lactating women with urinary iodine concentration (UIC) > 100 µg/l as a benchmark of iodine sufficiency (Lotscher, 2012). During lactation, the physiology of thyroid hormone production and UIC returns to normal. The iodine gets concentrated in the mammary gland for excretion in breast milk through a concentration gradient of 20 to 50 times that of plasma levels, through the increased expression of the sodium /iodine symporter (NIS) in the lactating breast cells. It should be also noted that colostrum contains higher iodine concentrations (approximately 200-400 µg/l) than mature milk (Lotscher, 2012; ICCIDD, 2007; Elting et al., 1986). Using UIC as an indicator of iodine status in this scenario can lead to underestimation of requirements. So, the increased requirement during lactation is to ensure that the infant gets optimal iodine from the breast milk to build reserves in the thyroid gland (ICCIDD, 2007).

Well documented studies conducted on healthy, preterm and full-term infants have reported that iodine intakes required to maintain a positive balance are 15 µg/kg/day in full-term newborns and 30 µg/kg/day in preterm babies (Ghirri et al, 2014, Zimmermann, 2009, Delange et al., 2004). Maintaining this balance is not only important to prevent consequences due to iodine deficiency, but also to avoid iodine overload. Iodine overload is equally harmful and so iodine supplementation is not recommended for neonates and infants. This is because neonates do not have a fully developed thyroid gland and so cannot regulate the uptake of iodine from the blood in response to excess iodine or iodine from external sources. This phenomenon has been termed as Wolff-Chaikoff effect (Ghirri et al., 2014). Dietary intake of 90 µg/day is recommended in the light of small iodine stores and rapid thyroidal turnover rate as compared to adults.

1.3 Causes of iodine deficiency

Various factors are known to predispose an individual towards development of iodine deficiency disorders.

(i) Low iodine intake – Low iodine intake could be attributed to two scenarios. Firstly, iodine content of the foods depends on the iodine content of the soil where these crops are grown. This kind of dietary deficiency is common in the population living in areas where the soil is deprived of iodine as a result of iodine washout by rain, past glaciation and leaching effects of snow and heavy rainfall (Vander-Reijden et al., 2017, World Health Organization (WHO), 2006). Consuming foods grown in iodine deficient soils in the long term, without incorporating an alternative in form of fortified foods or supplements can lead to iodine deficiency disorders. Secondly, following a certain dietary pattern such as low consumption or complete elimination of seafoods (vegan dietary patterns) can also account for low iodine intake and thereby insufficient iodine status.

(ii) Inadequate utilization – This relates to development of iodine deficiency in advent of high consumption of foods containing goitrogens (cassava, cabbage, cauliflower, brassica vegetables). This antagonist effect of goitrogens on the thyroid gland is due to the presence of thiocyanate, which inhibits the transport of thyroid iodide and at high concentrations competes with iodine in synthesis of thyroid hormones (WHO, 2006). Other inhibitory mechanisms of goitrogens on the thyroid gland reported (Vander-Reijden et al., 2017) include interference with oxidation of iodide to elemental iodine, inhibiting coupling of iodine in thyroglobulin and impairment of T_3 and T_4 production/release into circulation, thereby leading to deficient iodine status.

There is a substantial amount of work conducted over the years highlighting the significance of iodine in vulnerable populations (infants and young women) and across life stages. Iodine deficiency has been identified as one of the major causes of preventable brain damage and mental retardation. Multiple adverse health consequences have been associated with iodine deficiency usually termed as Iodine deficiency disorders (IDD) and these disorders can be observed from foetus to old age, as summarised in table 1.2.

Table 1. 2 Iodine deficiency disorders across specific life stages

Life stages /Age group	Iodine deficiency disorders
Foetal stage	Abortions Stillbirths Congenital Anomalies Increased Perinatal Mortality Increased Infant Mortality Neurological Cretinism Mental deficiency Deaf-mutism Spastic diplegia Squint Myxedematous Cretinism Mental deficiency Dwarfism Psychomotor Defects
Neonates	Neonatal goiter Neonatal hypothyroidism
Children and adolescents	Goiter Juvenile hypothyroidism Impaired mental function Retarded physical development
Adults	Goiter with complications Hypothyroidism Impaired mental function

Source: Zimmermann (2009), Kapil (2007), WHO (1994).

Studies conducted over the years have shed light on the adverse health consequences of iodine deficiency, specifically highlighting the significance of iodine during crucial and nutritionally demanding physiological states namely pregnancy, lactation, infancy and adolescence.

1.4 Impact of iodine deficiency during pregnancy and foetal development

According to Kapil (2007), the critical time for the impact of iodine deficiency is the middle of the second trimester (14-18 weeks of pregnancy), when the neurons of the cerebral cortex and basal ganglia are formed. Moreover, this is also the period for cochlea development (spiral cavity of the inner ear). Overall, this is a very dynamic phase during pregnancy involving formation and development of foetal organs and so an iodine deficit during this period can lead to slowing down of the metabolic activities of all the cells of the foetus and irreversible alterations in the development of the brain. This further explains the adverse consequences such as increased risk of spontaneous abortions, low birth weight, infant mortality and increased chances of neuromotor, behavioural and cognitive impairments in the offspring (Zimmermann, 2007).

There is a considerable amount of literature which explores the reasons and effects of severe iodine deficiency in pregnancy. An established consequence of severe iodine deficiency (indicated by medium urinary iodine concentrations of <20 µg/l) is congenital iodine deficiency syndrome (Bath and Rayman, 2013., Zimmermann, 2009, WHO, 2006). Two classic forms of congenital iodine deficiency syndrome have been identified (neurologic and myxedematous), however, studies have confirmed that neurologic syndrome is more common, and results in specific impairments such as deaf mutism, squint, spastic dysplasia and disorders of the stance and gait (Skeaff., 2011, Zimmermann, 2009). These symptoms occur when iodine intake is less than 25 µg/day.

Work solidifying the adverse consequences of iodine deficiency in pregnancy is being carried out in a long-term follow up study (O' Donnell, 2002). Two different groups of pregnant women were given iodine before the end of second trimester and before the end of third trimester respectively. Children born to these mothers were examined after two years and yet another group of children were recruited who were given iodine at age of two years. Analysis of psychomotor scores to understand the impact of iodine supplementation at different stages revealed that the scores for children whose mothers received iodine supplementation very early in the course of pregnancy (before end of second trimester) were higher than the scores of children born to women who received iodine later in the course of pregnancy. These findings help to strengthen the significance of iodine during early gestation and for children. In the context of congenital iodine deficiency syndrome, a classic sign of severe deficiency, Zimmermann (2009) in his review about iodine deficiency has cited an example of one of the early studies (Fierro-Benitez et al., 1988) where the impact of iodine supplementation was monitored in relation to birth of babies with congenital iodine deficiency syndrome in severely iodine deficient regions. This noteworthy study compared the effect in two villages where congenital iodine deficiency syndrome prevailed (8%). One village was given iodine treatment while the other was considered as iodine deficient control. Participants were all women of child bearing age, pregnant women and children. The iodine coverage was 90 percent with the treatment group receiving iodised oil injection at baseline and this treatment was followed at four-year intervals for a total span of 20 years. During this follow up series (Greene, 1994), the effects were monitored especially for the offspring born to these treated women. It was observed that the none of the new born babies were suffering from congenital iodine deficiency syndrome in the treated village and two years later, the IQ quotient of the first and second grade children were higher (10 points) in the treated village as compared to the control. Five years later, the participants in the treated village were categorised in order to compare the effect of iodine supplementation either before conception or at different stages in the course of pregnancy. It was concluded that, the children born to the mothers who received iodine injection before conception had significantly higher IQ than children born to mothers who received supplementation during pregnancy. This group of studies strongly establishes the critical significance of maintaining adequate iodine status for optimal neurological function

Thyroxine plays a key role during early foetal stage of life as it is required for growth, maturation, proliferation and migration of neurons and development of cytoarchitecture of the brain and neuronal differentiation in definitive regions of the brain during specific development windows (Bath and Rayman, 2013, Zimmermann, 2009, Williams, 2008, Kapil, 2007). The foetus converts maternal thyroxine into an active form that is triiodothyronine (T3) in order to achieve optimal development. A fall in active thyroxine leads to increased chances of perinatal mortality, stillbirths or death within one week of birth. Another crucial aspect that needs attention is that mother and foetus respond differently to iodine deficiency at different stages of pregnancy. If the mother is deficient due to inadequate dietary iodine intake, it can lead to iodine deficiency in the foetus. However, over the course of the period, the mother might regain the euthyroid stage having normal thyroid stimulating hormone (TSH) and T_3 concentrations. This is attributed to the auto-regulatory iodine homeostasis achieved by the mother, through increased iodine "trapping" in the thyroid gland, increased synthesis of T_3 hormones instead of T_4, hyperplasia and eventually goitre. However, these mechanisms are not fully developed in the foetus, thereby leading to localised hypothyroxinemia in specific parts of the developing foetal brain. Decreased thyroid hormones during initial stages of pregnancy may cause problems in visual attention, visual processing and gross motor skills. If this deficit of thyroid hormones continues then it can worsen the complications associated with visual skills, slowing the processing speed and fine motor skills (Skeaff., 2011, Zoellar and Rovet, 2004). This critical chronology of responses in iodine deficiency has been found to be responsible for neurodevelopmental damage. Since these alterations are due to differences in responses of TSH, neonatal TSH is used as an index of assessing iodine deficiency (Skeaff, 2011).

1.5 Iodine deficiency and its impact during early childhood

Inadequate dietary iodine during pregnancy may lead to maternal subclinical hypothyroidism resulting in a reduced supply of thyroid hormone to the foetus which my lead to long term consequences. Haddow et al., (1999) has linked maternal subclinical hypothyroidism to less favourable neurodevelopment outcomes when the children were assessed at seven years of age, following which congenital hypothyroid screening was recommended.

Based on this study (Haddow et al., 1999), yet another pilot study was conducted by Kibirige et al, (2004) at James Cook University in north-east England. In this study, urinary iodide excretions (UIE) were measured using ICP-MS in 227 women at 15 weeks gestation with an equal number of non-pregnant women as controls. UIE were expressed both in terms of iodine concentrations (µg/l) as well as iodine:creatinine ratio. The results for iodine:creatinine ratio and iodine concentrations revealed that 40% and 7% of pregnant women respectively suffered from borderline iodine deficiency. In order to assess the implications of this iodine deficiency on the foetus, screening for congenital hypothyroid was conducted according to the guidelines by National Neonatal Hypothyroidism Screening laboratory on the fifth postnatal day. The authors of this study concluded that more work in this area is required to establish the significance of congenital hypothyroid screening as well as screening for subclinical maternal hypothyroidism. However, these screenings can indirectly play a role in managing maternal iodine deficiency and thereby prevent its detrimental impact on the overall foetal development.

In view of the literature focussing on the detrimental effects of iodine deficiency on the cognitive outcomes, Bath et al., (2013) have worked on assessment of mild iodine deficiency during early pregnancy and its relation to adverse effects on child cognitive development. This study is worth mentioning as in this study, the authors selected the mother-child pairs who were already a part of the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort and analysed the urinary iodine and creatinine concentrations for 1040 first trimester pregnant women. Moreover, the IQ of the offspring of the selected mother was also measured at age eight years. A comparison was made between the iodine status of the mother and child IQ at age eight years and reading ability of the child at age nine years. The results demonstrated that the children of women with an iodine-to-creatinine ratio of less than 150 µg/g were more likely to have scores in the lowest quartile for verbal IQ, reading accuracy and reading comprehension than were those of mothers with a ratio of 150 µg/g or more. This data satisfactorily establishes direct relation between maternal iodine status and optimal neurological development of the offspring, thus highlighting the need to address iodine deficiency in the UK as an important public health issue.

In addition to this, the adverse consequences due to iodine deficiency differ significantly depending on the severity of iodine deficiency. In this context, WHO (2007) has recommended that total goitre rate should be used to define the severity of deficiency in the population at large.

Table 1. 3 Criteria for defining severity of iodine deficiency in the population

Goitre rate	**Iodine status of the population**
Below 5%	Iodine sufficiency
5.0-19.9%	Mild deficiency
20-29.9%	Moderate deficiency
Above 30%	Severe deficiency

Source: Ghirri et al., (2014), Zimmermann (2009), WHO (2007)

1.6 Concern regarding use of iodine supplements

While addressing the problem of iodine deficiency and exploring ways of improving iodine status of women of reproductive age group, it is important to note that, sudden increase in iodine levels after a prolonged period of deficiency can be equally harmful as the deficiency. Sudden increase in iodine intake through supplementation can lead to iodine-induced hyperthyroidism (IIH). This risk is elevated in case of people suffering from severe iodine deficiency (Speeckaert et al., 2011, Krohn et al., 2005, Todd et al., 1995, Galofre et al., 1994), especially adults (age > 40 years) with history of nodular goitre. The symptoms of IIH are not specific and so, it is not easily detectable, thereby increasing the risk for the individuals. Non-monitored use of iodine supplements to increase iodine intake can be a risk factor for autoimmune thyroiditis attributed to the presence of positive thyroid auto-antibodies in some individuals (Speeckaert et al., 2011). A study by Todd et al, (1995) also reported a sharp increase (from 2.8 in 100,000 inhabitants to 7.4 in 100,000 inhabitants) in incidence of IIH in Zimbabwe upon introduction of iodised salt. Furthermore, Sarwar et al., (2013), established that increasing iodine intake by introduction of iodised salt in people living in long standing iodine deficient regions (Azad Jammu and Kashmir region of northern Pakistan) could be a major risk factor for IIH. It has been postulated that the thyroid gland in people with thyroid disorders is often unable to process the iodine load when iodine intake is suddenly increased through use of iodine enriched foods (iodised salt, sea weeds – good source of iodine) or iodine supplements. Due to these above-mentioned reasons, the use of iodine supplements like Kelp tablets, iodine containing multivitamin tablets are contraindicated in pregnancy (Dairy Council, 2016) and the use should be carefully monitored under the guidance of medical professionals by adults.

1.7 Indicators of iodine status

Iodine nutrition at the community level is assessed by measurements of:

- Urinary iodine concentration (UIC)
- Thyroid size
- Thyroid stimulating hormone (TSH)
- Neonatal serum TSH

Urinary Iodine Concentration (UIC) - Each of these indicators are useful for specific purposes, however UIC is the most commonly used especially for studies conducted with large sample size or during population studies. It is an excellent indicator of current iodine intake (Zimmermann, 2009). Generally, along with UIC, urinary creatinine concentrations are also measured and this enables us to calculate the iodine:creatinine ratios thus give clarity about the iodine status of the selected cohort (Montenegro-Bethancourt, 2015).

Table 1. 4 Urinary iodine concentration and iodine intake

Iodine intake	Median urinary concentration (UI)				
	General population	Pregnant women	Lactating women	Children <2yr old	School aged children
Insufficient	<100 µg/L	<150 µg/L	<100 µg/L	<100 µg/L	<100 µg/L
Adequate	100-199 µg/L	150-249 µg/L	≥ 100 µg/L	≥ 100 µg/L	100-199 µg/L
More than adequate	200-299 µg/L	250-499 µg/L			200-299 µg/L
Excessive	>300 µg/L	≥ 500 µg/L		>180 µg/L	>300 µg/L

Source: Ghirri et al., (2014), Zimmermann, (2009), De.Benoist et al., (2008), Andersson et al, (2007).

UIC is measured in spot urine samples in the representative target group as using 24-hour samples in large scale population studies is cumbersome. It has been reported that the results of UIC and 24-hour samples correlate well.

However, there are few limitations of the method. The individual iodine intake is variable due to various reasons such as seasonal changes, reduced consumption of iodine rich food sources due to dietary preferences or allergy restrictions. This uncertainty can lead to higher variability of in spot UI for different individuals. Further, because of the nature of the calculations adopted, it is accepted that all individuals with spot UIC less than 100 µg/litre are iodine deficient could lead to misinterpretation. Therefore, 24-hour urine could be considered more reliable for interpreting the iodine status. Even though there is a window for debate on the pros and cons of the method, spot UI is widely accepted by research groups across the globe on the assumption that enormous sample sizes in population studies can minimize the errors to a considerable extent (Zimmermann, 2009).

Thyroid size

Thyroid size is measured to understand the level of iodine deficiency and is a very useful assessment tool especially in the areas of severe iodine deficiency. Goitre manifests in event of mild-to-moderate deficiency, however there are grades of goitre indicative of degree of deficiency. WHO (2007) and WHO (1997) have clearly defined goitre and classified each type grade wise. Accordingly, grade 0 is defined as a thyroid that is not palpable or visible. Grade 1 is a goitre that is palpable but not visible when the neck is in normal position whereas grade 2 goitre is a thyroid that is clearly visible when the neck is in normal position. Different methods of assessing goitre in different population sets have been well documented. The first method, which is very commonly used especially in areas of severe iodine deficiency, is neck inspection or palpation. According to this method, a thyroid is considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the individual being examined. This method is useful and non-invasive but cannot be used in population sets with mild deficiency due to poor sensitivity and specificity. So, in this case the second method of assessment is adopted, that is thyroid ultrasonography.

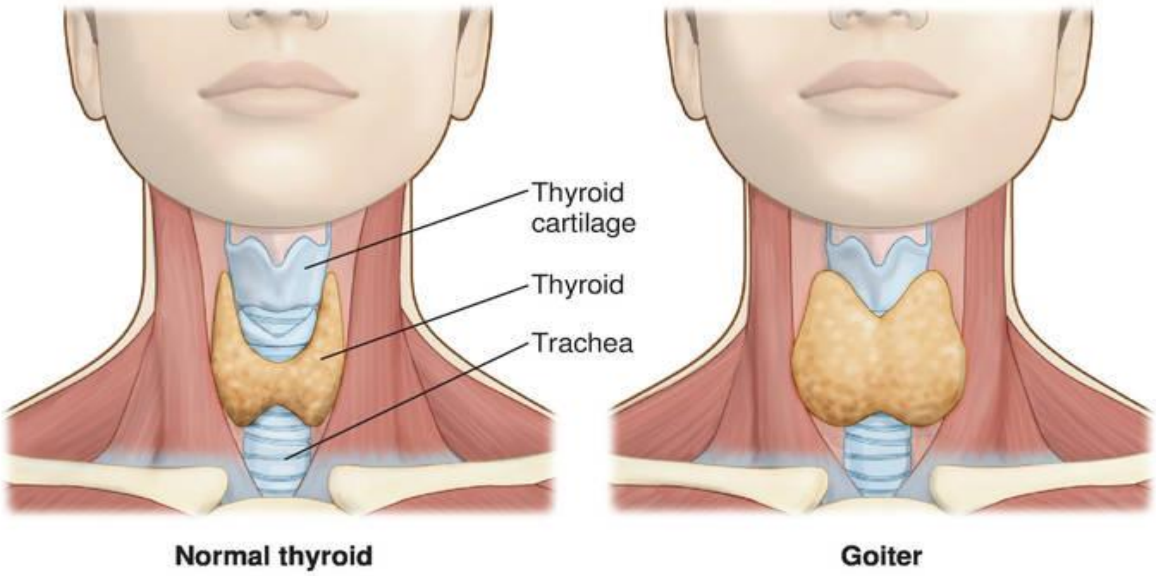


Figure 1. 1 Difference between the size of thyroid gland in healthy condition and during deficiency

Source: Farlex and Partners (2009)

Thyroid Ultrasonography

Thyroid ultrasonography is a non-invasive specialist method which is much more sensitive and rapid (2-3 minutes/person) than palpation of goitre. In this method, portable equipment is used for analysis and thus can be used in remote areas. The only crucial requirement for its optimal usage is availability of sufficient and scientifically established data about thyroid volume of iodine sufficient children, so that the data obtained regarding deficiency in that region can be interpreted to present results. The use of this method in various large-scale population studies is well documented. Delange et al., (1997), measured the thyroid volume by ultrasonography to assess the iodine status of school children (aged 7-15 years, n= 7599) in the past few years in 12 European countries. The authors utilized a mobile unit having ultrasonography equipment and gathered data from one to fifteen sites in The Netherlands, Belgium, Luxemburg, France, Germany, Austria, Italy, Poland, the Czech and Slovak Republics, Hungary and Romania. Results did compare well with the data for urinary iodine concentration thereby identifying iodine deficiency in the study regions. Due to the reliability and validity of the results obtained by thyroid ultrasonography, its use continues. Another study by Vitti et al., (1994) has also reported its use in measuring thyroid volume in children and compared with thyroid palpation for the assessment of the prevalence of goitre in an area of mild iodine deficiency. School children (6-14-year old) were from control areas (n = 2693; urinary iodine excretion, 110 micrograms/L) or from an area of mild iodine deficiency (IDA; n = 278; urinary iodine excretion, 72 micrograms/L). In the second group (IDA) who were mild iodine deficient the thyroid enlargement was measured by palpitation and results from both the methods were compared. The discrepancy between palpation and ultrasound was found in 23.9% of children living in an IDA, confirming that palpation is relatively inaccurate for assessing the prevalence of goitre in mild iodine deficiency. Moreover, this also reinforced the observation that thyroid volume measurement by ultrasound in children provides a useful tool for the assessment of goitre in mild iodine deficiency.

The usefulness of thyroid ultrasound is thoroughly proven; however, it is important to note that there are a few limitations to this method stated as follows

- It is subjective
- It requires judgement and experience
- Differences in technique can produce inter-observer errors in thyroid volume as high as 26%

  (Zimmermann, 2009, Zimmermann et al., 2001).

Thyroid stimulating hormone (TSH)

In order to understand the use of TSH levels for assessment of iodine status of an individual, it is important to understand the physiology of iodine uptake and release in the body (figure 1.2).

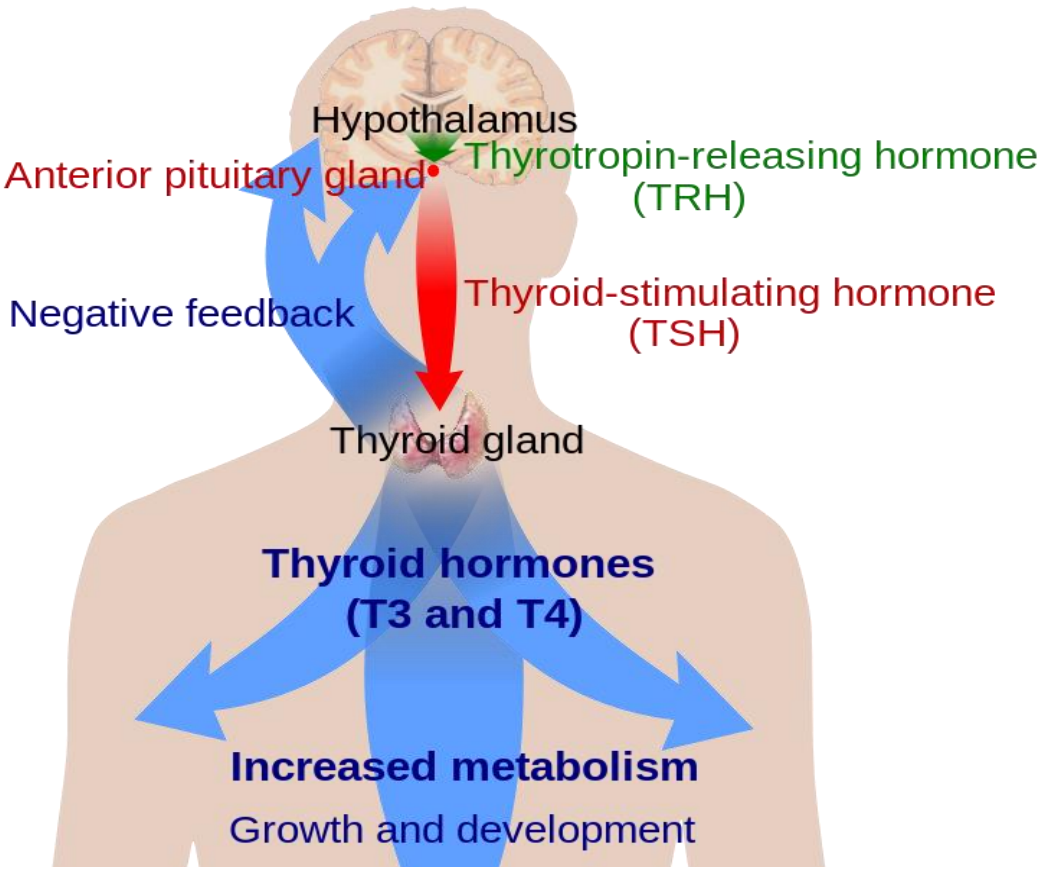


Figure 1. 2 Mechanism of production of thyroid hormones

Source: Hill et al., (1998)

Measurement of TSH values implies the level of circulating thyroid hormone, indicative of the iodine intake of the person. This is one of the most common biochemical tests suitable for assessing the iodine status across all age groups. However, the results may be misleading in elderly individuals where TSH levels are increased, but the values when analysed using laboratory procedure are within the normal range (Zimmermann 2009). This limitation needs to be considered while interpreting the results and thereby judging the degree of iodine deficiency.

Neonatal TSH Screening

The neonatal TSH screening test is an extremely important assessment tool for the newborn (Zimmermann, 2009, Glinoer et al., 2004, Copeland et al., 2002, Sullivan et al., 1997, Nordenberg et al., 1993). This is because it reflects iodine status during a period when the developing brain is particularly sensitive to iodine deficiency. Neonatal TSH screening can be used to detect even marginal deficiency when the newborn is three or four-days old (Ghirri et al 2014). TSH is used in many countries for routine newborn screening to detect congenital hypothyroidism (WHO, 2007). In terms of iodine status of the newborn, an important aspect that needs to be considered is that even though the thyroid gland of the newborn contains less iodine than an adult, it has a higher iodine turnover. If iodine deficiency is detected through this testing at this initial stage, then corrective measures can be adopted sooner, thereby reducing the adverse impact of iodine deficiency on the ability of the child to achieve the required milestones in the growing years.

With regards to various assessment tools for iodine status, it is important to note that the UIC is a marker for recent iodine intake, whilst goitre rate marks a long-term response to iodine deficiency (Ghirri et al., 2014). Furthermore, during pregnancy a small decrease in the concentration of serum free T_4, a moderate increase of thyroglobulin (Tg) levels or an increase in TSH or Tg levels in cord blood samples are not signs of iodine deficiency.

1.8 Historical perspective about Iodine deficiency in the UK

The prevalence of iodine deficiency in Britain has a strong imprint in the iodine history in Britain/Europe with presence of endemic goitre across various regions in the year 1836. Early records of goitre and congenital iodine deficiency syndrome, both associated with severe form of iodine deficiency were reported in English Counties, including Norfolk, Monmouthshire and Cornwall (Kelly and Snedden, 1960). Congenital iodine deficiency syndrome alone was highly prevalent in Chiselborough, Somerset (Pharoah et al., 1980) and this English goitre belt further extended to Cotswold and Chiltern Hills and then northwards into Derbyshire, Peak District, thus affecting both north and south Wales. The iodine deficiency or prevalence of goitre in Derbyshire- Peak district was again emphasized by Saiket et al much later in 2004 attributing it to geological factors resulting in low soil iodine content.

Due to the high prevalence of endemic goitre until the 1930's this region was described as 'Derbyshire neck' (Bath and Rayman, 2013., Saiket et al., 2004)

In the early nineteenth century, a survey was carried out among 12-year-old school children in England and Wales by school medical officers on the request of the Board of Education. A total of 375000 children were recruited and the results confirmed the high prevalence of visible goitre underlining the need for development of a national iodization programme. This survey is one of the prominent milestones and played a pivotal role in identifying the geographical pattern of the disease.

Significant work around iodine deficiency in the UK continued even during the war years (1939-1945) when visible goitre had become common among young women working in factories for war work. Considering this alarming situation, the Medical Research Council (MRC) carried out a number of surveys. It was found that 50% of adult women in Hook Norton (Oxfordshire), 43% of girls in Sherborne, (Dorset) and 26% of boys and girls in St. Albans (Hertfordshire) suffered from goitre, thus strongly establishing the case of iodine deficiency in the UK. Even though the need for an iodisation programme was identified, steps aiming towards this goal were not implemented. Research groups continued their work and towards 1990, a survey revealed that iodine deficiency symptoms, specifically thyroid enlargement in school children, had become undetectable and this was observed even in a traditionally iodine deficient area of South Wales. Phillips (1997) described this phenomenon as an 'Accidental public health triumph' clearly discussing the notable changes that could have helped to reduce iodine deficiency to such a level that not only the symptoms became non-detectable, there was a rise in concern regarding excess iodine intake. The timeline can be explained as in figure 1.3

1920

- Efforts were made to improve iodine status. Iodine supplementation of livestock could improve reproductive outcome. This research provided an incentive for iodine supplementation of dairy herds

1928

- Booths introduced iodised salt licks for the cattle

1937-1940

- In the year 1937, iodine enriched cattle feed was marketed by Spillers
- Before 1930, milk was a luxury. However after the establishment of Milk Marketing Board and the Milk Act (1934) and the National Milk Scheme 1940, milk was either provided free or at subsidised prices to expectant mothers and young children.
- This led to spectacular rise in milk iodine content especially during winters when cattle depend on artificial feeds.

1950

- By early 1950's the average milk consumption doubled. Winter milk alone fulfilled the recommended daily requirement (150 µg/day).

1952-1982

- Increase in iodine content of milk and related dairy products led to 3-fold increase in dietary intake thus leading to substantial decrease in iodine deficiency in Northern Europe
- This reduction in deficiency was not observed in Southern Europe and one of the main reasons was that in this region, pasture were more available and there was less dependence on artificial feeds

Figure 1. 3 Timeline - Accidental triumph over iodine deficiency in Britain/Europe

Source: Phillips (1997)

The reason for this “accidental public health triumph” was later found to be attributable to the adventitious entry of iodine in the food chain.

Iodine can enter the dairy food chain via a number of routes, namely:

a. The use of iodinated casein given to cows as a lactation promoter
b. Iodophor disinfectants used for cleaning teats dips, udder washes and bulk milk tankers leading to iodine contamination of milk
c. Iodine enrichment of meat and eggs due to spill over from use of iodine in animal feeds
d. Iodophor medication
e. Iodine-containing sterilizers of milking equipment

1.9 Re- emergence of Iodine deficiency in the UK

In recent years, it has been assumed that iodine deficiency exists only in developing countries and in areas with soils deficient in iodine and so iodination of salt and water was encouraged in developing countries where iodine deficiency was prevalent. However, contrary to the belief that the UK was iodine sufficient, recent research has revealed that there is widespread iodine insufficiency in Britain and other European countries (Rayman et al., 2008, Combat and Lean., 2014). As presented in table 1.1 , iodine requirements for optimal health varies depending on age, and physiological status (pregnant and lactating women) (WHO, 2007), however Iodine deficiency is defined as a urinary iodine concentration of below 100 µg/L. Literature exploring iodine status of the UK population is limited; however, there are a few studies which have shed some light on iodine status of women belonging to child bearing age in the UK. One such cross-sectional pilot study was conducted by Rayman et al (2008) in 30 women from Surrey, UK, with a median age of 23 years. 24-hour urinary collections were measured for their iodide concentrations. Findings revealed that 25.8% had mild iodine deficiency and one participant had moderate iodine deficiency. Out of 30 participants, only twelve had sufficient iodine levels. These results hold significance because if these iodine deficient women become pregnant, then it can lead to serious health consequences, not only for the women, but also for the offspring. Emphasizing the importance of iodine during adolescence and child bearing years, a cross-sectional study was conducted in girls (14-15 years) attending secondary school in nine UK centres (Aberdeen, Belfast, Birmingham, Cardiff, Dundee, Exeter, Glasgow, London, and Newcastle-upon-Tyne). Urinary iodine (737 participants) and tap water iodine concentrations were measured. In addition, dietary data using a validated food frequency questionnaire was obtained from 664 participants. The findings of the study revealed that the prevalence of iodine deficiency was highest in Belfast (85%, n=135). Tap water iodine concentrations were low or undetectable and were not significantly associated with urinary iodine concentrations. More recently, another study by the same group of researchers (Bath et al., 2014) emphasizes the alarming situation related to iodine deficiency in pregnant women worsened by lack of surveys or in-depth information about iodine status of pregnant women in the UK. In their cross-sectional study, the researchers recruited 100 pregnant women at the Royal Surrey County Hospital, Guildford, at their first-trimester visit and collected spot urine samples for measurement of UIC and creatinine concentrations. These women participants were also asked to complete a general information questionnaire and a food frequency questionnaire (FFQ). The findings revealed that the values of medium UIC(85·3 mg/l), iodine:creatinine ratio (122·9 mg/g) and 24 hour iodine excretion(151·2 mg/d) were all lower than the requirement criteria recommended by WHO, thus indicating mild to moderate iodine deficiency in the selected group of pregnant women. Also, positive correlation was observed between the iodine status of the women (indicated by the values) and dietary milk intake and consumption of iodine containing prenatal supplements, thus establishing the need for adequate nutritional guidance to women (pregnant and in child bearing age group) to improve their iodine status.

As research related to the emerging problem of iodine deficiency in the UK continues, more alarming statistics are revealed, and these reinforce the concern that the deficiency is prevalent in vulnerable populations widely spread across different areas in the UK, which makes it more important and challenging to find a solution to address this deficiency. More recently, Bath et al (2015), conducted an in-depth study to assess the iodine status of a cohort of UK pregnant women and explored critically the changes in this iodine status throughout the period of gestation. For this detailed study, 230 pregnant women already recruited to the Selenium in PRegnancy INTervention study were selected and spot urine samples were collected at 12, 20 and 35 weeks of gestation and analysed for their iodine and creatinine concentrations. Other related factors such as change in season, body mass index, daily milk intake and maternal age were recorded. Results revealed that the median urinary iodine concentration from urine samples collected at all time points (n = 662) was 56.8 mg/L, and the iodine-to-creatinine ratio was 116 mg/g, thus confirming that these pregnant women were mildly-to-moderately iodine deficient during all the trimesters. This clinical situation is not only harmful for the mother, but also increases the risk for the developing foetus and thereby chances of incidence of iodine deficiency in the new-born. Looking from a broader perspective, this study clearly demonstrates the re-emergence of iodine deficiency in the UK

Milk and dairy products contain substantial levels of iodine due to the accidental iodine contamination and so traditionally in the UK, regular milk consumption was instrumental in preventing iodine deficiency. However, as described above, recent studies have highlighted the emerging concern about iodine deficiency in the UK. One of the reasons for this scenario could be changes in animal feeds, which could result in a reduction in the iodine content of the milk. In this context, a review was conducted by de Jong (2007) which reported that dairy products and meat could be important sources of iodine in US diets if iodine containing cattle feed was used. It was concluded that changes and limitations in allowable levels of iodine in cattle feed could be one of the major reasons for reduction in dietary iodine levels. Similarly, another study was conducted by Flachowsky (2007) which focussed on iodine in animal nutrition and iodine transfer from feed to food of animal origin. Through dose-response studies, it was concluded that the transfer from feed into meat is below 1% of supplemented iodine, but it can be increased to about 30% in milk and eggs. Furthermore, the author highlighted that, due to high transfer of iodine, the EU commission decreased the maximum iodine levels for dairy cows and laying hens from 10 to 5 mg/kg feed. This helps to establish change in cattle feed content as one of the reasons for iodine deficiency in the UK.

1.10 Strategies to overcome iodine deficiencies

There are various ways of addressing the problem of any nutrient deficiency at population level such as Nutrition education, food diversification, supplementation and food fortification. Nutrition education and food diversification are very useful as part of a long-term strategy and it might take a decade or so to witness the impact of this strategy in reducing the prevalent deficiency in general or targeted populations. Supplementation is effective for short-term acute deficiency while fortification can be considered for achieving medium to long term objectives with selection of appropriate vehicle for fortification (Van Stuijvenberg et al., 1999). Among all the above-mentioned strategies, fortification has been suggested as an effective method as it can be conveniently implemented at small scale specific target groups as well as at population scale.

Literature further suggests that the addition of iodine to foods should be limited to commonly fortified foods like salt, bread, water and milk. Research bodies (WHO and FAO, 2006) have clearly discussed guidelines for choosing an appropriate food vehicle for iodine fortification. According to these guidelines iodate and iodide are the two chemical forms of iodine that are used for fortification, usually in the form of potassium salt and sometimes as sodium salt or calcium salt. The following table(Table 1.5) highlights the iodine content of different iodine fortificant salts.

Table 1. 5 Different forms of iodine fortificants

Iodine fortificant	**Percentage of iodine**
Potassium iodide	76.5
Potassium iodate	59.5
Calcium iodide	86.5
Calcium iodate	65.0
Sodium iodide	68.0
Sodium iodate	64.0

Source: WHO and FAO, 2006

Potassium iodide has been commonly used as an iodine additive in bread and salt especially in various European and North American countries. However, it has been suggested that iodine in form of iodate is much better if used in food as compared to the iodide form. This is attributed to the better stability of iodates in foodstuffs. Iodates are less water soluble than iodides, more resistant to oxidation and evaporation. This quality of iodates makes them suitable for use in countries with humid or adverse climatic conditions. In contrast to Iodates, iodine in the form of iodides is extremely sensitive to moisture, humidity, exposure to sunlight, heat or to the presence of impurities in the substances (salt, bread flour, water) to which these are added, thus causing increased iodine losses on processing (WHO and FAO., 2006, WHO, 1996, WHO, 1991).

1.10.1 Food sources and vehicles for iodine fortification

(I) Food sources of iodine

The richest dietary sources of iodine are marine products (fish, shellfish and molluscs), eggs, milk and milk products. In the UK, milk and milk products are important sources of dietary iodine. It has been established that iodine can enter the food chain via sanitising solutions, iodophors or iodinated cattle feeds. Research has shown that organic milk has 35-40 % lower iodine than conventional milk (British Dietetic Association, 2016). However, research groups (Bath et al; 2014, Haldimann et al., 2005, Julsham et al., 2001; Sieber et al., 1999), have also reported that data on iodine contents of food items are relatively scarce, except for milk and fish. Currently there is lack of an established scientific database which can be used for obtaining updated information about iodine content of different food products in the UK and so the data for iodine content of milk and milk products (table 1.6) has been obtained from various authentic, scientific journal articles and published leaflets from scientific bodies like BDA and SACN.

Table 1. 6 Iodine content of milk and milk products in the UK

Name of product	Portion size	Iodine content (mcg)	Notes	Source information
Milk samples				
Cow's milk	200ml	50-100	Depending on season, higher value in winter	BDA - The Association of UK Dieticians, Food Fact Sheet-Iodine, May 2016
Organic cow's milk	200ml	30-60	Depending on season, higher value in winter	BDA - The Association of UK Dieticians, Food Fact Sheet-Iodine, May 2016
Cow's milk	mean of 33 samples	194.8 µg/kg		FORDYCE, F.M. 2003. Database of the Iodine Content of Food and Diets Populated with Data from Published Literature. British Geological Survey, Commissioned Report, CR/03/84N. 50pp
Milk		690 ng/g		M. Haldimann et al., Journal of Food Composition and Analysis 18 (2005) 461-471
Conventional milk (cows)	200g	50-80 mcg	Depending on season, higher value in winter	Bath SC, Rayman MP. BDA Food Fact Sheet - Iodine. Available at: www.bda.uk.com/foodfacts/Iodine.pdf(accessed 30 July 2013): British Dietetic Association, 2013.
Organic cow's milk	200g	30-65	Depending on season, higher value in winter	Bath SC, Rayman MP. BDA Food Fact Sheet - Iodine. Available at: www.bda.uk.com/foodfacts/Iodine.pdf(accessed 30 July 2013): British Dietetic Association, 2013.

Whole milk, pasteurised, average		31 µg/100g	Average of summer and winter milk	(FSA, 2002, SACN, February 2014)
Semi-skimmed milk, pasteurised, average		30 µg/100g	Average of summer and winter milk	(FSA, 2002, SACN, February 2014)
Skimmed milk, pasteurised, average		30 µg/100g	Average of summer and winter milk	(FSA, 2002, SACN, February 2014)
Cheese samples				
Cheese	40 g	15		BDA - The Association of UK Dieticians, Food Fact Sheet-Iodine, May 2016
Cheese samples	mean of 11 samples	77.1 µg/kg		FORDYCE, F.M. 2003. Database of the Iodine Content of Food and Diets Populated with Data from Published Literature. British Geological Survey, Commissioned Report, CR/03/84N. 50pp
Cheese		473 ng/g		M. Haldimann et al., Journal of Food Composition and Analysis 18 (2005) 461-471
Cheese (cows)	40 g	15 mcg		Bath SC, Rayman MP. BDA Food Fact Sheet - Iodine. Available at: www.bda.uk.com/foodfacts/Iodine.pdf(accessed 30 July 2013): British Dietetic Association, 2013.
Cheddar cheese		30 µg/100g	Mild and mature English Cheddar	(FSA, 2002, SACN, February 2014)
Cheese, cottage, 2% fat	1/2 cup	26 - 71µg		Kapil. U Health Consequences of Iodine Deficiency December 2007 vol 7(3), P.267-272
Cheese, cheddar,	30g	5-23µg		Kapil. U Health Consequences of Iodine Deficiency December 2007 vol 7(3), P.267-272
Salt samples				

Iodised salt	mean of 18 samples	21041.1 µg/kg		FORDYCE, F.M. 2003. Database of the Iodine Content of Food and Diets Populated with Data from Published Literature. British Geological Survey, Commissioned Report, CR/03/84N. 50pp
Salt	mean of 9 samples	1787.5 µg/kg		FORDYCE, F.M. 2003. Database of the Iodine Content of Food and Diets Populated with Data from Published Literature. British Geological Survey, Commissioned Report, CR/03/84N. 50pp
Sea salt		50 µg/100g		(FSA, 2002, SACN, February 2014)
Salt, iodized	1 teaspoonful	400µg		Kapil .U Health Consequences of Iodine Deficiency December 2007 vol 7(3), P.267-272
Iodine fortified foods				
Iodised bread		20000 µg/kg		FORDYCE, F.M. 2003. Database of the Iodine Content of Food and Diets Populated with Data from Published Literature. British Geological Survey, Commissioned Report, CR/03/84N. 50pp
Water purification tablet		8000 µg/kg		FORDYCE, F.M. 2003. Database of the Iodine Content of Food and Diets Populated with Data from Published Literature. British Geological Survey, Commissioned Report, CR/03/84N. 50pp

Seaweed is a concentrated source of iodine and so its use should be monitored carefully in association with the iodine status of an individual. Iodine content of foods can vary considerably depending on the geographical location (iodine content of soil), season and farming practices. Apart from these sources, in many developing countries and countries with mandatory legislation for iodine fortification (Australia, New Zealand) or Universal salt iodisation, iodised salt is one of the most important dietary sources for iodine intake. Also, iodised oils are used in supplements outside Europe (EFSA ANS Panel, 2013).

(II) Food vehicles for iodine fortification

There is a substantial amount of literature on various food vehicles used for iodine fortification. However, the most common vehicles include salt, bread, water, milk and milk products.

(a)**Salt**- Fortifying table salt with iodine has been proven to be an effective and inexpensive way of improving iodine status in many developing countries. According to statistics published in Lancet, 2008, the use of iodised salt in developing countries has increased from 20% to 70%, while this consumption in the UK and Ireland is less than 5% (Vanderpump et al., 2011, Lancet, 2008). Understanding the usefulness of iodised salt in overcoming iodine deficiency, many developing countries have implemented Universal Salt Iodisation (USI).

Salt is iodized by the addition of fixed amounts of potassium iodide or iodate, as either a dry solid or an aqueous solution, at the point of production or import (Diosady et al., 1998). The WHO and FAO guidelines (2006) on food fortification have highlighted specific reasons for the suitability of salt as a fortificant for enhancing dietary iodine intake and for control of iodine deficiency disorders.

Firstly, salt is one of the most commonly consumed food items and its consumption is fairly stable throughout the year. This is because it is used as a condiment and so chances of overconsumption are rare. Secondly, salt iodisation technology is available at a reasonable cost. Worldwide, the estimated annual cost of salt iodisation is 0.02-0.05 US $ per child covered. This financial investment in salt iodisation is beneficial as a food fortification strategy, as this investment is much cheaper than the cost per child death averted due to deficiency (US$ 1000) (Eastmann and Zimmermann, 2018). Moreover, salt iodisation programmes are easier for implementation at national level. This has been proved by the success of salt iodisation program in Switzerland, which despite voluntary use of iodised salt, enabled people to achieve optimal iodine status thereby making Switzerland an iodine sufficient country (FCN, 2013). Even though more recently there have been changes in use of iodised salt, this success story due to use of iodised salt in foods (cheese and other processed foods) cannot be overlooked. Furthermore, addition of iodine to salt does not have any adverse impact on its colour, taste or odour. Finally, the quality of iodised salt can be monitored at stage of production, retail and household levels.

(b) **Water** - Literature has explored the use of water as a potential vehicle for iodine fortification. One of the main reasons for this consideration is based on the fact that water is consumed daily by people irrespective of differences in age, gender, residential location or economic status. There are different ways of water iodisation. Direct addition of concentrated iodine solution (as potassium iodide or iodate) in proportion to the amount of water is one of the non-invasive and inexpensive ways of water iodisation at large scale. However, the ratio of water:iodine has not been clearly defined. Another method of introducing iodide in water systems has been reported by Eastmann and Zimmermann (2018). According to this study, iodide could be added to the running water in pipes. In this method there is a canister filled with coarse crystals of iodine through which water is passed by a differential pressure mechanism. This was found to be a highly efficient system and when a similar version was put to practice in the Sarawak region of Malaysia, it resulted in reduction of goitre rate from 61% to 30% within a short span of 9 months. Improvement in thyroid function was also reported (Eastman and Zimmermann, 2018, Squatrito et al., 1986, Maberly et al., 1981). Furthermore, in the case of hand pumps and open wells, iodine in porous polymer containers can be introduced into the water supply. This leads to slow release of potassium iodide into the water and thereby has an impact on the iodine status of the population. However, it has been suggested that this method can become challenging and expensive as the porous containers used in this system have limited shelf life and so must be replaced every year (WHO and FAO, 2006). Cao et al, (1994) conducted some work on iodisation of irrigation water in Southern Xinjiang, China. The results revealed that there was an increase in iodine uptake by plants. From these results, the authors postulated that this increased iodine in rice plants could lead to enhanced iodine status of chicken and sheep. Furthermore, there will be a subsequent increase in the iodine content of eggs and thereby improved iodine status of population. This study presents a long-term effect of iodisation of water for irrigation and since this observation was made more than 20 years ago, more work should be conducted on similar lines to establish its impact on improving iodine status of the population.

Despite various advantages of using water as a carrier for iodine for improving iodine status of the deficient population there are a number of limitations. The sources of drinking water are numerous and ubiquitous and so it is challenging to ensure all these sources are adequately iodised regularly. Apart from this, another major limitation relates to the stability of iodine in water, which has been reported to be no longer than 24 hours. This also implies that the water has to be iodised at regular intervals in order to ensure that the levels of iodine in water are maintained.

(c) **Milk** - Milk has been considered as an important source of iodine in UK diets due to the accidental presence of iodine through use of iodophors in milk sterilizing plants or iodinated cattle feed increasing the iodine content of milk. However, this iodine content can vary considerably due to changes in practices. In the light of this information, Philips (1997) has confirmed that iodine enriched milk has become a major adventitious source of iodine in many countries in Northern Europe and the UK.

In addition to these main potential vehicles for iodine fortification, different research groups have explored the feasibility of use of other food vehicles such as Bread, Biscuits and Sugar.

Bread – Literature on use of bread or baked products as vehicles for iodine fortification is limited but there are a few studies, which have explored the use of baked products (biscuits) in understanding their effectiveness in improving the iodine status of vulnerable populations. Gerasimov et al, (1997) reported successful use of bread iodisation in Russia. These findings are important because bread is a staple diet in some European countries (Russia, Tasmania) and so targeting bread as a fortificant can ensure regular supply of iodine through bread consumption. (WHO and FAO, 2006).

On similar lines, it has been reported that in the Netherlands, bread was considered as an important source of dietary iodine. This is because the salt used in bread production (baker's salt) been found to be enriched with iodine since 1942. However, there was a change in legislation from mandatory to voluntary in 1984 and this led to subsequent decline in iodine in bread salt. However, in 1997 the Dutch authorities decided to increase the iodine content of bread salt from 45 ppm to 70-85 ppm. This was coupled with using iodised salt in grain-based preparations (de Jong, 2007). This scenario implies that bread could be a good fortificant for improving iodine status. Furthermore, a study by Van Stuijvenberg et al (1999) determined the effect of micronutrient-fortified biscuits on the micronutrient status of primary school children. The shortbread-based biscuits (cookies) were designed to provide 50% of the recommended dietary allowances of iron (5 mg ferrous fumarate), iodine (60 mg potassium iodate), and β-carotene (2.1 mg) for children aged 7-10 years. Considering the uncertainty of iodine due to baking procedures, a sugar drink was provided which contained 60 mg I. Micronutrient status was assessed in 115 children aged 6–11 years before and after consumption of biscuits (fortified with iron, iodine, and β-carotene) for 43 weeks over a 12-month period and was compared with that in a control group (n = 113) who consumed non-fortified biscuits. In addition to these, Cognitive function, growth, and morbidity were also assessed. Results revealed that consumption of fortified biscuits led to significant improvements in micronutrient status of the children. One aspect that needs to be considered in terms of this study is that this improvement could be due to intake of iodine as well as due to the combined positive effect of iodine, iron and β-carotene. However, more studies using iodine fortified baked products can provide better clarity towards use of bread, biscuits or baked products as a vehicle for iodine fortification.

Sugar - The use of sugar as a vehicle for iodine supplementation was explored in a study of iodine deficiency in the Sudan. Iodinated sugar was manufactured by addition to sugar solution prior to crystallisation in an evapocrystallizer or sprayed on the conveyor of cured sugar before it entered the dryers. This sugar was given to members of 18 and 60 families in mildly (urinary iodine < 5.1 micrograms/dl) and moderately (urinary iodine < 3 micrograms/dl) iodine deficient areas, respectively, over a one-month and a six-month period, respectively. Results reported positive improvements, where. the rates of goitre decreased, urinary iodine levels increased significantly (from 5.1 to 14.4 micrograms/dl and from 3 to 9.8 micrograms/dl, respectively) implicating that there is a possibility of use of sugar as a food vehicle for iodisation. However, aspects such as adverse health consequences of long-term consumption of sugar products needs to be considered (Eltom et al., 1995).

Use of iodised brine solution in food processing - In Switzerland, iodised salt has been regularly used in cheese production (FCN, 2012) and so, cheese could be considered as one of the most common food vehicles for improving iodine intake of the population. In this context, researchers have explored the use of iodised salt and brine solution containing iodised salt for preparation and processing of a variety of foods such as meat products, cheese and dairy products, canned vegetables (tomato juice, green beans, whole kernel sweet maize, sauerkraut), bulk sauerkraut, white bread, pickled olives and potato chips. The results revealed that, salt iodised either with potassium iodide or with potassium iodate has no influence on the quality of these foods. Literature (Blankenship et al., 2018, West and Merx, 1995) focussed on use of iodised salt in cheese and dairy products have emphasised that, iodized salt (integral ingredient in cheese manufacturing) does not have any impact on the organoleptic properties and technological characteristics of hard and soft cheeses. One of the earlier studies, cited by Hostettler (1953), investigated the effect of potassium iodide from salt containing 3.8 mg I/kg (the level officially allowed at the time) and 38 mg I/kg on the quality of Emmental cheese. The iodized salt was used to make the brine in which the cheese stayed for two days. When the quality of the cheese was evaluated after 4 months, it was found that there was no impact of either levels of iodine on the quality of the cheese. Furthermore, West and Merx (1995) in their review conducted for UNICEF, New York, have referred to another study, which discussed the influence of iodised salt on the quality of Gruyere cheese. After 77 days and 8 months of ripening, no difference between the iodized and non-iodized cheese could be detected. The postulated mechanism for this non-detectable impact of iodised salt (in brine solutions) put forth by researchers (West and Marx, 1995; Hostettler,1953) was that, if the salt content of cheese is 20 g/kg and the KI content of the salt is equivalent to 3.8 mg I/kg, the level of iodine in Emmental cheese, including iodine derived from milk, would be approximately 148 µg I/kg and so it was unlikely to observe any impact on the organoleptic properties of cheese at such low concentrations of iodine.

1.10.2 Concern regarding iodine intake of vegan population - Veganism is a growing trend in the UK population. According to the latest research by the Vegan Society, conducted in 2016, there are estimated to be around 540,000 vegans in Great Britain. It is estimated that this is up from 150,000 in 2006, and that there are twice as many women than men who are vegan. Another survey indicated that the number of vegans in Great Britain quadrupled between

2014 and 2018. In 2018, there were 600,000 vegans or 1.16% of the population, 276,000 (0.46%) in 2016 and 150,000 (0.25%) in 2014 (Ipsos Mori survey, commissioned by The Vegan Society, 2018, and The Food & You surveys, organised by the Food Standards Agency (FSA) and the National Centre for Social Science Research, Natcen). Therefore, it is important to consider iodine vehicles that would be acceptable to the vegan population.

Studies have highlighted that due to the avoidance of animal products there can be significant differences in nutrient intakes between meat-eaters and vegans (Fallon et al., 2018, Davey et al., 2003). Milk and milk products are good sources of micronutrients like calcium and iodine and the results of the study by Fallon et al. (2018), have highlighted that adult females in the north-west of England are at risk of low intakes of Vitamin D, iron and iodine when compared to the RNI. In addition, the exclusion of animal products indicates lower intakes of selenium and iodine. Adequate iodine levels are important for optimal thyroid hormone production and iodine deficiency may be of particular concern for adult females of childbearing age (Bath et al., 2008).

Iodine fortification of food products especially dairy (as it is a good source of iodine) has been established as one of the important strategies for improving iodine status of the population.

Dairy substitutes for vegan population - Recently variety of cheese substitutes are available in different popular supermarkets such as Sainsbury's and Morrisons. Cheese analogue is a substitute for milk cheese, which is similar in composition, appearance, characteristics and even in its intended use. In cheese analogues, the milk protein and milk fat are partly or wholly replaced by vegetable proteins (i.e. peanut protein, soybean protein) and vegetable fats and oils (i.e. partly hydrogenated vegetable fat like soybean and palm). Analogue pizza cheese is manufactured in a manner similar to that for processed cheese manufacture, which finds application in baking as a topping on pizza and as slices in stuffed burgers (Chavan and Jana, 2007). Fox et al., (2000), have reported manufacture of analogues of a wide variety of natural cheeses (e.g. Cheddar, Monterey Jack, Mozzarella, Parmesan, Romano, Blue and Cream) and pasteurized cheese products and these substitutes have been used widely in in salads, sandwiches, spaghetti sprinkling, cheese sauces, cheese dips and ready-made meals (Fox et al., 2000). Cheese substitute can be suitably fabricated to have nutritional benefits and so its use can be further explored as vehicle for improving iodine intake, where the regular salt used in manufacturing process could be replaced with iodised salt (Chavan and Jana, 2007). Some examples of vegan cheese varieties available in supermarkets include Morrisons Moo-zarella sticks, Koko's vegan cheese fortified with calcium and vitamins D_2 and B_{12}, Camembert-Style Cashew Cheeze, Feta-Style Tofu Cheeze, Cream Cheeze, and Blue Cheeze belonging to brand Bella Cheeze.

1.11 Concern regarding increased salt intake and associated health problems

In addition to the problem of iodine deficiency in the UK, there is simultaneous concern regarding consumption of excess salt as part of a daily diet. This is important due to increase in consumption of processed foods by the young population and thereby increased consumption of salt (sodium), which in turn has been found to be one of the major reasons for hypertension and related diseases (Christopher and Wallace, 2014). In the light of the average urinary sodium levels, it was revealed that in Europe, the average salt intake is 8.6 g/day, while that recommended by the Department of Health is no more than 6 g/day (Christopher and Wallace, 2014).

1.11.1 Salt intake and hypertension

Studies over the years have established that high levels of sodium in the regular diet can lead to increased water retention in the body which predisposes to hypertension; a risk factor for cardiovascular disease (Cruz et al., 2011, Tuomilehto et al., 2001). Changes in salt intake are associated in general with corresponding changes in arterial blood pressure. One of the earlier associations between salt intake and blood pressure was established by Kempner (1948) where he studied the impact of rice diets with salt <0.5 g on 500 hypertensive patients. These diets mainly contained rice and fruits. The results revealed rice diets containing little salt improved blood pressure, decreased heart size, reversed inverted T waves in the electrocardiography (EKG) and ameliorated hypertensive retinopathy. This study is important because it increased the awareness about adverse consequences of high sodium intake and highlighted the need to consider salt restriction strategies in treatment of hypertensive patients. Since then, extensive work has been carried out providing strong evidence of association between high salt intakes and increased incidence of hypertension. More recently, Ha (2014), in a review, reported one of the important studies linking blood pressure and salt intake from an epidemiological point of view. This study called INTERSALT was one of the first large international epidemiologic studies on sodium intake and hypertension using a standardised method for measuring 24-hour urinary sodium. This study involved cross sectional assessment of 10,079 subjects aged 20-59 sampled from 52 centres around the world. Sodium excretion ranged from 0.2 mmol/24 hours (Yanomamo Indians, Brazil) to 242 mmol/24-hour (north China). Result analysis revealed a strong positive correlation between salt intake and blood pressure in individuals from 48 centres out of the total 52 centres. Forte et al. (1989) studied reducing the dietary salt intake on blood pressure on a community in Portugal, which recruited two communities within the same district, each with 800 inhabitants who had salt intakes of ~21 g/day and a 30% incidence of hypertension. In the intervention community, there was a widespread health education effort to reduce the dietary salt intake. By the end of the second year, there was a small rise in systolic blood pressure in the control community and a significant fall in both systolic and diastolic blood pressure in the low salt intake community. These results imply that reduction of salt intake coupled with increased awareness about salt reduction strategies can improve cardiac parameters. Another well documented landmark study reported in the review by Ha (2014) was the DASH (Dietary Approaches to Stop Hypertension) 12-week controlled feeding trial wherein 412 individuals with and without hypertension were provided with 3 different doses of sodium (150, 100 and 50 mmol/day) and a specific intake level was required to be maintained for 30 days. Moreover, participants were assigned to two diets (control and DASH). Interpreting results of sodium assessment in combination with the differences in blood pressure parameters, it was observed that when the participants were shifted from a high sodium diet to a normal sodium diet, the systolic blood pressure decreased by 2.1 mmHg, whereas in case of consumption of low sodium diets the systolic blood pressure further reduced solidifying the association that high sodium intake is strongly associated with increased incidence of hypertension.

1.11.2 Salt intake and Stroke

Stazzullo and colleagues (2009) conducted a systemic review to assess the association between level of habitual intake of salt and stroke or total cardiovascular outcome. This review consisted of 19 individual cohorts from 13 studies including 177,025 participants. The follow-up period for this in-depth study was 3.5 -19 years. During this period, a total of 11000 vascular events were reports and on analysing these data it was clearly evident that high salt intake led to greater risk of stroke and overall incidence of cardiovascular disease. Kristal et al, (2013) reviewed the role of salt intake accompanied by potassium intake on the cardiovascular outcomes. This systemic review included data from various randomised controlled trials and 52 publications focussed on the various adverse cardiovascular outcomes and salt intake from 1 January 1990 to 31 January 2013. Results revealed that high salt intake, not only increases blood pressure, but also plays a role in endothelial dysfunction, albuminuria and kidney disease progression, and cardiovascular morbidity and mortality in the general population. Moreover, positive effects of potassium intake were recorded and dietary potassium intake was important to reduce the adverse consequences showing a linkage to reduction in stroke rates and cardiovascular disease risk.

Other harmful effects associated with high salt intake include increasing risk of stomach cancer and osteoporosis (Hashem et al., 2014). Another aspect that needs to be considered in relation to increased sodium intake is its impact on calcium bioavailability. A high level of dietary sodium interferes with calcium bioavailability due to increased renal excretion of this mineral. It was reported that urinary excretion of calcium increases by 30-40 mg for each 2 g of sodium consumed (Cruz etal., 2011, Pereira et al., 2009).

In the light of this well-established data on adverse cardiovascular consequences associated with salt intake, extensive work has been carried out to focus on finding ways of reducing salt intake in the population. The UK has developed a voluntary salt reduction program (Hashem et al., 2014). Further, the Consensus Action on Salt and Health (CASH) has clearly stated that salt intake could be lowered by reducing the amount of salt added to processed foods and by reducing the salt used in cooking or at the table by 40 percent. To facilitate the implementation of the strategies, the Food Standards Agency has set a series of progressively lower salt targets over 80 categories of food (FSA, 2013, FSA, 2012). At the 66th world health assembly it was agreed that all countries should reduce their salt intake by 30 percent and work towards a target of 5 g/day by 2025 (Hashem et al., 2014).

1.12 Cheese consumption and salt intake

Approximately 75% of salt intake in the UK comes from commercially processed foods and, according to a National diet and Nutrition Survey, ‘Cheese’ is one of the top ten contributors for increased salt intake. An overview of statistics indicates that in the UK, milk and milk products account for 9% of salt intake and from this category of milk products, cheeses contributes to 44% of the salt consumption. Hashem et al (2014) investigated the salt content of different types of cheese sold in the UK supermarkets. The results revealed that the salt content of cheese was high, and it varied across different types of cheese and among different samples for the same type of cheese. These variations can be shown in table 1.7.

Table 1. 7 Comparison of salt levels in cheese varieties from supermarkets and branded varieties

	Supermarket (g/100g)		**Branded (g/100g)**	
Cheese type	**N**	**Mean±SD**	**N**	**Mean±SD**
Camembert	12	1.63±0.16	4	1.49±0.01
Cheddar/cheddar style	187	1.72±0.14	63	1.78±0.13
Other processed	14	2.48±0.38	8	2.01±0.44
Cheese spread	7	1.89±0.28	12	1.88±0.31
Cream cheese	14	0.68±0.24	5	0.86±0.13
Edam	12	2.38±0.28	4	2.01±0.28
Emmental	5	0.60±0.14	4	1.66±0.28
Feta	18	2.51±0.58	4	2.52±0.71
Goat's cheese	16	1.23±0.40	4	1.24±0.58
Mozzarella	18	0.65±0.33	4	1.16±0.35

Source: Hashem *et al* (2014)

In this context, it is essential to understand that salt is an integral part of cheese production. It has been reported that overlooking a few exceptions, cheeses generally contain 0.5-2% sodium chloride. However, the salt content of Blue types of cheeses is much higher (3-7%) (Cruz et al.,2011). Apart from these, there is also some work done on understanding the ratios of sodium chloride (NaCl) and sodium (Na) in different types of cheese. However, to understand the different ratios of salt in different types of cheese, it is essential to understand the exact cheese processing, cheese salting and the vital role played by salt in imparting different organoleptic and sensory qualities to the final cheese product.

1.12.1 Significance of salt in cheese processing

Salt is an inevitable part of cheese production. Salt is added to the cheese curd to inhibit the activity of the starter culture and to enhance the flavour and sensory profile of the final cheese. Literature (Cruz et al., 2011) has reported three different methods of salting.

(i) Immersion in brine solution consisting of sodium chloride with a predetermined concentration. This method of cheese salting is useful for Edam, Gouda and Swiss cheese.

(ii) Dry salting technique wherein salt is directly added to the formed gel prior to its molding and/or pressing. This technique is particularly implemented in the production of Cheddar cheeses and Stilton varieties

(iii) Surface salting, which involves addition of salt on the surface of the molded cheese and this method is applied during production of soft cheese varieties such as blue cheese.

The amount of salt added has been shown to influence the enzymatic activity during maturation and also enables enhancement of the sensory profile of cheese. One of the important roles of salt in cheese development is its impact on the microbial aspects of cheese. Salt plays a vital role in determination of water activity and indirectly affects the microbial growth, enzymatic and biochemical reactions during ripening (Guinne, 2004). Here it is important to note that the water activity (dependent on solutes) is specific to the type of cheese. For example, in fresh cheese varieties, water activity is predominantly associated with high concentrations of sodium chloride in the aqueous stage whereas in hard cheeses, low molecular weight peptides and free amino acids contribute towards lowering the water activity. Picciinali et al., (2012) have elucidated the mechanism, which gives better clarity regarding the impact of salt on microflora in the curd. Accordingly, increase in salt concentration reduces the speed of proteolysis followed by decline in microbial and enzyme reaction and this in turn has considerable impact on the process of ripening and flavour development. Additionally, salt also contributes towards optimal development sensory attributes of cheese including consistency, taste, texture and aroma. In terms of taste or aroma, the concentration of salt is crucial as both, extreme salt reduction or excessively high levels of salt, have shown to present an unpleasant perception of taste. Schroedor et al., (1988) studied the impact of salt reduction in Cheddar cheese varieties. It was observed that there were no detectable changes in Cheddar when the salt content was reduced from 4.1% to 3.1%. However, when the levels were reduced to 0.7%, the cheeses became excessively viscous with acidic, sour and exhibited unpleasant residual flavour owing to increased proteolysis. This finding not only justifies the significance of optimal levels of salt in cheese production, but also highlights the potential role of salt in maintaining appropriate cheese consistency. It has been reported that hard cheeses become crumbly or lose the required consistency in presence of extremely high levels of salt. This is attributed to the displacement of calcium in casein-calcium phosphate complex by sodium (Piccinalli et al., 2012).

Salt also has been shown to have an impact on weight loss during cheese production. There is a direct relationship between duration of brining and the weight loss of cheese during production. The longer the duration in brining solution, the greater is the loss in weight, however there is limited work in this area which has reported that the overall weight loss is less as the extent of the weight loss mechanism is reduced towards the end of the ripening period. Another action of salt on cheese is that it draws moisture from the surface of cheese and this leads to the formation of rind. This influence of salt on the rind formation has been reported (Piccinalli et al., 2012) wherein Appenzeller (type of swiss cheese) was dipped in brine for a different duration to understand the effects of high levels of salt on rind formation. Faults such as rough, spotted surfaces or a rind with swellings were observed on the cheese wheels that were dipped in brine for 18 hours and smeared with unsalted or lightly salted water. Conversely, the cheese samples, that were dipped in brine for three days and smeared with very salty water (15% NaCL) produced a cheesy smear without any faults on the surface of the rind. This implies that a longer duration in brine solution, accompanied by smearing with high levels of salt, is beneficial for optimal rind formation.

In light of regulations for reducing the sodium intake, several studies have explored using KCl (potassium chloride) instead of NaCl. In some cases, conflicting results have been observed in terms of the effect of replacing NaCl with KCl. Flatcher (2008) reported that KCI helped to maintain the salty taste and replacement of NaCl with KCl could potentially reduce the sodium content of food up to 25% without affecting the sensory acceptability of the food. In contrast, Guinne, (2004) warned about careful use of KCl at higher concentrations (>1%) indicating increased perception of sourness, increase in proteolysis, acidity, water activity and adverse decrease in firmness of the cheese samples. Collating all the information related to the problem of iodine deficiency coupled with health concerns due to increased salt consumption, regulations to decrease sodium intake and the requirement of having a commonly consumed food product for fortification has led to explore the potential usefulness of replacing regular salt with iodised salt in cheese production. However, there are various barriers and challenges for successful iodisation.

1.13 Challenges and barriers for salt iodisation

Iodine intake through iodised salt can be one of the inexpensive, non-invasive methods, however there are certain limitations attributed to the sensitive, unstable nature of iodine in various systems. Salt iodisation can be a useful technique for improving iodine status of the population, however the stability of iodine in salt needs to be considered. WHO and FAO (2006) have highlighted that the stability of iodine in salt depends on the water content, acidity and purity of the salt to which it is added. Another crucial aspect that needs to be considered is the losses of iodine during storage. Due to this, the salt must be dry, as pure as possible and this salt should be stored in appropriate packaging. During selection of packaging material, it is important to note that iodine as an element tends to migrate from the top to bottom of the container when the water content is high. Also, rapid evaporation of iodine takes place when the acidity is high. Possible interaction of iodine with the material of the packaging needs to be noted. If packaging consists of impervious linings, then there are higher chances of packaging material becoming damp and humid thereby initiating the mechanism of iodine migration from the salt to the fabric and subsequent evaporation of iodine. Eastman and Zimmermann (2018) have also confirmed the association between the type of packaging and iodine losses. According to them, jute bags have been used extensively for storage of salt, but this is a concern in humid climatic condition. This is because in humid conditions, salt tends to absorb lot of moisture and consequently the iodate could dissolve and if the storage bag is porous this salt solution will drop out steadily. In a multi-country study, iodine losses from salt due to combined effect of humidity and porous packaging (jute) have shown to be in range of 30-80 % over a period of six months (Eastman and Zimmermann., 2018, Diosady et al., 1998). It has been suggested that high density polyethylene bags or bags lined with continuous film that are resistant to damage could help in prevention of iodine losses (WHO and FAO, 2006).

Apart from instability and sensitiveness to packaging, another concern regarding use of iodine in salts or processed foods is its uncertainty of retention in these food systems. WHO (2001) has presented typical iodine losses in different circumstances and according to this iodine lost from salt is 20 percent from production to household. Another 20 percent is lost during cooking before consumption. Working on similar lines Goindi et al., (1995) studied 50 different Indian recipes prepared in a hospital kitchen using different cooking procedures. Authors reported that iodine losses ranged from 6 to 37% depending on the cooking technique. Accordingly boiling was found to cause 37% average loss while steaming, deep frying and pressure cooking resulted in 20 % loss of iodine. Shallow frying leads to 27 % iodine losses whereas the least percent loss was observed during roasting (6 %). These iodine losses indicate that the method of cooking can have considerable impact, though the duration of the cooking method responsible for the indicated losses has not been specified. This aspect of the impact of cooking method on the percentage of iodine losses was confirmed by yet another Polish study (Waszkowiak et al, 1999), wherein the meatballs containing iodised salt resulted in only 5.5% iodine loss when they were cooked using hot air as opposed to frying leading to 31.7% loss. Steaming vs boiling demonstrated 42.9% and 64.9% of losses respectively. In contrast to these observations, Chavasit et al., (2002) denied the impact of specific method of cooking for the losses but highlighted that the amount of iodine lost is dependent on the cooking utensils used and the presence of other ingredients such as reducing sugars, food additives and acidulants. WHO (2001) recommended that these losses during various processing techniques should be considered while deciding on the iodine concentration that is to be used for fortification in population settings. Accordingly, the iodine concentration at the point of production should be within the range 20-40 mg of iodine per kg of salt (20-40 ppm of iodine). These levels are crucial to meet the daily requirements of iodine (150 µg). Since the added iodine will be either potassium or sodium iodate, the median urinary iodine levels will vary from 100-200 µ/litre. Iodine fortification levels differ substantially. In European countries, levels range from 8-69 mg iodine per kg salt while in some African countries (Kenya, Guinea-Bissau) levels are as high as 100 mg iodine per kg salt. Other confounding factors that could limit optimal salt iodisation include variability in the amount of iodine added during the iodisation process and uneven distribution of iodine in iodised salt within batches and individual bags (Diosady et al., 1998).

Furthermore, hurdles in implementation of salt iodisation policy or adopting USI (Universal Salt Iodisation) are not limited to the physical or chemical properties of iodine or salt. There is an unsatisfactory legal framework and there are no specific guidelines for iodine fortification of foods. There are certain stringent national regulations, which limit the free movement of iodised salt and food products containing iodised salt across national borders. Although several health claims on iodine as a nutrient has been approved by European Food Safety Authority (EFSA), there is no regulatory framework that would permit harmonised communication regarding the purpose and the need to consume the added nutrient (Lox, 2017). Adding to the absence of clear legislative policy for fortification, there is resistance from manufacturers as well as consumers. Consumer's scepticism stems from various reasons such as dislike for food additives, a belief that there is no need for added nutrients when a balanced diet is consumed, a perception that the addition of iodised salt could lead to changes in taste or colour of the food. Resistance from manufacturers relates to several factors. Apart from the concerns regarding consumer acceptability, there is an understanding that this could lead to increased cost of production if some foods contain iodised salt while others were manufactured using regular table salt. Other reasons for reluctance towards use of iodised salt include lack of appropriate awareness about the significance of iodine, limited resources, lack of technical expertise, potential equipment and process overhauls and limited availability of iodised salt (FCN, 2013).

1.14 Legislation policies in the UK and different countries

Legislation status for use of iodised salt for household, bakeries or the food industry as a whole has been defined differently by various authorising bodies. WHO, UNICEF, ICCIDD (now called Iodine Global Network) are the 3 primary authorising bodies involved in reviewing literature regarding legislation existing in different individual countries in the world and subsequently creating a database which is updated at regular intervals.

Over the years, various research groups have published literature on legal specifications for fortification of various food products in different countries in efforts to establish acceptable levels of fortification and using different food vehicles to solve the problem of nutritional deficiencies. A review by Just M.de Jong in August 2007 was focussed on use of iodised salt and clearly listed the legislation status of various countries along with highlighting the barriers for fortification. However, in the light of new data, it was observed that the status of certain countries was updated to either voluntary or mandatory. However, there was lack of clear specification for categorising a certain country as having mandatory or voluntary legislation. Also, universal salt iodisation was not explained clearly with countries such as China, where USI was implemented in 1995. Another term that needs to be understood is “national legislation with Presidential decree” which was introduced in Indonesia in 1994. This states that there must be mandatory iodisation of all the salt available for human consumption. However, there is no clear differentiation between USI and Mandatory regulation.

The need to clearly define the use of terms like mandatory, voluntary, USI, presidential decree was understood when a review paper by Ohlhorst et al., (2012) states that there is voluntary legislation in Pakistan but USI is implemented. This information was updated in a review by UNICEF in July 2015, which states that there is provincial USI implementation and mandatory legislation in Pakistan and this greatly helped in interpretation and implementation of the legislation. Further evidence of discrepancy in the interpretation of legislation can be identified in the presidential decree legislation which mandates all salt consumed must be iodised but does not include use of iodine in livestock or animal feeds, which is a part of definition of USI (July 2015). This implies that there could be mandatory legislation, but not necessarily USI.

An updated review on national legislation for universal salt iodisation (USI) for South and East Asia and Pacific was published by UNICEF in July 2015. According to this, Universal Salt Iodisation is defined as Iodisation of all human and livestock salt, including salt used in the food industry. Another issue for salt iodisation programmes is whether fortification is required on a mandatory basis or not. To obtain more clarity, the WHO and FAO ‘Guidelines on food fortification with micronutrients’ have explained that “the fundamental distinction between mandatory and voluntary regulation as it applies to food fortification is the level of certainty over time that a particular category of food will contain a pre-determined amount of a micronutrient. By providing a higher level of certainty, mandatory fortification is more likely to deliver a sustained source of fortified food for consumption by relevant population groups and in turn, a public health benefit”. It has been further stated that household coverage with mandatory legislation is higher (49-72 %) as compared to voluntary legislation (40-49 %). However, despite this, the consumer demand of fortified food products is not sufficient to achieve universal consumption. Moreover, from this discussion, it is clear that the definition of mandatory legislation has been discussed in terms of fortification of various products and not about iodisation of salt or use of iodised salt in food processing.

Voluntary fortification of food products should be defined more clearly, by providing more precise guidelines and specifications for the type and amount of iodine added to products, and whether or not this includes the use of iodine in salt, food processing by food industry and in animal feeds.

In the context of interpreting the legislation, the difference between Mandatory and USI can be clearly seen in countries like Mongolia, Sri Lanka, Thailand and Vietnam, these are the countries where there is mandatory legislation but no USI. In examples like Malaysia, it has been stated that there is mandatory legislation in only two states (Sabah and Sarawak), but there is no USI and legislation for the whole of Malaysia is under development. There is another perspective towards USI when it is discussed in terms of existing legislation in Pakistan. Pakistan has been categorised as a country with USI and mandatory status, but this USI is prominently termed as 'Provincial Legislation as a USI for complete Pakistan' and is still in draft awaiting approval. Khyber Pakhtunkhwa and Baluchistan have the Pure Food Act to ban non-iodised edible salt. Gilgit-Balistan has the IDD Control Act (2011) and Sindh province has the Compulsory Iodisation of Salt Act.

Apart from these 3 ways of identifying legislation in different countries, the UNICEF review also sheds light on 3 types of existing legislations specifically in terms of iodisation of salt.

1. prohibits non-iodised salt or allows only iodised salt
2. requires the iodisation of all salt within the scope of the legislation
3. guides the production of iodised salt but allows non-iodised salt.

There are 12 countries that adhere to the first type of legislation thereby explicitly prohibiting the use of non-iodised salt or allowing only iodised salt to be produced, sold or traded. The wording of this law is varying considerably in different countries e.g.: In India's legislation states – 'Prohibition of sale of common salt, for direct human consumption unless the same is iodised' whereas in Mongolia it says 'Only iodised salt shall be marketed in the territory of Mongolia'. Here it becomes important to understand the underlying meaning while interpreting the legislation.

Furthermore, in certain countries, there is no specific legislation, but the Food Laws or Food Acts have incorporated the concept of iodisation of salt making and depending on this food law or act there is either mandatory or voluntary use of iodised salt. For example, in Singapore there is voluntary legislation for use of iodised salt, but it is a part of the Food Act and not a national legislation.

In this context, there are 4 ways of describing the salt iodisation legislation. These include

1. Stand Alone legislation
2. Under existing law
3. Food Standard
4. Food Act

UNICEF has explained these terms by providing examples about its functionality in various countries. Sri Lanka has issued 'Iodisation of Salt Regulation' under the Food Act. This specifies the requirements for 'iodised, edible common salt' including iodisation levels, specifies packaging and labelling requirements and states "no one may manufacture any type of edible common salt other than iodised or iodated common salt for purposes of human consumption". India and Malaysia are another two countries where the specifications for salt iodisation have been made part of the Food Standards and Food Act respectively.

1.15 Rationale for the present study

In view of the above literature, there are two emerging public health concerns including the emergence of iodine deficiency and concern regarding higher salt intake. Whilst the conflict between salt reduction and iodine salt fortification is noted, salt is an integral ingredient in some food items. Therefore, if commonly consumed food items in the UK are studied for their iodine content it might be possible to identify possible foods that require a certain level of salt and, therefore, could be a target for the use of iodised salt. Moreover, this also agrees with the literature, which states that addition of iodine should be limited to already fortified foodstuffs like salt. This was observed in a study conducted by Charlton et al., (2010) wherein 78 non-pregnant women aged 20-55 year were given a questionnaire to understand their attitudes regarding salt iodisation, consequences of iodine deficiency and also about the strategies that could be used to address iodine deficiency. In this regard, 81% supported the idea of use of salt as a potential vehicle for improving iodine intake of the population. Through examination of food consumption patterns in the UK, it was identified that cheese is one such product which forms an important part of the UK diet (Hashem et al., 2014). ‘Salting’ is an essential step in cheese production (Cruz et al., 2011). So, if the salt used in cheese production is replaced by iodised salt then this fortified cheese can be a useful food vehicle for improving iodine intake without increasing the amount of salt.

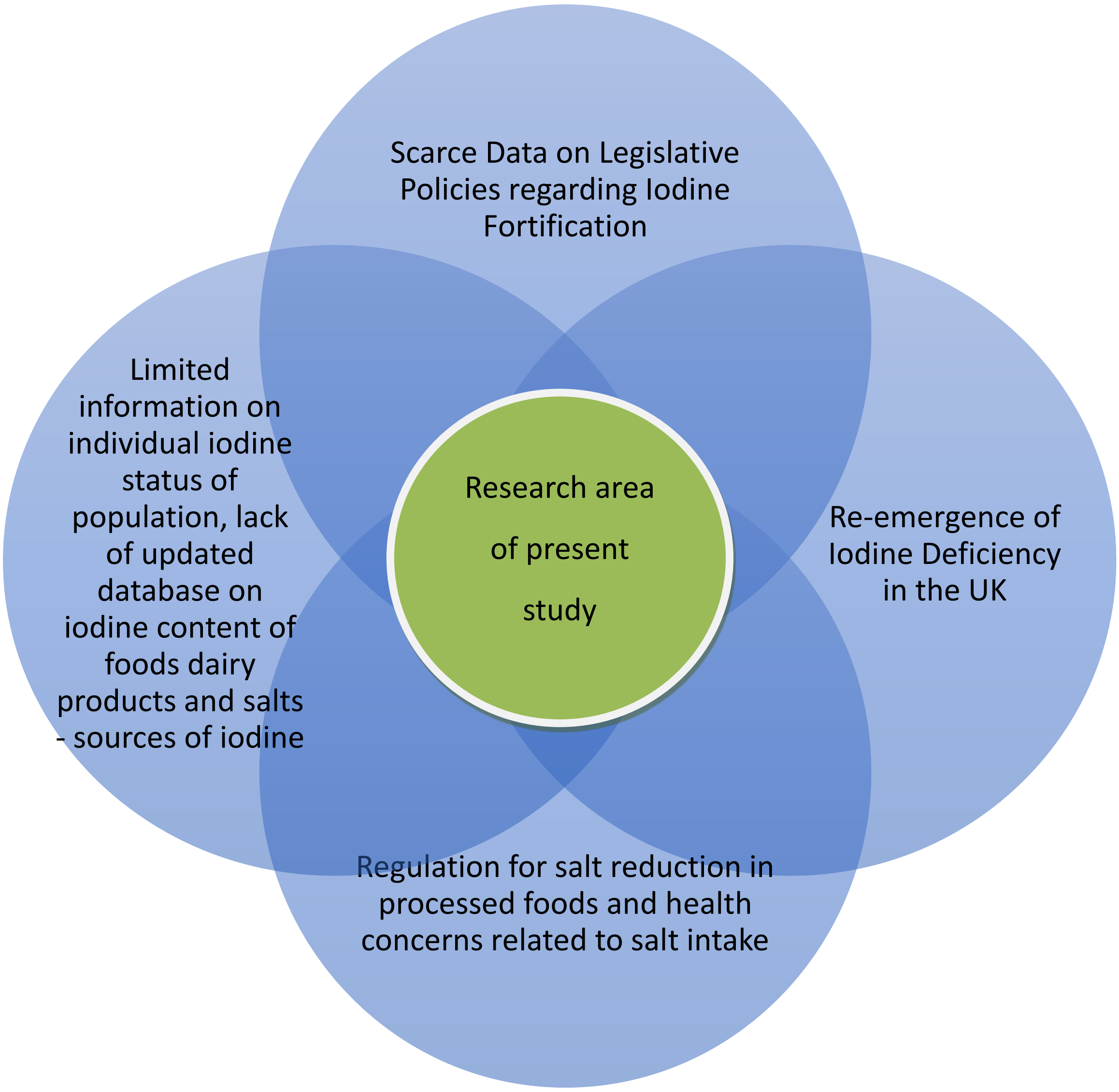


Figure 1. 4 Conceptual framework of the study

1.16 Aims of the study

1. To measure iodine content of the different categories of cheese (as defined by the British Cheese Board), as well as milk and salt available in UK supermarkets and to map this information, in relationship to the country of origin of the product.
2. To examine the relationship between iodine content and the legislation for iodine fortification in the country of origin for each cheese analysed in aim 1.
3. To explore the knowledge attitude and practices (KAP) of general consumers and obtain views on iodine fortification and use of iodised salt in food products.
4. Explore the impact of substituting non-fortified salt with iodine fortified salt in the production of cheese (a product where salt is integral to the recipe formulation), both in terms of the practical aspects of the manufacturing process (for example, cost and logistics) and in terms of the sensory quality and iodine content of the product.

1.17 Objectives for the study

(i) To develop and validate a laboratory method to measure iodine concentration in food products, in particular cheese, milk and salt.

(ii) To collect cheese samples that are representative of different categories of cheese and countries of origin available in the UK supermarkets and analyse them for their iodine content by using the validated method.

(iii) To develop and pilot KAP questionnaire

(a) Questionnaire-I - designed to explore the knowledge, attitudes and practise of general consumers regarding the health benefits of iodine enriched foods, consumption of iodine fortified foods (for example salt), awareness about the consequences of iodine deficiency.

(iv) Obtain information about attitudes of general consumers and food manufacturers regarding fortification of food with iodine, using the developed questionnaire

(v) To manufacture a new cheese with iodine- fortified, and non -fortified salt, and analyse it for its iodine content and to evaluate the cost of producing this cheese compared to regular cheese.

(vi) To undertake sensory evaluation of newly developed cheese to gain understanding of acceptability of cheese prepared using iodised salt.

Chapter 2: Methods

2.1 Overview of the chapter

The present study was developed in the light of growing evidence of iodine deficiency in vulnerable populations (teenagers, pregnant women and women of child -bearing age group) in the UK and the limited information available on iodine content of food products. The potential for the use of iodised salt in cheese making as a vehicle for increasing iodine intake was explored.

There were four specific aims identified for the present study. An appropriate blend of quantitative and qualitative methods were utilised in the process of accomplishing these specific aims.

The first two aims of this study were inter-linked in terms of the process of data collection and analysis. Firstly, using a qualitative approach, a detailed scoping exercise was conducted to identify different varieties of cheese available in UK supermarkets following a three step sequential pattern.

The available information (different categories of cheese, country of cheese production, country of production of milk and existing legislation in relation to iodine fortification, use of iodised salt in food manufacturing) was analysed and this formed the base for selection of cheese varieties for subsequent parts of the study. The selected cheese varieties were analysed for their iodine content using a well-established laboratory based quantitative method. Furthermore, this iodine content was again plotted graphically against legislative policies in the country of origin of the cheese varieties, thus integrating qualitative and quantitative aspects of the specific research aim.

The third aim of the study was to explore the knowledge attitude and practices (KAP) of general consumers and obtain views on iodine fortification and use of iodised salt in food products. This was accomplished by developing a questionnaire and analysing the responses of the participants (n=506) by grouping of responses and understanding the emerging themes.

There were two distinct parts for the final aim of the study. In the first part, the newly developed cheese varieties (cheddar and soft cheese with two different salt variations – based on information obtained in consumer survey) were analysed for its sensory acceptability using a questionnaire. The responses were analysed statistically to identify differences in preferences for various sensory attributes. In the second part of this aim, the iodine content of these newly developed cheese varieties was measured using a quantitative approach.

This chapter provides a detailed account of the methodology adopted in order to accomplish individual aims of the study.

2.2 Ethical Approval

The ethical approval for the study was granted in two phases (phase 1- for conducting consumer survey and phase 2 – for conducting sensory evaluation of newly developed cheese varieties) by the STEMH (Science, Technology, Engineering and Medicine) ethics committee at University of Central Lancashire (appendix 1 section 1.1 and 1.2)

2.3 Sources and databases for development of literature review

The topic for the research project was developed after identifying the gaps in knowledge and literature around iodine nutrition in the UK. In the initial stages of reviewing and collation of literature from various sources, it was ensured that the keywords used for the search were broad, such as iodine, iodine deficiency disorders, iodine requirements and population studies. This enabled the researcher to obtain a substantial amount of information regarding iodine deficiency, related consequences and different strategies adopted in different countries across the globe. It was found that iodine deficiency is not only prevalent in developing countries but also in some developed countries. Databases such as Google Scholar, Discovery search engine (university's database), Lancet journal articles and Science Direct were used for finding literature pertaining to specific topics such as iodine deficiency and cognitive health, iodine requirements during pregnancy, infancy. The information about the significance and recommended nutrient intake of iodine at different life stages (during pregnancy, lactation, infancy, adolescence and adulthood) was obtained from scientific journal articles (for example articles by Zimmermann ad his research team published in journals such as American Journal of Clinical Nutrition, Endocrine Reviews, Thyroid, Neuroendocrinology or articles by Rayman and Bath in Proceedings of Nutrition Society)

While conducting this search, the researcher ensured that the papers used for collating literature were not older than 10 years from the present date except for specific definitions or for understanding different incidents which helped the researcher in understanding the history and timeline for iodine deficiency in the UK.

After obtaining general information using the above-mentioned databases, a rough structure of thesis was prepared to ensure that several health and nutrition aspects related to iodine status and use of iodised salt in manufacturing were covered in appropriate depth.

The first part of the literature covered physiological significance of iodine for optimal health. It was observed that there were some key authors such as Prof. Michael Zimmermann, Bath and Rayman and their team at university of Surrey, Vanderpump and team who have been regularly publishing and updating information about the prevalence of iodine deficiency in the UK. The cross references reported in the papers published by these key research groups were also studied critically and incorporated in context of the present research. The second part of the literature focussed on scoping and mapping of cheese varieties available in different popular supermarkets. The official websites of popular supermarkets such as Morrisons (groceries.morrisons.com), Sainsbury's (www.sainsburys.co.uk) and Tesco (tesco.com) were searched thoroughly and information about name, variety and country of origin of cheese samples was mapped for further analysis. The cheese types available in different supermarkets were classified according to the classification provided by the British Cheese Board (www.cheeseboard.co.uk). The information about legislative policies regarding iodine fortification of foods and use of iodised salt in food manufacturing in different countries across the globe was obtained by conducting detailed search of all the scientific papers (Olivieri et al., 2017, Nystrom., 2016, Licha, 2015, Nazaimoon and Rufifah., 2010, Kunachowicz et al., 2002, Delange et al., 1993). In this search, papers older than 10 years from the present date were also included. This is because, it was important to understand how the policies and the permissible level of fortification of iodine have changed over the years. Furthermore, scientific newsletters published by Iodine Global Network (formed of three authorising bodies namely WHO, UNICEF and ICCIDD) and British Dietetic Association were also reviewed to obtain thorough understanding of the on-going discussions about different challenges related to implementation of iodine fortification policies in different countries in relation to its impact on the iodine status of the population.

The last past of the literature was focused on food sources of iodine, concern regarding salt intake, and food vehicles for iodine fortification. A database published by Fordyce (2003) was useful to obtain information about iodine content of milk and milk products. However, it was not an updated database and so more updated information was obtained from a journal article by Haddow et al, (2005). Moreover, BDA (2016) published fact sheet (www.bda.uk.com/food facts/iodine pdf) and Iodine leaflet published by Dairy Council UK were other valid and updated sources for obtaining information about iodine content of foods. A joint report by FAO and WHO (2006) and some reports by WHO (1991, 1994, 1996, 1999, 2001, 2006) were found to be useful for obtaining authentic and scientific information about iodine deficiency disorders in different parts of world and helpful strategies relating to iodisation of salt, recommended iodine levels and food vehicles for fortification. Journal articles published by key authors such as Christopher and Wallace (2014) and Cruz et al, (2011) provided substantial information about concern around salt intake and related health consequences. Reports published by CASH and FSA (2013) were other sources of accurate and updated information on regulation regarding reduced salt intake. The book Home-made cheese by Paul Thomas, Lorenz books (2016) was found to be good E-book for information about different roles of salt in cheese manufacturing.

2.4 Cheese mapping exercise - to achieve objectives (i) and (ii)

The cheese-mapping objective was accomplished by conducting an in-depth exercise encompassing a variety of smaller objectives thus enabling the researcher to achieve one of the primary aims of the study.

The flowchart below clearly depicts the multi-dimensional nature of this scoping activity

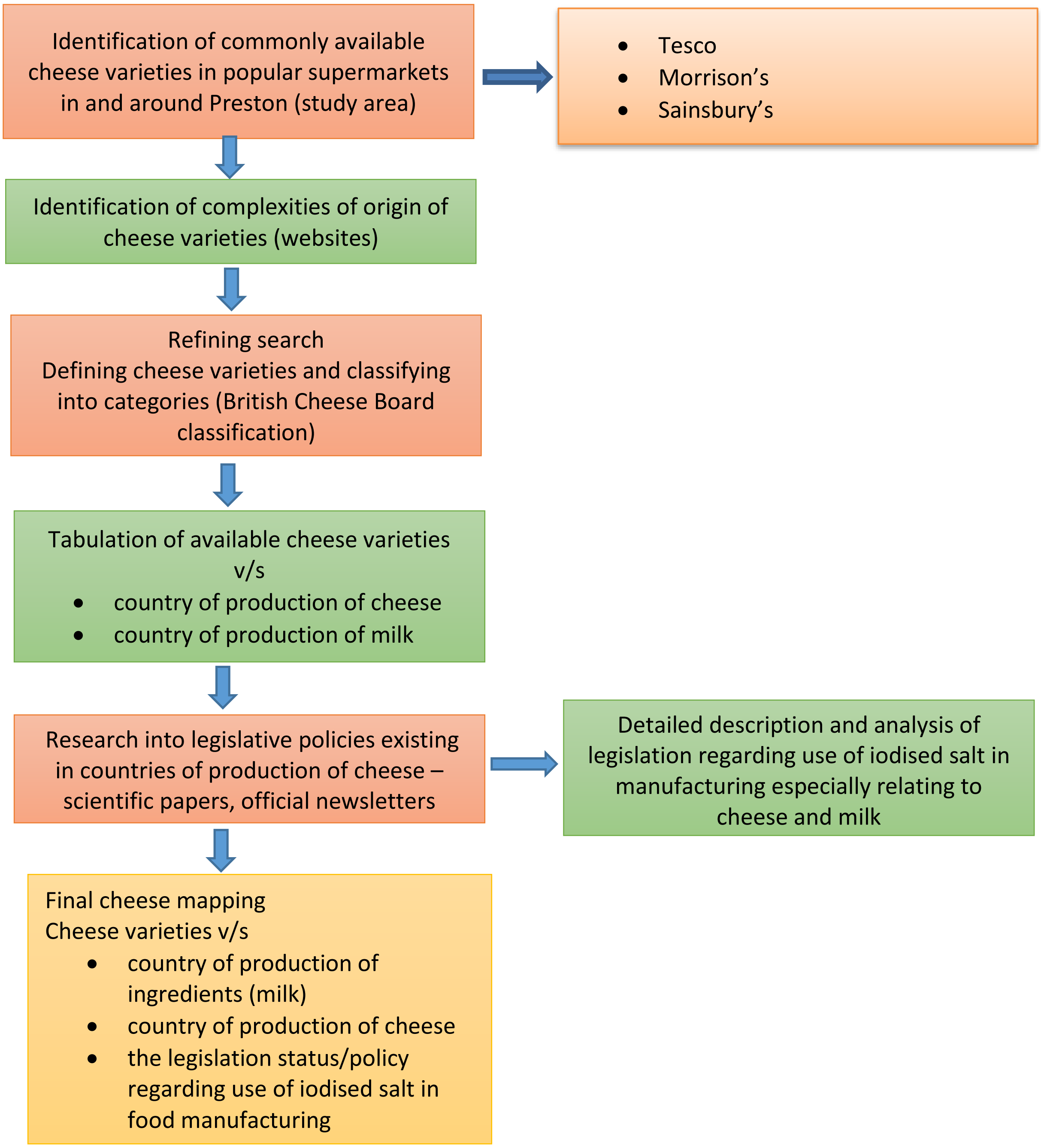


Figure 2. 1 Flowchart of cheese scoping

An exhaustive search of supermarket websites including tesco.com (2015), morrisons.com (2015); Sainsbury's.com (2015) was conducted in order to identify the different types of cheese varieties available in the UK and in other European countries. This global scanning of cheese varieties was vital, as apart from the cheese varieties that are manufactured in the UK, many varieties available in the UK supermarkets are either produced in other countries and packed in the UK or are imported from different countries depending on the demand and popularity of these varieties among the UK population. For example, Mature Cheddar cheese varieties available in some supermarkets are produced not only in the UK, but there are some cheddar varieties like Irish Cheddar from Ireland, Farmhouse Vintage cheddar from Canada or hard cheese produced in Italy using EU milk. This information highlighted the complexities related to differences in origin of cheese and its ingredients.

Cheese classification – There are different methods of classifying cheese varieties based on a number of criteria as follows

- the origin of the milk
- moisture content
- specific moulds and bacteria added
- varying lengths of ageing. (www.sciencelearn.org. accessed on 18 September 2018)

Different scientific bodies have categorised cheese according to these attributes. For example, the British Cheese Board (BCB) classifies cheese varieties based on their texture and the style of manufacture.

BCB is an educational body within Dairy UK, having information about over 700 named British cheeses produced in the UK. The board has members, which include small-scale to large-scale cheese makers, covering most parts of Great Britain and thus contribute to significant proportion of cheese made in the UK. Due to this, the information available from this database is constantly updated, reflecting the current practices related to cheese making. Therefore, this classification given by the British Cheese Board (BCB) was chosen.

To identify the cheese varieties available to the consumers in the UK, the official websites of 3 major National supermarkets namely Tesco, Morrisons, and Sainsbury's were researched. Relevant information relating to available cheese varieties, name of country of origin, country of production of cheese as well as its ingredients and any other cheese specific information was recorded. Once this information was obtained, a short list of available cheese varieties was compiled. Abiding to the method of classification given by BCB (based on the texture and the manufacturing style), they were sorted into seven different categories namely Fresh cheese (e.g. Cottage cheese, Mozarella), Soft cheese (e.g. Brie, Camembert), Semi-hard cheese (e.g. Port Salut), Hard cheese firm (e.g. Cheddar, Parmesan), Hard cheese crumbly (e.g. Cheshire, Caerphilly), Blue cheese (e.g. Shropshire blue, Blacksticks blue) and Blended cheese (e.g. Double gloucester with chives, Lancashire with garlic).

The available cheese varieties were then sorted against the existing policies regarding iodine fortification in countries of origin of cheese and its ingredients. To accomplish this objective, a thorough literature search for policies regarding iodine fortification of foods and use of iodized salt in food manufacturing existing in different countries across Europe was carried out. In this context, the older research papers (10 years older from present) were also included (Olivieri et al., 2017, Nystrom., 2016, Licha, 2015, Nazaimoon and Rufifah., 2010, Kunachowicz et al., 2002, Delange et al., 1993). This gave the scope to understand how the policies regarding fortification have changed over time. It was identified, that there are certain discrepancies in the terminologies used to describe the status of legislation (e.g. voluntary, mandatory) in different countries. This research exercise also brought out some challenges regarding implementation of these policies. So due to several confounding factors in interpretation of legislation related to iodine fortification of foods and probability of changing policies over a period of time, the cheese samples and their ingredients (milk) obtained from the different countries were mapped against the policy stated in the most updated scientific paper or authentic scientific newsletters. In this context, the confounding factors and possible discrepancies were tabulated simultaneously for valid interpretation of the information.

Considering all these criteria, a detailed table was formulated using an Excel Spreadsheet incorporating the list of commonly available and popularly consumed cheese varieties in the UK, mapped against the legislative policies for iodine fortification in the countries of origin of cheese and its ingredients. These specific varieties of cheese (the ones identified by the questionnaire) were then analysed for their iodine content in the laboratory.

2.5 Development of a quantitative method for analysis of iodine content of cheese

The process of development of a method for analysis of iodine content of cheese involved a series of trials adapting different approaches to formulate a method which would be valid, reliable and the results could be easily reproducible. For the purpose of the present research, the standard operating procedure (SOP) was requested from the original group of researchers (Michael Zimmermann and his team from Zurich, Switzerland) and was adapted to determine the iodine content of the commonly consumed varieties of cheese in the UK (details of the trials conducted at UCLan are given in the appendix 3, 3.1).

Principle - the iodine content was determined by using the ability of iodide to catalyse the reduction of yellow Ce(IV) to colourless Ce(III) in the presence of arsenious acid. The rate of colour disappearance is directly proportional to the iodide concentration:

$$2\,Ce^{4+} + As^{3+} \xrightarrow{I^-} 2Ce^{3+} + As^{5+}$$

(Source: Ohashi et al, 2000)

Challenges in the trials during development of the method

Many experimental runs over a period of 4 months were conducted to achieve an appropriate standard curve, which could subsequently form the basis for determination of iodine content of cheese samples. However, even after several trials it was not possible to obtain a consistent standard curve. The speed of the reaction taking place during the final stage of the analysis (Sandell-Kolthoff reaction) from yellow to colourless was faster than expected (28minutes). So, the change in colour reaction in the microplate was monitored and the plate was read at different time intervals (3 minutes, 5 minutes, and 10 minutes). In-depth discussions were conducted and the procedure for analysis was reviewed critically with the supervisory team as well as with one of the researchers contributing to developing the original SOP. Several modifications were made to overcome the challenges. Efforts were made to eliminate all possible sources of contamination.

A separate trial was conducted to test the purity of water to ensure that the nanopure water (18.2MΩ cm) was free of any traces of iodine. All the glassware was acid washed overnight and air-dried and stored in airtight plastic containers before use. All the chemicals were freshly made before the analysis. The template for pipetting standards and samples on to the well-plate given in the original method was modified. The shaking step (28 minutes) before the analysis was eliminated.

From these observations, it was apparent that the method was extremely sensitive and not reproducible in a wide range of settings. Therefore, in the process of identifying a more reliable method for iodine analysis, it was decided that these cheese samples should be analysed at a commercial laboratory to obtain the most accurate, reliable, reproducible results.

2.5.1 Determination of Iodine content of cheese samples at Fera Labs

Fera Science Limited – a research institute and a commercial laboratory was selected for iodine analysis of cheese samples for the present study.

Fera Science Ltd based in Sand Hutton, York (UK) is a science-based organization that works across the agri-food supply chain and is a national and international center of science excellence for interdisciplinary investigation and problem solving across plant and bee health, crop protection, sustainable agriculture, food and feed quality and chemical safety in the environment. They also collaborate with food companies, manufacturers and academic institutions for different laboratory-based projects. Fera labs are also regarded as a National Reference Laboratory in nine different areas. The food testing experts at Fera perform extensive mineral and trace element testing of food samples accredited to ISO17025.

While selecting the cheese varieties for iodine analysis, two main aspects were considered

Firstly, some cheese samples should belong to countries of origin where there is mandatory legislation status for iodine fortification and some should be from countries with voluntary legislation (List of countries is given in mapping diagram in chapter 3). Secondly, these cheese varieties were popularly consumed and available in the UK supermarkets (this information was derived from the consumer survey explained in chapter 4). Accordingly, a total of 30 samples, including 25 cheese samples, 4 salt samples and 1 milk powder sample were tested for their iodine content at Fera Labs, York (table no. 2.9). Salt and milk samples were selected for analysis as these were dietary sources of iodine and so it was important to know about iodine content of different salt and milk samples available in UK supermarkets.

Outline of the method adapted by Fera Labs

The iodine content was measured quantitatively using inductively coupled plasma-mass spectrometry (ICP-MS). To ensure that the results obtained by the method used for iodine analysis were valid, two certified reference materials (CRM) with known iodine content (3.38 mg/kg, 1.78 mg/kg respectively) were run simultaneously and these were treated in the same way as the other samples.

Here it is important to note that in this method, the reference materials were identified as follows

Certified reference material (CRM) - NIST 1549, Non-Fat Milk Powder (U.S Department of Commerce, National Institute of Standards and Technology, Gathersburg MD)

Proficiency test material (PTM) - ERM-BD151 Skim Milk Powder

Additionally, reagent blanks (n=4) were run at appropriate intervals.

Sample recovery test - For conducting sample recovery, iodine solution with concentration of 10 µg/ml was prepared and this solution was used to prepare an iodine-spiked solution. Spiked solution was prepared by adding 0.2 ml of this iodine solution to 0.5 g of sample.

<u>Preparation to obtain representative sample for analysis</u>

For each sample the entire sample was placed on to a clean plastic chopping board and cut into 6 roughly equal portions using a clean ceramic/stainless steel knife. The was done to obtain a representative sample from the entire block of cheese. Subsequently, from this, 3 pieces were selected and roughly homogenised in a food processor to achieve as small a crumb size as possible. The remaining sample was archived in case of any problems related to contamination or analysis.

<u>Sample Preparation for digestion</u>

0.5 g of sample/CRM/PTM/reagent blank/spiked solution was weighed accurately in acid washed plastic tubes. 2 ml of tetramethylammonium hydroxide (50% TMAH) and 3 ml of ultrapure water (18.2 MΏ) were added to the samples so that the total volume of mixture was 5 ml. This 5 ml mixture was subsequently vortexed (VWR Analog Vortex mixer, Fisher

Scientific, UK) and digested on the heating block (Grant QBT4, Grant Instruments, Cambridge UK) at 80°C for 4 hours (using attached timer for accuracy).

Once digested, the samples were allowed to cool down to room temperature. It is important to note that these digested aliquots are very stable and can be stored at room temperature (if required) for about 2-4 weeks before the actual analysis.

Preparation of Standard Curve

Iodine standard solution was used to prepare 7 standard dilutions (S7-S1) for plotting a standard curve. Initially a top standard (S7-200mg/ml) was prepared. For this, 1 ml of 10 µg/ml iodine solution was diluted to 10 ml with 10% TMAH giving the final concentration of 1 µg/ml. Subsequently 7 serial dilutions were made from this solution and values obtained were plotted graphically (figure 2.2.)

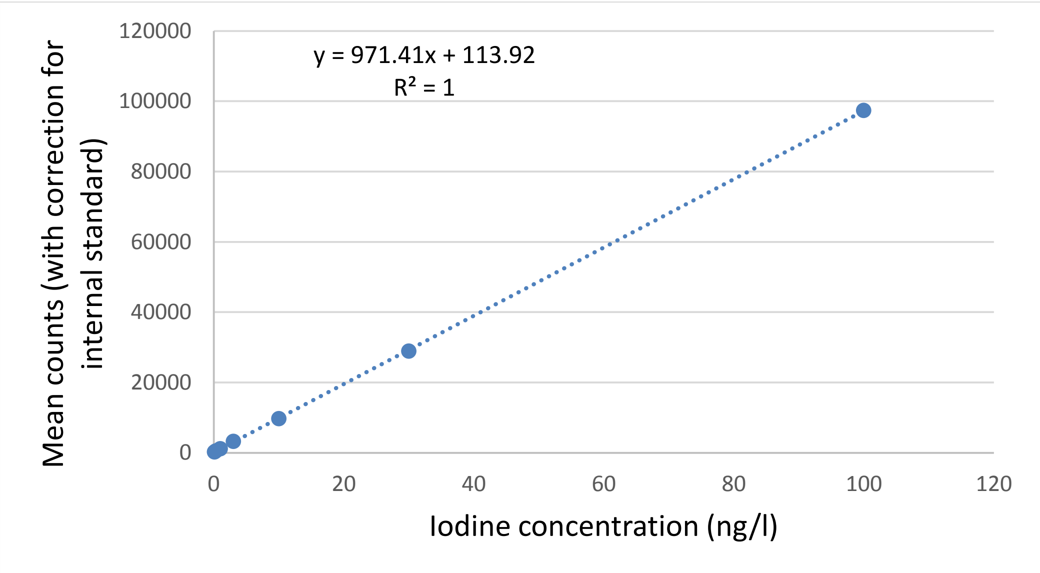


Figure 2. 2 An Example of a Standard curve for iodine using method by Fera Labs

Addition of Internal Standard – At the end of the digestion period, the samples were cooled down to room temperature and then a precise volume of the internal standard solution was added to each sample before preparing aliquots for final analysis.

In the present method, 'Antimony TE' was used as an internal standard. This is because it has a mass weight -121, exhibited greater stability in milk and milk powder matrices and is similar to iodine. This also compensates for the errors and changes in the instrument during analysis, thereby ensuring accurate reading of the iodine content of the samples (Huynh et al, 2015).

<u>Specific steps in preparation of sample for analysis</u>

1 ml of digested sample aliquots were accurately pipetted out in plastic tubes. To these, 4 ml of Internal Standard (spiked) was added and the volume was made upto 10 ml with ultrapure water (18.2 MΏ). Further, this solution was mixed thoroughly and centrifuged at 3000 rpm for 15 minutes. These sample solutions were then analysed for their iodine content using ICP-MS

A spike recovery for iodine was also carried out and 4 spiked samples were run during the entire procedure. The percent recovery for the spiked samples was 108%, establishing that the method adapted for iodine analysis of cheese, milk and salt samples was appropriate giving valid results. All the results obtained were corrected for reagent blank and spike recovery.

2.6 Development of Knowledge, Attitude and Practice (KAP) Questionnaire

The third objective of the research was to develop a knowledge attitude and practices (KAP) questionnaire for general consumers

2.6.1 Development of questionnaire for consumers

At the onset, efforts were made to understand the type and amount of information that was required to meet the objective of developing a survey questionnaire to explore knowledge attitudes and practices of consumers. Subsequently a thorough literature search was conducted to study the patterns and questions used in other studies conducted on similar lines. In a study by Charlton et al., (2010) 78 non-pregnant women aged 20-55 year were given a questionnaire to understand their attitudes regarding salt iodisation, consequences of iodine deficiency and about the strategies that could be used to address iodine deficiency. In this context, 81% supported the idea of use of salt as a potential vehicle for improving iodine intake of the population at large. Pires etal., (2017) conducted an evaluation survey for assessment of importance of iodine and need for supplementation in school age children in Portugal. It was an internet survey completed by 691 mothers of school-aged children under 18 years of age. The questionnaire used for this survey had consisted of three sections. In the first section, information was gathered about general characteristics (age, gender, height, weight) of the subjects, second section enquired about the health care provided to the child, eating habits in school or at home. The third section was focussed on obtaining information about mother's health such as supplementations taken during pregnancy, mother's knowledge about amount of iodine required during pregnancy, information about iodine as an essential nutrient and type of salt consumed as a part of daily diet. Although the objectives of these surveys were very different from the present study, the information gathered, the tools used (online survey method), analysis of the responses obtained were pivotal for formation of a skeleton of the consumer survey template in the present study.

Incorporating all the vital aspects covered in various surveys and interpreting them with the objectives of the survey in the present study, a questionnaire was developed. This questionnaire consisted of a total of 12 questions which were an appropriate blend of open and closed questions, designed to elicit precise attitudes of the consumers in a time-efficient manner. Since the aim of the survey was to obtain opinions about use of iodised salt in cheese production, the questions included dealt with 4 main aspects of nutrition namely iodine knowledge of the participants, type of salt consumed as a part of daily diet, frequency and type of cheese consumption and finally their views about nutrient fortification. This developed questionnaire is given in appendix 2 (2.1).

2.6.2 Questionnaire structure and analysis

In the beginning of the questionnaire participant's details (age and gender) were recorded. Following this, the questions began exploring the basic knowledge of the consumers about Iodine as a nutrient and then continued to become more detailed and specific to the consumers. Questions 1 to 7, 10 and 11 were expected to be answered Yes, No or Don't No. Questions 8 and 9 were designed to explore the frequency of cheese consumption (once in two weeks, once a week and so on) and the most consumed varieties of cheese by participants and so participants could answer according to their choice without any restriction on the length of the response. Finally, question 12 gathered participant's preference between iodised salt and unfortified table salt. The questionnaire can be found in appendix 2 (2.1).

2.6.3 Questionnaire pilot

A pilot study was conducted to test if the questions included in the questionnaire are easily and correctly interpreted by the participants, within an acceptable time frame. This pilot trial helped us to understand if it is possible to obtain the necessary information in order to accomplish the aim of the research project. The participants were recruited from among students and staff at UCLan. In total, 42 participants (both male and female) were recruited using opportunistic sampling technique.

2.6.4 Strategy for recruitment of participants for the consumer survey

A multifaceted strategy was adapted for recruitment of participants for the consumer survey. The participants were recruited through 3 different sources such as general consumers from different supermarkets, students, staff, other visitors during university events and online recruitment using Survey monkey software. These varied recruitment mechanisms helped us to obtain a good cross-section of population by reducing any kind of sampling bias related to age, gender, academic or professional background.

In total, 506 general cheese consumers were recruited for the present study.

2.6.4.1 Data collection from participants at supermarkets - The major supermarkets near and around Preston were identified and meetings were scheduled with the supermarket managers. In this meeting, the manager was provided with a covering letter informing about the study in appropriate depth (appendix 2, 2.2). Along with this letter, the procedure of the study and the takeaway materials for the potential participants of the study were verbally explained by the researcher. If the supermarket manager was willing to allow us to carry out the data collection at the selected supermarket, then an informed signed consent was obtained (appendix 2, 2.4) from the manger and the day and date for data collection was confirmed.

On the scheduled day of data collection, every potential participant who was willing to take part in the study, was given a copy of the participant information sheet and the information was also verbally explained by the researcher. If the participant was satisfied with the information provided and was willing to participate in the study, then he/she was asked to complete the consumer survey questionnaire. Depending on the convenience of the participant, either the questionnaire was completed by the participant or the researcher contributed towards completion of questionnaire, recording all the answers as given by the participant. After completion of the questionnaire, every participant was provided with a short iodine booklet published by Dairy Council (appendix 2, 2.12), to help them gain more information about iodine and its importance in maintaining optimal health.

2.6.4.2 Data collection at University - University students were recruited through convenient opportunistic sampling techniques during various on campus events such as student trips, Lancashire Science Festival, Welcome week. Lancashire Science Festival had family members visiting the university along with students from all across the UK and welcome week had newly enrolled students. The awareness about this study was created through word of mouth as well as putting up flyers about the study across different parts of the campus.

Concern regarding sampling bias during recruitment at University – When recruiting participants at University, efforts were made to recruit newly enrolled students or their families who had no to very little formal nutrition training use of iodised salt in food manufacturing. This was ensured by the researcher during verbal conversation while asking the participants about their opinion regarding participating in the study. Also, while recruiting UCLan staff (very small number only during these festival events), it was ensured that they did not have nutrition as their area of expertise. Despite these efforts, it is possible that there was some unintended bias and this could have affected the responses to the questions asked about 'knowledge of participants' and should be considered while interpreting the results of this study for general population.

2.6.4.3 Data collection through survey monkey programme - Survey monkey tool is an online survey development cloud-based software, which can be used for increasing the scope for recruitment of people from different areas of expertise. For the present study, once the survey monkey account was created, information about the study was added. The first page contained the 'Participant Information Sheet', which explained the entire project and procedure for participating in the study in clear and precise terms. If the participant was willing to participate in the study then there was a button 'next' which led the participant to the questionnaire and it also confirmed that the participant had read the sheet.

The developed questionnaire was entered into this software by clicking the link 'create a survey' allowed us to enter all the twelve questions listed in the developed questionnaire. Once all the questions were entered, it was to save them and click on 'create a link'. This link was then uploaded on various social media sites such as University's postgraduate society facebook page, researcher's personal facebook page and it was also emailed to the participants who were willing to participate in the study and would prefer to complete this survey online using the link. While using social media websites, it was ensured that the post explained the purpose of the study, who can participate in the study and further steps for a participant willing to complete the survey. Also, it was clearly explained that participants completing the survey must be based in the UK.

An advertisement flyer developed for creating awareness of the study while conducting consumer survey in different supermarkets has been provided in appendix 2, (2.8). This flyer also contained the link created using survey monkey software. Due to this, consumers who were willing to know more about the study and participate but did not have time to spare at the supermarket could complete this survey at their convenience. Posting information on the above-mentioned sites helped to increase awareness of the study and thereby in recruiting participants. However, it was not possible to capture the specific response (number of participants specifically from each site). This is because, the survey was completely anonymous and all the participants who wanted to complete the survey online, used the survey monkey link. So, it was possible to obtain the exact number of participants who completed the survey online, but it was not possible to differentiate between those who responded to the advert with the link from facebook page and those who responded to the flyer which was placed at the supermarkets.

The following tables (table 2.1 and 2.2) provides demographic information of the participants recruited for the consumer survey

Table 2. 1 Demographic characteristics of the consumers recruited from different sources

Name of supermarkets/ source	**Number of participants**
Aldi (Corporation Street)	60
Pound Stretcher (Corporation Street)	27
Sainsbury's (Bamber Bridge)	57
Booths Supermarkets (Penwortham)	63
Morrison’s (Riversway)	45
Recruitment on UCLan Open Days	55
Lancashire science festival	9
On-campus recruitment	12
Recruitment during Welcome week	61
Survey Monkey	117
Total	**506**

Table 2. 2 Characteristics of consumers based on gender and age group

Age group	Male	Female
18-25	73	97
26-35	21	66
36-44	14	39
45-60	36	62
60+	35	55
No answer for age and gender	8	

2.6.4.4 Data entry and analysis

The responses for the close -ended questions were ‘Yes’, ‘No’ and Don’t Know and these were coded as 1, 0 and 3 respectively for the purpose of analysis. Percentage was calculated for individual questions to interpret the overall opinion of the participants. The responses obtained for the open-ended questions were detailed and varied significantly between the participants. So, the common factors of these responses were analysed to understand the underlying meaning and subsequently the emerging themes were tabulated on an Excel spreadsheet. Further, all the responses were grouped under these individual themes for data analysis.

2.7 Cheese production at Food Technology Centre (Anglesey)

Working towards the aim 4 of the study to produce 2 varieties of cheese cheddar and soft cheese with/without iodised salt, the research team at UCLan collaborated with the cheese production team at Food Technology Centre (Llangefni, Wales).

2.7.1 Stepwise procedure for cheese production

The exact treatment or procedure for production of different types of cheese varies depending on the desired texture, flavour and shelf life of the final cheese. In the present study, 2 different categories of cheese (Cheddar – Hard firm type of cheese with longer shelf life and Soft cheese - Lactic cheese with soft, smooth texture with shorter shelf life) were produced. During each production session, certain steps and treatments applied were specific to that category of cheese, however there were certain steps in the cheese production procedure which were common to both the types of cheese.

In this section, the similarities in the methodology and differences specific to the type of cheese have been depicted using three different flowcharts

- Flowchart with common steps between cheddar cheese and soft cheese production (figure 2.3)
- Flowchart showing individual steps specific to cheddar cheese production (figure 2.4)
- Flowchart showing individual steps specific to soft cheese production (figure 2.5)

2.7.1.1 Common steps in Cheese production

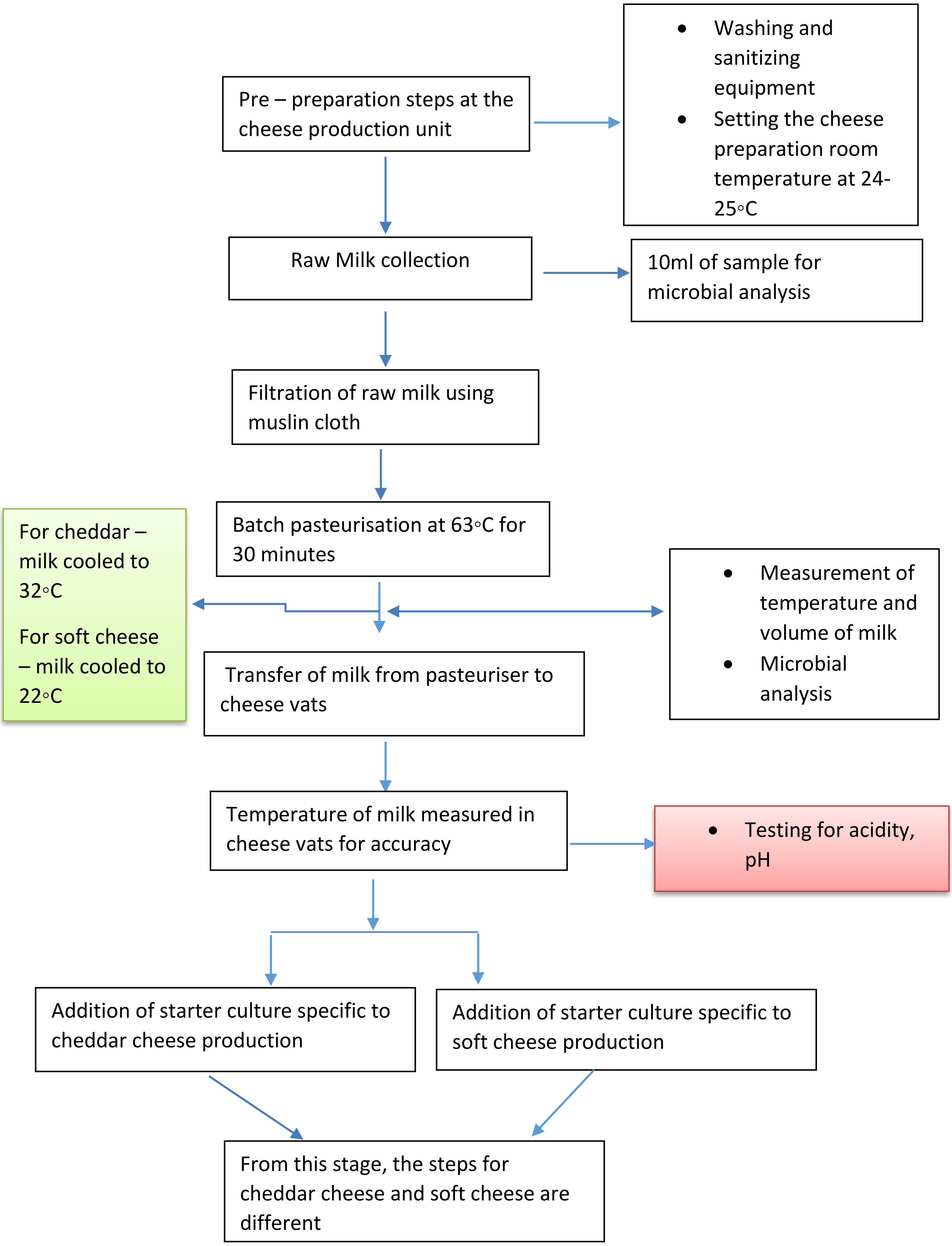
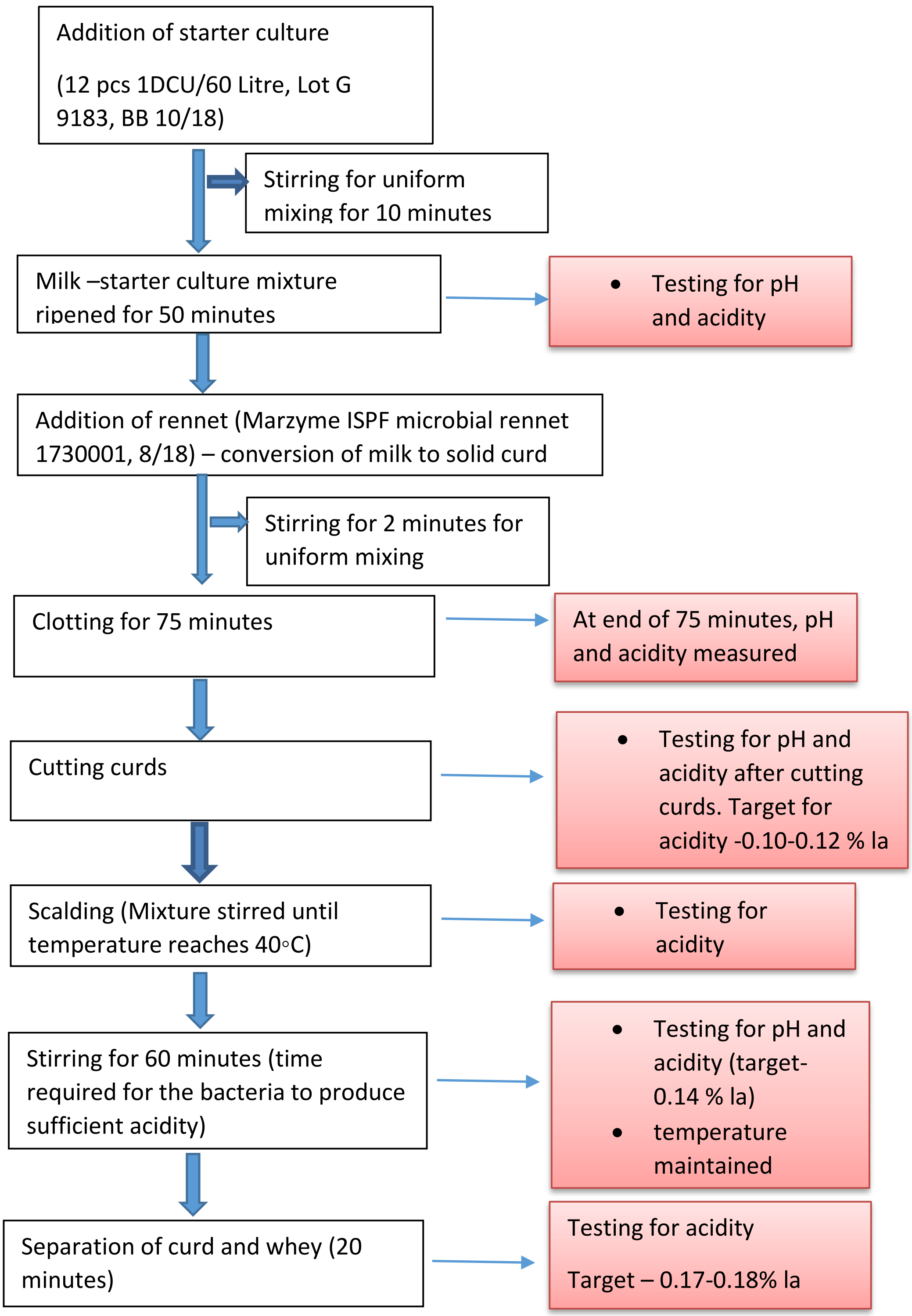


Figure 2. 3 Flowchart showing common steps between Cheddar cheese and soft cheese production

2.7.1.2 Individual steps in Cheddar Cheese production

In the present study, two batches of each variety of cheese (cheddar and soft) was made , one with iodised salt and one with regular table salt. In the section below, the differences in the production processes specific to the type of cheese are showcased using a flowchart, followed by in depth discussion about each step in the entire production cycle.



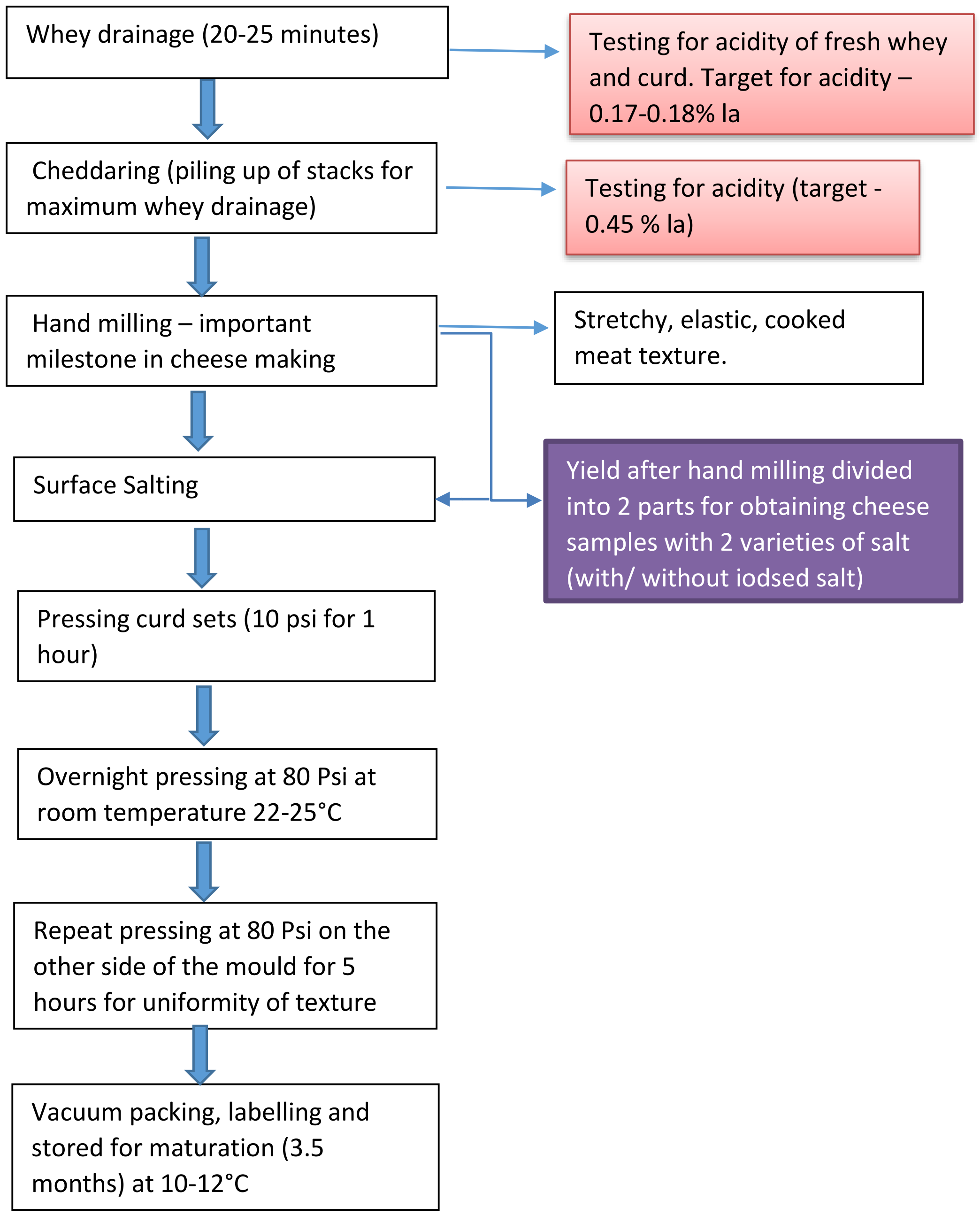
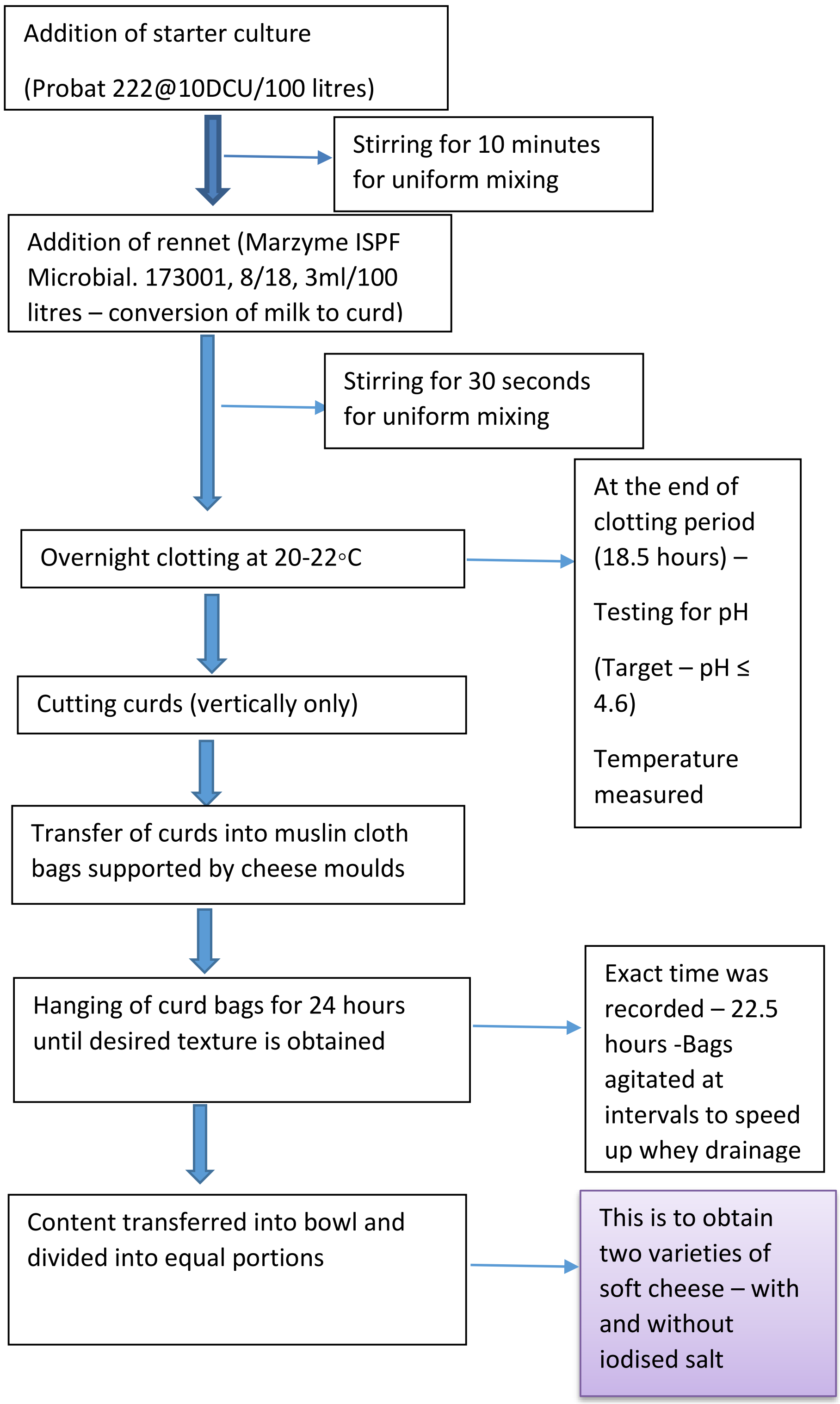


Figure 2. 4 Flowchart for individual steps in Cheddar cheese production

2.7.1.3 Individual steps in Soft Cheese production



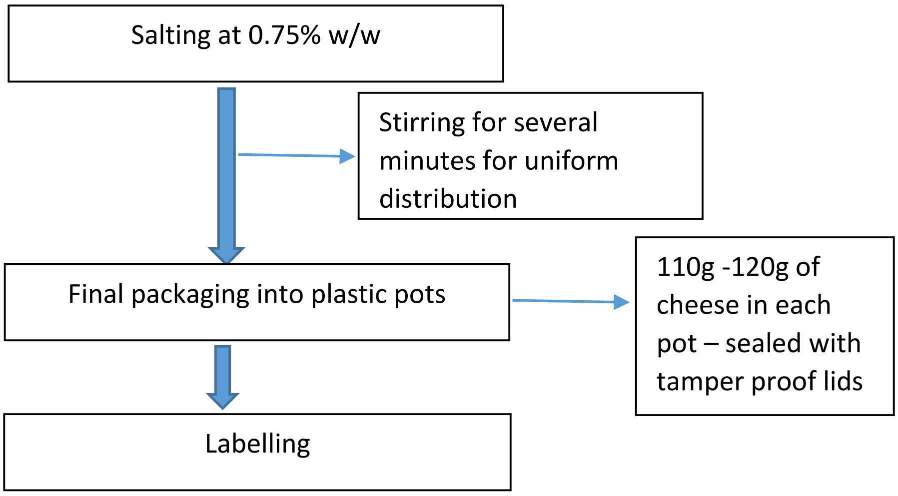


Figure 2. 5 Flowchart of individual steps in soft cheese production

2.7.2 Sensory evaluation of new cheese varieties

The final aim of the study (aim 4) was to explore the impact of substituting non-fortified salt with iodine- fortified salt in the production of cheese and to assess its effect on the sensory quality and iodine content of the final product. This was achieved by conducting sensory evaluation of newly developed cheese varieties (soft cheese with/without iodised salt and cheddar cheese with/without iodised salt) by recruiting general cheese consumers (to get unbiased perception about cheese attributes) and few sensory experts (to get specific scientific opinion about specific attributes and overall quality of the cheese varieties).

2.7.2.1 Development of Sensory Evaluation Template

The template for the sensory analysis of cheese samples was developed by adapting a Quantitative Descriptive Analysis (QDA) method. Here it is important to note that the method for QDA described in the literature (Papetti and Carelli, 2013; Lawless and Heymann, 2010; Stone and Sidel, 1993) is a standard method involving standard terms for describing attributes required for sensory analysis of cheese varieties. However, while developing the template for sensory evaluation for the present study, some aspects of this standard method were adapted. The intensity scale used for rating the response (like very much to dislike very much), the definitions used for describing different attributes related to sensory aspects of cheese (taste, texture, odour) and the spider diagram used for analysing the responses were similar to the standard method. The researcher also ensured that the template used for sensory evaluation was exactly same for both the non- expert participants and sensory experts.

During each sensory evaluation session, two cheese samples were to be evaluated and so these samples were given sample numbers for their identification (249 – for cheese sample produced with iodine- fortified salt and 198- for cheese sample produced using non-fortified (regular) table salt. The sensory evaluation template is given in appendix 2 (2.9). The first five questions were designed to obtain general information about the participant including age, gender characteristics as well as some information about the frequency and type of cheese and salt consumption. Subsequent questions were focused on obtaining information about consumer/ sensory expert opinions about the different parameters of cheese samples. A total of eight attributes related to sensory analysis of cheese varieties were selected for this study namely colour, appearance, saltiness, taste, odour, texture, level of moisture/mouth-feel and overall quality. In order to rate these main attributes a 7-point hedonic scale (like very much to dislike very much) was used (Singh-Ackbaraliand Maharaj, 2014). This hedonic scale is a standard rating scale that has been used for many years in sensory evaluation in the food industry and its use has been validated in scientific literature.

For attributes including taste, odour and texture, the participants were asked to detect specific characteristics. For example, in terms of taste, the participants were asked if they could detect sweetness, acidic taste, bitterness, aftertaste or any other taste. This method of indicating specific characteristics in the main sensory attribute is called profiling and it helps to obtain more in-depth opinions about the selected food sample.

At the end of the cheese tasting session, participants were asked if they could identify the two different types of cheese samples and distinguish between cheese sample produced with iodine-fortified and non-fortified salt and were also requested to indicate their preference between the two cheese samples.

2.7.3. Recruitment of participants for sensory analysis of cheese varieties

Site 1 – Cardiff Metropolitan University- In total seven expert sensory panellists were recruited for soft cheese evaluation and eight sensory experts were recruited for the sensory evaluation of Cheddar cheese samples at Cardiff Metropolitan University. This university has a centre called – Food Industry Centre which recruits sensory panellists regularly according to the requirement of the project. All the panellists had undergone threshold testing at the centre, which means they have been tested against a variety of tastes as per the International

Standards Organisation (ISO standards) and British National Standards adopted by European Community (BS EN), making them able to taste a wide range of products. The tastes and concentrations used in the threshold testing were compliant with : BS ISO 3972:2011 (Sensory analysis Methodology — Method of investigating sensitivity of taste) and BS EN ISO 8586:2014 (Sensory analysis – General Guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors).

Table 2. 3 Details of sensory experts recruited for sensory evaluation of cheese samples

Sensory Evaluation	**Total number of males**	**Total number of females**
For soft cheese samples	3	4
For cheddar cheese samples	1	7

Table 2. 4 Characteristics of sensory experts recruited for sensory evaluation of soft cheese samples

Age group	Male	Females
18-25	0	0
26-35	0	2
36-44	2	1
45-60	1	1
60+	0	0

Table 2. 5 Characteristics of sensory experts recruited for sensory evaluation of Cheddar cheese samples

Age group	Male	Females
18-25	0	0
26-35	0	2
36-44	1	5
45-60	0	0
60+	0	0

Site 2 – University of Central Lancashire– Non-expert participants (Student and Staff members) were recruited through opportunistic sampling techniques. Apart from the opportunistic recruitment, some undergraduate students attending nutrition classes were also invited and recruited after obtaining voluntary and informed consent for the sensory evaluation exercise. A total of 46 non-expert general cheese consumers were recruited for the sensory evaluation of soft cheese samples and 53 non-expert general cheese consumers were recruited for evaluation of Cheddar cheese samples. The following tables (table 2.6, 2.7 and 2.8) provide details of the participants in terms of gender and age group.

Table 2. 6 Details of non-expert participants for sensory evaluation of cheese samples

Sensory Evaluation	**Total number of males**	**Total number of females**
For soft cheese	20	26
For Cheddar cheese	17	34

Table 2. 7 Characteristics of non-expert participants recruited for sensory evaluation of soft cheese samples

Age group	Male	Female
18-25	3	10
26-35	11	6
36-44	3	2
45-60	2	8
60+	1	0

Table 2. 8 Characteristics of non-expert participants recruited for sensory evaluation of Cheddar cheese samples

Age group	Male	Female
18-25	7	7
26-35	5	8
36-44	3	5
45-60	2	14
60+	0	0

2.7.4 Determination of Iodine content of newly developed cheese samples at Fera Labs

The iodine content of newly developed cheese samples was determined at a commercial lab (Fera Labs, York) using the sample method as described in the section 2.5.1

Table 2. 9 Details of newly developed cheese samples analysed at Fera Labs

Cheese samples (n-=4)		
	Number of samples	Notes
Soft cheese with iodised salt (Cerebros)	2	Reason for analysis of 2 portions of samples was to get a representative sample from entire batch.
Soft cheese with regular salt (Saxa Table salt)	2	
Cheddar cheese with iodised salt (Cerebros)	2	
Cheddar cheese with regular salt (Saxa table salt)	2	
Salt samples (n=2)		
Table salt (Saxa)	1	These salt samples were used for cheese production
Extra Fine Iodised salt (Cerebros)	1	

2.7.5 Statistical Analysis

The responses obtained for sensory quality of the cheese varieties were statistically analysed to understand if there were any significant differences in the responses to different main attributes and specific characteristics in these main attributes. Since the responses were categorical in nature (Yes and No) a Chi square test was performed. Main attributes (Colour, Appearance, Taste, Texture and Overall Quality) were analysed using a two-way Chi square test to identify differences in the preference of one attribute over the other. Specific attributes - Sweetness, bitterness, acidic, aftertaste, hardness, chewiness, were analysed using one-way Chi square test to understand if one attribute is detected more prominently over the other in either of the cheese varieties. Finally, one-way Chi square test was also performed to find out if cheese with iodised salt was preferred more or less than cheese with regular table salt.

The present study consisted of five specific aims. The results of these individual aims are presented in chapters 3-6 as shown in figure 3.1. Each chapter includes results and a brief discussion related to the associated objectives.

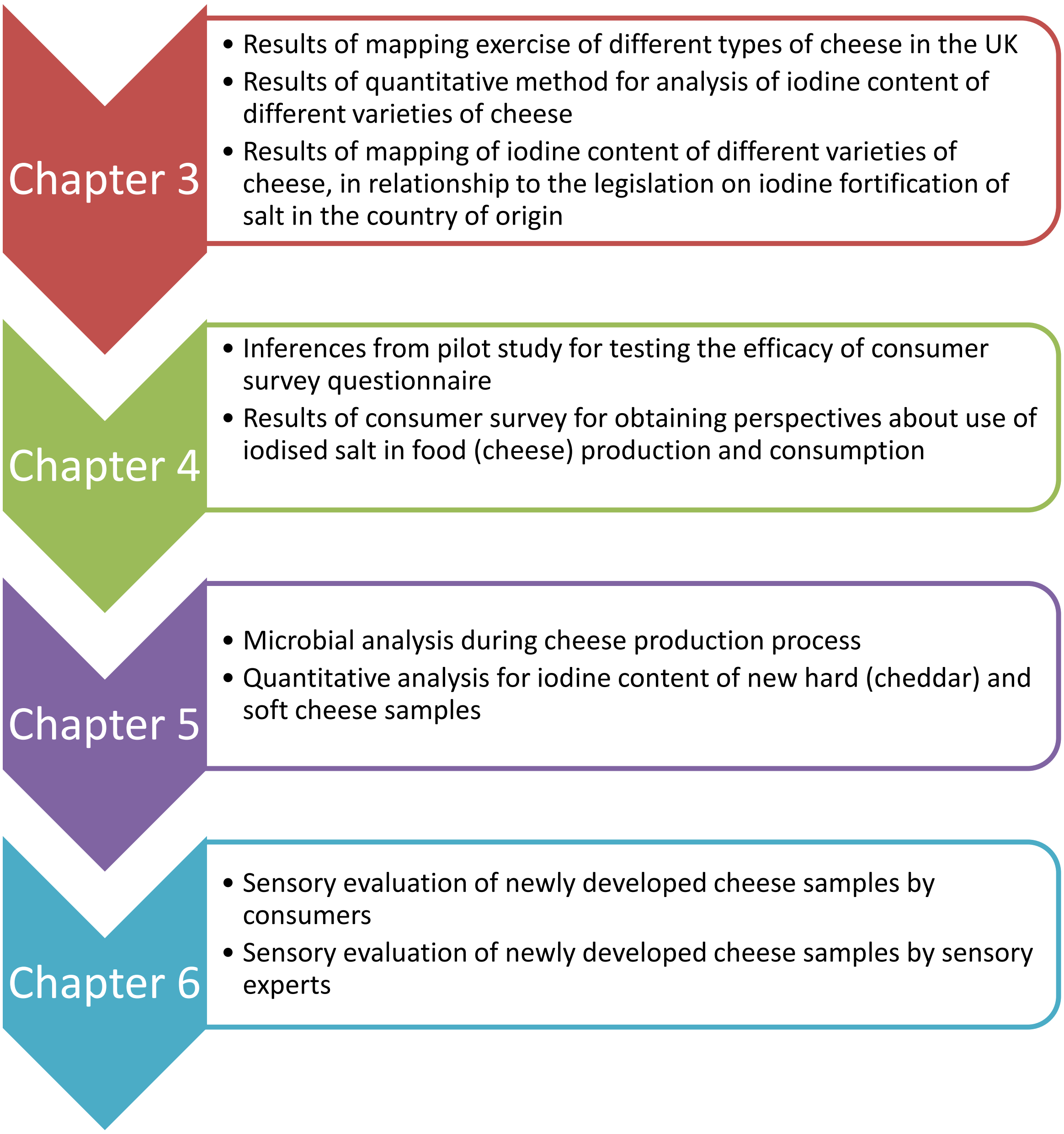


Figure 3. 1 Flowchart describing the content of chapters 3-6

Chapter 3 : Mapping of cheese varieties and quantitative analysis

3.1 Overview of this chapter

In this chapter the study results are presented in two sections

In the first section, the mapping exercise of different varieties of cheese samples easily available in some of the main UK supermarkets is presented in a tabular format. In some cases, the core ingredients had a different country of origin to the finished cheese. This is also noted in the table.

In the second section, the results obtained from quantitative analysis of commonly consumed cheese samples for their iodine content are presented. The selected samples from the cheese chart developed in the first section were analysed using an established ICP-MS method at Fera Labs, York. Along with cheese samples the core ingredients of cheese making process (milk and salt) samples were also tested for their iodine content. These data are tabulated with corresponding information of country of origin of production of cheese, its ingredients and legislative policy on iodine fortification of salt in the country of origin.

This chapter addresses aims 1and 2 of the thesis.

3.2 Scoping and mapping of different cheese varieties in the UK

One of the primary aims of the study was to measure the iodine content of different cheese varieties in the UK and examine this information in relation to the country of origin of cheese and legislative policies regarding iodine fortification of salt existing in these individual countries. In order to accomplish this, it was very important to collect and map the available information on all these aspects. In the context of this scenario, the mapping exercise was conducted in different parts.

The following flowchart depicts the steps involved in the scoping and mapping exercise

Step 1

- Broad classification, collation and tabulation of existing information about cheese varieties in supermarkets

Step 2

- Identification of different countries of cheese production and iodine fortification legislation in indvidual countries

Step 3

- Collation of the information obtained from steps 1,2 and development of detailed cheese map consisting information about specific categories of cheese, country of origin of cheese/milk and legislation for iodine fortification of foods

Figure 3. 2 Flowchart of steps in scoping and mapping exercise

3.2.1 Broad classification and plotting of information about cheese in supermarkets

To begin with, three popular supermarkets (Morrisons, Sainsbury's and Tesco) in and around Preston (study area) were selected. After conducting a general overview of the official websites of these supermarkets, all the information (related to the present study) about the varieties of cheese available in individual supermarkets were tabulated. An example of this basic information collection exercise is presented below (table 3.1)

Table 3. 1 An example of basic information about cheese varieties in supermarket (Step 1 of mapping exercise)

Name of cheese	Varieties (number of samples)	Country of origin
Cheddar cheese	Mild - 11, Medium – 5, Mature - 25 Extra Mature and Farmhouse - 11	Produced in the UK using British milk, Produced in EU using EU milk
Grated and sliced cheese	Grated cheese – 21 Sliced cheese - 27	All other types of Grated cheese- Made in the UK from EU milk or directly made in UK from British milk
Grated cheese	M Savers Grated Hard cheese	Made in Italy from EU and Swiss cheeses
Sliced cheese	Morrisons Edam cheese, Maasdam slices	Produced in Netherlands from Dutch milk
	Leerdammer cheese slices	Made in Holland
	Jarlsberg Original cheese slices	Made in Ireland to a Secret Norwegian Recipe
	Mlekovita Prymus Cheese slices (Swiss type full fat hard cheese)	Poland
	Mlekpol Salami Sliced cheese	Poland
	Mlekpol Krolewsk Sliced cheese	Poland
Regional cheese	Gloucester and Red Leicester – 12 Lancashire and Cheshire – 3	UK

	Wensleydale - 5	
Fruit and Flavoured cheese	Pineapple and Almond Cream cheese	Made in Germany from EU milk
Soft and Cottage Cheese	Soft cheese – 34 Cottage cheese – 10 Quark - 1	UK
	Full fat soft cheese	Produced from Austrian milk
	M Savers soft cheese	Germany
	Boursin	France
	Lowicz Cottage cheese	Poland
	Galbani Dolcelatte	Italy
Continental and Speciality cheese	White cheese – 19 Blue cheese – 10 Hard cheese – 1 Parmesan, Padano, Pecarino – 9 Mozarella, Marscapone, Ricotta – 9 Feta, Halloumi, Paneer-10	
White cheese	President Brie	France
	Go Dutch Edam, Go Dutch Gouda	Produced in EU and packed in the UK
	Swiss Le Gruyere	Produced in Switzerland from Swiss milk
	French Camembert, French Roule, Le Rustique Camembert, French Brie, Port Salut cheese, Morrisons Brie, Mini French Brie	France from French milk
	German Cambozola, M Savers Brie	Germany from German milk
	President Emmental cheese	Produced in France and Czech Republic
	Jarlsberg cheese -Medium Fat Hard	Produced in Norway and Packed in UK

The above-mentioned table provides an example of the data collected from a supermarket website (Morrisons). The information collected from the other two supermarkets selected for the present study was tabulated in a similar pattern. All the data collected can be found in appendix 3 (section 3.4).

3.2.2 Identification of different countries of cheese production and iodine fortification legislation in individual countries

After obtaining this basic information about cheese varieties in the UK, a list of countries of origin was prepared and the legislation policies regarding iodine fortification of foods in these countries were identified. Subsequently these countries were sorted according to the existing legislation for fortification. This is presented in table 3.2

Table 3. 2 Classification based on existing legislation regarding iodine fortification (Step 2 of mapping exercise)

Countries with voluntary Legislation	Examples types of cheese in different countries	Example brand names of cheese samples in supermarkets
UK	Mature cheddar	Pilgrims Choice
	British Blue Stilton	M & S
	Butlers Blacksticks (Preston)	Specially Selected
	Vintage cheddar	Farmhouse
France	Roquefort Blue cheese	Specially Selected, Tesco
	Creme de Saint Agur	
	M Signature Comte	Morrisons
Switzerland	Swiss Le Gruyere/Swiss Emmental	M Savers (Morrisons)
Spain	Spanish Machego	
Germany	Cambozola blue brie MSavers Blue cheese	Tesco Morrisons
Norway	Jarlsberg cheese	Morrisons
Poland	Mlekovita Prymus Cheese (Swiss type Hard Cheese)	
Czech Republic	President Emmental	President
Countries with Semi-Compulsory legislation	Examples types of cheese in different countries	Example brand names of cheese samples in supermarkets
Italy	Parmesan, Gongozola Piccante	Specially selected
Countries with mandatory legislation	Examples types of cheese in different countries	Example brand names of cheese samples in supermarkets
New Zealand	Mature Cheddar	Not easily available in the selected supermarkets*
Australia	Mature Cheddar	Pilgrims Choice*
Denmark	Danish Blue cheese	Castello Tesco Milbona (From Lidl)
Canada	Canadian Cheddar	Morrisons The Best Sainsbury's Finest brand from Tesco
	Canadian Cheddar	
	Vintage Canadian Cheddar	
Netherlands	Frico Gouda Wheel/Frico Edam/Port Salut	

Holland (Netherlands)	Leerdammer cheese	
Countries with no legislation	Name of cheese samples	Brand Name
Ireland	Irish Cheddar (Mature)	Pilgrims Choice
Cyprus	Cypriot Halloumi	

*- This implies that these cheese varieties are mentioned on the official website of the supermarket, but these samples are not available in the selected supermarket as these cheese varieties are ordered by the store only if there is specific demand for them from the customers.

As seen in table 3.2 all the cheese varieties in the selected three supermarkets (Morrisons, Sainsbury's and Tesco) were manufactured in 21 countries (UK, Denmark, Cyprus, France, Ireland, Italy, Germany, Norway, Greece, Czech Republic, Austria, Netherlands, Holland, Switzerland, Spain, Poland, Bulgaria, Limnotopos Kilkis, New Zealand, Australia, Canada). These countries were subsequently sorted according to the legislation policy existing in these countries. In context of the manufacturer information of cheese samples, a single variety of cheese from same country of origin is available from different brands. For example, Cheddar cheese produced in Canada is available from three different brands (Morrisons –The Best, Sainsbury's, Finest brand from Tesco) in the UK.

3.2.3 Details of cheese mapping

Considering all the information obtained from steps 1 and 2, the data were sorted according to the British Cheese Board classifications described in chapter 2 section 2.4 (table 3.3). This enabled the identification of a range of cheeses from the various countries and legislation types, for further analysis of iodine content in order to achieve objective 2.

Table 3. 3 Detailed cheese mapping exercise (Step 3)

Type of cheese	Sub-type	Sub-type	Country of production of cheese	Country of production of milk
Fresh	Castello pineapple halo		Denmark	Denmark
	Galbani Mozarella/Ricota		Italy	Italy
Soft	Somerset Brie		UK	UK
	Brie		France	EU milk
	St.Angur/Goat/Camembert		France	France
	Greek Feta Cheese		Greece	Greece
Semi-hard	Frico Edam Ball, Port Salut		Netherlands	Netherlands
	Morrisons Edam Cheese		Netherlands	Dutch milk
Hard-firm	Cheddar	Mature	UK,NZ, Ireland, Australia	UK,NZ, Ireland, Australia
	Cracker barrel cheddar	Mature Irish	Ireland	Packed in Belgium
		Mild white	UK	UK
		Farmhouse, vintage	Canada, UK	Canada, UK
	Butlers hard cheese	Farmhouse	UK	UK
	Parmesan		Italy	Italy
	Swiss Gruyere/Swiss Elemental		Switzerland	Switzerland
	Spanish Machego		Spain	Spain
	President Ememtal		Czech Republic	
	M Savers Hard Cheese		Italy	EU
	Frico Gouda Wheel		Netherlands	Netherlands
		Parm Reggiano	Italy	Italy
		Grana Padano	Italy	Italy
	Five countries	Derby	UK	UK
		Red Leicester		
		Cheshire		
		Gloucester		
		Cheddar(with herbs and fruits)		
	Cathedral city cheddar		Great Britain	British milk
Hard-crumbly	Beacon Fell Creamy Lancashire		UK	UK
	Caerphilly		UK	
	Wensleydale		UK	
	Sliced cheeses in Morrisons		Poland, Holland, Netherlands	
Blue	Blue Shropshire/Blue Stilton		UK	UK
	Butlers Blacksticks		Preston UK	Preston UK
	Cambozola blue brie		Germany	Germany
	Castello Danish Blue Cheese		Denmark	Denmark
	Roquefort/Saint Agur		France	France
	Gorgonzola Piccante		Italy	Italy

As seen in table 3.3 all the information from steps 1 and 2 is collated wherein the cheese varieties obtained from different supermarkets were listed and further sorted into specific categories including Fresh, Soft, Semi Hard, Hard firm, Hard crumbly and Blue. This classification was conducted according to the categories identified by the British Cheese Board (BCB) (British Cheese Board, 2018). The sub type column was incorporated to gather in depth information about a cheese variety, so that it can be further used for understanding the cheese processing technique. While doing so the focus was to ensure that the cheese samples selected for the present study were representative of different categories of cheese and belonging to a different country of origin. So, the information about country of origin of the cheese as well as its ingredient milk was also formulated in the table. As it is clearly seen in table 3.3 effort was made to include cheese samples from as many countries as possible. This was to obtain an appropriate spread of countries with different legislative policies. This mapping exercise formed the basis for identifying samples for further laboratory analysis and mapping against legislative policies for iodine fortification.

3.3 Mapping of iodine content of different varieties of cheese, in relationship to the legislation on iodine fortification of salt in the country of origin

Based on the above-mentioned steps of scoping and mapping exercise, the selected cheese samples from the mapping exercise were analysed for their iodine content using a well-established quantitative method (ICP-MS method) at a commercial laboratory (Fera Labs, York, UK), thereby achieving the first aim of the study.

Table 3. 4 Details of cheese, milk and salt samples for commercial labs (Fera Labs) for analysis of iodine content

Cheese samples (n=25)			
Name of sample	**Type of cheese**	**Country of production**	**Brand Information**
Swiss Le Gruyere	Hard	Switzerland	Morrisons
French Comte	Hard	France	The Best
Blue cheese	Blue	France	Saint Agur
French Roquefort	Blue	France	Specially Selected
Extra Mature Cheddar	Hard	UK	Pilgrim's Choice
Mature Welsh Cheddar	Hard	UK	Dragon
Vintage Cheddar	Hard	UK	Valley Spire
Mature Cheddar	Hard	UK	Cathedral City
Extra Mature Cheddar	Hard	UK	Davidstov
Farmhouse Cheddar		UK	The Best
Blue Stilton	Blue	UK	Morrisons
Blacksticks blue	Blue	UK	Butlers
Cannadian Cheddar	Hard	Canada*	The Best
Blue Cheese	Blue	Germany	Msavers
Cambozola Blue	Blue	Germany	M&S
Cheese slices (smoked over beechwood		Germany using AUS/NZ/EU milk	Milbona
Creamy Danish Blue	Blue	Denmark*	M&S
Danish Blue	Blue	Denmark*	Castello
Edam slices	Hard	Netherlands*	Tesco
Maasdam slices	Hard	Netherlands*	Emporium
Leerdammer Original	Hard	Holland*	Leerdammer
Jarlsberg Original	Hard	Norway	Jarlsberg
Emmental	Hard	Czech Republic	President
Machego D.O.P	Hard	Spain	The Best
Mature Machego	Hard	Spain	Roncero
Milk samples (n=1)			
Skimmed milk powder		UK	
Salt samples (n=4)			
Rock salt		UK	
Sea salt		UK	
Iodised salt		Pakistan (USI)	
Table salt		UK	

*- indicates that there is mandatory policy for iodine fortification in the country

The second aim of the study was accomplished by collating the laboratory data for iodine content of cheese samples and information obtained from the mapping exercise of the cheese varieties in the UK. The main aspect that was emphasized while working towards the second aim of the study was examining the iodine content of the cheese varieties in relation to the legislation for iodine fortification in the country of origin for each cheese analysed in aim 1. In accordance to this requirement, the study results were presented in two different tables.

Before interpreting the results obtained for iodine content of cheese samples belonging to countries with different legislation status (mandatory, voluntary), it is essential to clearly define the differences in the meaning of these terms.

The WHO and FAO 'Guidelines on food fortification with micronutrients' have explained that "the fundamental distinction between mandatory and voluntary regulation as it applies to food fortification is the level of certainty over time that a particular category of food will contain a pre-determined amount of a micronutrient. By providing a higher level of certainty, mandatory fortification is more likely to deliver a sustained source of fortified food for consumption by relevant population group and in turn, a public health benefit".

The following table (table 3.5) shows plotting of data for countries with mandatory legislation policy regarding iodine fortification of salt

Table 3. 5 Iodine content of cheese samples from countries with Mandatory Legislation status for iodine fortification

Name of sample	**Country of origin of ingredients**	**Brand Information**	**Amount of iodine (mg/kg)**	**Permissible iodine levels in the salt in the country of origin (mg/kg)**
Creamy Danish Blue	Denmark	M&S	0.16	8-13
Danish Blue	Denmark	Castello	0.17	8-13
Edam slices	Netherlands	Tesco	0.22	
Maasdam slices	Netherlands	Emporium	0.23	
Leerdammer Original	Holland (Netherlands)	Leerdammer	0.26	
Cannadian Cheddar	Canada	The Best	0.76	

Table 3. 6 Iodine content of cheese samples from countries with Voluntary Legislation for iodine fortification

Name of sample	**Country of origin of ingredients**	**Brand Information**	**Amount of iodine (mg/kg)**	**Permissible iodine levels in the salt in the country of origin (mg/kg)**
Swiss Le Gruyere	Switzerland	Morrisons	0.08	20
French Comte	France	The Best	0.09	10-15
Blue cheese	France	Saint Agur	0.27	10-15
French Roquefort	France	Specially Selected	0.64	10-15
Extra Mature Cheddar	UK	Pilgrim's Choice	0.12	10-22
Mature Welsh Cheddar	UK	Dragon	0.13	10-22
Vintage Cheddar	UK	Valley Spire	0.32	10-22
Mature Cheddar	UK	Cathedral City	0.55	10-22
Extra Mature Cheddar	UK	Davidstov	0.61	10-22
Farmhouse Cheddar	UK	The Best	0.92	10-22
Blue Stilton	UK	Morrisons	0.71	10-22
Blacksticks blue	UK	Butlers	0.48	10-22
Blue Cheese	Germany	Msavers	0.17	15-25
Cambozola Blue	Germany	M&S	0.14	15-25
Cheese slices (smoked over beechwood	Germany using AUS/NZ/EU milk	Milbona	0.19	N/A
Jarlsberg Original	Norway	Jarlsberg	0.24	5
Emmental	Czech Republic	President	0.42	No confirmed data available
Machego D.O.P	Spain	The Best	0.67	60
Mature Machego	Spain	Roncero	0.9	60

Cheese samples from three countries with mandatory legislation were analysed for their iodine content. Multiple samples were selected from a single country (for example three samples from Netherlands and two samples from Denmark) belonging to different brands to ensure that all the different samples available in the UK from that specific country are covered, thereby gaining better clarity for interpreting the iodine fortification scenario in the country. Overall, iodine content of cheese samples ranged from 0.16mg/kg for creamy Danish blue cheese (from Denmark) to 0.76mg/kg for Canadian cheddar.

Concerning the iodine content of cheese samples from countries with voluntary legislation status related to iodine fortification policies (table 3.6), samples were collected belonging to seven such countries with multiple samples from a single country to ensure that the samples were selected from different brands available across the UK.

The following diagram (figure 3.3) depicts the iodine content of hard/semi-hard and blue cheese samples from countries with mandatory and voluntary legislation for iodine fortification.

The mean values with the error bars (standard deviation) shows that iodine content of cheese from countries with mandatory legislation will be more consistent than cheese samples from countries with voluntary legislation. Moreover, wide variability was observed for iodine content of cheese samples (Hard and blue types) samples produced in countries with voluntary legislation for iodine fortification ranging from as low as 0.08 mg/kg (Swiss Le Gruyere) to as high as 0.92 mg/kg (Farmhouse Cheddar)

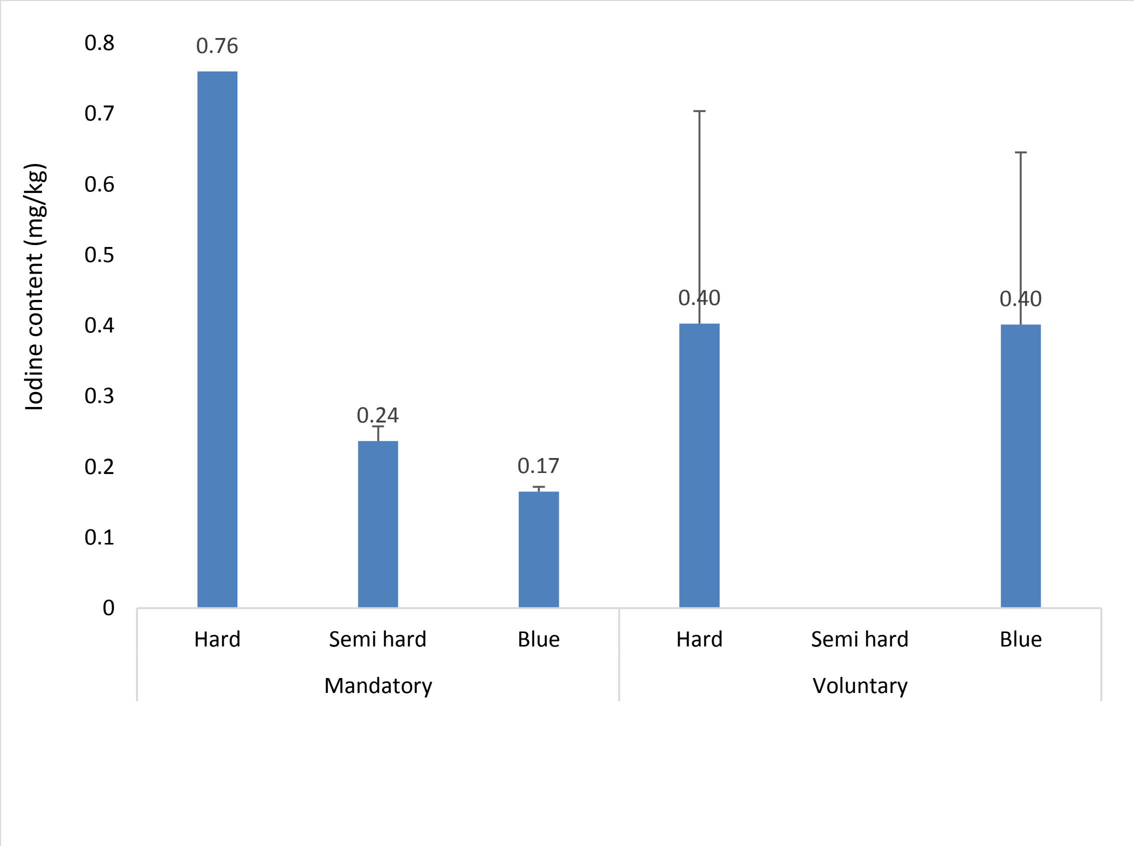


Figure 3. 3 Iodine content of cheese varieties from countries with mandatory and voluntary legislation

Table 3. 7 Iodine content of cheese samples from countries with mandatory legislation expressed in terms of portion size and recommended iodine intake

Name of the sample	Country of origin	Iodine content (mg/kg)	Iodine content (µg I) per adult portion (30g)	% Recommended Nutrient Intake* (iodine intake) per adult portion (30g)
Creamy Danish Blue	Denmark	0.16	4.8	3.2
Danish Blue	Denmark	0.17	5.1	3.4
Edam slices	Netherlands	0.22	6.6	4.4
Maasdam slices	Netherlands	0.23	6.9	4.6
Leerdammer Original	Holland (Netherlands)	0.26	7.8	5.2
Canadian Cheddar	Canada	0.76	22.8	15.2

*Recommended iodine intake for adults is 150 µg/day

As seen in table 3.7, When the iodine content of cheese samples from countries with mandatory legislation were expressed in terms of iodine content per portion size, it was found that Canadian Cheddar provided the highest amount of iodine (22.8 µg I), thereby meeting 15% of the daily recommended iodine intake for adults. The iodine content of cheese samples per adult portion size from Denmark and Netherlands was found to be similar ranging from 4.8 to 7.1 µg I.

Table 3. 8 Iodine content of cheese samples from countries with voluntary legislation expressed in terms of portion size and recommended iodine intake

Name of the sample	Country of origin	Iodine content (mg/kg)	Iodine content (µg I) per adult portion (30g)	% Recommended Nutrient Intake* (iodine intake) per adult portion (30g)
Swiss Le Gruyere	Switzerland	0.08	2.4	1.6
French Comte	France	0.09	2.7	1.8
Blue cheese	France	0.27	8.1	5.4
French Roquefort	France	0.64	19.2	12.8
Extra Mature Cheddar	UK	0.12	3.6	2.4
Mature Welsh Cheddar	UK	0.13	3.9	2.6
Vintage Cheddar	UK	0.32	9.6	6.4
Mature Cheddar	UK	0.55	16.5	11
Extra Mature Cheddar	UK	0.61	18.3	12.2
Farmhouse Cheddar	UK	0.92	27.6	18.4
Blue Stilton	UK	0.71	21.3	14.2
Blacksticks blue	UK	0.48	14.4	9.6
Blue Cheese	Germany	0.17	5.1	3.4
Cambozola Blue	Germany	0.14	4.2	2.8
Cheese slices (smoked over beechwood	Germany using AUS/NZ/EU milk	0.19	5.7	3.8
Jarlsberg Original	Norway	0.24	7.2	4.8
Emmental	Czech Republic	0.42	12.6	8.4
Machego D.O.P	Spain	0.67	20.1	13.4
Mature Machego	Spain	0.9		18

*Recommended iodine intake for adults is 150 µg/day

From table 3.8, it is evident that, Farmhouse Cheddar cheese (UK) has the highest iodine content (27.6 µg I) per adult potion size (30 g) meeting 18% of recommended iodine intake. Furthermore, cheese samples from Spain (Mature Manchego and Manchego D.O.P) also provide considerable amount of iodine per adult portion size, thereby meeting 18% and 13% of daily recommended iodine intake respectively.

Table 3. 9 Iodine content of milk and salt samples in the UK supermarkets

Name of sample	**Country of origin of ingredients**	**Brand Information**	**Amount of iodine (mg/kg)**	**Legislation status for Iodine fortification in the country of origin** **Voluntary = V**	**Permissible iodine levels in the salt in the country of origin**
Salt samples					
Sea salt	UK	Schwartz	< 0.01	V	10-22 ppm
Iodised salt	Pakistan	National	35.6	USI	
Rock salt	UK	Saxa	~ 0.02	V	10-22 ppm
Table salt	UK		~ 0.01	V	10-22 ppm
Milk samples					
Dried skimmed milk powder	UK		2.79	V	

As seen in table 3.9 the iodine content of all the selected salt samples produced in the UK is negligible. The iodised salt from Pakistan has iodine content of 35mg/kg.

3.4 Discussion of key findings of the chapter

The information available on the iodine intake and status of the population in the UK is sparse (Bath et al., 2014). In 2003 a database for iodine content of foods was published, which was populated from the available literature at that time (Fordyce, 2003) and so more updated information on the iodine content of foods is necessary to gain better clarity about the dietary intake of the population. In the present study, a mapping and scoping exercise was conducted to identify cheese varieties differing in country of production, available in popular supermarkets in the UK. The iodine analysis of the selected cheese varieties revealed that there is wide variation in the iodine content of cheese varieties. When the iodine content was discussed in relation to the legislative policies (voluntary, mandatory) regarding iodine fortification in the country of production of cheese, it was found that there is no clear and direct relation between the policy and iodine content. The iodine content from cheese from countries with mandatory legislation was less variable. However, the sample size for cheese varieties selected for analysis was small (n=25). Also, when the results were discussed in terms of the number of samples analysed from countries with mandatory v/s voluntary legislation, then it was evident that the number of samples from countries with mandatory legislation were very small (n=1, 3 and 2) for hard, semi-hard and blue cheese respectively. Due to this small sample size, it was not possible to interpret accurately regarding the variability in the iodine content of both (mandatory v/s voluntary) groups. Milk and salt samples (good sources of iodine if fortified) were also analysed for their iodine content. It was found that iodine content of all the selected salt samples produced in the UK was negligible.

Chapter 4: Results of Consumer Survey

4.1 Overview of the chapter

Working towards accomplishment of the third aim of the study to explore the knowledge attitude and practices (KAP) of general consumers regarding the use of iodised salt in food products, an in-depth consumer survey was conducted using a consumer survey questionnaire. As a part of this survey, data was obtained from 506 consumers recruited from 10 different sources including supermarkets, university students, public engagement events at University and online survey monkey software.

4.2 Results of Consumer Survey for perspective on use of iodised salt in food (cheese) production and consumption

4.2.1 Question-wise analysis of the questionnaire

The first four questions were designed to understand the basic knowledge of the participants regarding the significance of iodine for normal health and wellbeing. This has been presented in figure 4.1

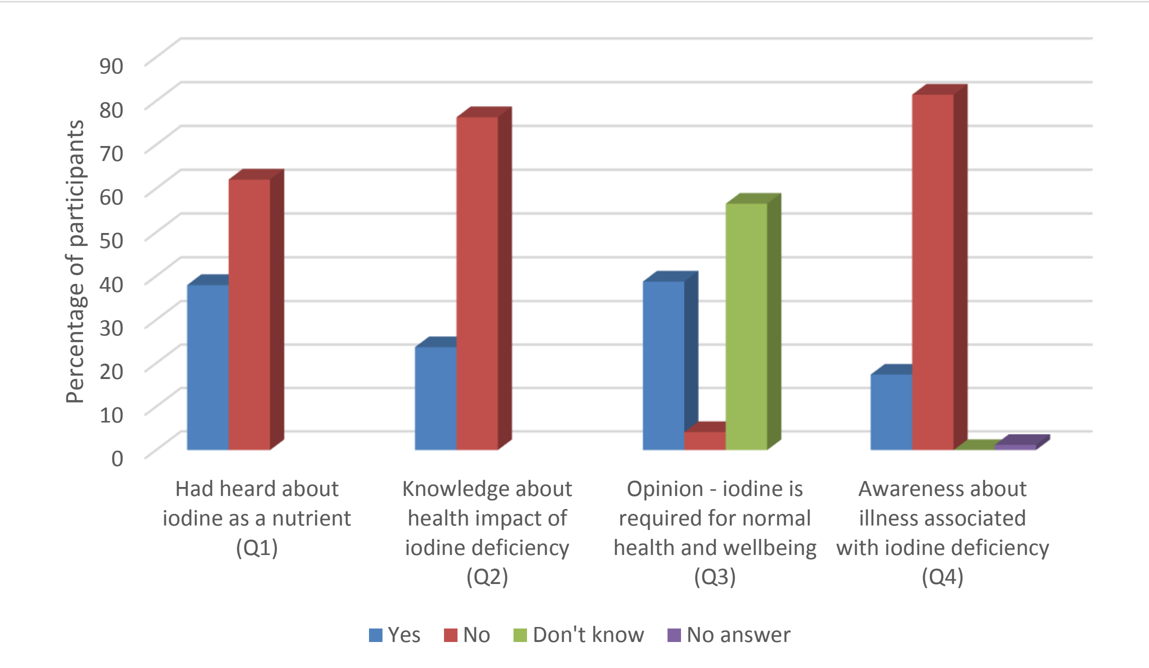


Figure 4. 1 Basic knowledge about iodine nutrition and deficiency

Figure 4.1 shows that 37% of participants had heard about iodine as a nutrient, but a lower number (23%) had an awareness about possible health impacts of iodine deficiency. Further, when the participants were asked about their opinion on the requirement of iodine for normal health and wellbeing, it was revealed that more than half of the participants (56%) responded 'Don't Know', while only 38% responded positively.

Following this, it was important to gather opinion on more specific perceptions about the importance of iodine for optimal health. So, in the second part of question three, the participants were asked to rate on a scale of 1 to 5 how important it is to maintain sufficient levels of iodine in the body.

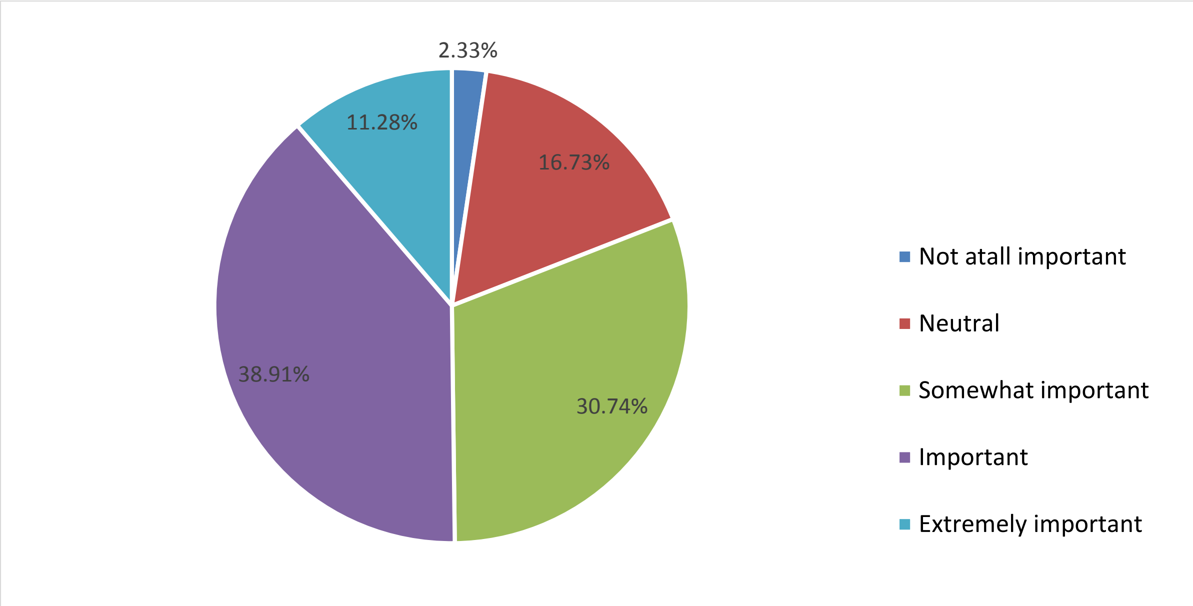


Figure 4. 2 Pie chart showing opinion about importance of sufficient iodine levels in the body

As seen in figure 4.2, 39% of the participants regarded it as 'important' and a similar percentage of participants (30%) felt that iodine was 'somewhat important' for normal health. Another noticeable aspect observed in these responses is that only 11% considered that iodine was 'Extremely important' for maintaining optimal levels of iodine in the body, thus emphasizing the need to increase the awareness about iodine nutrition. Here it is important to note that the data from 257 participants has been presented in the pie chart as 74 participants did not answer this question. Also, this question was not applicable for 175 participants as they answered 'No' in the first part of the question 3 implying that these did not perceive iodine was important for normal health and wellbeing.

To gain more clarity about the depth of knowledge of the participants, Question 4 enquired about the awareness of participants relating to illnesses associated with iodine deficiency. Figure 4.1 shows that a very high percentage of participants (81%) were not aware about any illness associated with iodine deficiency.

4.2.1.1 Awareness about iodised salt in the supermarkets

Question 5 was formulated to gather information about the overall awareness about the addition of iodine as a fortificant in salt and question 6 in continuation explored whether or not the participants had encountered any brands of iodised salt in the supermarkets. Question 7 asked about the details of the type of salt consumed on a daily basis (Figure 4.3).

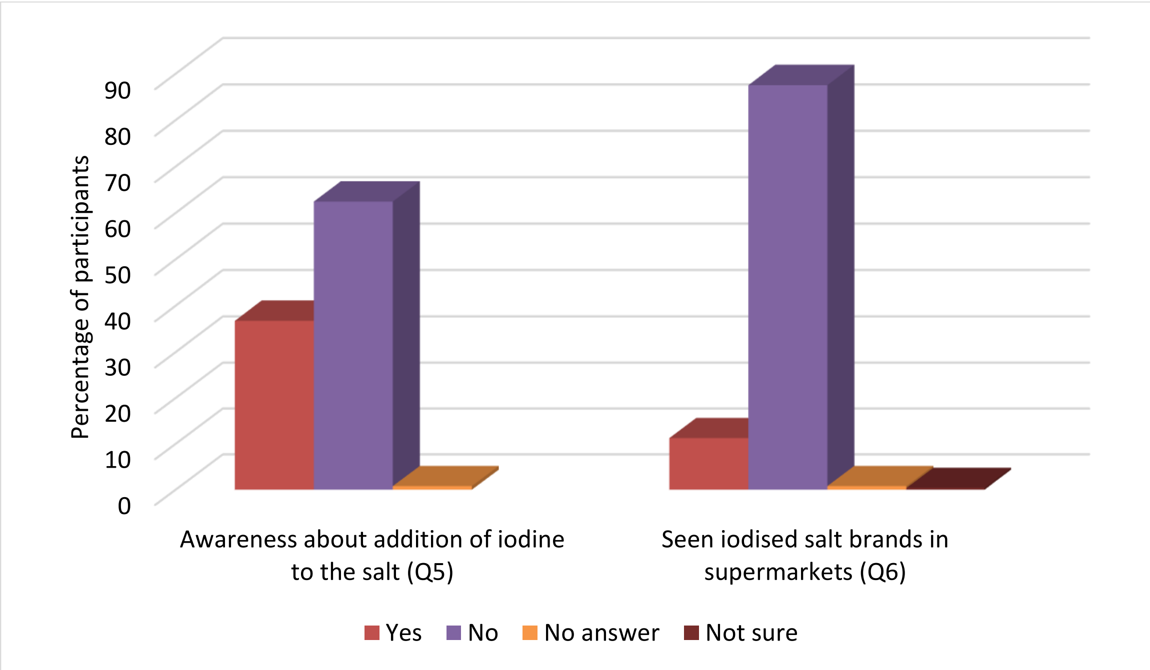


Figure 4. 3 Awareness about iodine addition to salt and iodised salt brands

It was found that only 36% of the participants had the knowledge that iodine could be added to salt and even less (11%) had come across brands of iodised salt in supermarkets.

4.2.1.2 Frequency and consumption pattern of different types of cheese

Proceeding further, questions 8 and 9 were focussed on knowing the frequency of cheese consumption and most frequently consumed varieties of cheese respectively. The results obtained have been represented in form of a bar chart and a pie chart with percentages indicating varying popularity and frequency of consumption of different types of cheese (Figure 4.4 and Figure 4.5).

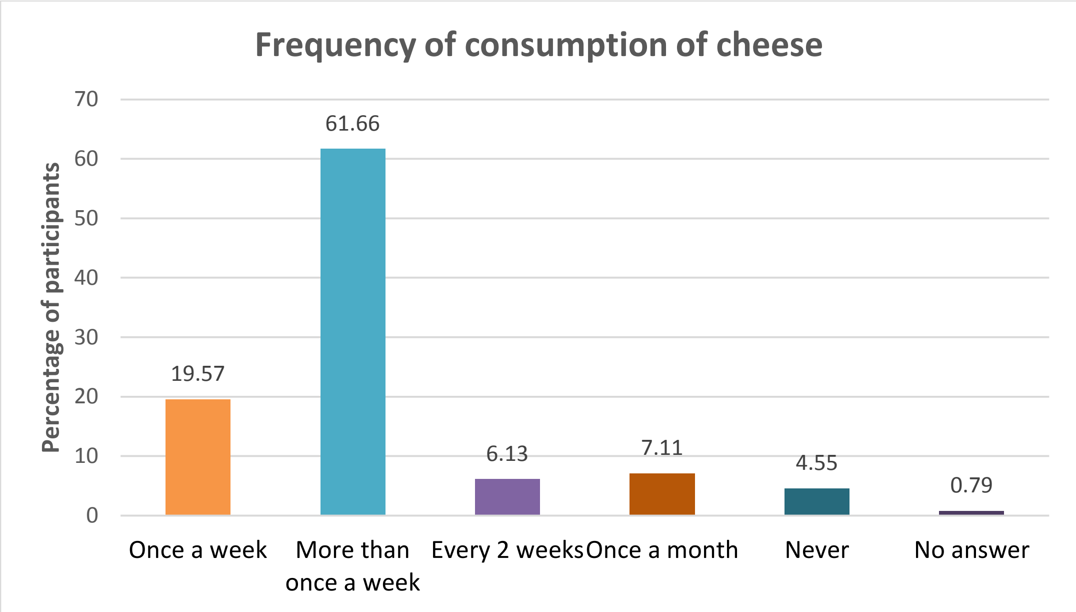


Figure 4. 4 Bar diagram showing frequency of consumption of cheese by the participants

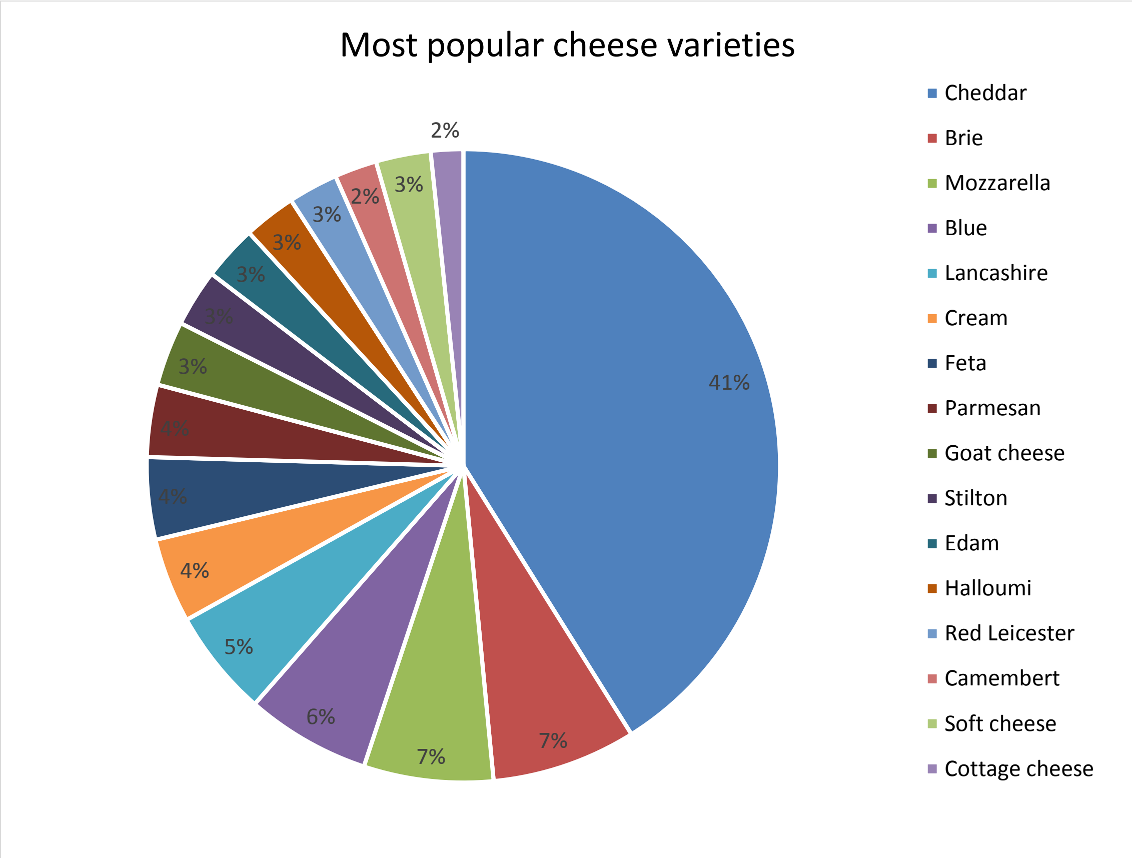


Figure 4. 5 Pie chart showing popularly consumed varieties of cheese

It is evident from the figure 4.4 that the highest percentage of the participants (61%) consumed cheese more than once a week and this is followed by 19.57% of participants consuming cheese once a week. Figure 4.5 clearly highlights that Cheddar is the most popularly consumed type of cheese occupying 41% (n=323) on the pie chart. The diagram also sheds light on a wide variety of cheese that is consumed by the participants ranging from the most popular cheddar (41%) to least frequently consumed camembert and cottage cheese. Here it is important to note that the pie chart depicts most frequently consumed cheese varieties and so those cheese varieties that were consumed by less than 10 people have not been included in the diagram.

Cheese varieties including Wensleydale, Machego, Roquefort, Smoked cheese, Gouda, Jarlsberg, Cambozola, Leerdammer, Emmental, Cheshire and Double Gloucester were consumed by 1-7 participants. Additionally, another aspect regarding cheese consumption is that a high number of participants (n=84) consume cheese as a part of a recipe (pizza with cheddar or mozzarella mix as topping, Greek feta cheese in salads, cheese sandwiches, cheese in lasagne).

After gathering vital information on iodine nutrition, salt and cheese consumption pattern the subsequent questions were focussed on obtaining data about consumer acceptability on various aspects including nutrient fortification, opinion on sensory differences (taste, texture) and their salt preferences.

4.2.1.3 Opinion on fortifying foods with vital nutrients and its impact on sensory qualities

Question 10 asked the participants if they liked the idea of fortifying commonly consumed foods with vital nutrients (vitamin D, iron). Moreover, this was an open-ended question, which prompted the participants to state the reasons if they responded negatively. The responses were varied. The majority of the participants (50%) liked the idea of fortification of foods with vital nutrients, 30.83% were not sure and so the response was ’Don’t know’ and only 17% participants responded negatively to this question. The following pie chart (figure 4.6) clearly depicts the various reasons stated by the participants who did not like the idea of fortification of foods.

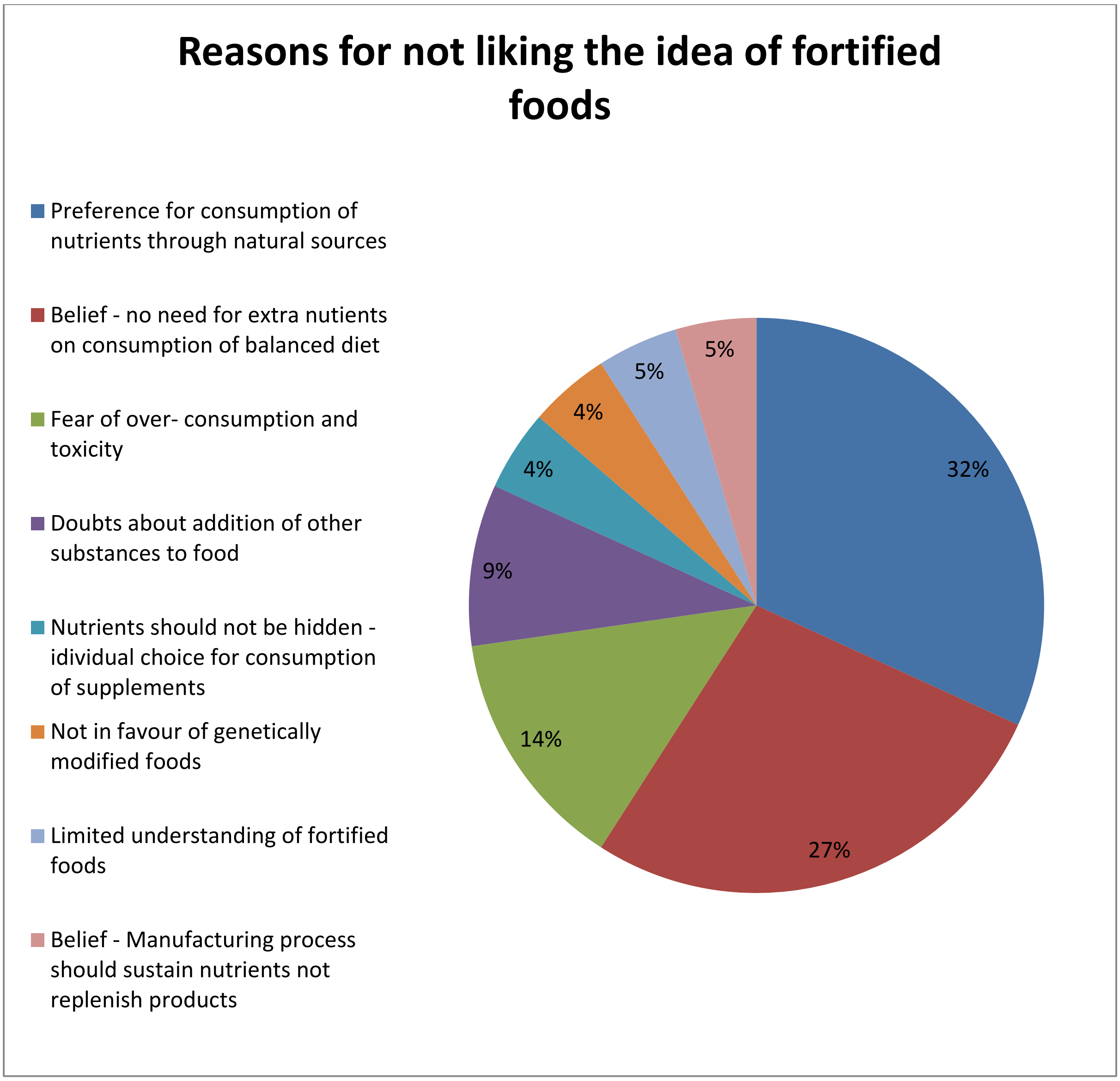


Figure 4. 6 Reasons for not liking the idea of fortified foods

Some of the prominent reasons for not liking fortified foods were: Liking food with natural nutrients, personal choice, products should be left without any enhancement, don't like any addition in the foods, prefer not to eat processed foods.

Question 11 – 'Do you think fortified foods differ in taste/texture/cost as compared to non-fortified food?' continued on the similar lines as question 10 and gathered more in-depth opinion about the perception of consumers towards fortified foods (Figure 4.7).

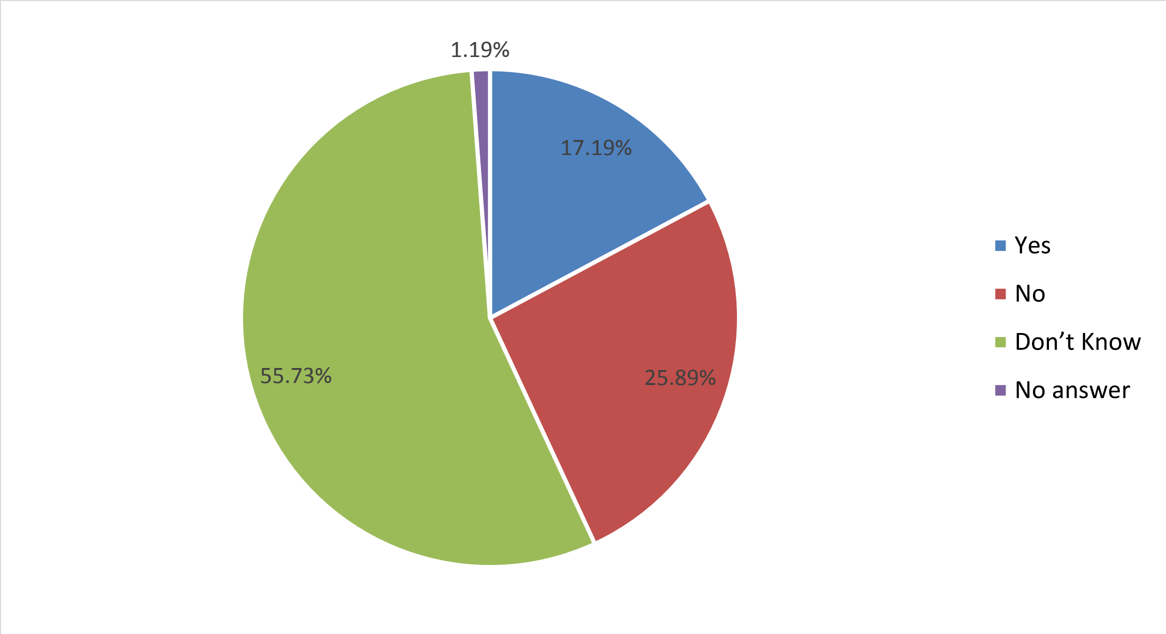


Figure 4. 7 Pie chart showing the opinion on difference in sensory qualities between fortified and non-fortified foods

4.2.1.4 Choosing between consumption of iodised salt and regular table salt

The final question (Question 12) enquired about the participant's preference between consumption of regular salt or iodised salt as a part of daily diet. This was an open-ended question where the participants were expected to state the reason for their choice (regular or iodised salt). The results obtained indicated that percent responses for each choice (regular salt and iodised salt) were very varied. This has been presented in the diagram below (Figure 4.8).

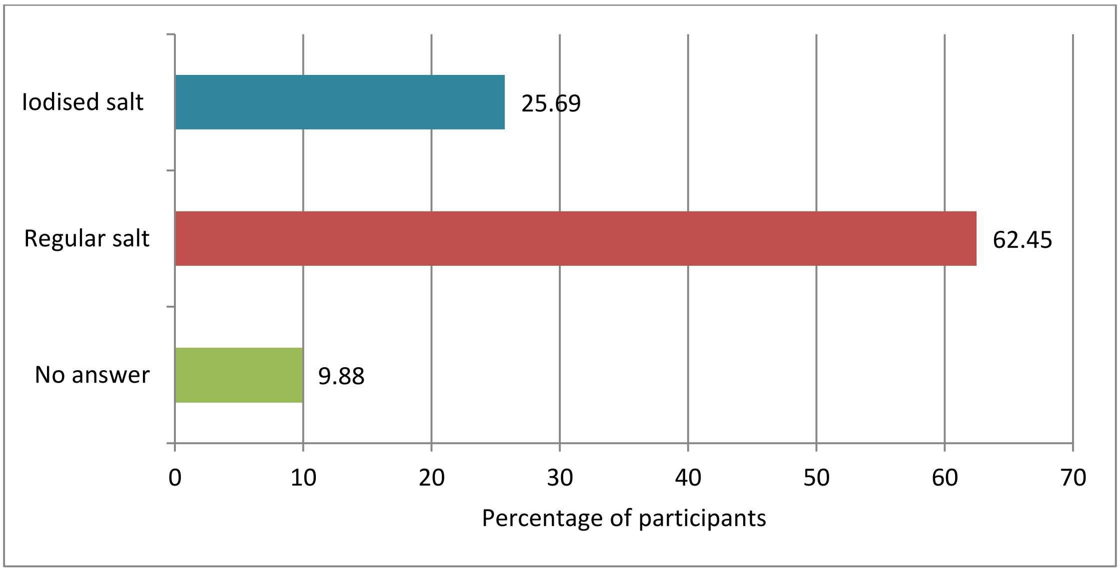


Figure 4. 8 Percent preference for consumption of iodised salt and regular table salt

It is clear from the above figure 4.8 that a high number (62%) of participants preferred regular table salt over iodised salt (preferred by 26% of participants). However, 10% of participants did not answer the question at all. In addition to these participants, who stated their preference between the two types of salt, there were 10 participants did not choose either of the salt, instead explained their response in the blank space provided in the template where participants were asked to provide their reasons for their choice of salt. Accordingly, six participants stated that they do not use salt as part of their daily diet, while three other participants were undecided about their preference and one participant did not have a preference about the type of salt.

In the second part of the question, the participants were also asked to state the reason for their choice between iodised salt and regular table salt. A wide range of reasons could be identified through an interesting blend of responses. These responses were sorted in a way which helped us to identify specific themes or the most common reasons for their individual choices. The following pie chart (figure 4.9 and figure 4.10) highlights the prominent reasons given by participants for their choice of salt.

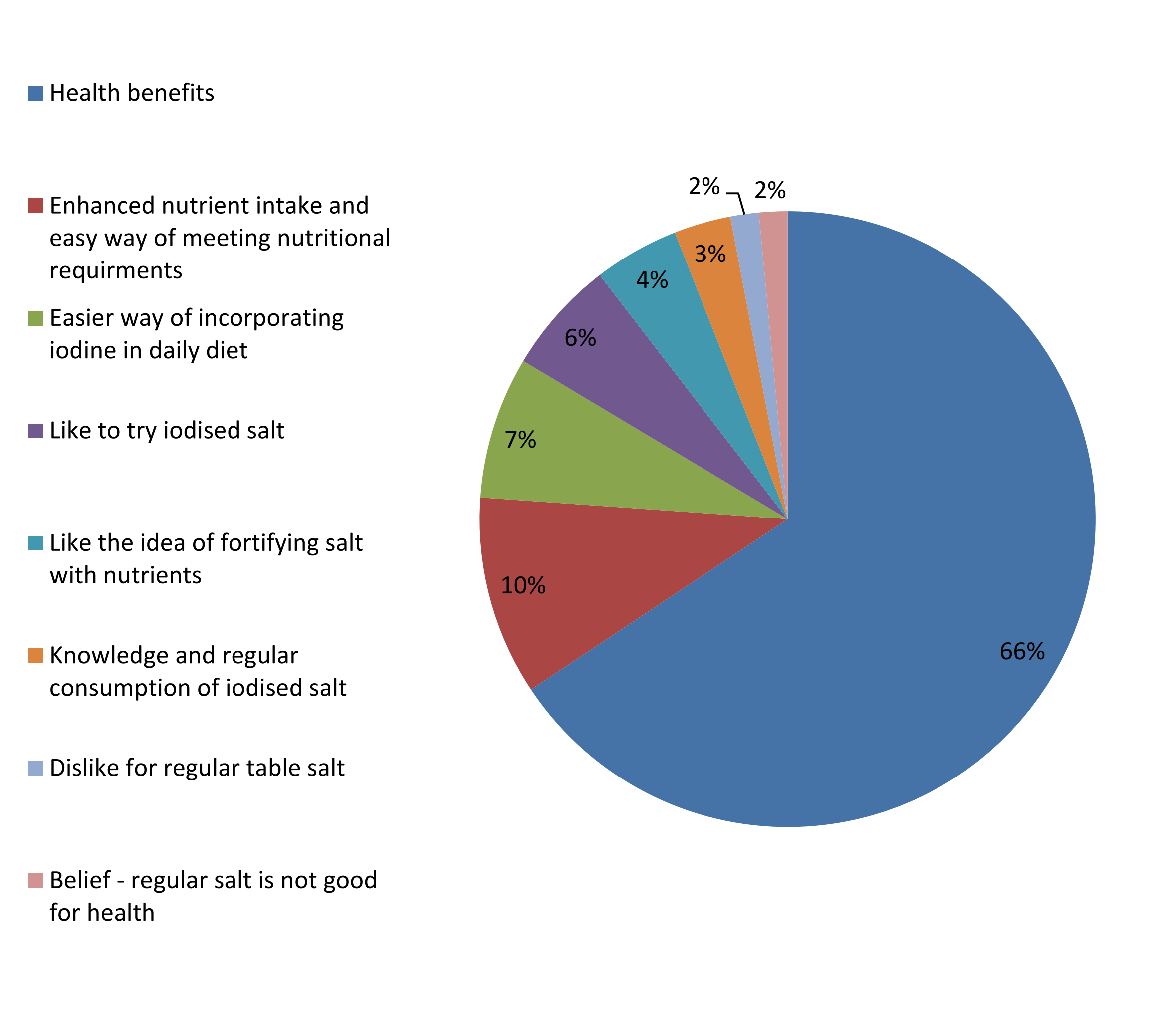


Figure 4. 9 Reasons for preferring iodised salt

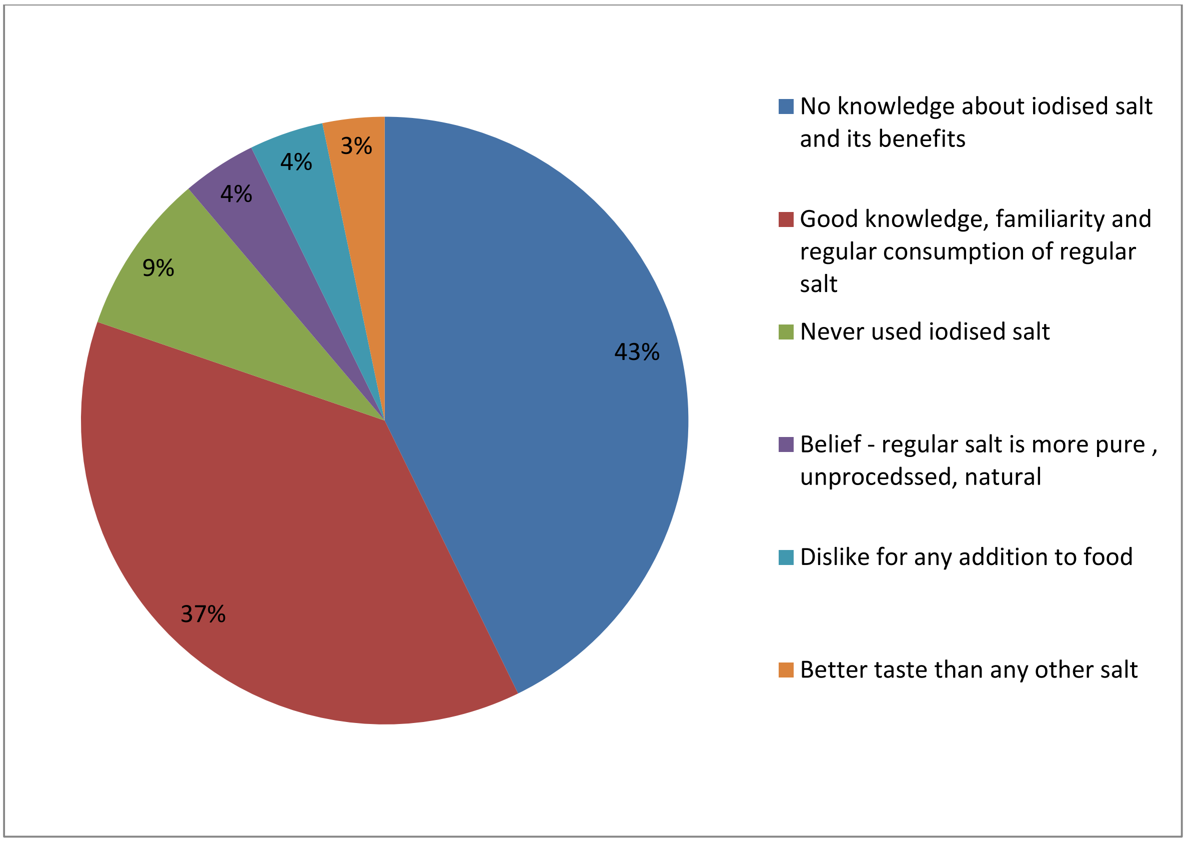


Figure 4. 10 Reasons for preferring regular table salt

From these diagrams (figure 4.9 and 4.10) it is clearly evident that participants who preferred iodised salt stated 'Health benefits' as one of the most important reason for the consumption the type of salt. This sheds light on the awareness of some general cheese consumers about the beneficial role of iodine for normal health and wellbeing. The participants who selected 'regular table salt' stated varied reasons for their choice of salt. The results reveal that the 'no knowledge about iodised salt and its benefits' and good knowledge or familiarity with regular table salt were some of the prominent reasons given by considerable percentage of participants (43% and 37%) respectively. This also shows that if the amount of awareness about iodine nutrition is increased in the general population then it is possible that the choice of type of salt might change over a period of time.

Here it is important to note that the pie chart covers the most common reasons given by the participants and so reasons given by less than 5 people have not been included in this chart. Carefulness due to medications, presence of plenty of iodine in daily diet, possible absence of iodine deficiency, cost of salt, easier option for purchase were some of the other reasons given by 1-4 participants.

4.3 Discussion of key findings of the chapter

In order to explore use of cheese as a potential vehicle for increasing iodine intake (via use of iodised salt), it is necessary to understand the level of awareness of the importance of iodine as a nutrient, perspectives about consumption of fortified foods and cheese and salt consumption patterns.

The consumer survey concurs with national surveys (Kantar Worldpanel, 2018, Defra, British Cheese board, Dairy UK, 2014) that have reported that Cheddar cheese is the most popularly consumed cheese in the UK and is therefore a potentially useful vehicle for iodine fortification. The frequency of consumption was found to be 'more than once a week' by the majority of the participants, which indicates that if this cheese is fortified with iodine then it can ensure regular intake of dietary intake of iodine. 50% of participants liked the idea of fortifying foods with nutrients while a similar percentage were 'not sure' indicating limited knowledge about health benefits of fortified foods. In terms of preference for type of salt as a part of their daily diet, regular table salt was preferred over iodised salt. No knowledge about iodised salt and familiarity with regular table salt topped the list of reasons for the choice of salt by the consumers. These findings have clearly established the need for improving awareness about the significance of iodine for normal health and wellbeing.

4.4 Limitations of the consumer survey

- In order to reduce sampling bias, a random sampling technique was adopted by recruiting consumers from different supermarkets (ranging from economically affordable- Aldi, Poundstretcher, to high-end supermarkets such as Booths, Sainsbury's) from different areas in and around Preston. It was assumed that this random sampling technique will help in obtaining good cross-section of population and reduce sampling bias however exact information regarding academic qualifications, occupation or socio-economic background was not collected in the developed survey template. So, it is important to note that the results are obtained from random sample of population, but these results cannot be interpreted in relation to an individual's knowledge or economic status.
- Use of complex words (deficiency, fortified) in the questionnaire is another aspect which could have affected the results obtained to a certain extent. For example: In the

Question 2 of the questionnaire, the participant was asked whether he/she is aware of health impact of iodine deficiency or in question 10, the participant is asked if he/ she likes the idea of fortified foods. The answers of these questions could be affected if the person does not know the meaning of the word ‘deficiency’ or fortified’. The accuracy of the results can be increased by using terms that would be easily understood or by rephrasing the question in simpler language. The researcher was willing to explain and answer any queries that the participant might have, but if the participant does not have sufficient opportunity (participant does not have sufficient time for thorough discussion with the researcher, or if the survey is completed online), so, the impact of use of scientific word should be considered.

- Another limitation of this survey is that in question 11 the participant was to comment yes/no if they think that fortified foods will differ in taste, texture and cost as compared to non-fortified foods. This is a limitation as while answering this question the participant would be unable to answer if the individual feels that he/she want to answer ‘Yes’ for difference in taste and texture but ‘No’ for the cost of the product. This aspect should be considered, and the question should be divided into three separate questions for all the three aspects for the comparison between fortified and non- fortified foods.
- Furthermore, for answering question 12, the participants were asked to guess which cheese contained iodised salt. The limitation of this question was that a ‘guess’ does not provide a clear understanding of the preference of the participant. The researcher could have asked a qualitative question about overall preference, and the reasons for the same. The question could be rephrased and the participants could be asked whether the two cheeses tasted different and also to describe how they were different from each other. This would have given some more information on whether the participants thought that they tasted different.

Chapter 5: Cheese production and analysis

5.1 Overview of chapter

The consumer survey established that Cheddar cheese and soft cheese were among the most popularly consumed cheese varieties and therefore, these varieties were selected for the present study. The flowcharts in chapter 2 (figure 2.3, 2.4 and 2.5) provide a brief account of the common pre- production steps for both the cheese varieties as well as individual steps specific to a selected variety of cheese (cheddar and soft).

This chapter describes the exact application of the individual steps involved in production of specific variety of cheese. The amount of core ingredients and calculations for various cheese specific ingredients (starter culture, rennet) have been explained in appropriate depth and this information is supported by use of photographs highlighting providing visual presentation of different stages in cheese production.

In the second section of this chapter, the results of the quantitative analysis (microbial and nutrient analysis) of the raw material of the cheese production (milk) and of the final cheese samples are presented in tabular format (table no. 5.1, 5.2 and 5.3).

During the cheese making process, it was identified that there are some key parameters, namely pH, acidity and temperature which were required to be tested continuously, at appropriate intervals and play a crucial role in the development of quality of the end product. Since these components are not only common between cheese varieties but also inevitable to the cheese making process, it becomes important to understand the fundamental roles of these parameters (also termed as process check parameters) in different stages of the cheese making process.

5.2 Significance of the key process check parameters in cheese production methodology

pH – It is considered as one of the most important factors which helps to ensure that all the characteristic developments in the cheese samples are taking place at their optimal level. This is because pH has fundamental influence on every character of cheese and this can be elucidated as follows

- The speed with which curd coagulates – a lower pH at the coagulation stage shortens the setting time
- The drainage of whey – lower- pH cheese will tend to retain more moisture. Rennet is more soluble at low pH and, therefore, the amount retained in the curd increases with decreasing pH at draining.
- The retention of calcium – low- pH cheeses will lose more calcium in the whey, decreasing elasticity of cheese
- The activity of ripening enzymes and ripening microflora – depends largely on pH. (Thomas, 2016)
- Cheese moisture, mineral content, texture and flavour are all influenced directly by the activity of free hydrogen ions (i.e. pH).
- pH also helps to control spoilage and pathogenic bacteria

Acidity – In context of cheese making, acidity is not described in terms of pH. It is determined using Titratable Acidity (TA), expressed as percentage of lactic acid. TA is a measure of the total quantity of lactic acid present in conjugation with the buffering ability of the casein. The value obtained after titration cannot be converted to pH as it does not take into consideration the buffering effect of the casein. Another important aspect to be highlighted is that, acidity is one of the most important process checks as it helps the manufacturer to decide the subsequent step in production and the functional roles of certain amounts of acidity are different at each stage of cheese processing.

- Initially during ripening, after addition of starter culture, it helps us to confirm that the starter culture is working properly as there is a slight increase in the acidity (the bacteria converts lactose into lactic acid)
- Acidity testing while cutting curds informs us about the rate of acidity development (which is carefully controlled by the level of starter culture and temperature).
- Amount of acidity produced is affected by temperature and so monitoring temperature is equally important.
- The amount of acidity development is also important for the final characteristics of the curd and cheese.

Temperature – The temperature has a significant role in optimal coagulation of milk to form curds. Curd formation is indirectly responsible for the formation of firm good quality cheese varieties. The optimum coagulation temperature for most cheese is 30-32°C, the exception is Swiss which is set at 37°C. At temperatures, less than 30°C the gel is weak and difficult to cut without excessive yield loss. At temperature, less than 20°C coagulation does not occur, but the milk can coagulate quickly if warmed to the appropriate temperature. Also, appropriate temperature drives optimal whey drainage and then more firm cheese blocks can be formed.

Difference and interlink between acidity and pH

Since acidity, in the context of cheese making, is not described in terms of pH, it is important to understand how these two parameters can have an impact on cheese processing individually, as well as together at each specific stage. The difference between TA and pH is due to their effect on cutting. Up to the time of cutting, TA of the milk increases with the development of acidity by the culture. After cutting curds, the TA of the whey is much lower. This does not mean that acid development stopped. This is because the titratable H^+ ions associated with the milk proteins are no longer present in the whey. In a nutshell, pH is a good indicator of the initial quality of milk, while acidity can help to reflect changes occurring throughout the process of cheese making.

Overall, it can be concluded that pH, acidity and temperature are interlinked at different stages of cheese processing. In totality, these parameters can have significant impact on the quality of curd and final cheese development and so it is imperative to monitor them at specific intervals during the cheese production cycle

Starter cultures and related terms used in their description

Packets of starter cultures for different varieties of cheese are sold to the cheese manufacturers on the basis of their units of activity and the methods used for production of cheese indicates how many units of activity per volume of milk is needed, specific to the exact type of cheese that is being manufactured. Some methods used in cheese making need faster acidification than others, thus according to this requirement, different starter cultures are used.

<u>Specific terms related to the use of Starter culture</u>: The information about the nature of the starter culture is labelled on the packets as 1U or 1DCU. Here 'U' means units of activity of starter culture. In the present study, the starter culture used for Cheddar cheese production was labelled as 1DCU/60 litres while that used for soft cheese production was 10 DCU/100 litres. Here 'DCU' is used for cultures made by the company 'Danisco' and so this DCU means Danisco Culture Units.

The cheese production at FTC was carried out under close guidance of the food technologist at the centre.

5.3 Food safety measures before entry into the production area

The food technologist at the centre (The Food Technology Centre, Anglesey) provided detailed guidance on health safety, food hygiene and food safety measures. This included the wearing of a head to toe lab coat with head cap and long rubber boots. After adopting the required safety clothing, hands were washed thoroughly, wiped with clean hand tissue and hand sanitizer solution applied. This detailed procedure for safety clothing and hand hygiene was critical as bare hands (without gloves) were used for the subsequent cheese production.

5.4 Common production steps for cheese varieties (cheddar and soft cheese)

As seen in the flowchart (section 2.7.1.1, figure 2.3), there are number of pre-production steps that are common for production of both the varieties of cheese samples (cheddar and soft with and without iodised salt) for the present study. These steps are elucidated in appropriate depth in the description given below.

Milk collection

Approximately 80 litres of milk was obtained from a regular milk supplier for the centre (Glanbia Cheese Limited) one day before the scheduled cheese production. The tankers from this company regularly deposit large quantities of milk, collected from several small farms. This milk was stored in appropriate plastic containers (figure 5.1).

Figure 5. 1 Raw milk collection containers

On the following day, the cheese production process started at 9.00 am and continued till 5pm incorporating various crucial steps at different time intervals.

Microbial testing for raw milk and pasteurised milk

Before beginning any part of the production process, the raw milk obtained from the supplier was sampled (4 bottles, each containing 20ml of milk) and this milk was tested for total viable count (TVC). Milk samples were also collected after pasteurisation. These pasteurised milk samples were tested for six microbial parameters namely TVC, Ecoli, *Listeria*, S.aureus, Enterobacteriaceae and *Salmonella*.

Figure 5. 2 Bottle for collection of milk for microbial analysis

Washing and sanitizing

All the containers, glassware and equipment were dipped in 0.25% hypochloride solution for 15-20 minutes, after which they were rinsed thoroughly under running tap water.

Figure 5. 3 Sanitizing and detergent washing area

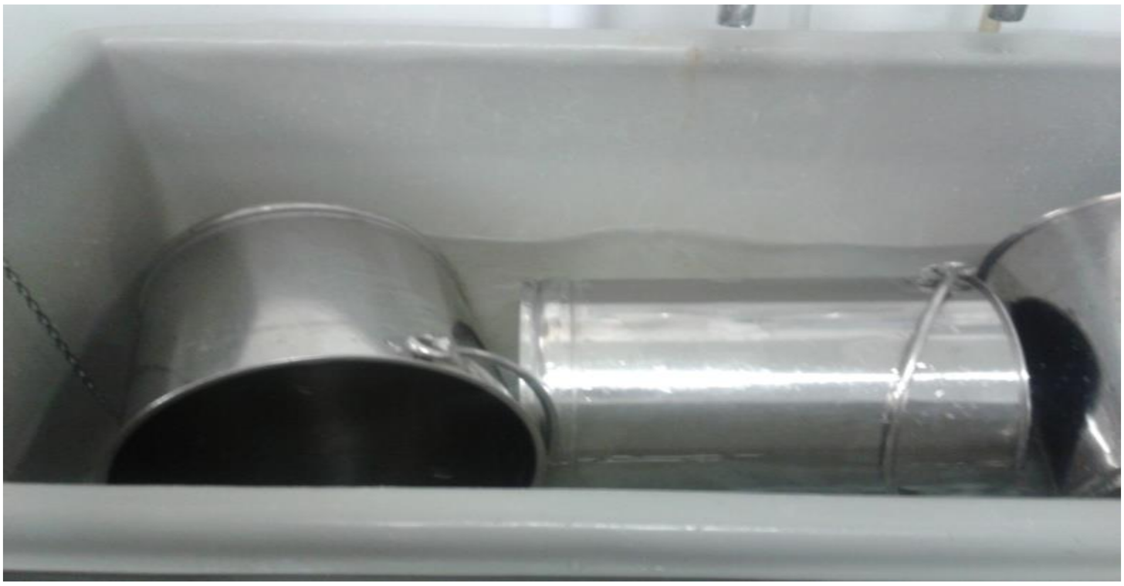


Figure 5. 4 Containers for transporting milk from the pasteuriser to the cheese making vats

Figure 5. 5 Small cheese moulds

Figure 5. 6 Cheese vat washed using sanitizing solution and thorough cleaning with tap water

Figure 5. 7 Cheese vat filled with water for heating it to specific temperature

Room setting and filtration of raw milk

The cheese preparation room temperature was set at 24-25°C. The temperature of raw milk was measured and recorded at 4°C, following which the milk was passed through a filter to remove any impurities or particles.

Figure 5. 8 Sieve and muslin cloth for raw milk filtration

Batch-pasteurisation of raw milk

The milk was pasteurised at 63°C for 30 minutes in a batch pasteuriser (maximum capacity- 120 litres). A constant temperature is essential for effective pasteurization, monitored using a thermo-regulator. In addition, digital thermometer (TO234LC, Digitron UK) was also attached to the pasteuriser, so that the temperature measurement could be as accurate as possible (figure 5.9).

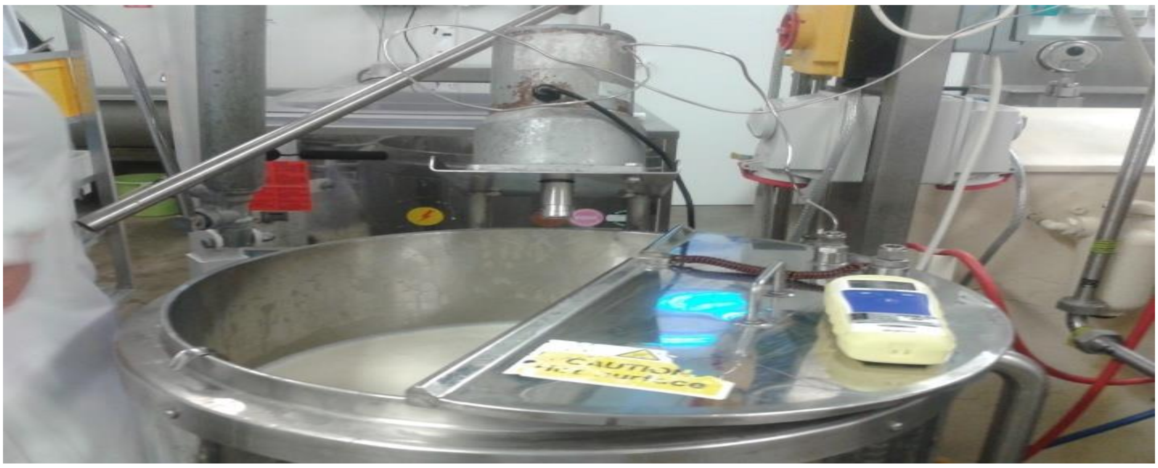


Figure 5. 9 Batch pasteuriser

If the temperature of milk falls below 63°C during the pasteurisation process, it is necessary to repeat the 30-minute heating cycle.

Figure 5. 10 Batch pasteuriser with temperature of the milk being measured

Figure 5. 11 Digital thermometer

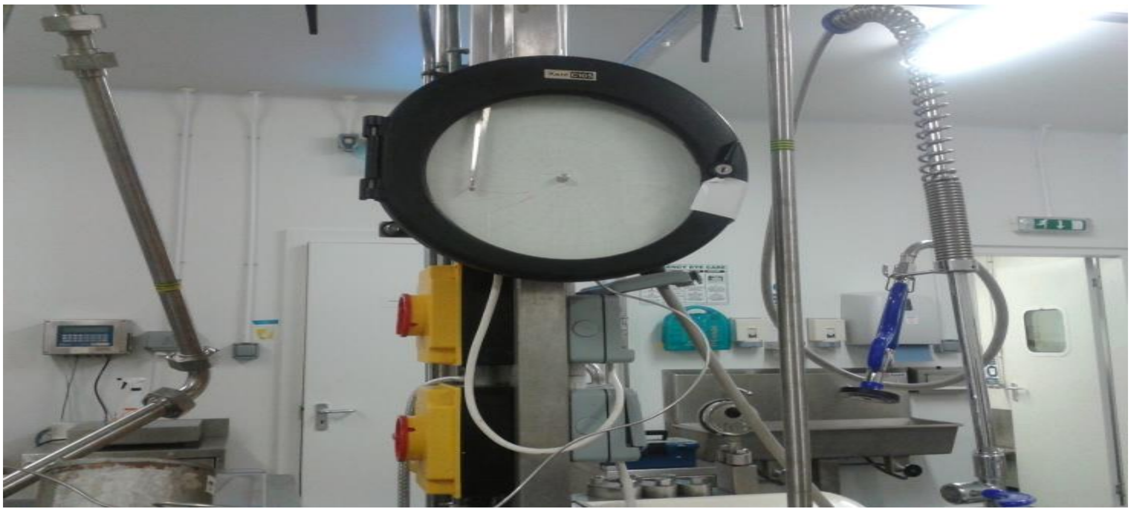


Figure 5. 12 Thermo-regulator

Transfer of milk from the pasteuriser to cheese vats

Following successful pasteurisation, the hot water in the pasteuriser was replaced with cold water in order to cool the milk from 63°C to 32°C (for cheddar cheese) and to 22°C (for soft cheese). Once the required temperature was attained, the volume of the milk was recorded to enable the calculation of starter culture and salt to be added at a later stage, and the milk was transferred into cheese vats.

Figure 5. 13 Tap of the pasteuriser from where milk from pasteuriser is transferred into the milk container to be transported into cheese vats

Testing for acidity, pH and addition of starter culture

Following transfer into the cheese vats, the temperature of milk was about 32°C (for cheddar cheese) and 22°C (for soft cheese). At this point in the process, the 10 ml of milk was sampled for testing for acidity and pH (using pH meter – Mettler Toledo Seven 2GO PH/MV) (figure 5.14).

Acidity was recorded as 0.22 % lactic acid and pH was 6.70, which falls within the acceptable range (expected pH – 6.5-6.8, acidity – 0.11-0.18% lactic acid) for cheddar cheese while the pH for soft cheese was recorded as 6.68.

Figure 5. 14 pH meter

The starter culture PCS 12 (1 dcu/60 litres) was accurately weighed (0.96g and 0.90g respectively) using an accurate analytical scale (SHS Super Hybrid Sensor and A and D Company limited), added to the individual vats (for 2 varieties of cheddar cheese). The starter culture used for soft cheese was Probat 222 @ 10 DCU/100 litres (Lot number 4113109954 10U = 1.5g). These starter culture- milk mixtures in the individual cheese vats were stirred for 10 minutes for uniform dispersion. (figures 5.15, 5.16). The weight of starter culture required was calculated as described in section 5.4.1

Figure 5. 15 Starter culture (PCS 12)

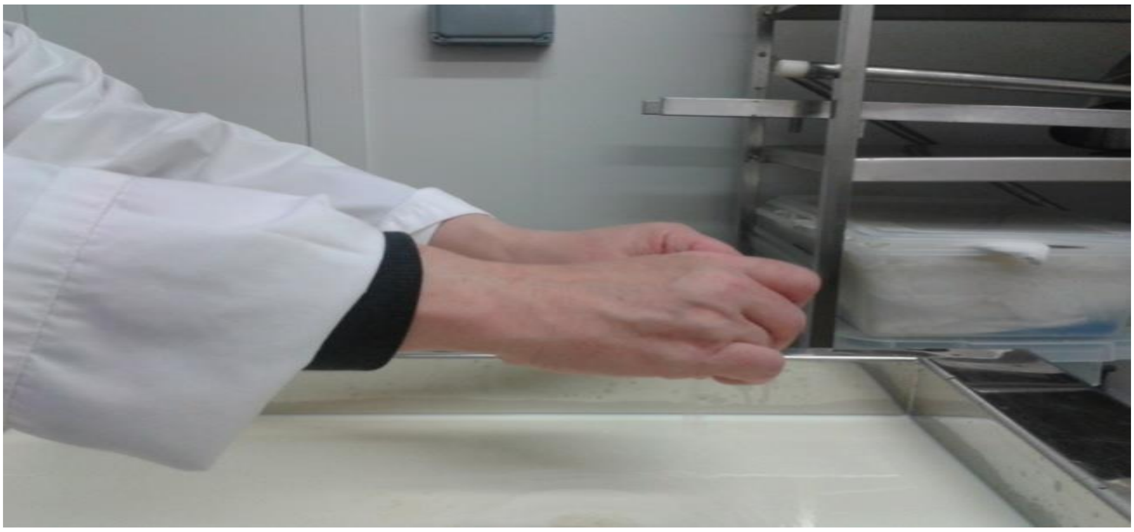


Figure 5. 16 Addition of starter culture

5.4.1 Calculation of starter culture for Cheddar cheese production

The starter culture used for Cheddar cheese production was PCS 12 Lot G9183, BB 10/18

Values on packet - 10 U = 13.175g, so for 1U = 1.3175g

1U = 60 litres

So, with this information we have to calculate the Units in and then the grams of starter culture that should be added in the specific amount of milk for this production session.

60 litres = 1U, so for 1litre – 1/60 U

We have 43.8litre of milk in one of the cheese vats – so it amounts to 0.734U

1U = 1.3175g

So, 0.734 U = 0.962g

This implies that in the cheese vats containing 43.8 litres and 41.2 litres of milk, 0.962g and 0.905g of starter culture respectively was added

The calculation for starter culture for soft cheese is similar to cheddar cheese depending on the Units of activity of starter culture and volume of milk.

After addition of starter culture, the steps in production (heat treatments, process checks, time for maturation and duration until the final product is packed and labelled) vary depending on the type of cheese manufactured.

Details about the individual steps after addition of starter culture for Cheddar cheese production

Ripening period for the starter culture

The milk and starter culture mixture was allowed to ripen for 50 minutes.

During this time span, the acidity and pH was measured by sampling 10 ml of milk accurately with help of a syringe (BD).

The pH was measured using pH meter (Mettler Toledo Seven 2GO PH/MV) and the acidity was measured using titration method with phenolphthalein indicator.

Determination of titratable acidity

10 ml of milk (or whey once it was clotted) was measured accurately using a syringe and this was added to a 100 ml clear plastic beaker (glass beaker should be avoided as much as possible in the dairy). Following this, the plastic beaker was placed on a white tile (white tile is important as it is easy to see the colour change) and 4-5 drops of phenolphthalein indicator were added using a plastic pipette. Subsequently, 0.111M sodium hydroxide solution was added from a burette, and the beaker was swirled to observe the colour change. The end-point was attained when the solution changed colour to pale pink and this colour remained stable for a longer duration. At this stage, the volume of sodium hydroxide required for the

titration was recorded. To get the final acidity value, this final volume obtained after titration (ml) was divided by 10 and the result was recorded as percentage lactic acid.

Here it is important to note that acidity and pH are two crucial parameters in the process of cheese making and if not monitored properly can adversely impact the quality of the final product. So, acidity and pH were tested throughout the process at appropriate intervals.

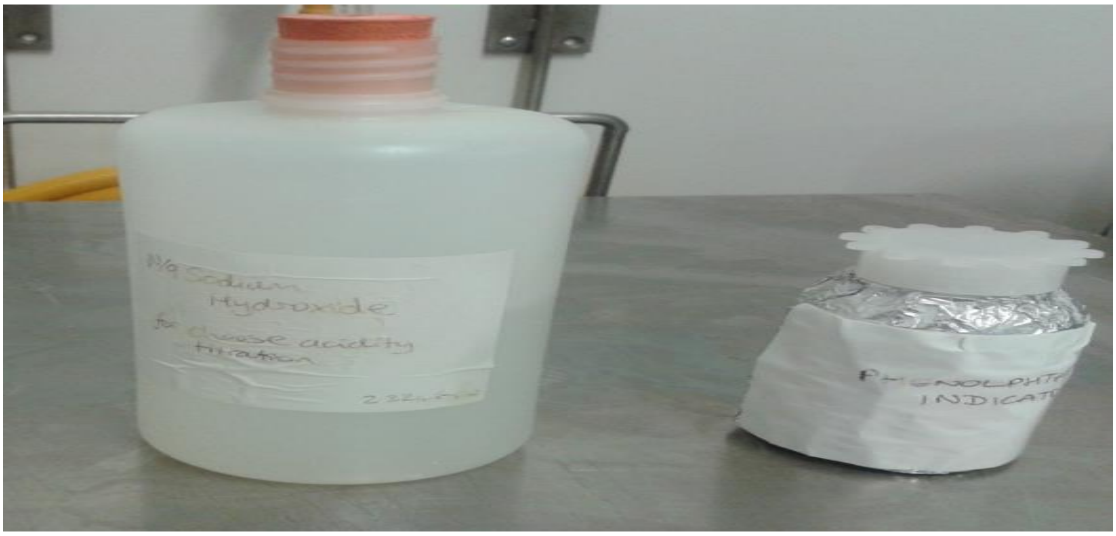


Figure 5. 17 Indicator and alkali solution for titration

Figure 5. 18 Titration apparatus for acidity measurement

Addition of rennet

At the end of ripening period, the pH, acidity and temperature of the milk samples from the individual vats were measured and recorded. Rennet was added to the vat, based on the volume of cheese culture as described in the following calculation. Here it is important to note that the measured rennet was not diluted before addition to the vat. However, while adding rennet to the vat, the contents of the vat were continuously stirred, so that the rennet can be uniformly distributed in milk.

Figure 5. 19 Measurement of rennet

Figure 5. 20 Standard rennet procured for semi hard cheese production

5.4.2 Calculation for amount of rennet in individual vats

Standard value for rennet addition - 25 ml per100 litres of milk

Vat 1 contained 43.8 litres, therefore 11 ml rennet was added.

Vat 2 contained 41.2 litres, therefore, 10.3ml of rennet was added.

Ripening for formation of curds

Following addition of rennet, the milk samples were stirred continuously for exactly 2 minutes to ensure uniform distribution of rennet. At this point, the heating element was switched off and the vats were covered and left to clot (form curd) for 75 minutes (figure 5.21)

Figure 5. 21 Ripening period of 75 minutes after addition of rennet

Cutting of curds

Exactly at the end of 75 minutes the curds were cut. Cutting of curds was carried out using stainless steel curd cutters (two types of cutters) to cut the curds horizontally and vertically so that it can lead to formation of clear curd cubes (figure 5.23, 5.24). Simultaneously the pH, acidity and temperature was measured and recorded. Further the curds were allowed to settle for 5 minutes. (figure 5.25)

Figure 5. 22 Stainless steel curd cutters

Figure 5. 23 Curd is cut using stainless steel curd cutters

Figure 5. 24 View of the curd in the vat after cutting curds in horizontal and vertical direction

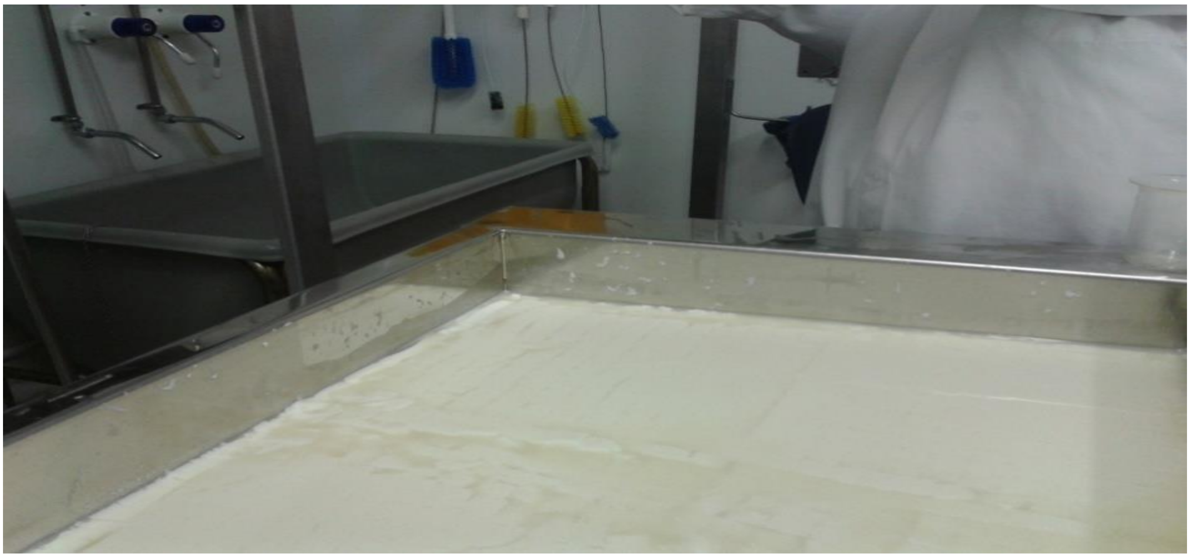


Figure 5. 25 Another view of the curd after cutting curds in horizontal and vertical direction

Scalding

Moving further in the production process, the heating element was switched on and the curd-milk mixture was stirred and scald to 40°C and then this was followed by continuous stirring for 60 minutes. During this 1-hour time-span, it was ensured that the temperature of the curd-whey mixture was maintained at 40°C. This 1 hour is the time required for the bacteria to produce sufficient levels of acidity.

This amount of the acidity has significant impact on the final characteristics (texture, flavour and overall quality) of curds and cheese and so was measured at short intervals (approximately every 5-10 minutes). The expected target for acidity was 0.14%. Once this acidity was attained, the stirring process was stopped and the curds were allowed to settle for 5 minutes.

Figure 5. 26 Whey sample extracted using syringe (BD) for testing acidity and pH

Figure 5. 27 Stirring the curds continuously at 40°C until the optimal acidity value is obtained

Figure 5. 28 Temperature is checked while stirring at close intervals

Acidity testing for the cut curds (whey) and draining of whey to separate curds

At the end of this short settling time, the curds were pushed 1/3rd of the way up the vat. At this stage, the curds were appropriately squeezed and pushed to separate whey out of the curds and small balls of curds (cricket ball size) were formed. For this, stainless steel cutters were utilized, however a muslin cloth was around, as the main purpose of this was to push the curd on one side of the vat, so that the whey can be easily separated (figures 5.29, 5.30, 5.31).

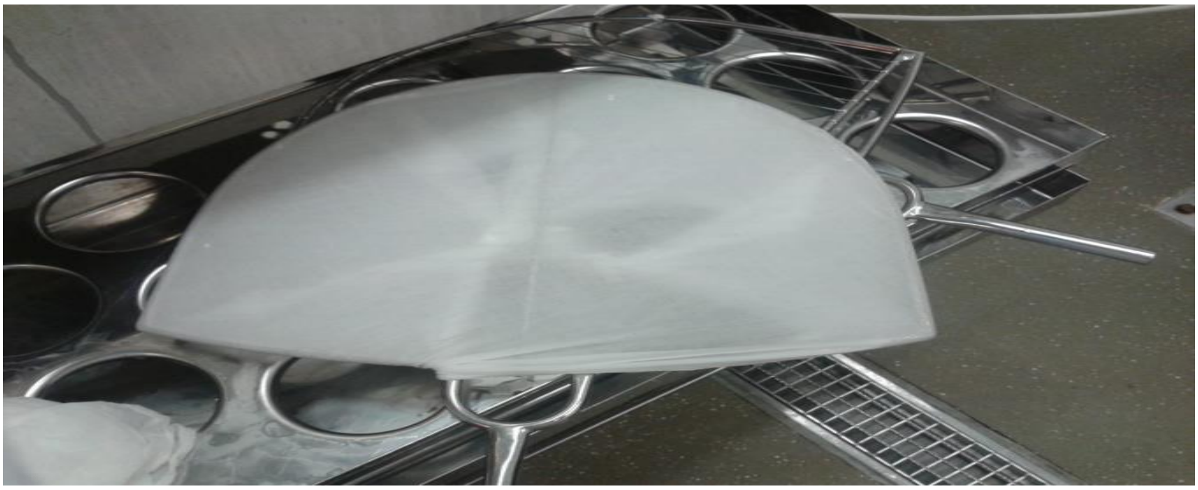


Figure 5. 29 Curd cutter prepared by wrapping muslin cloth for curd-whey separation

Figure 5. 30 Whey is separated from the curd

Figure 5. 31 Whey is passed through a sieve to separate curd mixed with whey

Figure 5. 32 Curd is pushed 1/3 way up on one side of the vat to separate whey and curd

This was continued for about 20 minutes and after this the acidity was measured (target value – 0.17-0.18% lactic acid). The curds were cut in two parts (stacks) in such a way that a channel was created in between two blocks of curds, so that the maximum amount of whey could be drained out.

Figure 5. 33 Channel created between two big curd blocks to release all the trapped whey more efficiently

Acidity testing was one of the most crucial steps in cheese production (as described in section 5.2) and so again, it was important to test the acidity of fresh whey that was coming out through the channel created in between the curd blocks.

This whey draining process was continued for 20-25 minutes depending on the acidity values obtained after testing the curd and whey. By this time, it was observed that the curd was increasing in its firmness and improving in its hand feel and texture.

Figure 5. 34 Separating whey from the curd blocks

Figure 5. 35 Transfer of whey from the vat to another container (either discarded or reused)

Since the curds were developing firm texture these were cut into 9-inch blocks and were turned to extract as much whey as possible.

Figure 5. 36 Curd blocks

Figure 5. 37 Curd piles being turned to separate as much whey as possible

Figure 5. 38 Another view of curd piles being turned to separate as much whey as possible

During this curd piling, the fresh whey was tested for acidity (target value -0.22-0.25% lactic acid). In the present production session, the acidity was recorded as 0.38% lactic acid for both the cheese vats. Even though this value was slightly higher than the expected range, it was suggested that it can be accepted as in subsequent steps in the production the acidity would be following an upward trend.

Combining curds from individual cheese vats

Once the optimal value for acidity was obtained, the curds from two individual cheese vats were combined in one vat and all the individual blocks of curd were piled one above the other.

Figure 5. 39 Cheddaring (piling cheese blocks one above the other)

This piling of blocks helps to give more firm structure / shape to the curd as well as ensures more efficient draining of the whey. At this stage, the acidity of the fresh whey coming out of curds was measured and the piles were turned after 10-15 minutes. At the end of 15 minutes the piles were again turned in the opposite direction and the whey was tested for acidity. This turning and piling process of the curds was continued until optimal acidity value was obtained (expected value = 0.45% lactic acid). The acidity of the curds in the present study was found to be 0.50 % lactic acid.

Figure 5. 40 Turning the piled cheese blocks upside down to change positions

Hand-milling of curds

The milling process by hand is an important milestone in the cheese production process that needs to be noted. At this stage, the texture and feel of the curd underwent a marked change, becoming stretchy or elastic (cooked meat texture).

Figure 5. 41 Hand milling of the curds

Figure 5. 42 Cooked meat like texture of curd due to the combined reaction of milk, starter culture and rennet

At the end of milling process all the curds were thoroughly combined and weighed accurately using the analytical scale (SHS Super Hybrid Sensor and A and D Company limited). The total weight was found to be 11.3 kg.

Addition of salt

The final stage in the cheese production process is the addition of salt. The aim of the present study was to develop two varieties of cheddar cheese, one with regular table salt and the other with iodised salt. The firm, stretchy blocks were divided into two equal portions of 5.6 kg. The salt was added to a final value of 2.3% by weight, thus 130 g of either, regular table salt (SAXA) or iodised salt (Cerebros) was sprinkled on the surface of the curds in the individual vats and mixed thoroughly, to ensure uniform distribution throughout the curd. This mixture was allowed to settle for 15 minutes, then remixed after another 5 minutes and then again after 10 minutes.

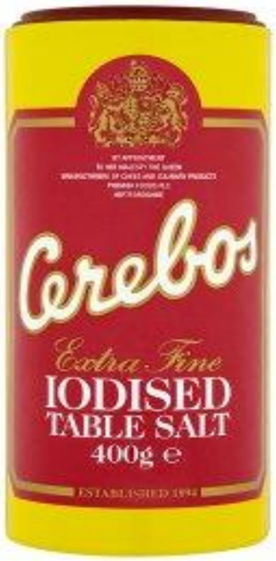


Figure 5. 43 Iodised salt used for production of Cheddar and soft cheese

Figure 5. 44 Regular table salt used for production of Cheddar and soft cheese

Curd Pressing

Two different cheese curd sets (one with regular table salt and other with iodised salt) were transferred into stainless moulds (1 mould for each vat) for pressing. These moulds were pressed at 10 psi for approximately 1 hour (figure 5.48, 5.49). The excess whey released from the curd (figure 5.50) has a high protein content and can be dehydrated for use in high protein powders or recipes.

Figure 5. 45 Moulds being prepared by lining them by muslin cloth

Figure 5. 46 Curd transferred into stainless steel moulds

Figure 5. 47 Preparing moulds with curd for pressing in curd pressing machine

Figure 5. 48 Moulds loaded on the curd pressing machine

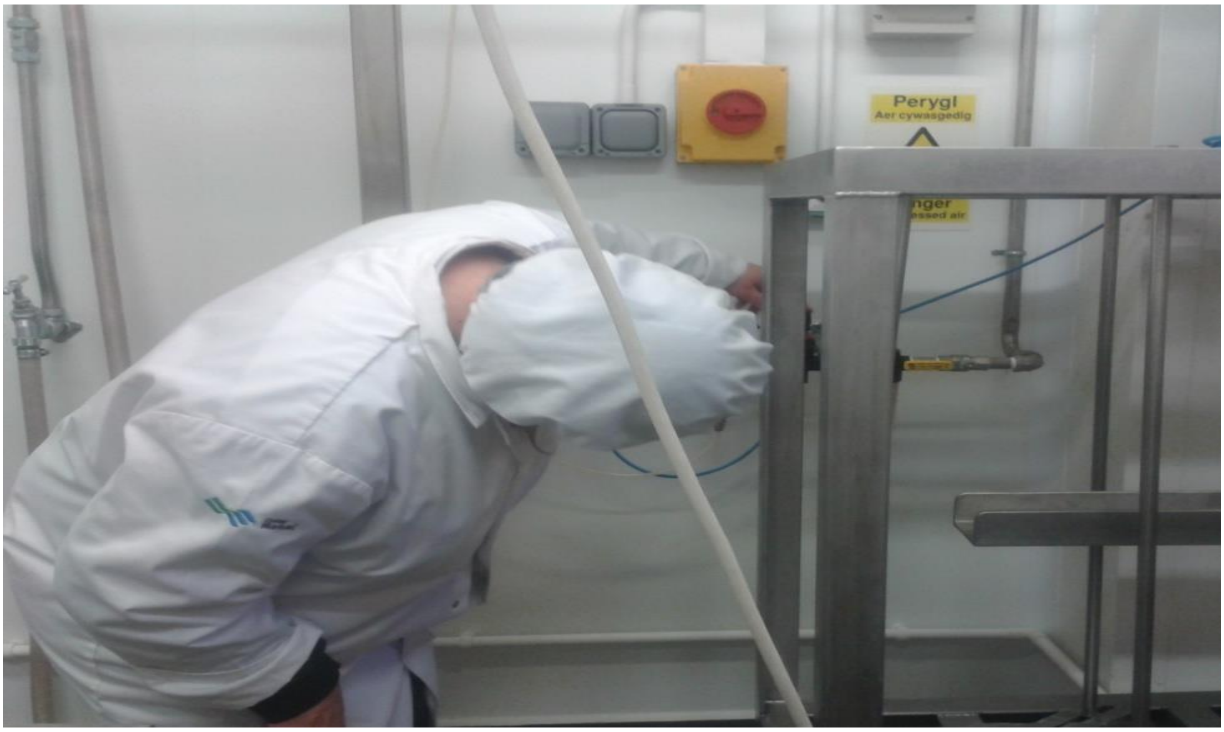


Figure 5. 49 Pressure is being set at 10 psi for 1 hour

Figure 5. 50 Excess whey released from the moulds upon pressing

Overnight pressing at 80psi

After pressing the curds for 1 hour at 10 psi, these curds were checked for uniform, smooth texture and the pressing was continued overnight at 80 psi. This increased pressure is important because as the curd becomes thicker and firm, draining of whey becomes difficult. So, in order to drain out all the possible whey, the pressure was increased. In the morning, the cheese moulds were checked for their firmness and smooth texture. These curd blocks were then turned in the opposite direction to ensure that the curd blocks were pressed uniformly from both the sides. After turning, the moulds were again pressed for 5 hours at 80 psi, then removed from the moulds and weighed. The total yield recorded was 4.9 kg for cheese with iodised salt and 4.8 kg for cheese with regular table salt. Following this, the samples were vacuum packed, appropriately labelled and then stored for ripening (flavour development) at 10-12°C

Figure 5. 51 Curd moulds pressed overnight at 80 psi

Figure 5. 52 Final cheese after uniform pressing from both the sides

Figure 5. 53 Another view of final cheese pressed uniformly from both the sides

Figure 5. 54 Final vacuum-packed cheese samples (2 types - one with iodised salt and other with regular table salt)

The maturation period for Cheddar varies from 3 months to 18 months or more (mild is usually sold at 3 months, medium 5-6 months, mature cheddar 9 months, extra mature 15 months and vintage 18 months plus) and this period helps in development of better flavour. For the present study, there were time constraints and so the cheddar cheese samples were tasted at short time intervals for observing the flavour development. The first block of vacuum packed cheese was tasted after maturation period of 1 month and 10 days. As part of this tasting procedure, the food technologist at FTC opened the individual blocks of cheese (with and without iodised salt) with a sterilised knife and cut slices of cheese towards the edges. Cutting at edges was decided for convenience. It was suggested that cutting at the edges or at the centre would not affect the taste or flavour as generally the whole block of cheese would mature uniformly. Once the required block of cheese was taken out for tasting, the remaining sample of cheese was re-vacuum packed. The cheese samples were subsequently cut into uniform cubes for tasting. Approximately, 5 staff members tasted them and individual opinions were verbally discussed. It was agreed that, the cheese samples had attained good taste but the flavour was very mild. The cheese samples were then stored for further maturation, as this would help in enhancement of flavour, taste, texture. It was assumed that this longer maturation period would also enable the researcher to monitor any specific differences between cheese with and without iodised salt. The process of tasting samples was repeated again after 2 months. It was observed that, the taste, texture, flavour, and appearance improved. At the end of 3.5 months, it was concluded that the newly developed cheddar cheese samples could be categorised as 'mild cheddar' and were appropriate for further laboratory and sensory analysis.

5.5 Details about the individual steps after addition of starter culture for Soft cheese production

Addition of starter culture and rennet

Once the temperature is appropriate, this is the stage when starter culture is added. To produce these soft cheese samples, 0.666 g of Probat 222 @10 DCU/100 litres (Lot number 4113109954 10 U = 1.5 g) was added and uniformly mixed in the milk. In order to ensure this uniform dispersion, this milk and starter culture mixture was stirred for 10 minutes. At the end of this stirring period, 1.33 ml of rennet (Marzyme ISPF Microbial. 173001, 8/18, 3 ml/100 litres) was added and this mixture was again stirred properly for 30 seconds. This entire mixture of milk, starter culture and rennet was now covered and left aside to clot overnight at 20-22°C.

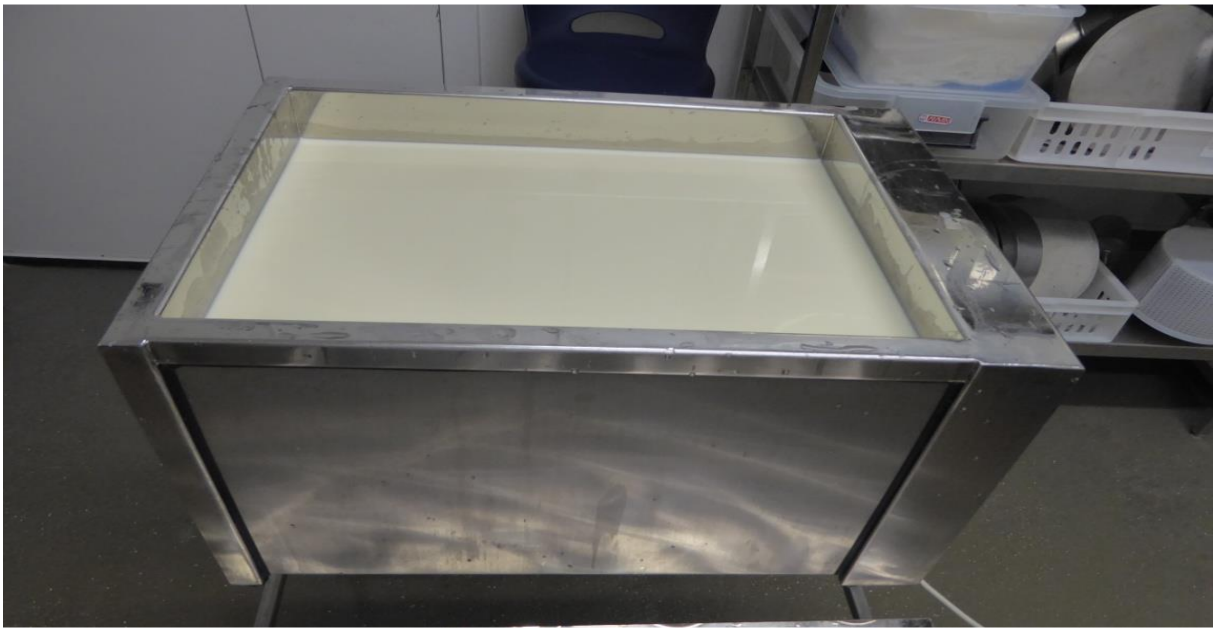


Figure 5. 55 Milk in the vat with starter culture and rennet, left overnight

The overnight clotting time for formation of curds was recorded as 18.5 hours

At the start of the second day of the cheese production process the pH (must be $\leq$ 4.6) and the temperature of the mixture was measured and it was recorded as 4.54 and 21.2°C respectively.

Cutting curds

Immediately after the measurement of the above-mentioned parameters, the curds were cut using stainless steel curd cutter vertically. Here it is important to note that the curds were cut only vertically in both directions (not horizontally). This is because unlike cheddar cheese, curds for soft cheese require only columns to be formed and so only vertical cuts are sufficient.

Figure 5. 56 Cutting the curd, vertical cutters only to make columns

The curds were gently scooped using a ladle into muslin cheese cloth supported in the moulds. In total 6 such cloths (look like bags) were formed.

Figure 5. 57 Gentle scooping of the curd into cloths

Figure 5. 58 Filing curd into cloths

Subsequently, these cloths (bags) were hung to drain for up to 24 hours until the desired texture is achieved.

Figure 5. 59 Hanging the cloths to drain whey overnight soon after filling

However, it is important to agitate the bag at intervals for optimal whey drainage

Figure 5. 60 Another view of hanging cloths to drain whey overnight

For this particular production session, the exact time for which the curds were hung (figures 5.59, 5.60, 5.61) was recorded as 22.5 hours.

Figure 5. 61 Cloths hanging

On the third day, all the content from the bags was transferred to a big bowl and weighed accurately using an appropriate analytical balance.

Salting

The total yield was recorded as 11.3 kg (25%). For the present study it was required to develop two different variations of soft cheese, so the entire content was divided into two equal portions weighing 5.7 kg each. Iodised salt (Cerebros Iodised extra fine table salt 7279W, 20:58) was added to one portion and non-iodised salt (ASDA Saxa Fine salt 72NIW 17133) was added to the other portion, at 0.75% (w/w), followed by stirring for several minutes with a spoon. According to the calculation expressed in terms of grams, exactly 42.4g of salt was added to each portion.

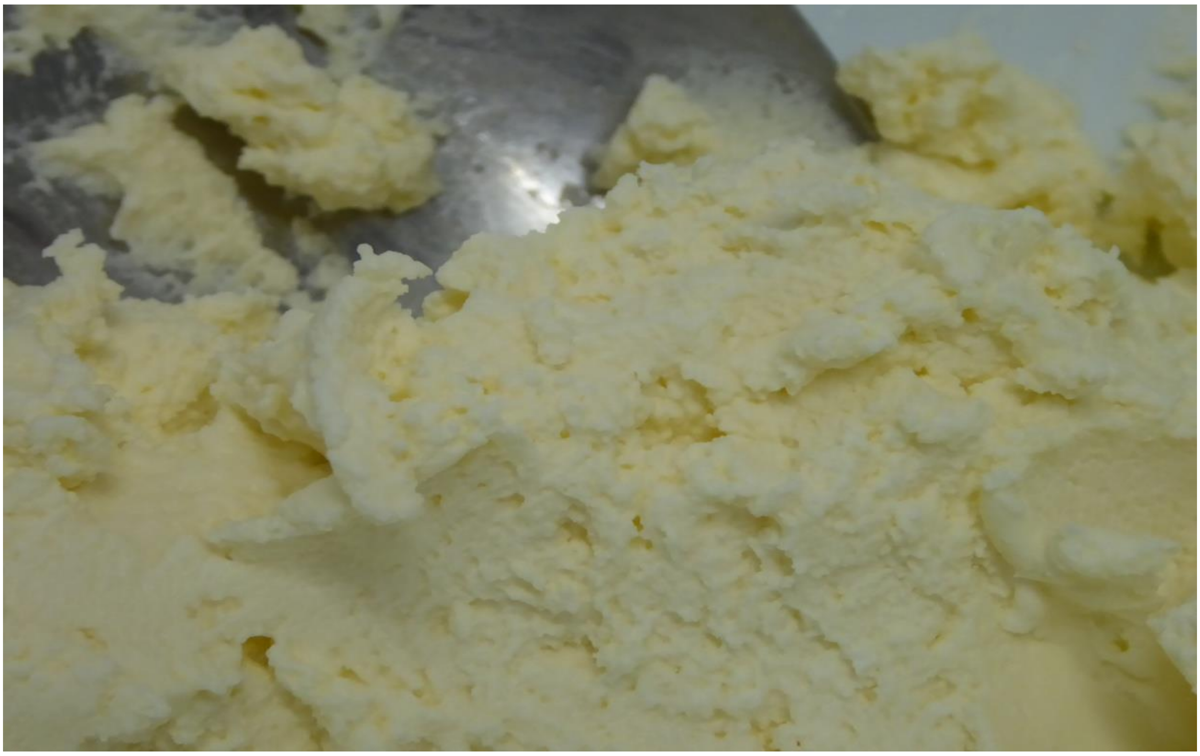


Figure 5. 62 Cheese emptied out into bowl and salt is stirred in

The final cheese samples formed were filled into plastic pots (capacity of pot = 120 ml and weight of empty pot = 8.3 g) using spoon.

Figure 5. 63 Cheese filled into pots using spoon

Approximately 110-120 g of cheese was filled in each pot and then were sealed by pushing tamper-proof lids.

Figure 5. 64 Final soft cheese pots

In total 48 pots filled for the entire amount of cheese of each variety (cheese with iodised salt and cheese with regular table salt).

Finally, sticky labels were attached clearly highlighting the name of the product, name of the allergens present in the product, name of manufacturer and Use by date.

5.6 Results of microbial testing of raw and pasteurised milk

The raw milk obtained from the supplier was sampled 4*20ml and was tested for aerobic plate count (APC). Milk samples were also collected after pasteurisation. These pasteurised milk samples were tested for six microbial parameters namely Aerobic plate count *Escherichia coli*, *Listeria*, Coagulase-positive *Staphylococci*, Enterobacteriaceae and *Salmonella spp*.

For the cheddar cheese, on 28/11/17 the raw milk was tested at <100 cfu/g on APC. The raw milk is normally tested only for APC, as there is a legal requirement for raw cow's milk immediately before processing to have a plate count of less than 300,000/ml. (EU Reg 853/2004). For the soft cheese tested on 06/12/17, the raw milk APC was $1.03x10^4$ cfu/g.

Table 5. 1 Microbial results of pasteurised milk during soft cheese and Cheddar cheese production

Sample	**Date**	**Aerobic plate count (APC) cfu/g**	**Entero-bacteriaceae cfu/g**	***E. coli* cfu/g**	**Coagulase-positive Staphylococci cfu/g**	***Salmonella* spp. cfu/g**	***Listeria* spp. cfu/g**
Pasteurised milk for cheddar cheese	28/11/17	<100	<1	<1	<2	absent	absent
Pasteurised milk for soft cheese production	6/12/17	$3.8x10^3$	1	<1	<2	absent	absent

The pasteurised milk was a bit higher than expected on APC, for freshly pasteurised milk, but it was acceptable to use as it was below the HPA guideline levels (satisfactory $<10^5$ cfu/g, borderline up to 10^7 cfu/g, unsatisfactory $>10^7$ cfu/g) and no other microorganisms were showing levels of concern. This could be attributed to the use of open buckets and vats during production, thereby leading to some risk of some cross- contamination. However, during production of the above-mentioned cheese varieties, optimal efforts were made to ensure all equipment was washed and sanitised properly, and pasteurisation temperatures were recorded on the thermograph and checked with probes.

5.7 Results of microbial testing of newly developed cheese samples

Table 5. 2 Microbial test results for soft cheese and Cheddar cheese (cfu/g)

Sample	Entero-bacteriaceae cfu/g	*E. coli* cfu/g	Coagulase-positive *Staphylococci* cfu/g	Yeasts cfu/g	Moulds cfu/g	*Salmonella* spp. cfu/g	*Listeria* spp. cfu/g
Soft cheese with non-iodised salt (1 day old)	<10	<10	<20	<10	<10	Not tested*	Not detected
Cheddar with iodised salt (3.5 months)	<10	<10	<10	3.8×10^4	<10	Not detected	Not detected
Cheddar with non-iodised salt (3.5 months)	<10	<10	<10	240	30	Not detected	Not detected

*- sample was not tested as *Salmonella* spp is generally tested in raw milk

Both a soft, spreadable cheese and a hard Cheddar type cheese were prepared using both non-iodised and iodised salt. The cheeses were vacuum packed and matured at 10-12°C. Table 5.1 provides the results of the microbiological testing carried out on the batches of cheese. This was carried out to confirm the safety of cheeses for consumption in the sensory trials.

These were assessed against EC Regulation 2073/2005, as amended by 365/2010, The Specialist Cheesemakers Code of Best Practice: Appendices 2 & 3, rev. Sept 2012, and the SCA's Summary of HPA's Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. The results were all satisfactory. Yeasts were detected in the cheddar at 3.5 months but these can be naturally present in maturing cheese and are not an issue unless they reach higher levels (at least $>10^6$ cfu/g), which could potentially cause a yeasty fermentation odour and off-flavours.

Only the soft cheese, made without iodised salt, was selected for this test because the aim of this analysis was to confirm if the developed samples were safe for consumption, rather than seeing if the 2 types of salt had caused any immediate change in levels of microorganisms.

Moreover, it was the same batch of cheese divided in two for the two types of salt, so it was sufficient to test one of them.

The developed cheese samples were not tested for the presence of *salmonella* as the raw milk was already tested for it. If *Listeria* and *Salmonella* were present, it would be more likely due to presence in the milk than from cross contamination during making. The details of the methods used for microbial analysis are provided in the appendix 3 (3.2).

5.8 Laboratory analysis of iodine content of newly developed soft cheese and cheddar cheese varieties

To accomplish the final aim of the study (aim 4), the newly developed cheese varieties (cheddar, soft cheese) with/without iodised salt were analysed for their iodine content using the same method used to analyse different cheese varieties from UK supermarkets (section 2.5.1).

Table 5. 3 Iodine content of newly developed cheese varieties with/without iodised salt

Name of the sample	Iodine content (mg/kg) Mean ± SD	Iodine content (µg I) per adult portion size (per 30 g)	% RNI met by single portion (30 g)
Soft cheese with Iodised salt (n=2)	0.855 ± 0.091	25.5	17
Soft cheese with Non-Iodised salt (n=2)	0.72 ± 0.05	21.6	14.4
Cheddar cheese with Iodised salt (n=2)	0.76 ± 0.01	22.8	15.2
Cheddar cheese with Non-Iodised salt (n=2)	0.47 ± 0.01	14.1	9.4

As seen in the above (table 5.3) duplicate samples from each cheese variety (with 2 different salt variations) were analysed for their iodine content. These results reflect that the iodine content of cheddar cheese samples was less variable than that of the soft cheese samples. This could be attributed to the different production methods of the two cheese types.

Firstly, during soft cheese production, a weighed amount of salt is added in the curd and then mixed thoroughly by hand. There is a possibility that this hand mixing could lead to inadequate stirring and uneven distribution of salt. Secondly, the cheddar cheese samples had a long time (3.5 months) to mature, which allowed the salt to diffuse evenly in the sample. This time was not given for the soft cheese samples before packaging for analysis.

The data presented in Table 5.3 show that the soft cheese with iodised salt provided the highest iodine content of the cheeses analysed, fulfilling 17% of the daily iodine requirement for adults.

Additionally, the salts used for manufacturing these new cheese samples (regular table salt for cheese with non-fortified salt and iodised salt for cheese with iodine-fortified salt) were also analysed. It was found that iodised salt contained 13.8 mg/kg of iodine while the regular table salt contained negligible amounts of iodine (< 0.01 mg/kg).

5.9 Discussion of key findings of the chapter

Based on the information obtained through cheese scoping/mapping and consumer survey it was found that cheese (especially Cheddar and soft cheese) was one of the most regularly consumed products which had salt as an integral part of manufacturing and therefore it can be a potentially useful vehicle for improving iodine status by replacing regular table salt with iodised salt. Cheddar cheese and soft cheese varieties were produced with two salt variations (using iodised salt and regular table salt). Since these cheese varieties differ in manufacturing process, (salt is added at different stages in processing) it was important to analyse the effect of processing on the iodine content of the final product. It was demonstrated that the iodine content of cheese can be increased by the use of iodised salt in the manufacturing process, both in soft and hard cheese production.

Chapter 6: Sensory evaluation of newly developed cheese varieties

6.1 Overview of chapter

The final aim of the study was to understand the sensory acceptability of the use of iodised salt compared with regular salt in 2 types of cheese produced as described in chapter 5. Sensory evaluation of these cheese samples was conducted by recruiting sensory expert panellists (E) and non-expert general cheese consumers (NE).

This chapter describes the development of an evaluation template, suitable for obtaining information from expert sensory panellists as well as non-expert general cheese consumers. The exact steps involved in this template designing have been explained in appropriate depth in the first part of this chapter.

The second section of this chapter explains the detailed sensory evaluation procedure that was undertaken at two study locations (at Cardiff Metropolitan University and University of Central Lancashire).

Finally, in the last part of this chapter, an analysis of the results/responses obtained from the non-expert consumers and sensory experts is presented using tables and graphically using bar charts, pie charts and spider diagrams.

This chapter will conclude with a brief discussion of the findings.

6.2 Details of the sensory evaluation trial

At Cardiff Metropolitan University - The selected panel was requested to taste the samples and then complete the sensory evaluation template described in section 2.7.2.1 (appendix 2. 2.9). After completion of the individual tasting session, the panellists provided a combined report for both the cheese samples detailing the overall quality of the cheese samples. For this report, the Campden Overall Eating Recommended Scoring Scale was used as a reference to describe the quality of the product (appendix 2 – 2.13).

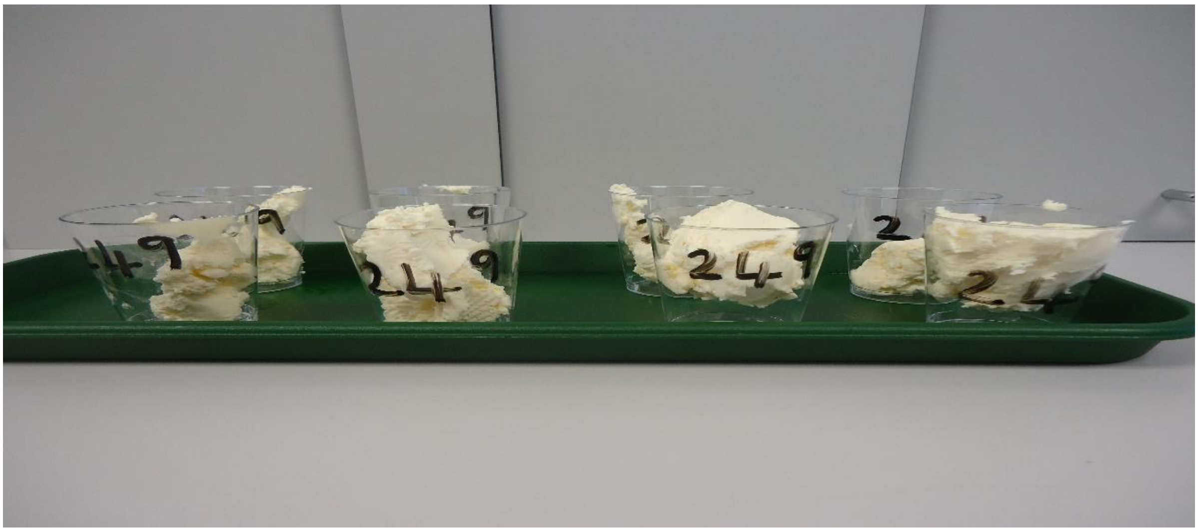


Figure 6. 1 Arrangement of soft cheese samples for sensory evaluation at Cardiff Metropolitan University

Figure 6. 2 Arrangement of Cheddar cheese samples for sensory evaluation at Cardiff Metropolitan University

At University of Central Lancashire Campus - A small room was booked in the selected area of the university campus (Foster refectory area –Scholar bar room). Inside this room, desks were set up for individual participants. Each desk had two cheese samples placed in shot size plastic cups with disposable spoons with cheese samples. A small glass of water was provided as water had to be consumed between two samples. Consumption of water between samples was important as it helped the participant to get rid of any taste/aftertaste associated with the sample consumed, thus prevent the impact of previously tasted samples on the sensory perception of the other sample. The staff and students entering the refectory were invited to participate in the study. Information was also provided both written (appendix 2, 2.10) and verbally by the researcher and all the queries relating to participation were answered. Willing participants signed an informed consent form (appendix 2, 2.11) and were provided with the sensory evaluation template (appendix 2, 2.9) to complete, along with a list of definitions for the sensory terms used in the evaluation template to ensure that the meaning of descriptors was understood and interpreted accurately.

Figure 6. 3 Arrangement of soft cheese samples for sensory evaluation at University of Central Lancashire

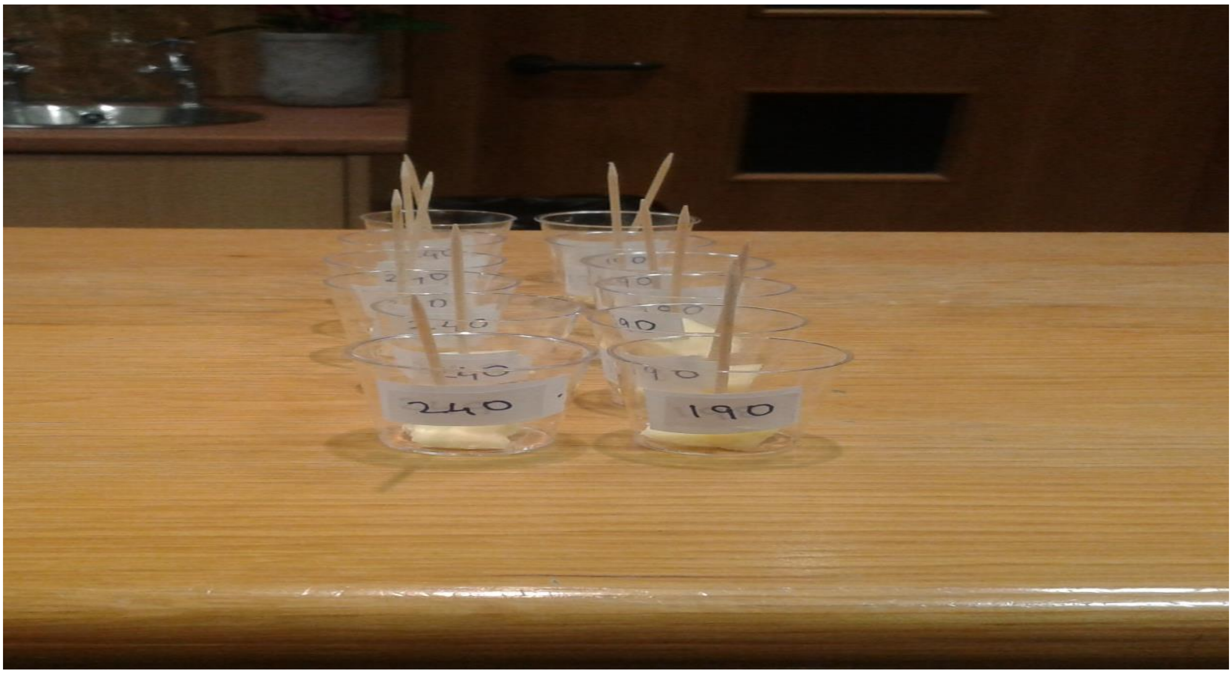


Figure 6. 4 Arrangement of Cheddar cheese samples for sensory evaluation at University of Central Lancashire

Figure 6. 5 Arrangement for participants for sensory evaluation at University of Central Lancashire

In classroom situation at University of Central Lancashire – A risk assessment was completed (appendix number) for conducting sensory evaluation exercise in the classroom situation. Undergraduate students attending nutrition classes were invited to take part in this sensory testing of cheese samples. It was clearly explained to the students that their participation in this exercise was completely voluntary and those students who did not wish to participate were invited to observe as an educational experience. Subsequently a table was set up with the cheese samples required to undergo sensory testing. The protocol for providing participant information sheet, consent form and then sensory evaluation template was exactly the same as for the participants who were recruited through opportunistic sampling technique.

Impact of shelf life of cheese samples on the recruitment of participants

The shelf life of soft cheese samples was 10-12 days and this provided the researcher with a short time to recruit participants. Contrary to this, Cheddar cheese samples had a longer shelf life and it was possible to store them and provide them to the participants for safe consumption up to 3-4 weeks from the date of manufacture. This longer time-span for conducting sensory evaluation was helpful to recruit more participants. The food technologist provided 'Use by date' on the label for each variety of cheese.

In the following section, the responses of consumers and experts for both the varieties of cheese samples have been tabulated quantitatively.

In the beginning of the sensory template, general information was obtained relating to frequency of consumption of salt, type of salt consumed regularly, stage (during cooking, at table) when salt is added to the food. This information has been presented using bar charts figure 6.6 to figure 6.11)

The following diagrams (figure 6.6 and figure 6.7) provides a clear comparison of frequency of cheese consumption between non-expert consumers (NE) and Sensory experts (E) during two sets of sensory evaluation. During both the sensory evaluation sessions (for soft cheese and Cheddar cheese), the majority of participants (both experts and non-experts) consumed cheese more than once a week. Also, the percent responses from both experts and non-experts were similar for both the evaluation sessions

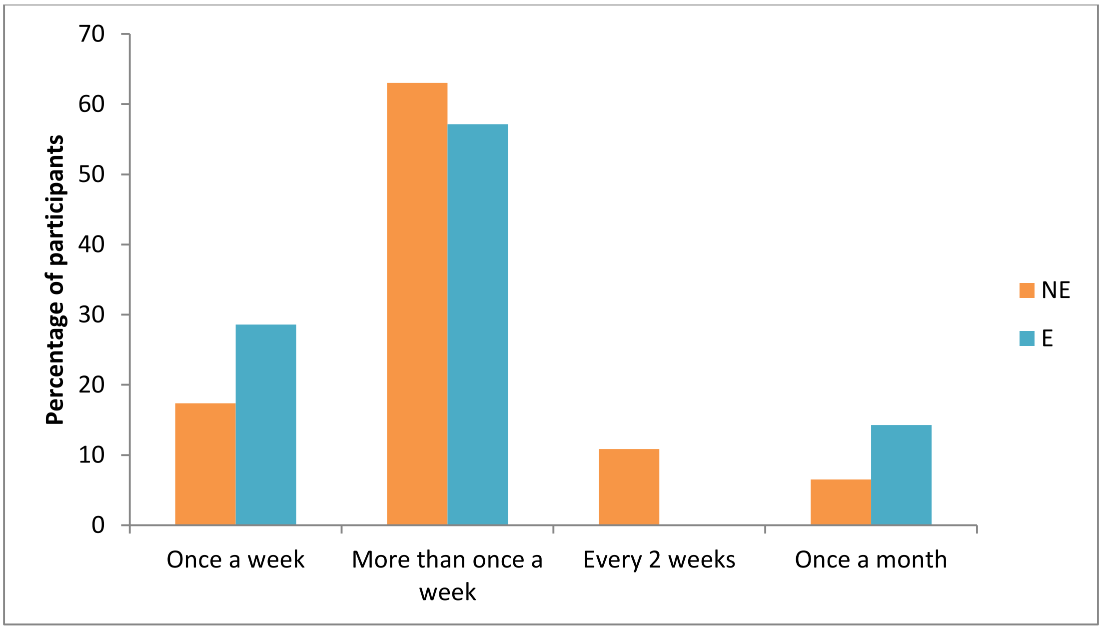


Figure 6. 6 Frequency of cheese consumption by non-expert consumers (NE) and sensory experts (E) for soft cheese evaluation

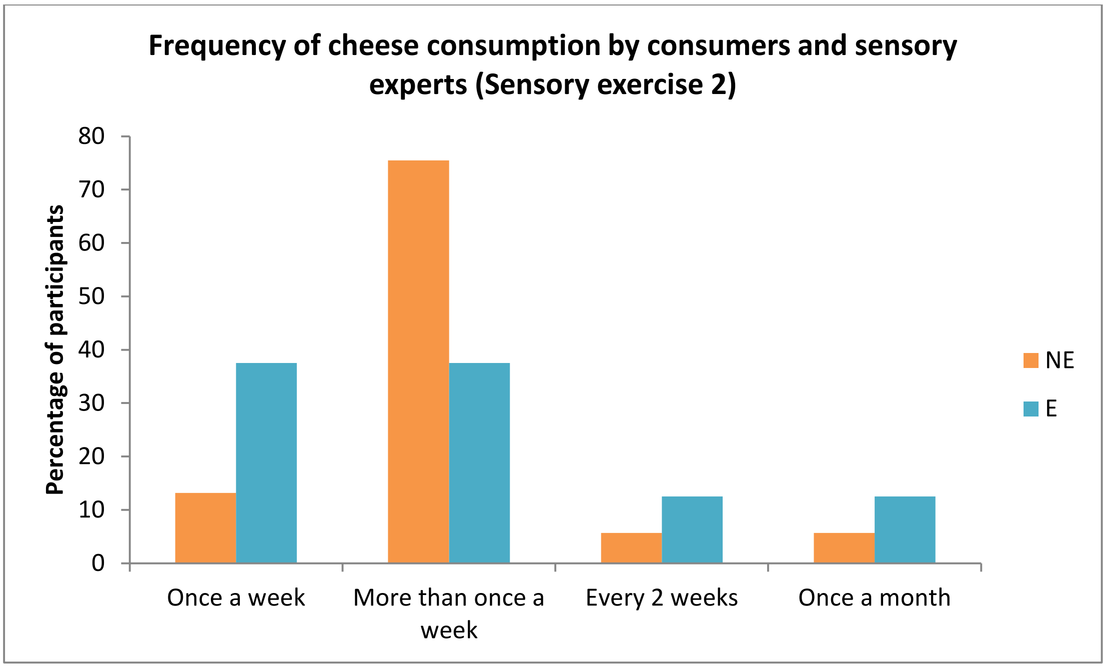


Figure 6. 7 Frequency of cheese consumption by non-expert consumers (NE) and sensory experts (E) for soft cheese evaluation

Further in to the template, the participants (non-expert consumers and sensory expert panel) were enquired about the type of salt they consumed in their daily diet.

It was revealed that all the sensory expert panellists (n=7 for soft cheese evaluation and n=8 for Cheddar cheese evaluation) consumed regular table salt in their daily diet.

The responses from the non-expert consumers have been depicted in the following diagrams (figure 6.8 and figure 6.9). Regular table salt was consumed by highest percentage of participants (78% and 65% respectively) during both the cheese sensory evaluation sessions. Further, there were few participants (n=3 and n=5) during soft cheese and cheddar cheese evaluation respectively who did not use any salt during food preparation.

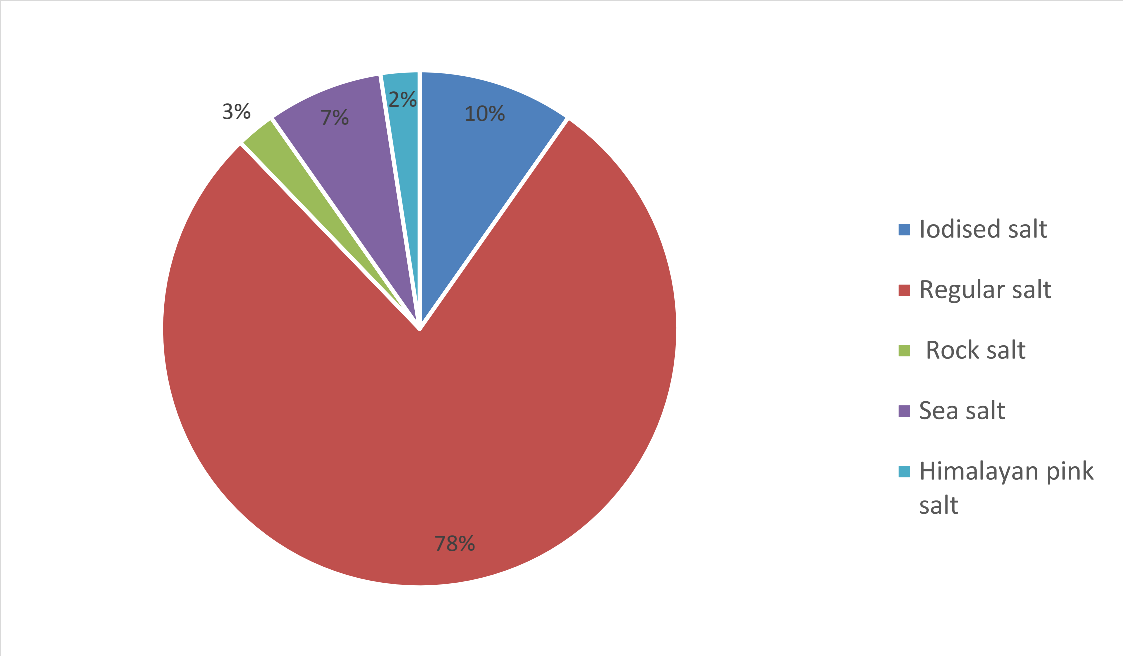


Figure 6. 8 Types of salt consumed by participants (non-expert consumers) as a part of daily diet (soft cheese sensory evaluation)

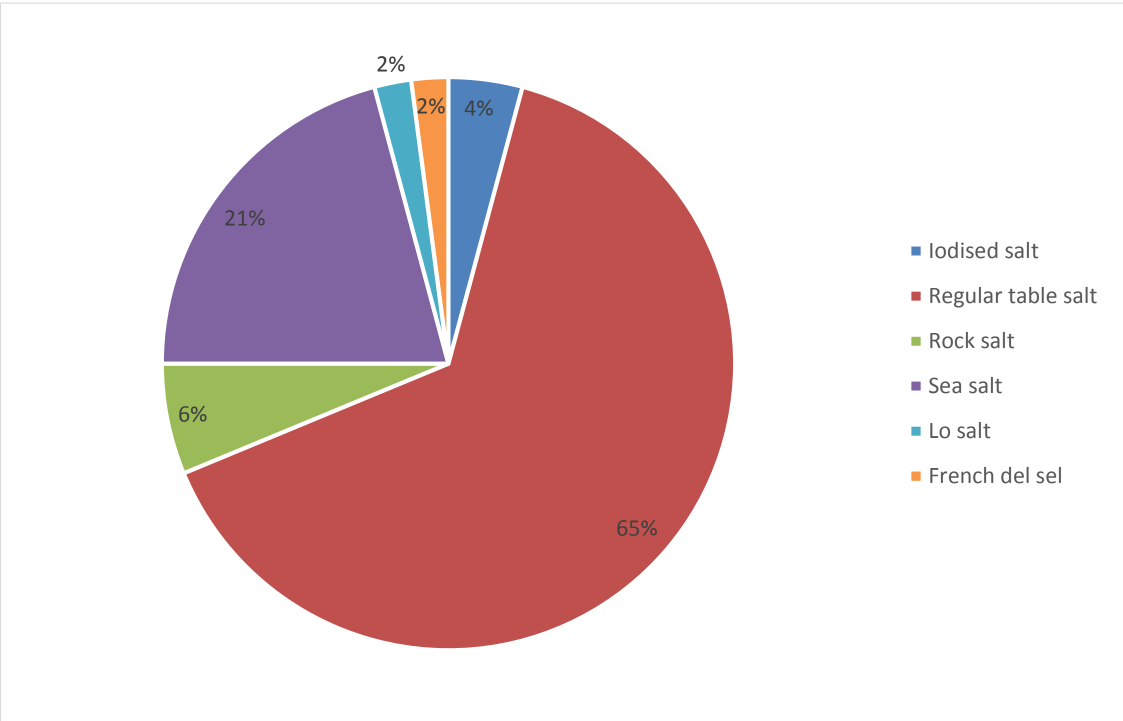


Figure 6. 9 Types of salt consumed by participants (non-expert consumers) as a part of daily diet (Cheddar cheese sensory evaluation)

Apart from the type of salt, it is imperative to understand the stage of cooking when this salt is added to the food as it affects the availability of iodine in the final product. So, in continuation with the question on type of salt, the participants were asked ‘when do they add salt to their food - during cooking, sprinkle on top at the end of cooking or at table before/after eating’ (figure 6.10 and figure 6.11).

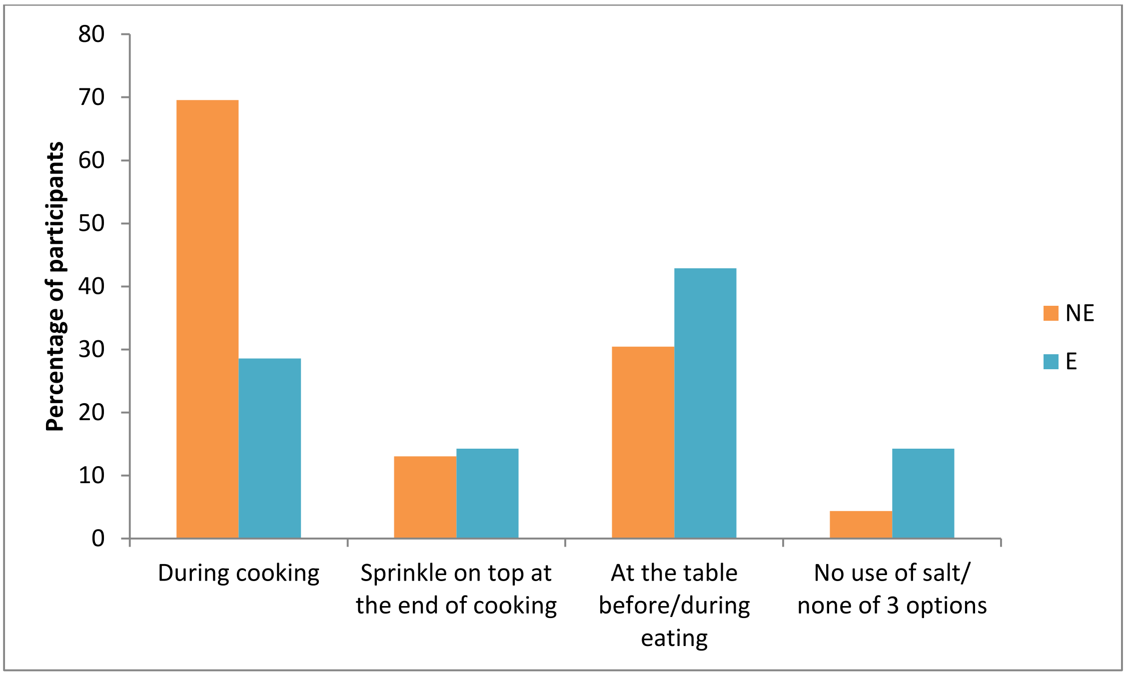


Figure 6. 10 Time of addition of salt to food by non-expert consumers (NE) and sensory experts (E) for soft cheese evaluation

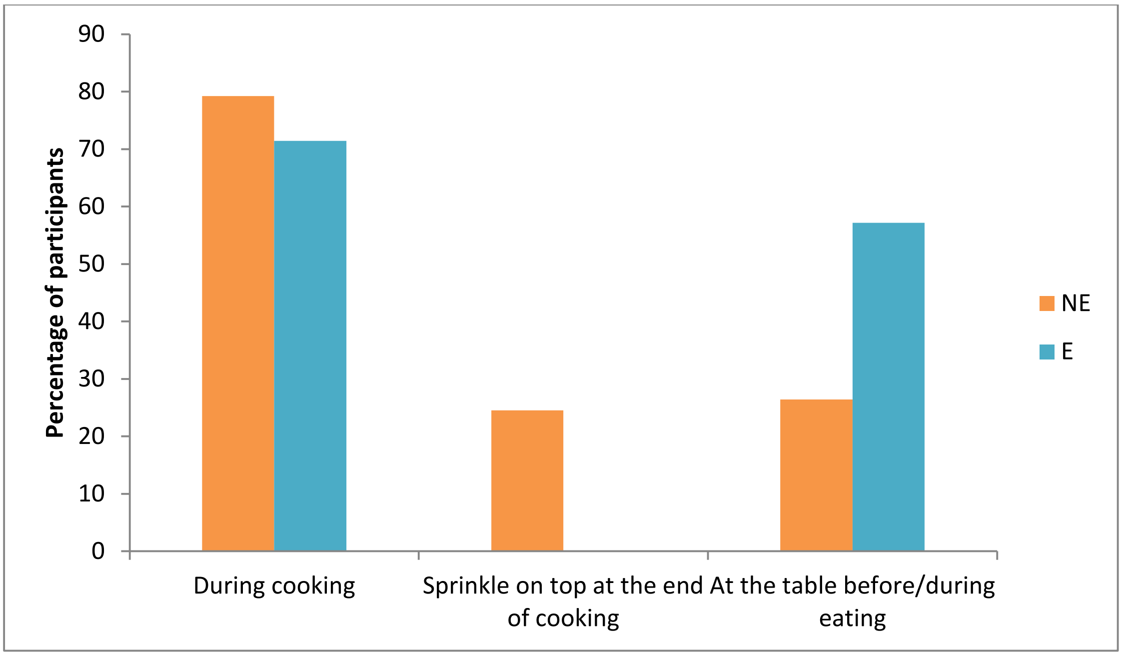


Figure 6. 11 Time of addition of salt to food by non-expert consumers (NE) and sensory experts (E) for Cheddar cheese evaluation

After obtaining in depth information about the frequency of consumption of cheese, type and stage of addition of salt, the subsequent part of the template focussed on obtaining responses about different sensory attributes of the newly developed cheese varieties.

Interpreting information from figures 6.6, 6.7, 6.10 and 6.11, it was observed that there were clear differences in the cheese consumption and salt use patterns of sensory experts compared with those of the non-expert consumers. The non-expert participants consumed cheese more frequently as compared to the cheese consumption by sensory experts. So, in context of the present study, this would have been beneficial as there would have been a possibility that they could be encouraged to consumed iodine-fortified cheese and thus would indirectly enable them to improve their iodine intake. Furthermore, if a comparison is made based on how they used salt, then it is evident that high percentage (79%) of non-expert participants added salt during cooking. The sensory experts have reported adding salt during cooking, but a high percentage (57%) of these experts have reported adding salt at the table before or during eating. This practice of adding salt at the table is beneficial (helps to retain iodine if iodised salt is used) thereby reflecting better knowledge and understanding of the experts and highlighting the need for nutrition education to create awareness about appropriate cooking practices and their impact on the nutrients in the food.

A total of 8 attributes related to sensory analysis of cheese varieties were selected for this study namely colour, appearance, saltiness, taste, odour, texture, level of moisture/mouth-feel and overall quality. In order to rate these main attributes, a 7-point hedonic scale (like very much to dislike very much) was used (Singh-Ackbaraliand Maharaj, 2014). These responses were quantified and have been presented in table number 6.1, 6.2, 6.3 and 6.4.

In continuation, the participants also indicated if they could identify specific characteristics relating to the main profiling attributes (taste, texture, odour). The responses relating to specific attributes such as sweetness, bitterness, creamy odour, acidic taste, hardness, chewiness have been depicted using bar charts.

Table 6. 1 Results of Sensory Evaluation of newly developed Soft cheese (with iodine fortified salt) samples by non-expert consumers (NE) and sensory experts (E)

Number of participants in each category – NE = 46 E = 7

Attributes for Soft cheese with iodine fortified salt	Like very much Number (%)		Like moderately Number (%)		Like slightly Number (%)		Neither like nor dislike Number (%)		Dislike slightly Number (%)		Dislike moderately Number (%)		Dislike very much Number (%)	
	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E
Colour	14(30.4)	3(42.8)	14(30.4)	2 (28.6)	7(15.2)	0	10(21.7)	2(28.6)	1(2.2)	0	0	0	0	0
Appearance	16(30.8)	2(28.5)	11(23.9)	4 (57.1)	8(17.4)	0	7(15.2)	1(14.3)	4(8.7)	0	0	0	0	0
Taste	11(23.9)	2(33.3)	20(43.5)	4 (66.7)	6(13.0)	0	1(2.2)	0	5(10.9)	0	3(6.5)	0	0	0
Odour	6(13.0)	1(14.3)	11(23.9)	2 (28.6)	8(17.4)	2 (28.6)	19(41.3)	2(28.6)	1(2.2)	0	1(2.2)	0	0	0
Texture	15(32.6)	3(42.8)	14(30.4)	2 (28.6)	10(21.7)	1 (14.3)	6(13.0)	1(14.3)	1 (2.2)	0	0	0	0	0
Overall quality	12(26.1)	3(42.8)	20(43.5)	2 (28.6)	6 (13.0)	2 (28.6)	3(6.5)	0	4(8.7)	0	1 (2.2)	0	0	0

The responses obtained about intensity of likability for both varieties of soft cheese were statistically analysed using a two-way Chi-square test. The specific Chi square test values and p values for all the attributes have been presented in appendix 3.3.

Table 6. 2 Results of Sensory Evaluation of newly developed soft cheese (with non-fortified salt) samples by non-expert consumers (NE) and sensory experts (E)

Number of participants in each category – NE = 46 E = 7

Attributes for Soft cheese with non-fortified salt	Like very much Number (%)		Like moderately Number (%)		Like slightly Number (%)		Neither like nor dislike Number (%)		Dislike slightly Number (%)		Dislike moderately Number (%)		Dislike very much Number (%)	
	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E
Colour	10(21.7)	3(42.8)	18(39.1)	1 (14.3)	6(13.0)	0	10(21.8)	2 (28.6)	2(4.3)	1(14.3)	0	0	0	0
Appearance	15(32.6)	2(28.6)	13(28.2)	3 (42.8)	6(13.0)	0	8(17.4)	2 (28.6)	4(8.7)	0	0	0	0	0
Taste	9(19.6)	2(33.3)	21(45.6)	1 (16.7)	9(19.5)	3 (50)	1 (2.2)	0	3(6.5)	0	3(6.5)	0	0	0
Odour	6(13.0)	0	11(23.9)	2 (28.5)	8(17.4)	1(14.3)	17(36.9)	4 (57.1)	3(6.5)	0	1(2.2)	0	0	0
Texture	15(32.6)	1(14.3)	17(36.9)	4 (57.1)	9(19.6)	1(14.3)	3(6.5)	1 (14.3)	0	0	2(4.3)	0	0	0
Overall quality	10(21.7)	3(42.8)	20(43.5)	3 (42.8)	9(19.6)	0	1(2.2)	1 (14.3)	3(6.5)	0	3(6.5)	0	0	0

From the above tables (6.1 and 6.2) it can be concluded that the overall quality of both the cheese samples was liked similarly by both consumers and experts as indicated by high number of participants responding ‘like very much’ or ‘like moderately’. Also, none of the experts indicated disliking of any intensity for soft cheese with iodised salt and only one expert disliked the colour of soft cheese with non-iodised salt thereby establishing overall acceptance of both the soft cheese variations. A two-way Chi-square test was performed to understand whether there were any significant differences in the responses of sensory experts for soft cheese with and without iodised salt. Results revealed that sensory experts did not perceive the main attributes (colour, taste, texture and odour) of soft with iodised salt any differently than soft cheese with non-fortified salt. The specific Chi square test values and p values for all the attributes have been presented in appendix 3.3.

In terms of consumer responses, the perception about different parameters (colour, taste, texture, appearance) was similar. The overall quality of the soft cheese samples with both the salt variations were liked by high percentage of participants (21.7% and 43.5% respectively for soft cheese with regular salt and 26.1% and 43.5% for soft cheese iodine fortified salt) indicating ‘like very much and ‘like moderately’ for the overall quality as opposed to only 1-4 participants recording ‘dislike’. Also, it is clearly evident that the consumer responses to the individual attributes and overall quality of both the soft cheese samples were very similar.

Table 6. 3 Results of Sensory Evaluation of newly developed Cheddar cheese (with non-fortified salt) samples by non-expert consumers (NE) and sensory experts (E)

Number of participants in each category – NE = 53 E = 8

Attributes for Cheddar cheese with regular table salt	Like very much Number (%)		Like moderately Number (%)		Like slightly Number (%)		Neither like nor dislike Number (%)		Dislike slightly Number (%)		Dislike moderately Number (%)		Dislike very much Number (%)	
	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E
Colour	19(36.5)	1(12.5)	15(28.8)	5(62.5)	12 (23.1)	1 (12.5)	6(11.5)	1 (12.5)	0	0	0	0	0	0
Appearance	21(40.4)	3(37.5)	15(28.8)	3(37.5)	7 (13.5)	1 (12.5)	7(13.5)	1 (12.5)	2(3.8)	0	0	0	0	0
Taste	20(38.5)	1(12.5)	12(23.1)	3(37.5)	13(25)	3 (37.5)	4(7.7)	1 (12.5)	1(1.9)	0	1(1.92)	0	1(1.92)	0
Odour	6(11.32)	3 (37.5)	18(34)	2(25)	9(17)	2 (37.5)	15(28.3)	0	3(5.7)	0	1(1.88)	0	1(1.88)	0
Texture	12(23.1)	3 (37.5)	25(48.1)	1(12.5)	5(9.6)	3 (37.5)	5(9.6)	1 (12.5)	3(5.8)	0	0	0	2(3.84)	0
Overall quality	13(24.5)	1 (12.5)	22(41.5)	4(50)	10 (18.9)	3 (37.5)	3(5.7)	0	2(3.8)	0	2(3.77)	0	1(1.88)	0

Table 6. 4 Results of Sensory Evaluation of newly developed Cheddar cheese (with iodine fortified salt) samples by non-expert consumers (NE) and sensory experts (E)

Number of participants in each category – NE = 53 E = 8

Attributes for Cheddar cheese with iodised salt	Like very much Number (%)		Like moderately Number (%)		Like slightly Number (%)		Neither like nor dislike Number (%)		Dislike slightly Number (%)		Dislike moderately Number (%)		Dislike very much Number (%)	
	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E
Colour	19(36.5)	3(37.5)	14(26.9)	4 (50)	11 (21.1)	0	7(13.4)	1(12.5)	1(1.9)	0	0	0	0	0
Appearance	17(32.7)	3(37.5)	19(36.5)	4 (50)	9(17.3)	0	5(9.6)	1(12.5)	2(3.8)	0	0	0	0	0
Taste	12(23.1)	1(12.5)	16(30.8)	2 (25)	13(25)	4 (50)	5(9.6)	0	4(7.7)	1(12.5)	1(1.9)	0	1(2)	0
Odour	6(11.3)	3(37.5)	18(34)	3(37.5)	9(17)	1(12.5)	16 (30.2)	0	3(5.7)	1(12.5)	1(1.9)	0	0	0
Texture	9(17)	3(37.5)	22(41.5)	1 (12.5)	9(17)	2 (25)	4(7.5)	2 (25)	7 (13.2)	0	1(1.9)	0	1(1.9)	0
Overall quality	11(20.7)	2 (25)	22(41.5)	4 (50)	9(17)	1 (12.5)	5(9.4)	0	4(7.5)	0	1(1.9)	1 (12.5)	1(1.9)	0

As seen in table 6.3 and 6.4, the colour, appearance and taste of both the cheddar cheese samples (with and without iodised salt) was rated as 'Liked very much' by similar percentage of general consumers. The overall quality of the cheese samples was also moderately liked by high percentage (41.5%) of respondents and this response was exactly same for both the variations of cheddar cheese. Another notable aspect was that the majority of sensory experts perceived all the main sensory attributes of cheese in the range of 'Like very much' to Like slightly' with only one expert maintaining a neutral stand on all the five sensory parameters.

The responses obtained about intensity of likability for both varieties of Cheddar cheese were statistically analysed using a two–way Chi-square test. The specific Chi square test values and p values for all the attributes have been presented in appendix 3.3.

In order to observe the sensory profiles and understand the intensities of responses for likability of various attributes, the responses have been depicted using spider diagrams.

Procedure for development of individual spoke points for different attributes

Firstly, in the evaluation template, the responses were from Like very much to dislike very much. These responses were converted into a rating scale where like very much is rated '7' and dislike very much is rated '1'. This conversion represents the frequency of all the scores (1 to 7). In order to generate a single score, it was important to multiply each score by the frequency. So for example, for colour, the score 30.4 was multiplied by 7, 30.4 by 6, 15.2 by 5 and so on all the way along, then all individual scores were added up to obtain a total score at the end of the row. This provided an overall score for colour from all the participants. On the spider diagram, the overall score for colour is 564.6. This was then the point on the Colour "spoke" of the diagram. The final scores were used for plotting the spider diagrams (figures 6.12, 6.13). These diagrams also provide direct comparison between the two varieties of cheese (cheddar and soft cheese with 2 salt variations. The numbers on the diagram are the range of final scores for different attributes.

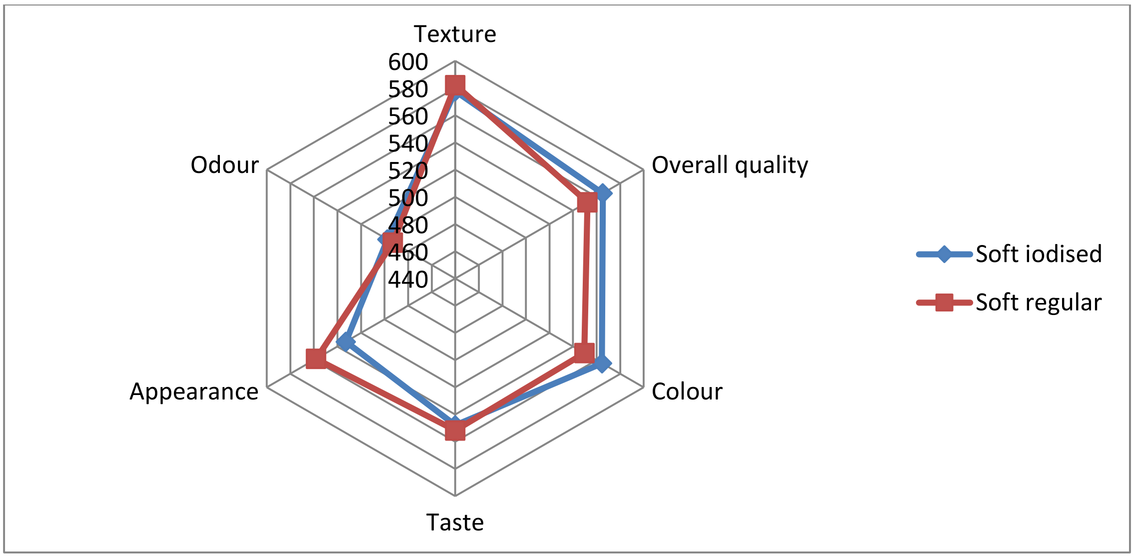


Figure 6. 12 Spider-diagram showing responses for soft cheese with/without iodised salt

Figure 6. 13 Spider-diagram showing responses for Cheddar cheese with/without iodised salt

6.2.1 Statistical Analysis – A two-way chi square test was performed using statistical software (SPSS v24-IBM) to understand if there are any statistical variations in the consumer responses to the main attributes (colour, appearance, taste, texture, odour, overall quality) for both variations of cheese. Also, one-way Chi square test was performed for understanding the statistical significance between the responses for specific attributes such as sweetness, bitterness for cheese samples with both the salt variations. The data was also analysed to establish the similarity in the overall responses provided by non-expert consumers as well as experts.

Results revealed that there was no statistical difference (P>0.05) in the preference of attribute over other. Also, there was no statistical difference (P>0.05) in the intensity of likability for different parameters. The specific Chi square values and p values for all the parameters have been presented in the appendix 3.3.

6.2.2 Identification of specific characteristics related to 'Taste' of cheese samples

While describing 'Taste' of cheese samples, it important to note that this term covers a range of specific attributes such as 'Sweetness', 'Bitterness', 'Acidic taste' and After-taste (Lawless and Heymann, 2010).

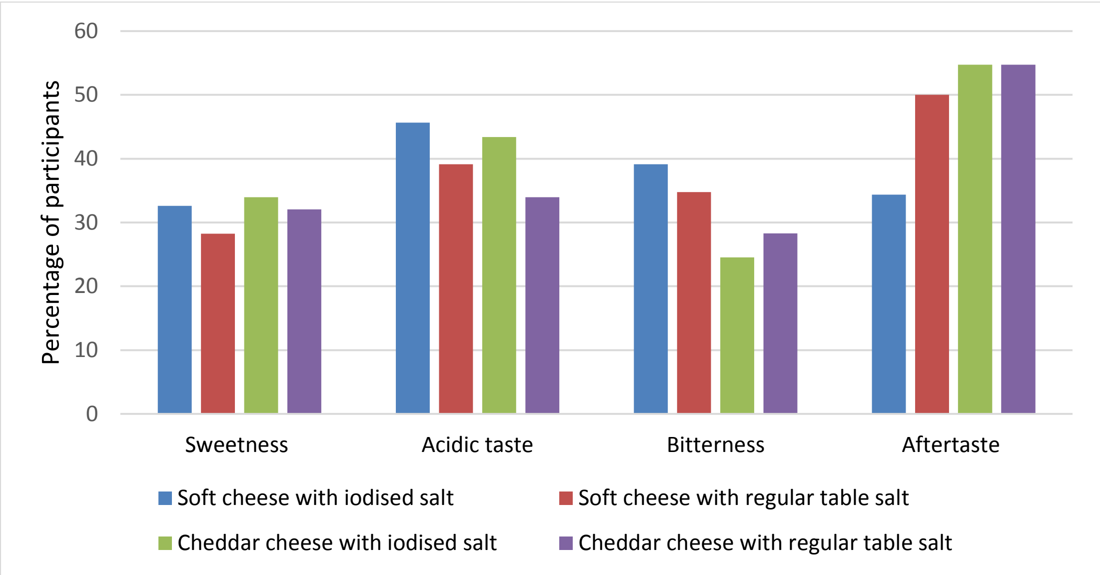


Figure 6. 14 Non-expert consumer opinion about different attributes related to 'Taste' of different cheese samples

As seen in figure 6.14, there is absence of a clear pattern in the perception of different taste attributes due to iodine fortification of salt. The percentage of participants detecting sweetness and after taste is very similar for all the four cheese samples. However, it can be observed that cheese samples (soft and cheddar) with iodised salt have more acidic taste than cheese samples with non-fortified regular table salt. In order to understand, if there were any variations in the responses to cheese with or without iodised salt, one-way Chi square test was performed. It was revealed that there were no statistical differences (p>0.05) in responses, which implies that sweetness, acidic taste, bitterness and aftertaste for cheese with iodised salt was not perceived any differently from cheese without iodised salt. The chi square values and p values for these attributes have been presented in appendix 3.3.

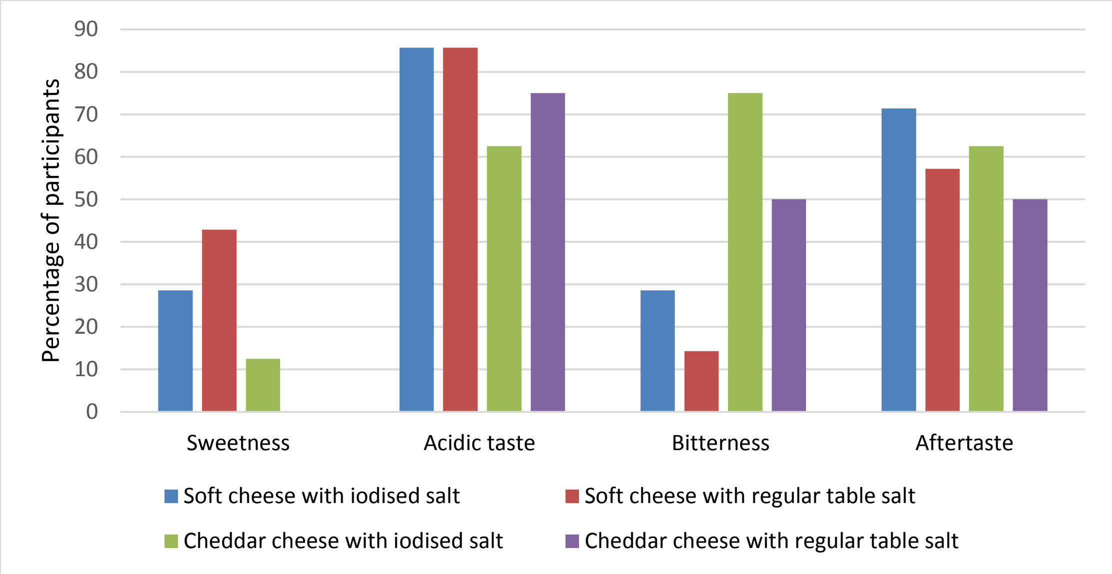


Figure 6. 15 Opinion of sensory experts about different attributes related to 'Taste' of different cheese samples

A total of 7 experts conducted sensory analysis of soft cheese samples while cheddar cheese samples with variations of type of salt was tasted by 8 sensory experts (figure 6.15). Accordingly, soft cheese with and without iodised salt was found to be more acidic in taste than cheddar samples. Another aspect that stands out clearly is that Cheddar cheese with iodised salt was found to be most bitter and this explains less sweetness as indicated by only 12.5% of experts. Moreover, Cheddar cheese with regular salt was not at all sweet. Apart from these specific taste parameters, experts have also highlighted some of the other tastes detected during this sensory assessment including creamy and astringent taste for soft cheese samples and metallic flavour, sourness, dairy taste for Cheddar cheese samples. Despite these varied tastes recorded by experts during the evaluation, there were no statistically significant variations ($p>0.05$). The Chi square values and p values indicating no significant difference have been presented in the appendix 3.3. The experts did not perceive the sweetness, acidic taste, bitterness or after taste of cheese samples with iodised salt any differently than cheese samples without iodised salt.

6.2.3 Identification of specific characteristics related to 'Odour' of cheese samples

According to the QDA method (Papetti and Carelli, 2013) the specific profiling attributes related to Odour include creamy odour, acidic odour, buttery odour and flowery/fruity odour.

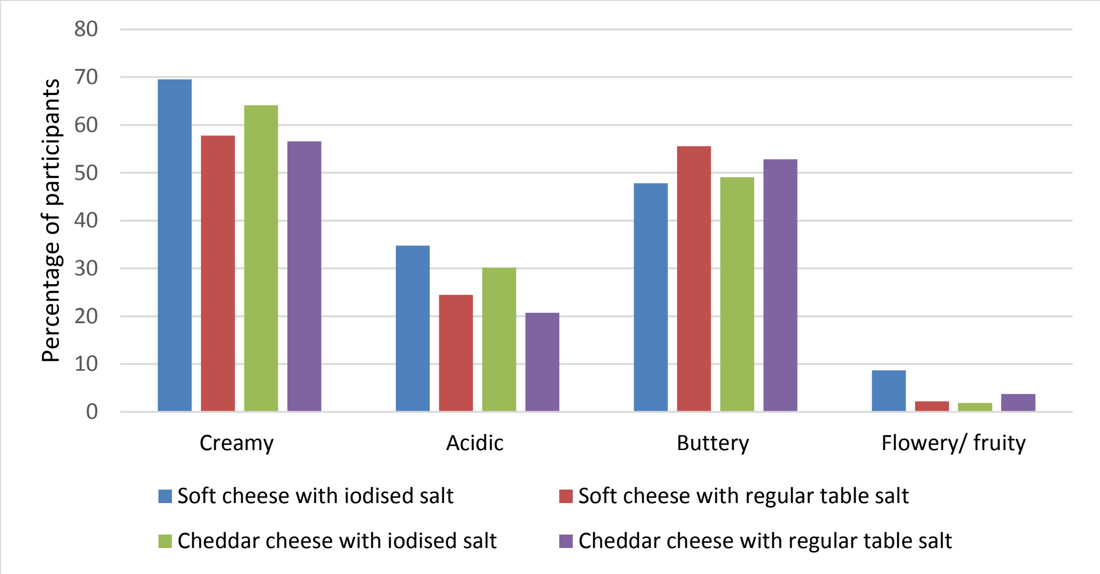


Figure 6. 16 Non-expert consumer opinion about different attributes related to 'Odour' of different cheese samples

From consumer responses for different variations of soft and cheddar cheese (figure 6.16), it is evident that creamy odour was identified by a large percentage of participants for both the varieties of cheese (irrespective of type of salt). On comparing cheese varieties based on type of salt, it was revealed that both the cheese varieties (soft and cheddar) manufactured using iodised salt had creamier odour than the varieties made without iodised salt. A similar pattern was observed in terms of acidic odour where cheese samples with iodised salt exhibited more acidic odour than those made without iodised salt. In contrast to this, cheese samples with regular table salt had butterier odour than with iodised salt. Flowery/fruity odour was identified by very small percentage (1-8 percent) of participants for both the cheese varieties. Apart from these main sensory attributes, 6 participants identified some other types of odours such as ‘Cheddar cheese odour’ (n=2), ‘too smelly’ and ‘pungent’. Out of the remaining two participants, one did experience some odour but could not specify while other did not have any odour experience.

When the varied odour experiences of non-expert consumers were analysed statistically using a one-way Chi-square test, no statistical variations were observed ($p>0.05$) between responses for cheese with iodised salt or without iodised salt. The Chi square test values and p values indicating no significant difference have been presented in appendix 3.3. This implies that use of iodised salt in cheese did not impact the perception of odour any differently from the perception of cheese without iodised salt.

Apart from the consumers, a panel of sensory experts also rated the odour of cheese samples (figure 6.17).

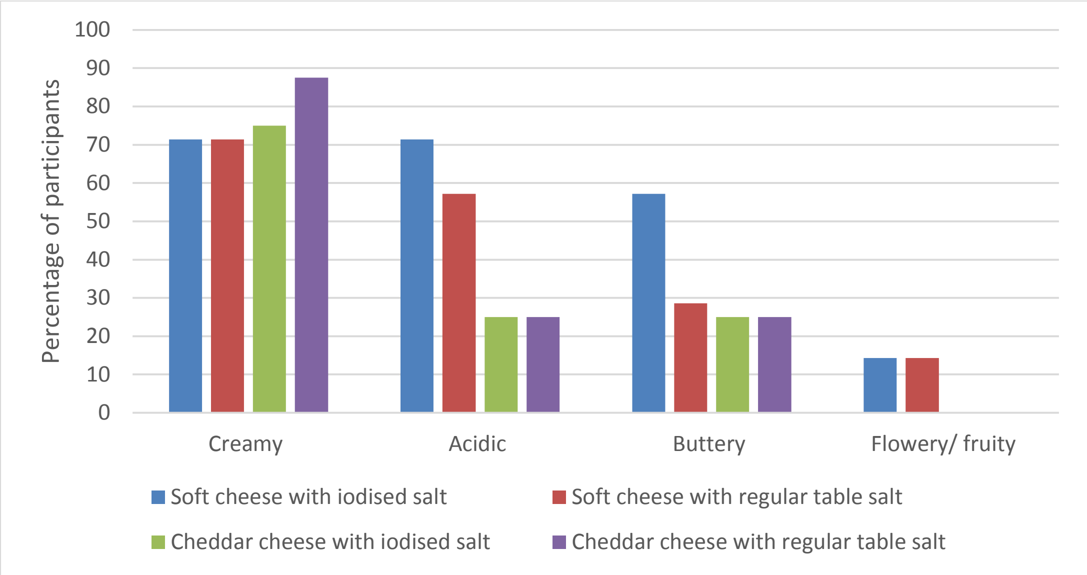


Figure 6. 17 Expert opinion about different attributes related to 'Odour' of different cheese samples

There is absence of any clear pattern in the perception of odour for cheese varieties with/without iodised salt. Creamy odour was prominently identified in all the cheese varieties by similar numbers of experts, however Cheddar cheese with regular table salt was found to have a creamy odour by 87.5% of the experts (n=7) which was in contrast to the perception by the non-expert consumers. Further, soft cheese with iodised salt was identified to have more acidic and buttery odour than any other cheese varieties. However, there were no statistical variations (one-way Chi square test p>0.05, the specific p values for all the attributes for odour have been presented in appendix 3.3) in the odour experience irrespective of use of iodised salt in cheese production and this implies that there is no impact of presence of iodised salt on the odour of cheese.

6.2.4 Identification of specific characteristics related to ‘Texture’ of cheese samples

The attributes related to ‘Texture’ of cheese includes ‘Hardness’, ‘Chewiness’, ‘Rubbery’ and ‘Grainy’. Following diagram (figure 6.18) depicts the consumer perception about the individual texture properties of four cheese varieties

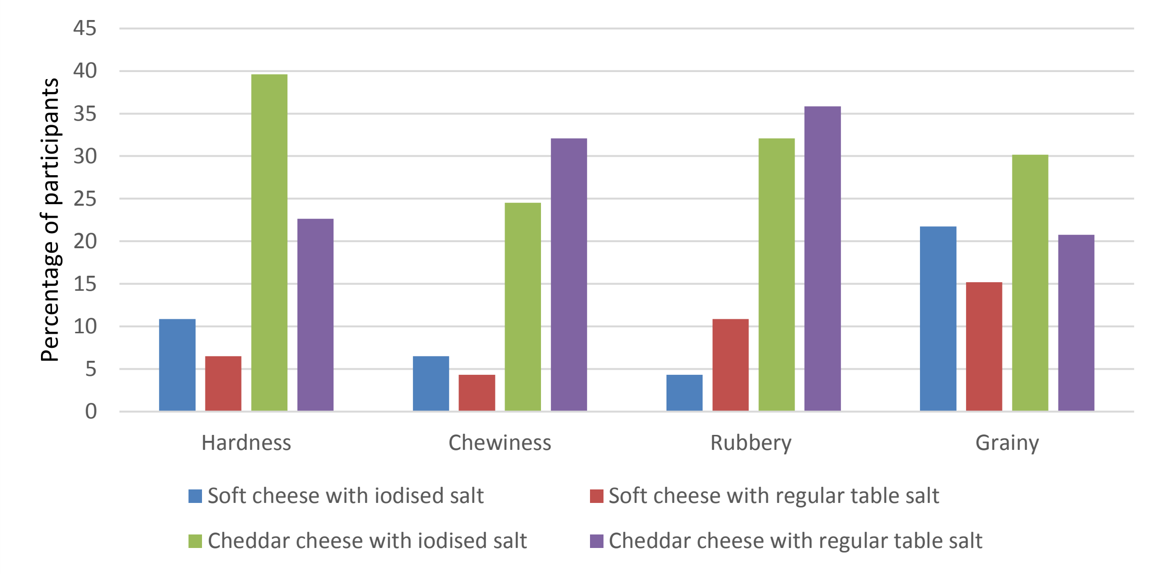


Figure 6. 18 Non-expert consumer opinion about different attributes related to 'Texture' of different cheese samples

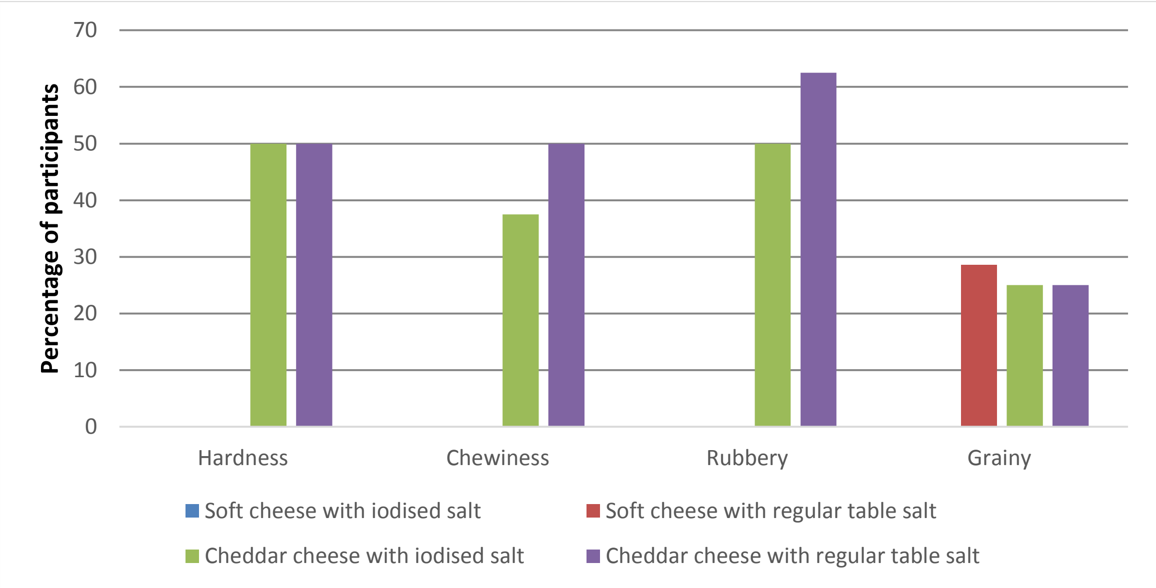


Figure 6. 19 Perception of sensory experts about different attributes related to 'Texture' of different cheese samples

Consumer perceptions about textural attributes of different cheese varieties were mixed with no clear pattern in view (figure 6.18). However, Cheddar cheese with iodised salt was found to be harder, chewy, rubbery and grainy than any other cheese varieties.

In contrast to this, a distinct trend was visible in the responses of sensory experts (figure 6.19) where Cheddar cheese with/without iodised salt was rated as 'hard, chewy and grainy by similar percentage of participants. Cheddar cheese with regular table salt was found to be rubberier than with iodised salt by majority of the participants (62%). Hardness, Chewiness and rubbery texture was appropriately not detected in any of soft cheese samples and only 28% of experts (n=2) could detect grainy texture in soft cheese with regular salt.

Non-expert consumers did not perceive the texture of cheese varieties with iodised salt any differently than cheese varieties without iodised salt and this was confirmed by statistical analysis (one-way Chi square test) where no significant differences were observed (p>0.05). A similar pattern was evident in the responses of experts indicating that presence of iodised salt in cheese varieties does not have any impact on the textural perception of those varieties. The specific Chi square test values and p values confirming no significant differences in the perception of texture for cheese with and without iodised salt by both non-expert consumers and sensory experts have been presented in the appendix 3.3.

In addition to the main profiling attributes (colour, appearance, taste, texture) and related specific attributes (sweetness, bitterness, hardness, chewiness), saltiness and level of moisture (dryness or mouth feel of cheese) also play a vital role in the overall acceptability of cheese samples and so the participants were asked to rate these aspects related to the developed cheese varieties.

Table 6. 5 Saltiness of cheese varieties by non-expert consumers (NE)

Type of cheese	**Not at all salty (%)**	**Slightly salty (%)**	**Moderately salty (%)**	**Very salty (%)**
Soft cheese with iodised salt	7.39	58.69	19.56	4.34
Soft cheese with regular table salt	19.56	32.60	36.95	10.86
Cheddar cheese with iodised salt	15.09	56.60	18.86	5.66
Cheddar cheese with regular table salt	18.86	37.73	37.73	3.77

As seen in the above table 6.6, all the four cheese samples were rated either ‘slightly salty’ or ‘moderately salty’ by large percentage of participants

Similar mixed response trend was observed in the perception of experts relating to level of saltiness opinion about the level of saltiness. The majority (n=3 and n=5 respectively) rated the cheddar cheese samples and soft cheese with iodised salt as moderately salty. Soft cheese made with regular table salt was found to be 'slightly salty' and 'very salty' by an equal number of participants (n=2).

6.2.5 Level of Dryness (level of moisture or mouth feel) of cheese samples

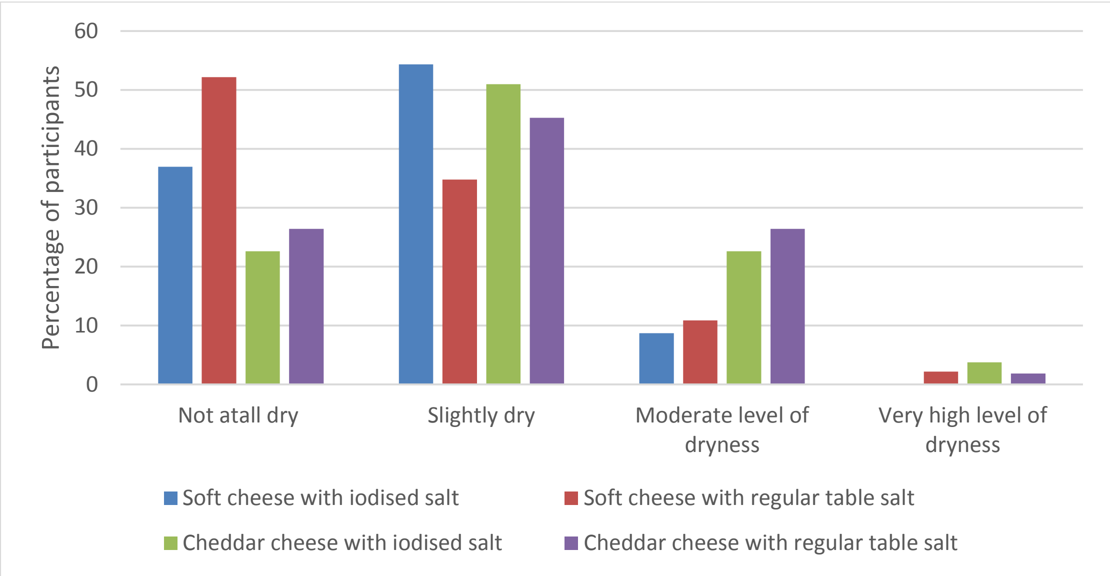


Figure 6.19 Non-expert consumer perception about level of dryness in different cheese varieties

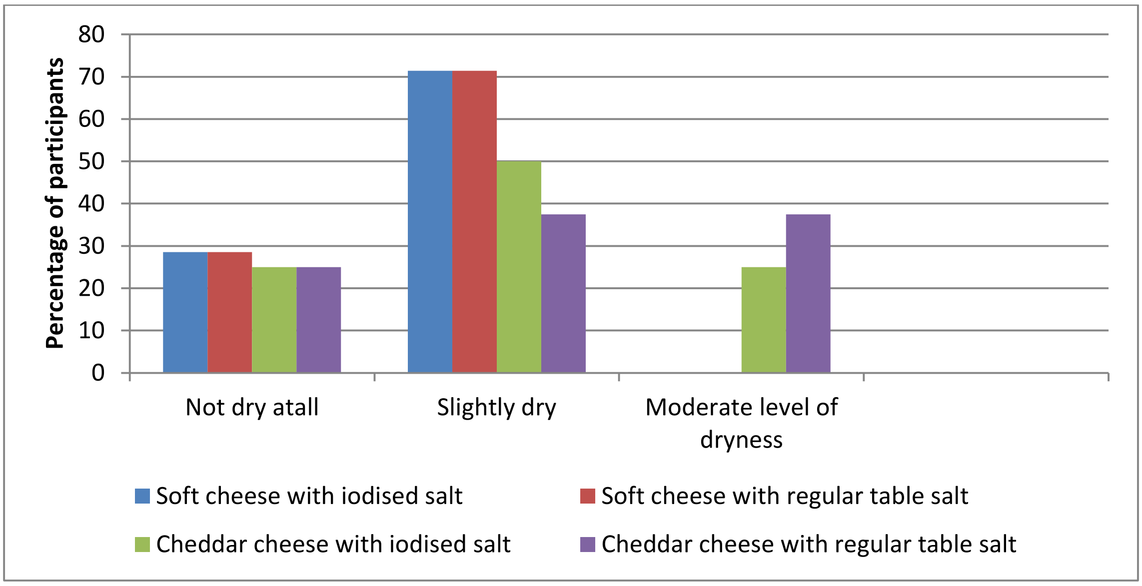


Figure 6. 20 Perception of sensory experts about level of dryness in different cheese varieties

In the last part of the sensory evaluation exercise, the participants (non-expert consumers and sensory experts) were asked if they could identify which cheese sample contained iodised salt between the two samples tasted during the sensory exercise. In continuation, the participants were further asked to state their personal choice/preference between the two cheese samples.

6.2.6 Identification of cheese samples made with /without iodised salt by non-expert general cheese consumers

The sensory evaluation exercise for soft cheese samples was completed by 46 participants. Out of these, 12 participants correctly identified the cheese samples prepared with and without iodised salt. A high number of participants (n=23) could not make out any difference in type of salt in these cheese samples and so responded 'Don't Know' for this question and one participant did not answer this question at all. Similarly, the sensory evaluation for Cheddar cheese samples was undertaken by 53 participants. Out of these 26 participants correctly identified the cheese samples made with iodised salt and one with regular table salt. Further 13 participants could not identify any difference between the samples and so responded 'Don't know' for this question. Moreover, 2 participants did not answer this question at all.

6.2.7 Identification of cheese samples made with /without iodised salt by sensory expert panellists

The sensory evaluation for soft cheese samples was undertaken by a panel of 7 sensory experts. Out of these, 5 participants could correctly differentiate the cheese samples made with/without iodised salt. Also, one expert could not make out any difference between the samples and so responded 'Don't know' for this question.

Similarly, the sensory evaluation exercise for Cheddar cheese samples was completed by 8 sensory experts. Out of these only three experts could correctly identify the samples made using iodised salt and regular table salt. Also, one expert could not make out any difference between the samples and so responded 'Don't know' for this question.

6.2.8 Preference between cheese samples by sensory experts

With respect to soft cheese samples, 85% of sensory experts preferred soft cheese made with iodised salt over cheese made with regular table salt. In terms of Cheddar cheese samples, the preference was exactly the same where 50 % of participants preferred cheese with iodised salt and other 50% preferred cheese with regular table salt

6.2.9 Preference between cheese samples by non-expert general cheese consumers

The consumer preference for soft cheese samples with both the salt variations was similar with soft cheese with iodised salt preferred by slightly higher percent of participants (58%), in comparison with soft cheese with regular table salt, which was preferred by 43% of participants. Here it is important to note that these percent preferences were calculated for 41 participants. This is because, out of the total 46 participants, 3 participants did not have any preference and so they either responded as 'no preference' or did not answer the question at all. Also, one participant could not make any choice and so did not answer the question.

On similar lines the consumer preference for cheddar cheese samples was found to be very similar with 44% (n=23) of participants preferring Cheddar cheese with iodised salt whereas 48% (n=25) preferred Cheddar cheese with regular table salt. Out of the total 53 participants who undertook this evaluation, one participant did not answer the question and so the percent preferences were calculated for 52 participants.

6.3 Detailed sensory report for cheese varieties by sensory experts

Completion of the individual sensory evaluation exercise for all the cheese varieties, was followed by a detailed panel discussion wherein an extensive report was prepared (appendix 2 – 2.14)

In this report, all the attributes (colour, external appearance, flavour/taste, texture, aroma) related to the developed cheese varieties were discussed thoroughly and detailed comments and overall scores about the quality of cheese samples were provided by consensus among all the panel members using a Campden Overall Eating Recommended Scoring Scale (Table 6.7) as a reference to describe the quality of the product.

Table 6. 6 Campden Overall Eating Recommended Scoring Scale

Score	Product Quality band	Description
10	Excellent	For commercial and technical reasons, manufactured food products in this zone are very rare.
9	Very Good	Products in this band are of high achievement, reflecting special attention to raw materials and technology and usually commanding a price premium
8	Good	
7	Fairly Good	Products that fall into this zone are considered to offer marketplace quality. Within this broad band, it is likely that specific products will have been positioned for a particular market. Historically, majority of new products have fallen in this band
6	Satisfactory	
5	Acceptable	
4	Not quite acceptable	Products in this band are considered to be disappointing and failing to meet their description
3	Poor	It is inconceivable that any manufacturer would aim to market product in this zone. We would assume technical failure or product abuse
2	Bad	
1	Inedible	

6.4 Results of assessment ratings/scores by sensory expert panellists for newly developed cheese varieties

Table 6. 7 Scores of different cheese varieties by sensory experts

Total number of Panellists 7- for soft cheese 8 - for cheddar cheese	Score for External appearance	Score for internal appearance	Score for flavour/taste	Score for aroma	Score for texture	Score for colour	Score for eating enjoyment
Soft cheese with iodised salt	9	-	8	8	7	-	8
Soft cheese with non-iodised salt	9	-	7	8	6	-	7
Cheddar cheese with iodised salt	8	8	5	7	6.5	8	6.5
Cheddar cheese with non-iodised salt	8	8	6	8	6.5	8	6.5

As seen in the above table the external appearance of both the cheese varieties was rated very highly (good to very good) by the experts. In terms of flavour, texture and aroma of soft varieties, soft cheese with iodised salt was preferred over soft cheese with regular table salt. The external, internal appearance and colour and aroma of the Cheddar cheese varieties was liked very much indicated by a high score of 8 for these attributes. Finally, in terms of overall eating experience, soft cheese varieties were preferred over cheddar cheese samples.

6.5 Discussion of key findings of the chapter

The sensory acceptability of the newly developed Cheese varieties (Cheddar and soft with two salt variations) was analysed by conducting a sensory evaluation trial. This sensory tasting of cheese was carried out by recruiting non-expert consumers and sensory experts. It was found that all the main attributes (colour, appearance, taste, texture, odour and overall quality) of all the four cheese samples were liked similarly by non-expert consumers and sensory experts. The responses for specific characteristics like sweetness, bitterness, aftertaste, creamy, buttery odour, saltiness for cheese with iodised salt were not statistically different than cheese with regular table salt. From these observations, it can be concluded that the use of iodised salt in place of non-iodised salt in soft and hard cheese production does not result in any detectable differences in the taste or perception of various sensory attributes of cheese, thus this should not be a barrier to the introduction of iodised salt into cheese production.

6.6 Limitations of the sensory evaluation protocol for the newly developed cheese varieties

While conducting the sensory evaluation protocol, the researcher adapted some aspects of the standard method of conducting sensory analysis (Papetti and Carelli, 2013), however there were some clear differences in methodology and these could have impacted the responses obtained during the evaluation conducted in the present study.

Firstly, the language used for recording responses for different attributes of cheese could be improved after conducting detailed discussion with trained sensory panellist. By undertaking the language development exercise, it would be possible to understand, if the questions asked are easily and correctly interpreted by the respondents. Also, the attributes used for description of cheese varieties could be improved. For example, while developing questions about the texture of cheese varieties, terms like 'hardness' and 'grainy' are appropriate for recording responses for Cheddar cheese, but these should not be used for describing soft cheese varieties. Secondly, the samples provided to the non-expert participants at UCLan and the sensory experts at Cardiff Metropolitan University were different in size and presentation (figures 6.1, 6.2, 6.3 and 6.4). Furthermore, there were differences in the seating arrangement, lighting of the room and complete setup of the samples (placement of the samples and glass of water on the table) across the two sites. These differences could have affected the responses of the participants. This scenario could be improved by having a mutual agreement between both the sites to ensure all the above-mentioned aspects are exactly same, thereby increasing accuracy of the entire sensory evaluation exercise.

Chapter 7: Discussion

7.1 Overview of the chapter

This chapter begins with a brief summary of the overall findings of the study, followed by a. discussion of the results obtained from this research in light of existing literature. Strengths and limitations of the research will be discussed. The last part of this chapter provides a graphical presentation and discussion of the current situation regarding iodine deficiency in the UK, with recommendations for future research to inform policy.

7.2 Summary of the key findings of the present study

The overall aim of the present study was to explore the use of iodised salt in cheese production to improve the iodine status in the UK. In order to accomplish this, 4 smaller interconnected aims were identified namely development of method and subsequent analysis of iodine content of cheese samples in the UK, examining these results in relation to the legislative policies regarding iodine fortification practised in the country of production of cheese, understanding about the knowledge, attitudes and practices of consumers regarding iodine nutrition and fortification through a survey and then finally manufacturing popularly consumed cheese varieties (soft cheese and cheddar – from the consumer survey) using iodised salt and comparing it with cheese made with non-fortified regular table salt in terms of iodine content and overall sensory acceptability.

The findings of this programme of research can be summarised as follows. Firstly, it was shown that the iodine content of cheese produced in countries with mandatory legislative policy for iodine fortification was not necessarily higher than that of cheese manufactured in countries with voluntary legislation, but it was less variable. In addition, this research demonstrated that the iodine content of cheese can be increased using iodised salt in the manufacturing process, both in soft and hard cheese production. Secondly, from the standpoint of finding a suitable food vehicle for iodine fortification, the consumer survey revealed that cheddar cheese is the most popularly consumed cheese in the UK and is therefore a potentially useful vehicle for iodine fortification. The survey also highlighted a lack of awareness about importance of iodine for optimal health and a lack of availability of iodised salt in UK supermarkets. Finally, from the information gathered during sensory evaluation, it can be concluded that the use of iodised salt in place of regular table salt in soft and hard cheese production does not result in any detectable differences in the taste or perception of various sensory attributes of cheese, thus this should not a barrier to the introduction of iodised salt into cheese production.

7.3 Cheese mapping and scoping

The first aim of the present study was to measure the iodine content of the different categories of cheese available in UK supermarkets and to explore whether or not the iodine content was related to the iodine fortification policy of the country of origin. The results of this cheese scoping and mapping has contributed towards updating the currently available limited database on iodine content of various foods in the UK. The cheese scoping/mapping results of the present study also become important in the light of the work conducted by Haldimann et al., (2005), who have also reported that data on iodine contents of food items are relatively scarce, except for milk and fish (Julsham et al., 2001; Sieber et al., 1999). This problem of limited information has been more recently pointed out by Bath et al. (2014). Working along lines of the present study, efforts were made towards development of a database for the iodine content of foods in the UK by Fordyce (2003). However, these values have been populated from the available information at that time and so some of the values for cheese samples date back to 1952 when the use of iodophor disinfectants in the dairy industry was more common. The values on iodine content of iodised salt samples have been reported from literature published in 1992 and so it is possible that there have been changes in this data and this needs to be updated. This information clearly establishes the importance of cheese scoping and mapping conducted in the present study.

7.4 Quantitative method for iodine analysis of cheese varieties from UK supermarkets

In the present study, several trials were conducted for development of a valid and reliable method for iodine analysis in cheese. However, various inconsistencies were observed in the overall process of setting up the method as well as in the results obtained. These inconsistencies in the results could be attributed to various aspects related to the process and ingredients used for analysis. Zimmermann (2009) has discussed a reliable method for determination of iodine concentration in urine samples. However, when efforts were made to adapt this method for iodine analysis of cheese samples in the present study, it was not possible to obtain valid reproducible results. This could be attributed to the complexities of the food matrix (complex interactions between the components of the food). Cheeses contain more organic substances than urine, such as fats and proteins that can interfere with the spectroscopic analysis and reduce the recovery of iodine from the samples. Another potential hurdle in analysis could be the higher fat content of cheese samples causing difficulty in acid digestion which is crucial for preparation of samples for analysis. In the context of food matrices and problems related to sample preparation, observations made by Winger et al, (2008) are in complete agreement with the present study wherein it has been reported that though modern techniques are available for detection of low levels of iodine, sample preparation techniques are inadequate and insufficiently robust to detect low levels of iodine and covalently bound forms of iodine in the complex food matrices. Furthermore, these researchers have also pointed out an important knowledge gap which is non-clarity about identification of different chemical forms of iodine (iodate, iodide, bound form) within a specific food matrix at a given point in time and suggested that more work needs to be conducted.

The difficulties encountered in analysis in the present study were similar to the challenges reported by other researchers working with the development of analytical methods for iodine content of foods. Winger et al., (2008) have reported that the determination of different iodine species in foods (and other biological samples) is extremely complicated and requires substantial investment in technical equipment for regular operation. The need for technical expertise, especially related to appropriate functioning and modification of ICP-MS instrument for iodine analysis, was also identified in the present study.

Due to all the above-mentioned challenges in development of a method for iodine analysis, the selected cheese samples were analysed at a commercial laboratory (Fera Labs, York, UK).

7.5 Examination of cheese iodine content in relation to legislative policies in the country of origin of cheese

Cheese samples from three countries with mandatory legislation were analysed for their iodine content. Multiple samples were selected from a single country (for example 3 samples from the Netherlands and 2 samples from Denmark) belonging to different brands to ensure that all the different samples available in the UK from that specific country were covered, thereby gaining better clarity for interpreting the iodine fortification scenario in the country.

Wide variation was observed in the iodine content of cheese samples from countries with voluntary legislation. The iodine content of hard cheeses varied from as low as 0.12 mg/kg (Extra Mature Cheddar) to as high as 0.92 mg/kg for Farmhouse cheddar despite being from the same country of origin (UK). In addition, the iodine content of cheese also varied depending on the type of cheese (manufacturing process). For example, French Comte and French Roquefort, both cheese samples have the same country of origin, but French Comte is a hard cheese (iodine content – 0.09mg/kg) while French Roquefort is a type of blue cheese (iodine content – 0.64mg/kg). Other factors that may affect the iodine content of cheese include seasonal variations in milk composition, and the iodine content of animal fodder.

There is limited literature available on the iodine content of cheese varieties from the UK and across the globe. Different research groups have compiled the data available on the iodine content of cheese samples at the time of their study. These data can vary if more updated information becomes available. According to the reports available in SACN (2014) and FSA (2002) the iodine content of mild and mature Cheddar cheese samples was found to be in range of 0.4-0.5mg/kg. Similarly, in the work undertaken by Bath and Rayman (2013) and Haldimann et al., (2005) the reported iodine content of cheese samples ranges from 0.37-0.47mg/kg. However, in this database there is no information about the type or source of cheese. A more recently published report by the British Dietetic Association (2016) has noted that iodine content of cheese is 0.37mg/kg.

In the present study the iodine content of cheese samples produced in countries with voluntary legislation demonstrated a wide range from 0.09mg/kg to 0.92mg/kg. Comparing these results with the values reported in the literature, it is evident that that iodine content of Blacksticks® Blue cheese, Mature cheddar and Vintage Cheddar from the UK and Emmental from the Czech Republic compares well with the values reported by SACN (2014) and FSA (2002). Iodine values obtained in the present study also fall in the same range of values as reported by Bath and Rayman (2013) and Haldimann et al., (2005).

Interpreting the results obtained for cheese samples from countries with Mandatory legislative policy, it can be concluded that the iodine content is very low (0.16-0.26mg/kg). However, there is an exception for Canadian Cheddar (0.76mg/kg) wherein the iodine content has been found to be higher than the average range of iodine values established in the literature.

From the overview of the results obtained for iodine content of cheese samples from either countries with voluntary legislation or mandatory legislation, there are 3 cheese samples (Cheddar cheese samples from UK and Canada and Swiss Le Gruyere cheese from Switzerland) which emerge as distinctively different and it is important to understand the reasons for these results. Firstly, the iodine content of Swiss Le Gruyere, a type of hard cheese produced in Switzerland has been found to be lowest in the selected samples analysed in the present study. This result is an important finding of the present study in the light of the remarkable successful triumph of Switzerland's salt iodisation program and related prevention of iodine deficiency in the country with voluntary legislative policy for iodine fortification (FCN, 2013). Ongoing research in this area of nutrition has pointed out various potential reasons for this observation. Seasonal variations leading to lower iodine content in summer milk could be one of the prominent reasons. Apart from this seasonal effect, it has been seen that the relative amounts of iodine entering the milk can vary considerably from farm to farm. Other factors include differences in cow's breed, lactation stage of the animal and feeding practice. Most of the cow's fodder belongs to plant origin and the iodine content of this plant matter can vary considerably in composition depending on the iodine content of the soil and water indirectly affecting the milk iodine concentrations (Stevenson et al., 2018, FCN,2013). Examining this from the point of view of existing legislative policy for iodine fortification, it has been postulated that Switzerland's changing legal and political framework for iodine fortification, that has resulted in a reduction of the use of iodised salt in food manufacturing especially in cheese production, could have had an impact on the iodine content of cheese produced in Switzerland.

Piccinalli et al., (2012) have reported the reasons for stopping the use of iodised salt in cheese production. Firstly, there is very small diffusion of iodine into the cheese. This is highlighted in a study (Hani, 1997), which explains that the diffusion of iodine to the centre of a cheese is relatively weak, so that the majority remains in the rind zone. When this rind portion is removed before consumption, part of the iodine is removed with the rind, which is not consumed. Understanding this iodine diffusion mechanism, while conducting analysis in the present study, it was ensured that a representative sample was obtained from the entire block of cheese by cutting the block into 6 roughly equal portions, selecting 3 random portions and then passing it roughly through a food processor to obtain a homogenised sample, thereby reducing under or over-estimation of iodine content related to concentration of iodine in specific parts of the cheese samples. Secondly, there is a requirement in many countries that the foods that contain iodised salt are labelled as such. Some countries, such as France, have a ban on the import of foods containing iodised salt. This ban in France is based on the report entitled "Development of nutritional effects of the introduction of iodised food components" produced by the French food safety authority AFSSA (Agence Française de Sécurité Sanitaire des Aliments). This report highlights the importance of monitoring the systematic use of iodised salt in processed food to reduce the risk of the population being exposed to intakes that exceed the upper safety limit for iodine.

Analysis of Cheddar cheese in the present study revealed that the samples from the UK and Canada contained the highest concentrations of iodine. Interpreting these results from the perspective of existing legislative policies in the country of origin, it is clear the Canada has mandatory legislation for iodine fortification of salt and the higher iodine content of Canadian Cheddar reflects this legislative policy. It also strongly confirms the guidelines provided by WHO and FAO (2006), which states that 'with high level of certainty mandatory fortification is more likely to deliver a sustained source of fortified food for consumption'. Contrary to this the UK adheres to voluntary legislation for iodine fortification of foods and so it is interesting to encounter high iodine values for Cheddar cheese from the UK. Two potential aspects could have contributed to this result. Firstly, the iodine concentration of milk used for the cheese production could have been higher- higher content during winters and use of iodophors in disinfecting agents, teat dippings in milking plants are prominent and well-established reasons throughout research (Vander-Reijden et al., 2017, Flachowsky, 2007, Haldimann et al., 2005). Secondly, it is important to confirm if iodised salt was used to produce this cheese or whether it was fortified with nutrients during manufacturing. This is because if cheese is made using iodised salt and if the outer portion of the cheese is selected for analysis, then it will reflect higher iodine content for the selected cheese sample. However, more work in this specific area of cheese processing needs to be conducted to support this theory. Furthermore, to date there is no database, which has interpreted the iodine content of food items in relation to the legislative policies (voluntary v/s mandatory), regarding iodine fortification or use of iodised salt in food manufacturing. The results of the present study have contributed towards filling this research gap.

7.6 Consumer survey on use of iodised salt in food production and consumption

Recent studies have reported the re-emergence of iodine deficiency in the UK, particularly in pregnant women (Rayman and Bath, 2015). However, there is a paucity of information about iodine status of the population in the UK, coupled with lack of guidance and awareness about the significance of iodine for health. The need for an in-depth consumer survey to gather public opinion about various aspects related to iodine nutrition, knowledge, awareness about use of iodised salt was addressed in the present study. The results of the survey highlighted the limited public awareness of the importance of iodine for health and their personal iodine intake levels. Combet et al., (2015) undertook a cross-sectional survey to explore the public awareness of iodine dietary requirements. They reported that only 12 % of the participants were aware about the dietary requirements and 28% had some knowledge about ways to achieve this recommended value. More recently Bouga et al., (2018) undertook a study to explore perceptions, awareness, and experiences of nutrition during pregnancy, focusing on iodine. This study revealed a lack of awareness about the importance, food sources, and recommendations for iodine intake. These observations made in the literature are in close agreement with the observations made in the present study.

Considering the significant role of iodine throughout the lifecycle, especially among vulnerable populations (pregnant women, women of reproductive age and young children), it is extremely important to initiate adequate steps to increase awareness about iodine requirements and consequences of deficiency because if young women, unaware of their suboptimal iodine status, become pregnant in near future then it could lead to adverse consequences not only for the women but also for the growing foetus. This scenario implies that there is need for more research in the form of consumer surveys (at local or national level) aimed at increasing awareness about iodine nutrition (Bath et al., 2014, Bath and Rayman, 2013, Haldimann et al., 2005).

The present study explored awareness about the addition of iodine to foods and availability of iodised salt in the supermarkets. The results revealed a limited awareness about iodised salt and simultaneously shed light on the limited availability of iodised salt brands in supermarkets. Here it is important to note that the limited availability and limited awareness are inter-connected, where limited awareness leads to limited demand and thereby leading to limited availability. These observations extrapolated from the present survey compare well with the conclusions put forth in various related studies wherein it is stated that low awareness about iodine nutrition is coupled with low availability of iodised salt in UK supermarkets (Bath et al., 2014, Bath and Rayman., 2013).

Information gathered regarding the frequency and type of cheese consumption among the consumers revealed that cheese is consumed more than once a week by 61% of the participants and, among the different cheese varieties, cheddar is the most popularly consumed type of cheese (41%, n=323). This popularity of cheese compares well with the observation made by British cheese board, Dairy, UK, which has clearly established that Cheddar is Britain's favourite cheese. The cheese map developed for the British Cheese board clearly identifies that 56% of the total cheese sold in UK is Cheddar (Defra, British Cheese board, Dairy UK, 2014). Furthermore, the preference of consumers for Cheddar cheese over other cheese varieties has been reinforced by the regularly published Mintel market reports which points out that Cheddar continues to be the best-selling cheese over the years (Mintel, September 2016). This has been attributed to the fact that it is not only locally produced, but it also has a long-standing historical hold on the public consciousness.

When views were obtained on the idea of fortification of food with nutrients for health benefits, it was observed that even though 50% of participants liked the idea, there were other participants who were not sure or did not know about this. Here it is important to understand various reasons for not liking the idea of fortification and some of the prominent ones included 'Liking the food in natural form', fear about over- consumption and toxicity and limited understanding of fortified foods. From these responses, it can be clearly seen that fear of toxicity and fear of addition of external substances or toxicity could have stemmed from the limited understanding about the process and benefits of fortified foods and so it is imperative to plan nutrition education strategies to enhance the overall understanding of the population at large.

To get more in-depth perspective of consumers and understand the reasons for liking or not liking the idea of fortified foods, participants were asked about the impact of fortification on sensory attributes and cost of the foods. Responses revealed that 55% of the participants did not know about the impact, thereby reinstating the need for increasing awareness about food fortification, especially regarding safety of consumption of iodine- fortified salt. The final question explored the consumer's preference between regular table salt and iodised salt and were asked to state the reasons for their choice. It was found that regular table salt (62%) was preferred more than iodised salt (43%) because they had no knowledge about iodised salt and so continued using the familiar regular table salt. These data reinstate the need for increasing awareness about benefits of iodised salt over regular table salt. There is a possibility that these preferences might change after adequate nutrition education.

Apart from the consumers, it is important to obtain the perspectives of food manufacturers regarding fortification and challenges involved in processing due to fortification. Remarkable work on increasing awareness about the use of iodised salt in food products (cheese) was conducted by UNICEF Regional Office for CEE/CIS and UNICEF Country office in Moldova wherein a tour was organised by Swiss producers for the Moldovan government officials to witness the functioning and feasibility of use of iodised salt in food products. Some of the main challenges highlighted by the officials regarding use of iodised salt in food production included resistance from the food industry especially manufacturers and concern regarding the negative impact of iodised salt on the overall quality of the final product. Another concern raised by the food producers was the increase in the cost of the product and process of inspection if these fortified foods are exported across the globe. During the course of the present study, brief conversations were conducted with cheese manufacturers and food technologists to enquire about their views on use of iodised salt in cheese production. These have not been formally presented in the preceding chapters because the data were limited and mostly anecdotal, however they are worthy of bringing in to the discussion here.

Manufacturers and food technologist were informed about the potential low dietary intake of iodine in general population in the UK. To gauge the existing scenario related to use of salt in food processing, manufacturers were asked if their company produces any product where salt is an integral part of its formulation and if Iodised salt is used in any of their products. It was revealed that they neither used iodised salt in any of these cheese samples nor in any other product because there is no clarity as to whether or not iodised salt should be used in the UK. When reasons for the lack of use of iodised salt were explored, ‘No demand or benefits to the product’ was one of the most common reasons while some others were not sure about the reason. Further, they also thought that presence of iodine in cheese production might limit starter culture activity, making it impossible to make the cheese properly. All the manufacturers and food technologist liked the idea of fortification. Also, there was lack of awareness about possible low dietary iodine intake in the population.

Perspectives from general consumers and manufacturers helped in providing better clarity about the barriers for salt iodine fortification at a national level and also shed light on aspects to be considered for planning iodine nutrition education programmes for the manufacturers as well as general population.

7.7 Production of new cheese varieties using iodised salt

The present study, primarily aimed at improving dietary iodine intake in the UK through use of iodised salt, was based on two public health concerns; one regarding the potential re-emergence of iodine deficiency in vulnerable populations in the UK (Rayman and Bath, 2015, and the other regarding the risks associated with high salt intakes associated with increased risk of cardiovascular disease (Hashem et al., 2014). Replacing regular salt with iodised salt in cheese making may go some way towards addressing this conflict, since salt is an integral part of the cheese making process.

Cheese was selected as the vehicle for incorporation of iodine as it was found to be one of the most commonly consumed products. A considerable amount of evidence has established cheese as a frequently consumed product in the UK diet and globally. World trends regarding cheese consumption reported by Cruz et al (2011) states that cheese consumption has increased manifold in many countries over the years regardless of the socio-economic condition of the nation. In UK, the popularity can be assessed by increasing sales trend reported by Mintel Market reports, for example hard cheese sales have increased from 350.23 tonnes in 2011 to 382.20 tonnes in 2016. This upward trend has been reported for all varieties of cheese. Cheddar remains the best-selling cheese whereas soft cheeses also have a high demand in the UK. The versatility of cheese with varied consistencies, colours, flavours offer a large array of possibilities for its use in a variety of cuisine (Cruz et al., 2011) thereby clearly establishing cheese as one of the popularly consumed products in the UK. However, despite its regular consumption as a part of daily diet, cheese is high in salt content contributing to 44 percent of salt consumption in UK diets (Hashem et al., 2014). Salt is an integral part of the cheese manufacturing process and overlooking few exceptions cheeses contain 0.5-2 percent sodium chloride. This data shows the relevance of cheese as a food vehicle wherein regular table salt could be replaced with iodised salt, indirectly improving dietary iodine intake.

In the light of this literature, the present study developed two new cheese varieties (Cheddar and Soft cheese) and replaced regular table salt with iodised salt in cheese production, so that these cheese varieties could be used as a food vehicle to improve iodine intake and thereby contribute towards overcoming the problem of iodine deficiency without increasing the salt content of the product.

7.8 Iodine content of newly developed cheese varieties with/without iodine fortified salt

A total of 4 samples, including 2 soft cheese samples with /without iodised salt and 2 cheddar cheese samples with/ without iodised salt were analysed at a commercial laboratory (Fera Labs, York, UK). The values obtained for 2 samples of the same variety were very similar and this could be attributed to use of appropriate method for analysis. The values of iodine content of cheddar cheese samples obtained in the present study (0.46-0.48 mg/kg) are in complete agreement with the existing literature (SACN,2014, Bath and Rayman., 2013, Haldimann et al., 2005, FSA, 2002).

Scarce data is available for the iodine content of soft cheese samples, however Jahreis et al., (2001) reported the iodine content of camembert and brie to be 0.74mg/kg and 0.41mg/kg respectively. These data are comparable with values obtained for soft cheese varieties (0.68mg/kg - 0.76mg/kg) in the present study. In addition, the salt used in the production of the cheeses (soft, cheddar) was also analysed for iodine content. The iodised salt (Cerebros) was estimated to contain a minimum of 11.5mg/kg (according to the label on the container) and the values obtained on analysis are 13.8mg/kg which is a reasonable agreement, particularly bearing in mind that the labelled value may account for some loss of iodine during transport and storage (Eastman and Zimmermann, 2018; WHO and FAO, 2006). Furthermore, Fordyce,2003 compiled a database collating iodine values of various food stuffs from a variety of sources. According to this database iodised salt contained 21.04mg/kg of iodine, normal salt contained 1.79mg/kg of iodine whereas sea salt has been found to contain 0.5mg/kg of iodine (SACN., 2014, FSA., 2002). These values are higher in comparison to the iodine content of regular table salt (ASDA) analysed in the present study which has revealed negligible levels (<0.01 mg/kg) of iodine.

Overall, the results of iodine analysis of newly developed cheese varieties establish that it is possible to increase the iodine content of cheese by using iodised salt in the manufacturing process, both in soft and hard cheese production.

7.9 Sensory evaluation of newly developed cheese varieties by consumers and sensory experts

While consuming food, multiple sensations arise from the individual flavour, taste, texture or temperature, influencing the perception of the quality and acceptability of the product. Scientific mechanisms postulated by various research groups (Cruz et al., 2011, Saint-Eve et al., 2009, Kilcast and Rider,2007, Guichard, 2002) implied that the sensations experienced may interact either at perceptible levels (smell, palate, chewing) or at physicochemical levels (pH, acidity, protein content, fat content). The final perception and acceptability of a certain product is a result of the integration of all this sensory information experienced during consumption. This mechanism plays a role in the consumer preference for specific cheese varieties over others, which is governed largely by its sensory attributes like taste, flavour, texture or mouth-feel. Some consumers prefer strong cheese varieties while others prefer milder versions. The salt content of the cheese can also influence the rheology and texture of the cheese. It has been suggested that salted cheese varieties tend to have firmer shape while salt free samples tend to be creamier, soft, pasty depending on the specific type and age of maturation (Cruz et al., 2011, Guinee and Fox, 2004). This part of the study was designed to understand sensory acceptability of replacing regular salt with iodised salt in the newly developed soft and hard cheese varieties. This was achieved using a sensory evaluation template which was designed based on a QDA method which includes 16 clearly defined profiling attributes (Papetti and Carelli, 2013). Here, it is important to note that the method for QDA described in the literature (Papetti and Carelli, 2013) is a standard method for sensory analysis of cheese varieties. However, while developing the template for sensory evaluation for the present study, some aspects of this standard method were adapted. Due to this, there were some clear differences in the overall methodology adapted for undertaking the sensory evaluation protocol at both the sites such as variability in sample size, lack of language development for describing different attributes of the cheese and differences in the overall setup for conducting the tasting session. It is likely that these differences could have affected the responses of the participants and so these limitations should be considered while interpreting the results. Consumers rated cheese samples on 6 main parameters including colour, appearance, taste, texture, odour and overall quality using a 7-point hedonic rating scale ('like very much' to 'dislike very much') (Singh-Ackbaraliand Maharaj, 2014). Additionally, consumers were asked if they could detect any specific characteristics related to the main sensory attributes. For example, in terms of taste, the participants were asked if they could detect sweetness, acidic taste, bitterness, after-taste or any other taste. In this context, literature has strongly established that one of the most important functions of salt in cheese processing is that it can form a foundation for perception of other flavours, as it works as an enhancer of taste in some cases, whereas it can act as inhibitor in others. It has also been demonstrated that salt can not only promote higher perception of aromas, but also in some cases help to mask the bitterness in processed foods (Cruz et al, 2011, Hayes et al, 2010, Kilcast and Rider, 2007). So obtaining information about individual parameters related to taste, texture and odour enabled the researchers to understand the impact of iodised salt not only sensory attributes but also on the organoleptic qualities of the final cheese product; both in combination can influence the overall acceptability of the final cheese.

In the present study, sensory evaluation of cheddar cheese and soft cheese was conducted by non-expert consumers as well as sensory experts. The overall quality of all the four cheese samples with salt variations was liked similarly by non-expert consumers and sensory experts indicating overall acceptance. These responses compare well with the literature which states that iodised salt (KI or KIO_3) does not have any adverse impact on the sensory quality of dairy products especially soft cheeses and hard cheeses (IDD Newsletter, Aug 2018). Understanding the consumer responses in terms of taste, sweetness was found to be similar for both the cheese varieties irrespective of type of salt. However, certain differences were encountered with regards to detection of acidic taste. Cheese samples with iodised salt were found to be more acidic in taste than cheese samples with regular table salt. This acidic taste could be attributed to the breakdown of lactose into lactic acid. This process is known to have an impact on the taste or flavour components of cheese. The starter culture breaks up the lactose and engulfs the glucose from the lactose sugar molecule turning it into lactic acid. The acidic taste could also be due to the growth of lactic acid bacteria (Papetti and Carelli, 2013). The effect of the presence of iodine on the formation acid taste has not been documented well and so it is difficult to establish any association between presence of iodised salt and acidic taste of the cheese. The assessment of taste parameters by sensory experts was different from the consumer responses. Accordingly, soft cheese samples were found to be more acidic in taste whereas bitterness was highly detected in cheddar cheese samples by the experts. Here it is important to note that sweetness was least detected in cheddar cheese which is explained by the prominent experience of a bitter taste in these samples. In the context of bitterness in cheddar cheese samples, the presence of salt or salty taste plays a role in masking the bitterness in various processed foods (Piccinalli et al., 2012, Cruz et al., 2011). In the present study, the consumers and experts agreed that the cheeses are in the range of slight salty to moderately salty, which may explain the hightened perception of other tastes such as bitterness.

With regards to specific characteristics related to the odour of cheese samples, the consumer responses revealed that cheese varieties with iodised salt were creamier and had a more acidic odour than cheese varieties manufactured without iodine–fortified salt. However, these responses were in sharp contrast to the responses of sensory experts wherein creamy odour was detected in cheese varieties with non-fortified salt. Consumer perceptions about textural attributes of different cheese varieties were mixed with no clear pattern. From an overview it was observed that cheddar cheese with iodised salt was found to be harder, chewy, rubbery and grainy than any other cheese samples. This could be attributed to difference in processing of cheddar cheese and improvement in the textural qualities during maturation. This observation is in close agreement with findings from work by Papetti and Carelli, 2013 which reports improvement in sensory profiles of cheese from month zero to months 9 due to metabolic processes that occur during ripening. The increase in chewiness in cheddar cheese might be due to the change in the average size of fat globules, distance between fat globules and variation in the size of globules during the ripening period (Richardson and Booth, 1993).

Towards the end of the sensory template, participants were asked if they could differentiate and identify cheese with /without iodised salt. Also, they were asked to state their preference between cheese samples. Responses demonstrated that the majority of the participants could not differentiate between cheese with or without iodised salt. There was also no notable difference in preference of cheese with/ without iodised salt.

In the light of the discussion about various consumer and sensory expert views regarding sensory acceptability of cheese varieties, it is important to highlight that despite differences in responses between consumers and experts, these differences were not statistically significant. There was no statistical difference ($p>0.05$) in the preference of one attribute over the other. Also, respondents did not perceive soft cheese or cheddar with iodised salt any differently ($p>0.05$) than soft or cheddar cheese made with non-iodised salt for any of the attributes (Sweetness, bitterness, acidic, aftertaste, hardness, chewiness, rubbery, grainy and so on). The specific Chi square test values and p values are provided in the appendix 3.3. This enabled the researcher to establish that the presence of iodine in cheese production does not have any adverse impact on the sensory acceptability of the final product, thus enhancing the suitability of the use of cheese with iodised salt as a vehicle for improving iodine intake and consequently iodine status of the population in the UK.

7.10 Overall contribution of iodine-fortified cheese for improving iodine status of the population

Considering the concurrent public health concerns (iodine deficiency and the regulation to reduce salt intake), the present study explored the use of iodised salt in cheese manufacturing, thereby improving iodine intake of the population without increasing the salt intake. It was found that replacing non-iodised salt with iodised salt in the production of both soft and hard cheese increased the iodine content of the cheese by 21% and 60% respectively. Cheese also forms an important part of the diet in the UK population with Cheddar topping the list of popularly consumed cheese varieties (Kanter Panel, 2018). This popularity of cheddar cheese has been established in the present study with 61% of respondents consuming cheese more than once a week. If this cheese consumption pattern is studied in terms of iodine content of cheese varieties, then it will be possible to understand the contribution of cheese containing iodine towards fulfilling the recommended daily requirement for iodine. However, while doing so, it is important to consider the dairy recommendations for adults by National Health Service (NHS), UK. According to the Eatwell guide (NHS, UK), it is recommended that adults (Aged 18 years and above) should include 2-3 daily servings of dairy (milk and milk products or dairy substitutes) and emphasis is on using healthier dairy products (reduced fat content) for better health (example: choosing skimmed milk, low fat yogurt, reduced fat cheese varieties, unsweetened calcium-fortified dairy alternatives). When cheese varieties from popular supermarkets belonging to different countries were analysed for their iodine content, it was found that five cheese samples from the UK namely Farmhouse Cheddar, Blue Stilton, Extra Mature Cheddar, Mature Cheddar and Blacksticks blue provided considerable amount of iodine per adult portion size (30 g) with Farmhouse Cheddar fulfilling the highest percentage (18%) of daily requirement of iodine for adults. However, these were individual samples of cheese from different brands and so more work should be conducted with large number of samples to establish these results. Furthermore, if iodine content of newly developed cheese varieties manufactured using iodised salt was considered, then abiding to the recommendations, consumption of minimum of 2 servings of soft cheese and cheddar cheese would be able to fulfil 34 % and 30 % of daily recommended intake of iodine respectively. In contrast, 2 servings of soft cheese and cheddar cheese with regular table salt helped in meeting 28% and 18% of the requirement clearly indicating 7 % and 12% increase in iodine intake respectively with consumption of cheese with iodine fortified salt. Using this approach of fortifying cheese with iodine, not only helps to meet the iodine requirements, but also provides a healthier dairy option without increasing the fat and salt content. Additionally, this concept of replacing regular salt with iodised salt is not only beneficial for regular cheese consumers, but it can also be adapted for developing cheese substitutes (salt used in manufacturing cheese analogues could be replaced with iodised salt), thereby helping in reducing the risk of iodine deficiency in the population following vegan dietary pattern or avoiding dairy for specific health conditions.

7.11 Conclusion

The present study established that the iodine content of cheese can be increased by using iodised salt in the manufacturing process, both in soft and hard cheese production (content increased by 21% in soft cheese and by 60% in hard cheese) and use of iodised salt in cheese production does not result in any detectable differences in the taste or perception of various sensory attributes of cheese. Collating these findings, it can be concluded that it is possible to use cheese with iodised salt as a potential vehicle for improving iodine status of the population in the UK. Moreover, the resistance towards use of fortified foods by general consumers and food industry professionals can be overcome by increasing awareness about benefits of iodine and safety issues regarding use of iodised salt through various nutrition education programmes.

7.12 Strengths of the study

The present study made valuable contributions to literature in following ways

Firstly, the iodine content of various common cheese varieties available in UK supermarkets was measured and this content was examined in relation to the legislation for iodine fortification policy in the country of production of cheese. In the light of early work in this area, iodine content of cheese products have been determined in previous studies, however, this has not been discussed from the perspective of voluntary or mandatory iodisation of food ingredients and processed foods in the individual countries and so the present research was a valuable addition to the literature.

There is currently no published research on the knowledge, attitudes and practice of consumers on the use of iodised salt in cooking and food products. In this research, a completely new consumer survey questionnaire was designed and it was possible to obtain some crucial information about the cheese consumption pattern, consumer preferences regarding use of fortified foods especially iodised salt in cheese production. This survey indirectly explored the use of cheese as a food vehicle for overcoming the emerging problem of iodine insufficiency in the UK.

Finally, two new cheese types (Cheddar and Soft) were prepared by using iodised salt instead of normal table salt. Comparison of cheese samples with and without iodised salt for understanding sensory acceptability by consumers revealed that the acceptability of cheese with iodised salt was similar to cheese with regular table salt, thus enabling the use of cheese with iodised salt as a potential vehicle to increase iodine intake in the UK

7.13 Limitations of the study

One of the primary objectives was to develop a valid, reliable and a robust method for measuring iodine content of the foods in the University laboratories. Several challenges were encountered in this process of method development. Some of the primary reasons for not being able to develop a successful method included the level of sensitivity of the method, the volatile chemical nature of iodine and requirement for specialist expertise in the analytical instrument. Since the samples were analysed at the commercial laboratory, the number of samples had to be limited due to the high cost of analysis per sample.

In addition to the consumer survey, efforts were made to interact with manufacturers to obtain their perspectives on use of iodised salt in cheese production. A total of 20 dairy managers, cheese makers were contacted through telephonic and electronic medium. Initial documents containing the required briefing about the study were sent by email. However, very few manufacturers responded (n=3), and those that did cited time constraints and unwillingness to collaborate on a project as reasons for not providing any information relating to this survey. So, even though this study provides better clarity about consumer perspectives and sensory acceptability about development of cheese varieties with iodine –fortified salt, more research is required regarding the attitudes and practices of food industry professionals.

7.14 Recommendations for future work

This research has highlighted a lack of awareness about iodine nutrition and limited knowledge about significance of iodine for normal health in the UK. This has led to the formation of a vicious cycle wherein due to a lack of awareness there is lack of demand for iodised salt and thereby low availability of iodised salt in the supermarkets. In order to solve these interlinked issues, it is important to raise consumer awareness through nutrition education strategies. To begin with, it is important to ensure that there is appropriate emphasis on creating awareness about general nutrients for optimal health at primary school as a part of school curriculum. Furthermore, public health campaigns could be organised at regular intervals by authorised health and nutrition professionals to create awareness among general population. In these campaigns, it is important to help people identify the authentic sources for improving their knowledge about existing health issues and prevention strategies. There is lack of consistency in the health messages passed on to the general population and so it will be helpful if Public Health England could develop a strategy wherein the valid health messages could reach the general population. This could be done by creating various channels such as monthly newsletters, events such as Health Mela, presenting an opportunity for the general public to communicate with professionals.

There is also limited data on iodine content of various foods and especially dairy products (milk, cheese) and salt as these are some important sources of dietary iodine. However, in order to achieve this a rapid, inexpensive method for easy and accurate determination of iodine content is required.

Sensory evaluation of cheese samples in the present study revealed that all the cheese samples, with or without iodised salt, were acceptable. However, the cheddar cheese samples were ripened for only a period of three months due to the time constraints of the present study, and it will be interesting to assess the sensory acceptability after 6 months or a year as there is high possibility that the sensory characteristics and iodine content may change over the maturation period.

The iodine fortified cheese varieties developed in the present research have the potential to improve the iodine intake of the general UK population. However, it would not be suitable for people following vegan dietary pattern. Veganism is a growing trend, the number of vegans in Great Britain quadrupled between 2014 and 2018 amounting to 1.16 percent of the population (www.vegansociety.com, accessed on 4 september 2019). Due to the avoidance of dairy and dairy products (good sources of micronutrients like iodine, calcium, vitamin B12), may increase the vulnerability of this section of population for development of iodine deficiency. Iodine plays a crucial role in thyroid hormone production, for proper bone and brain development during pregnancy and infancy. Iodine fortification of dairy alternatives will present an opportunity for the vegan population to improve their iodine status without altering their dietary choices. Cheese substitutes like analogue pizza cheese can be manufactured replacing regular table salt with iodised salt without changing the manufacturing process. Also, plant milks like soya milk, almond milk, coconut milk consumed by the vegan population could be fortified with iodine. Currently there are very few plant milk drinks which have been fortified with iodine (potassium iodide) such as Soya Growing UP Drink for 1-3+ (from the brand Alpro) providing 24.5 µg I per 100 ml and Koko Dairy Free Super

Drink contains 13 µg I per 100 ml. However, there are other varieties of almond milk, soya milk and pea milk, which do not contain iodine and so if all these varieties were fortified with iodine, then it could be helpful in providing the vegan population with similar health benefits as obtained on consumption of natural dairy milk.

The key findings of the research described in this thesis have been used to generate a visual representation of the relationship between factors that influence the design of a fortification strategy to increase dietary iodine intake in the UK (figure 7.1).

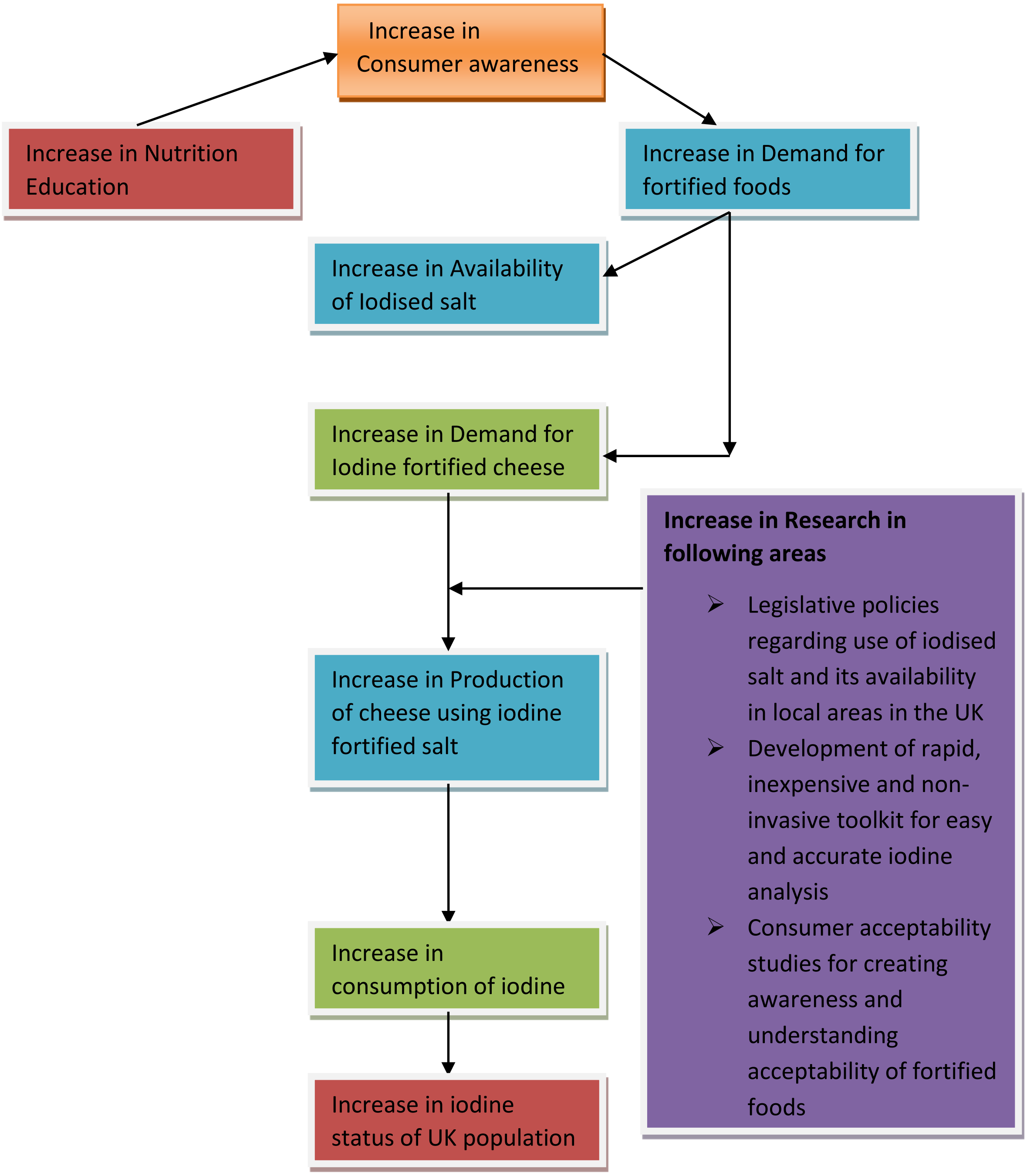


Figure 7. 1 Visual presentation of the recommendations from the discussion of findings of the study

As seen in the figure 7.1, there are various interlinked factors, which play vital roles in the overall success of the fortification strategy for improving the dietary iodine intake in the population. Increasing consumer awareness through adequate consumer education about the nutritional significance of iodine, consequences in case of deficit and benefits of food fortification strategy is one of the most milestones towards development of food vehicles for fortification. Consumer education could be enhanced by utilising appropriate routes Nutrition education can be improved by introducing nutrition modules in school curriculum. Public health nutritionists and Dieticians could inform their clients about importance of iodine across life cycle during their consultations or by arranging interactive nutrition education workshops for children and women. Furthermore, General Physicians (GP’s), nurses, midwives and health visitors in the community could be trained to create awareness about iodine among the target population (women of reproductive age group and young children). Information about importance of maintaining optimal level of iodine (during pregnancy and for the neonate), dietary sources of iodine, possible benefits of replacing regular table salt with iodised salt in daily diet could be included in the healthy eating booklet that is given to pregnant women during their hospital visits. This increased awareness would help in reducing the general sceptism towards fortified foods and this indirectly could help in increasing consumer acceptance towards replacing regular salt with iodised salt in foods (cheese varieties). Additionally, the research branch of the strategy needs to be strengthened through consumer acceptability studies for food fortification. Apart from the consumer acceptance, there are complexities related to the inadequate legal framework for food fortification policies in the UK. There are also other national trade barriers for export and cost of iodisation, which has increased the reluctance for fortified foods (especially salt iodisation) among manufacturers. Therefore, if all these interlinked branches, consumers (increase demand due to the impact of education), manufacturers and policy makers (for development of appropriate policies guiding food fortification mechanisms) work together, then the concept of using iodised salt in cheese manufacturing would be beneficial strategy for improving dietary iodine intake and thereby iodine status of the population in the UK.

The present study has made valuable contributions by highlighting the need to create an updated database for information about iodine content of commonly consumed products in the UK (milk and cheese). It has been possible to identify gaps in knowledge about the awareness and perception of the general UK population relating to the nutritional significance of iodine and iodine fortified products, by developing a completely new consumer questionnaire. Researchers can use this questionnaire in future for assessing consumer perceptions in large-scale population studies. Additionally, this research has also shed light on various national barriers for salt iodisation in the UK and discussed the possibilities of finding solutions through interactions with the policy makers and key authorities from the food industry (for example : dairy manufacturers). Finally, this study made a novel contribution to the body of work around food fortification, by developing iodine fortified cheese varieties. The overall acceptability of these newly developed cheese varieties with increased iodine content has shown that it is possible to consider cheese as a useful food vehicle for implementing iodine fortification strategy in the UK.

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Appendices

Appendix 1

1.1 Ethics approval – part 1 of the study

1.2 Ethics approval – part 2 of the study

1.3 Data protection checklist

1.4 Off-campus risk assessment for study in supermarkets

1.5 On-campus risk assessment for conducting sensory evaluation

1.6 Off- campus risk assessment for visit to Anglesey for cheese production

1.7 Risk assessment form for laboratory analysis

1.8 COSHH assessment form for laboratory analysis

1.1 Ethical approval for part 1 of the study

1.2 Ethical approval for part 2 of the study

1.3 Data protection checklist for the study

Data protection checklist: Teaching, research, knowledge transfer, consultancy and related activities

All activities which involve personal data of any kind, in any way, must comply with the Data Protection Act 1998 (DPA). This checklist will outline the requirements of the DPA and the measures you must take when processing personal data; it will also provide a mechanism for recording the steps you will take to ensure the personal data you are using are safeguarded and the reputation of the University is upheld.

Ensuring personal data are processed fairly and lawfully with due regard for individuals' privacy and ensuring that personal data remain secure are paramount. Demonstrating that we have considered the requirements of the DPA when conducting our activities will provide assurances to students, employees and business partners that their personal data is protected at UCLan. Organisations can be fined up to £500,000 for breaches of the DPA which are considered to be as a result of negligence or recklessness; therefore it is important that we get it right from the outset. If it is possible to use anonymised data so that individuals cannot be identified from it and still achieve your aims, this is always the preferred method of operating. Truly anonymised data (which cannot be reconstructed or linked to any other data you hold or may hold in the future to enable you to identify individuals from it) does not constitute personal data because it cannot be used to identify individuals.

What is *personal data*? Personal data are data relating to a living individual who can be identified from those data (or from those data and other information in our possession or likely to come into our possession). Personal data can be factual (such as name, address, date of birth) or can be an opinion (such as a professional opinion as to the causes of an individual's behavioural problems). Information can be personal data even if it does not include a person's name or other obvious identifiers; for example, a paragraph describing a specific event involving an individual or a set of characteristics relating to a particular individual may not include their name, but would clearly identify them from the set of circumstances or characteristics being described or represented. If you are unsure whether or not your activity involves personal data, please contact the Information Governance Officer to discuss on DPFOIA@uclan.ac.uk.

What is *processing*? The DPA is concerned with the processing of personal data. Processing means obtaining, recording or holding the information or data or carrying out any operation or set of operations on the information or data, including – (a) organisation, adaptation or alteration of the information or data, (b) retrieval, consultation or use of the information or data,

(c) disclosure of the information or data by transmission, dissemination or otherwise making available, or
(d) alignment, combination, blocking, erasure or destruction of the information or data.

If your proposed activity involves processing personal data, you must complete the following checklist. If you are unable to answer *Yes* to each applicable question, you must contact the Information Governance Officer for advice before proceeding. If you require any further information or guidance to enable you to answer *Yes* to each question, please contact the Information Governance Officer: DPFOIA@uclan.ac.uk.

Type of activity:	**Qualitative and Quantitative Research**
Activity name/title:	Use of iodised salt in food manufacturing to improve iodine status in the UK

Processing personal data fairly
The DPA requires us to process personal data fairly and lawfully. In practice, it means that you must: • have legitimate grounds for collecting and using the personal data; • not use the data in ways that have unjustified adverse effects on the individuals concerned; • be transparent about how you intend to use the data, and give individuals appropriate *privacy notices* when collecting their personal data; • handle people's personal data only in ways they would reasonably expect; and • make sure you do not do anything unlawful with the data.

Have you checked and confirmed that the intended uses of personal data in your activity have a legal basis?	Yes
If your activity involves *sensitive personal data*, have you checked and confirmed that you can satisfy a condition for processing this kind of personal data from the DPA? Sensitive personal data includes data about racial or ethnic origin; political opinions; religious or similar beliefs; trade union membership; physical or mental health or condition; sexual life; commission or alleged commission of any offences; or any proceedings for any offence committed or alleged to have been committed.	Not applicable
If the intended use of the personal data would or would be likely to have an adverse effect on one or more individuals, have you considered and documented why that adverse effect is justified?	Not applicable
Have you documented why you are collecting the specific items of information to demonstrate that you have legitimate grounds for doing so e.g. if you are carrying	Yes

out research into how students' music preferences affect their degree classification and also collecting participants' shoe sizes, can you show you have a legitimate need for this information?	
Have you written an appropriate privacy notice to provide to individuals at the point you collect their personal data? A privacy notice tells individuals how we will use their personal data once we have it. It should contain your or your organisation's identity, as appropriate; the purpose or purposes for which you intend to process the information; and any extra information you need to give individuals in the circumstances to enable you to process the information fairly, such as whether or not the information will be disclosed to a third party. If you need assistance drafting a privacy notice, the Information Commissioner's Office (ICO) has produced a Privacy Notices Code of Practice.	Yes

Consent

One of the conditions from the DPA which you can satisfy to enable you to process personal data is 'consent'. Consent is defined by the European Data Protection Directive as '…*any freely given specific and informed indication of his wishes by which the data subject signifies his agreement to personal data relating to him being processed*.'

The ICO maintains that the fact that an individual must 'signify' their agreement means that there must be some active communication between the parties. An individual may 'signify' agreement other than in writing, but organisations should not infer consent if an individual does not respond to a communication e.g. from a customer's failure to return a form or respond to a leaflet.

Consent must also be appropriate to the age and capacity of the individual and to the particular circumstances of the case. For example, if you intend to continue to hold or use personal data after your relationship with the individual ends, then the consent should cover this. Even when consent has been given, it will not necessarily last forever. Although in most cases consent will last for as long as the processing to which it relates continues, you should recognise that the individual may withdraw consent, depending on the nature of the consent given and the circumstances in which you are collecting or using the information. Withdrawing consent does not affect the validity of anything already done on the understanding that consent had been given. You must realise that consent must be*informed* and be freely given; this means it can be withdrawn at any time and you must have a process in place to manage this. If you are doing something which you are required to do by law and the individual has no choice about it, do not ask for their consent as this is misleading because you must do it by law anyway, whether or not they consent to it.

Consent can either be explicit or implied:

- *Explicit consent* is where an individual actively opts in to an activity e.g. Tick this box and sign here if you consent to us using your information in this way, then return this form.

• *Implied consent* is where you tell an individual what will happen to their information unless they tell you they object e.g. Please sign and return this form. We will use your information for the additional purposes outlined in our privacy notice unless you tell us not to by ticking this box. **If you are processing *sensitive personal data* and relying on consent as your basis for doing so, you must obtain explicit informed consent from individuals**.

If you are planning to obtain consent from individuals before using their personal data, have you checked and confirmed that consent is necessary and is the most appropriate basis for your processing?	Yes
If you are processing sensitive personal data, have you planned to obtain individuals' explicit consent?	Not applicable
If you are relying on individuals' consent as a basis for using their personal data, have you developed a process for managing the withdrawal of consent?	Yes
If you are obtaining consent, you must ensure that the individual understands their rights and is capable of giving consent; this is assessed on a case-by-case basis. If you are processing personal data about younger individuals or those with reduced capacity, have you put a process in place to obtain consent from parents, guardians or legal representatives, if appropriate?	Not applicable

Security
Ensuring personal data are secure at all times is extremely important. Organisations can now be fined up to £500,000 for breaches of security involving personal data where those breaches are considered to have been due to negligence, recklessness or as a result of an issue which should reasonably have been foreseen. The DPA requires us to ensure that *appropriate technical and organisational measures shall be taken against unauthorised or unlawful processing of personal data and against accidental loss or destruction of, or damage to, personal data*. It is important that any personal data you collect or use during your activities remains secure until it is destroyed, which includes ensuring that only those who are authorised to access and use the data can do so. **For further guidance on information security, please see the data protection pages of the UCLan website and the LIS IT Security Policy available on the intranet.**

If you are intending to publish information which could identify individuals, have you made those individuals aware that this will happen via your privacy notice and obtained their consent, if appropriate?	Not applicable
Will papers, files, audio visual recordings, CDs, USB (memory) sticks, microfiche or other media which contain personal data be kept in locked cabinets, cupboards, drawers etc. when the offices are vacated?	Yes

Do all individuals who will have access to or be using the personal data understand that it must not be provided to any unauthorised person (which includes disclosing information to family members or other representatives of data subjects, unless the data subject has given consent for us to do this)?	Yes
Do all individuals who will have access to or be using the personal data understand their responsibilities under the DPA and have they received data protection training?	Yes
Do you have appropriate procedures in place to ensure the security of the personal dataif it is removed from UCLan offices for any reason? Electronic data must only be removed if it is stored on encrypted devices or media e.g. an encrypted disc or USB stick, an encrypted laptop etc. Alternatively it can be accessed remotely via a secure connection. If an unencrypted device containing personal data is lost or stolen, it is likely to lead to a substantial fine for a breach of the DPA. Non-electronic records must be rigorously safeguarded at all times and not left unattended or in view of unauthorised people. Laptops, USB sticks and other devices, papers or any other form of personal data must not be left in cars.	Yes
Will the personal data be stored on the UCLan network in a secure location with restricted access, to prevent unauthorised parties who have no right or need accessing the data?	Yes
Are all individuals who will have access to or use the personal data aware that personal information should not be stored off the UCLan network and should only be stored on equipment owned or leased by UCLan, unless exceptional circumstances apply? Storage under such exceptional circumstances must include the use of appropriate security measures. No personal information should be stored on any removable media e.g. USB sticks, CDs or devices e.g. laptops, smartphones unless they are encrypted.	Yes
Are all individuals who will have access to or use the personal data aware that any information accessed via remote working methods such as Outlook Web Access, UCLan Global or similar must be treated securely in line with relevant legislation and all University guidelines?UCLan business information, including personal data, should not be stored on personal, non-UCLan equipment or devices unless exceptional circumstances apply.	Yes
Are all individuals who will have access to or use the personal data aware that email is not a secure method of communication and can easily be sent to the wrong recipient and do they know how to encrypt documents so that they can be attached to an email and sent securely?N.B. Encryption passwords must be provided separately and never included in the same email as the encrypted attachment.	Yes
Are all individuals who will have access to or use the personal data aware that all non-electronic material which contains personal data and has been authorised for disposal must be disposed of via the University's confidential waste service (including handwritten notes, computer print-outs etc.)?	Yes

Are all individuals who will have access to or use the personal data aware that any paper documents, electronic media or hardware which has been designated for disposal must be kept in a secure location until it has been appropriately destroyed and any information it contains is no longer accessible or recoverable? Electronic media and hardware should be disposed of in line with LIS guidelines and procedures.	Yes
Can you confirm that the personal data will not be transferred overseas? This includes via email and by virtue of using 'cloud' providers which store your data on their servers based overseas.	Yes

Third parties acting on behalf of UCLan
Under some circumstances, it will be necessary or desirable to work with organisations external to UCLan, such as charities, research organisations, private companies, other public sector organisations, contractors, service providers or any other types of third parties. If a third party is acting on our behalf e.g. providing a service for us or on our behalf and that activity involves the third party accessing, collecting or otherwise processing personal data, they are a *data processor* under the DPA. A well-recognised example of a data processor relationship is a UK bank using an overseas company to provide its call centre. The overseas company has access to the UK bank's customer information in order to provide the call centre service, but it can only use that data for the purposes of providing the call centre service because this is the service they are providing under contract on behalf of the UK bank. The DPA contains specific requirements we must adhere to when we use a data processor: • we must choose a data processor which provides sufficient guarantees about its security measures to protect the personal data it will process for us; • we must take reasonable steps to check that those security measures are being put into practice; and • there must be a written contract setting out what the data processor is allowed to do with the personal data. The contract must also require the data processor to take the same security measures we would have to take if we were processing the data ourselves.

If you are using a data processor or you need help deciding if the proposed arrangement does involve a data processor, have you taken advice from the Information Governance Officer?	Not applicable
If you are using a data processor, have you taken advice on information security from the Information Governance Officer and the Information Security Officer?	Not applicable
If you are using a data processor, have you taken advice from the Contracts team in SDS or from Purchasing (as appropriate) to ensure you have sufficient contractual arrangements in place to cover the use of a data processor?	Not applicable

If you are using a data processor, can you confirm that a contract will be signed by all parties which meets all the requirements of the DPA as set out above?	Not applicable
Can you confirm that we have been provided with sufficient guarantees about the security measures the data processor has in place and that you have a process in place to confirm that these are being followed?	Not applicable

Once this form has been completed, it should be attached to your ethics checklist and submitted as directed. If your activity does not require further ethical approval, this form should be retained with your project documentation as a record of your considerations and data protection compliance. If you require any further advice or guidance to help you complete this checklist, please contact the Information Governance Officer: DPFOIA@uclan.ac.uk.

1.4 Off-campus risk assessment for study in supermarkets

1.5 On-campus risk assessment for conducting sensory evaluation

1.6 Off-campus risk assessment for visit to the Food Technology Centre, Anglesey for cheese production

1.7 Risk assessment form for laboratory analysis

1.8 COSHH assessment form for laboratory analysis

Appendix 2

2.1 Questionnaire for consumer survey

2.2 Covering letter for supermarket managers

2.3 Business information sheet

2.4 Business consent form

2.5 Participant information sheet

2.6 Participant information sheet for online survey (using survey monkey program)

2.7 Individual consent form for consumer survey

2.8 Advertisement flyer for consumer survey

2.9 Sensory evaluation template

2.10 Participant information sheet for sensory evaluation

2.11 Individual consent form for sensory evaluation

2.12 Iodine information leaflet by dairy council

2.13 Campden Overall Eating Recommended Scoring Scale

2.14 Extensive sensory report by sensory experts for newly developed cheese varieties

2.1 Questionnaire for consumer survey

Questionnaire for obtaining consumer opinions on using iodised salt in cheese production

By completing this questionnaire and returning it to the principal researcher you give your voluntary consent to be a part of the research project and that the information collected can be used for further analysis as a part of the project

Please answer the questions as fully and as honestly as possible

Participant details (Please ✓ as appropriate)

I Age group

18-25 ☐ 26 – 35 ☐ 36-44 ☐ 45-60 ☐ 60+ ☐

II Gender

Male ☐ Female ☐ efer Not to disclose ☐

Please answer the following questions by using (✓) where appropriate

Questions

1. Have you heard about Iodine as a nutrient?
   Yes ☐ No ☐
2. Do you know about any health impact of iodine deficiency?
   Yes ☐ No ☐
3. Do you think Iodine is required for normal health and wellbeing?
   Yes ☐ No ☐ Don't Know ☐

If yes , please specify on a scale of 1 to 5 how important it is to maintain sufficient levels of iodine in the body

1 = Not at all important
2 = Neutral
3 = Somewhat important
4 = Important
5 = Extremely important

Please indicate your preference by writing the number in the given box ☐

4. Are you aware of any illness associated with iodine deficiency (lack of optimal levels of iodine)?

   Yes ☐ No ☐

   If yes please specify

   ----

5. Are you aware that iodine could be added to salt (iodised salt)

   Yes ☐ No ☐

6. Have you come across any brands of iodised salt in the supermarkets?

   Yes ☐ No ☐

   If yes then, can you please give details?

   ----

7. Please state the type of salt (regular table salt or any other branded/fortified salt) that is consumed every day? Please give details.

   ----

8. How often do you consume cheese or cheese products? (eg. Cheese and biscuits, Slices in burgers, cheese pizzas, in sandwiches, salad bowls with cheese toppings, as a part of a recipe, cheese spreads, lasagne etc)

   Once a week ☐ More than once a week ☐ Every 2 weeks ☐

   Once a month ☐ Never ☐

9. Please list the type of cheese(eg. cheddar, brie, blue cheese, cream cheese, cheese spreads) or cheese products (eg. Cheese burgers, cheese pizzas, salad bowl with cheese toppings, as a part of recipe, lasagne) consumed most frequently by you? Please give details

10. Do you like the idea of fortifying commonly consumed foods with vital nutrients (eg. Vitamin D fortified milk, Iron fortified rice)

    Yes ☐ No ☐ Don't Know ☐

    If No please can you briefly state the reasons?

11. Do you think fortified foods differ in taste/texture/cost as compared to non-fortified foods?

    Yes ☐ No ☐ Dont Know ☐

12. If given a choice between regular table salt and iodised salt which of the two would you prefer?

    Regular table salt ☐ Iodised salt ☐

Please give reason for your choice

2.2 Covering letter for supermarket manager

2.3 Business information sheet

2.4 Business consent form

2.5 Participant information sheet

PARTICIPANT INFORMATION SHEET

Project Title: Use of iodised salt in food manufacturing to improve iodine status in the UK

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.
Please feel free to contact us if there is anything that is not clear or if you would like more information. The contact information is given at the end of the form.

Take time to decide whether or not you wish to take part.

Purpose of the Study

My name is Miss Suruchi Pradhan. I am a student studying nutrition at University of Central Lancashire and this present research is being conducted as a part of my PhD degree in Nutrition under supervision of Professor Nicola Lowe (Professor of Nutritional Sciences at UCLAN).

The current research will help to gain an understanding of the knowledge, attitudes and practice of manufacturers and consumers on the use of iodised salt in cooking and food products. Moreover, this information will be useful to inform future public health policy.

Eligibility

There is no specific eligibility inclusion or exclusion criteria and it is completely voluntary to take part in the study. If you decide to take part, you will be given a questionnaire to complete. Returning the completed questionnaire to the researcher will be considered your voluntary consent for taking part in the study. You are free to withdraw at any time without giving a reason, up to the time when you hand the completed questionnaire to the researcher. The completed questionnaire is anonymous therefore it is not possible to withdraw after the questionnaire has been returned to the researcher.

Study Procedures

If you decide to take part in the study, then you will be asked to complete a short questionnaire. It is not necessary to be an expert in the field of nutrition to complete this questionnaire. This questionnaire consists of 18 questions in total and will take around 5 minutes to complete it. 13 questions are tick box and only 5 questions will enquire about your opinion about various aspects related to nutrition and fortification for achieving optimal health.

If you decide to take part in the study, please answer all the questions as fully as honestly as possible.

Benefits

There are no benefits to be gained by taking part in this study but it is hoped that the information gained may be of benefit to people in the future.

Ethical Approval
Ethical approval for this study has been granted byResearch Ethics Committee at University of Central Lancashire.

Further Information
Researchers contact details
Miss Suruchi Pradhan : 01772 894914 Email ID – spradhan@uclan.ac.uk
Professor Nicola Lowe: 01772 893599 Email ID – nmlowe@uclan.ac.uk

If you have any concerns about the way in which the study has been conducted, you should contact University Officer for Ethics.
Contact details of the Ethics officer – Email ID – OfficerForEthics@uclan.ac.uk

'Thank you for considering taking part in this study and taking the time to read this sheet'.

2.6 Participant information sheet for online survey (using survey monkey program)

Project Title: Use of iodised salt in food manufacturing to improve iodine status in the UK

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Please feel free to contact us if there is anything that is not clear or if you would like more information. The contact information is given at the end of the form.

Take time to decide whether or not you wish to take part.

Purpose of the Study

My name is Miss Suruchi Pradhan. I am a student studying nutrition at University of Central Lancashire and this present research is being conducted as a part of my PhD degree in Nutrition under supervision of Professor Nicola Lowe (Professor of Nutritional Sciences at UCLAN).

The current research will help to gain an understanding of the knowledge, attitudes and practice of manufacturers and consumers on the use of iodised salt in cooking and food products. Moreover, this information will be useful to inform future public health policy.

Eligibility and withdrawal

There is no specific eligibility inclusion or exclusion criteria and it is completely voluntary to take part in the study. If you decide to take part, you are still free to withdraw at any time without giving a reason, up to the time when the questionnaire is completed and until the time you press 'Submit'. Once the questionnaire is submitted, it cannot be withdrawn because there is no link between the questionnaire and the participant.

Study Procedures

If you decide to take part in the study, then please complete a short questionnaire by clicking 'Next'.
It is not necessary to be an expert in the field of nutrition to complete this questionnaire.
This questionnaire consists of 18 questions in total and will take around 5 minutes to complete it. Out of these, 13 questions are tick box and only 5 questions will enquire about your opinion about various aspects related to nutrition and fortification for achieving optimal health.
If you decide to take part in the study, please answer all the questions as fully as honestly as possible.

Benefits

There are no benefits to be gained by taking part in this study but it is hoped that the information gained may be of benefit to people in the future.

Ethical Approval

Ethical approval for this study has been granted by Research Ethics committee at University of Central Lancashire

Further Information

Researchers contact details

Miss Suruchi Pradhan : 01772 894914 Email ID – spradhan@uclan.ac.uk

Professor Nicola Lowe: 01772 893599 Email ID – nmlowe@uclan.ac.uk

If you have any concerns about the way in which the study has been conducted, you should contact University Officer for Ethics.

Contact details of the Ethics officer – Email ID – OfficerForEthics@uclan.ac.uk

'Thank you for considering taking part in this study and taking the time to read this sheet'.

2.7 Individual consent form for consumer survey

CONSENT FORM

Use of iodised salt in food manufacturing to improve iodine status in the UK

Name : Suruchi Pradhan **Position** : Research student at University of Central Lancashire

Researchers contact details

Miss Suruchi Pradhan : 01772 894914 Professor Nicola Lowe: 01772 893599

Email ID – spradhan@uclan.ac.uk Email ID – nmlowe@uclan.ac.uk

Please read the following statements and initial the boxes to indicate your agreement

I confirm that I have read and understand the information sheet, dated …………. for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, until data collection is complete.	☐
I agree that my data gathered in this study may be stored (after it has been anonymised) at UCLan and may be used for future research.	☐
I understand that it will not be possible to withdraw my data from the study after final analysis has been undertaken.	☐
I understand that all the contact details/email address will be separated from the questionnaire by the researcher, and that no link between the completed survey and the email address will be kept, to ensure maintain the anonymity of the survey.	☐
I agree to take part in the above study.	☐

Name of Participant	Date	Signature
________________________	______________________________	___________________
Name of Researcher	Date	Signature
________________________	______________________________	___________________

2.8 Advertisement flyer for consumer study

Participants Needed!!!

For an interesting research project on food fortification to improve nutritional status

Are you aware about fortifying foods with vital nutrients?

Do you like different

to know more about fortified foods?

or

If you are just interested in knowing about nutrition for optimal health..... then...

Please come along and be a part of this interesting PhD research project

If you decide to be the part of this research then……..

You will have to complete a short questionnaire about

- Your preferences regarding selecting different types of cheese varieties
- The type of table salt that is consumed regularly
- Your opinion about consuming 'fortified foods' for improving nutritional status

It will take only 5 minutes for completing the questionnaire.

If you are interested in participating, then please follow the link given at the bottom of the page or please feel free to

Contact:

Miss Suruchi Pradhan – Research student

Contact No. - 01772 894914 / DB-329 Email – spradhan@uclan.ac.uk

'Thank you for considering taking part in this study and taking the time to read this sheet'.

https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey	https://www.surveymonkey.co.uk/r/iodine_survey

2.9 Sensory evaluation template

University of Central Lancashire

Sensory Evaluation Template

The purpose of this questionnaire is to obtain opinion of general consumers about acceptability of various cheese samples.

Note – This exercise **cannot** be completed if you are **Lactose Intolerant**

Please answer the questions as fully and as honestly as possible
Participant details (Please ✓ as appropriate)

1. Age group

18-25 ☐ 26 – 35 ☐ 36-44 ☐ 45-60 ☐ 60+ ☐

2. Gender

Male ☐ Female ☐ Prefer not to say ☐

3. How often do you consume cheese or cheese products(e.g. Slices in burgers, cheese pizzas, in sandwiches, salad bowls with cheese toppings, as a part of a recipe, cheese spreads, lasagne etc.)

Once a week ☐ More than once a week ☐ Every 2 weeks ☐

Once a month ☐ Never ☐

4. Please state the type of salt (regular table salt or any other branded/fortified salt) that is consumed everyday? Please give details.

----

5. If you use salt in daily meals, then please state when do you add salt in your preparations

During cooking ☐ Sprinkle on the top at the end of cooking ☐

At the table before /during eating ☐

Some Instructions for the sensory evaluation exercise:

Please taste the cheese samples and please indicate your opinion about the characteristics of the cheese samples by ticking (✓) appropriate boxes.

For more clarity in understanding the terms used for describing different attributes related to cheese samples, you will be provided with the list of terms and definition clearly explaining the meaning of the term (e.g. Creamy odour, flowers/ fruity odour).

Please rate the **'Colour'** of cheese samples

Sample No.	Like very much	Like moderately	Like slightly	Neither like nor dislike (Neutral)	Dislike slightly	Dislike moderately	Dislike very much
249							
198							

Please rate the **'Appearance'** of cheese samples

Sample No.	Like very much	Like moderately	Like slightly	Neither like nor dislike (Neutral)	Dislike slightly	Dislike moderately	Dislike very much
249							
198							

Please rate the **'Saltiness'** of cheese samples

Sample Number	Not at all salty	Slightly salty	Moderately salty	Very salty
249				
198				

Please rate the **'Taste'** of cheese samples

Sample No.	Like very much	Like moderately	Like slightly	Neither like nor dislike (Neutral)	Dislike slightly	Dislike moderately	Dislike very much
249							
198							

In context of taste, please indicate using (✓) if you can detect specific characteristics related to taste in the given cheese samples

Sample Number	Sweetness		Acidic taste		Bitterness		Aftertaste		Others	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
249										
198										

If you have indicated 'Others' in the above question, please give details

____

Please rate the **'Odour'** of cheese samples

Sample No.	Like very much	Like moderately	Like slightly	Neither like nor dislike (Neutral)	Dislike slightly	Dislike moderately	Dislike very much
249							
198							

In context of odour, please indicate using (✓) if you can detect specific characteristicsin the given cheese samples

Sample Number	Creamy odour		Acidic odour		Buttery odour		Flowers/fruity odour		Others	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
249										
198										

If you have indicated 'Others' in the above question, please give details

____

Please rate the **'Texture'** of cheese samples

Sample No.	Like very much	Like moderately	Like slightly	Neither like nor dislike (Neutral)	Dislike slightly	Dislike moderately	Dislike very much
249							
198							

In context of texture, please indicate using (✓) if you can detect specific characteristics in the given cheese samples

Sample Number	Hardness		Chewiness		Rubbery		Grainy		Others	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
249										
198										

Please rate the level of **'Dryness (level of moisture or mouth-feel)'** of the cheese samples

Sample Number	Not dry at all	Slightly dry mouth feel	Moderate level of dryness	Very high level of dryness
249				
198				

Please rate the **'Overall quality'** of cheese samples

Sample No.	Like very much	Like moderately	Like slightly	Neither like nor dislike (Neutral)	Dislike slightly	Dislike moderately	Dislike very much
249							
198							

Out of the 2 cheese samples provided please indicate (✓) which sample/samples do you think contains iodised salt and regular table salt respectively

Sample number	Iodised salt	Regular table salt	Don't Know
249			
198			

Out of the two cheese samples provided please indicate (✓) which sample you prefer?

Sample 249 ☐ Sample 198 ☐

2.10 Participant information sheet for sensory evaluation

PARTICIPANT INFORMATION SHEET FOR SENSORY EVALUATION

Project Title: Use of iodised salt in food manufacturing to improve iodine status in the UK

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.
Please feel free to contact us if there is anything that is not clear or if you would like more information. The contact information is given at the end of the form.

Take time to decide whether or not you wish to take part.

Background/Rationale for the present study

Iodine is one of the essential minerals required for normal health and wellbeing. Initially, it was assumed that iodine deficiency existed only in developing countries and in areas with soils deficient in iodine and so fortification of salt with iodine was encouraged. Recent research has revealed that there is widespread iodine insufficiency in Britain due to changes in animal feed and milking practices (Rayman et al., 2008, Combat and Lean., 2014). Iodine deficiency during pregnancy can have adverse postnatal consequences (Khazan et al., 2013).

Fortifying table salt with Iodine has proved to be an effective and inexpensive way of improving Iodine status at a population level in developing country settings (WHO, 2014). However, in the UK, where there is a concurrent concern about high salt (sodium) intakes, there is a potential conflict with promoting the use of iodised salt to improve Iodine status, and reducing salt intake to reduce the risks due to hypertension. One way of dealing with this scenario is using iodised salt in cheese, a product that naturally contributes salt to the diet, could help in reducing iodine deficiency in the population without promoting additional salt intake.

Purpose of the Study

My name is Miss Suruchi Pradhan. I am a student studying nutrition at University of Central Lancashire and this present research is being conductedas a part of my PhD degree in

Nutrition under supervision of Professor Nicola Lowe (Professor of Nutritional Sciences at UCLAN).

The current research will help to gain an understanding of the sensory preferences and attitudes of manufacturers and consumers on the use of iodised salt in cheese samples. Moreover, this information will be useful to inform future public health policy.

Why I have been selected to participate?

This study aims to obtain opinions, preferences about the sensory acceptability of the newly developed cheese samples for the present study. Therefore we are inviting participants who like and consume cheese and cheese products in everyday life irrespective of age, gender, areas of occupational expertise.

Eligibility

Since this sensory evaluation involves consumption of cheese samples, **people with lactose intolerance (dairy allergy) are not eligible to take part in the study.** All the data in form of sensory evaluation questionnaire will be anonymous and it is completely voluntary to take part in the study.

If you decide to take part you will be given this information sheet and you will be requested to sign a voluntary consent form. You are free to withdraw at any time without giving a reason, up to the time when the questionnaire is completed and collected for the research project.

Study Procedures

This study is about sensory evaluation of different cheese samples. If you decide to take part in the study, then you will be presented with2different cheese samples and you will be requested to taste these samples. You will be given a glass of water to be consumed in between two samples. Upon tasting you will be asked to complete a sensory evaluation questionnaire. This evaluation template involves rating the cheese samples on different sensory attributes like colour, taste, texture and so on using a rating scale ranging from like very much to dislike very much.

It is not necessary to be an expert in the field of nutrition to complete this evaluation. There are no correct or incorrect answers for this evaluation as it completely depends on participant's taste preferences. It is completely voluntary to take part in this study

If you decide to take part in the study, please answer all the questions as fully as honestly as possible.

Benefits

There are no benefits to be gained by taking part in this study but it is hoped that the information gained may be of benefit to people in the future. This research has the potential to inform public health policy makers regarding the use of iodine in cheese making to improve the iodine status of the general UK population.

Ethical Approval

Ethical approval for this study has been granted by Research Ethics Committee at University of Central Lancashire.

Further Information

Researchers contact details
Miss SuruchiPradhan : 01772 894914   Email – spradhan@uclan.ac.uk
Professor Nicola Lowe: 01772 893599   Email – nmlowe@uclan.ac.uk

If you have any concerns about the way in which the study has been conducted, you should contact University Officer for Ethics.
Contact details of the Ethics officer –   Email ID – OfficerForEthics@uclan.ac.uk

'Thank you for considering taking part in this study and taking the time to read this sheet'.

2.11 Individual consent form for sensory evaluation

CONSENT FORM

Use of iodised salt in food manufacturing to improve iodine status in the UK

Name: Suruchi Pradhan **Position**: Research student at University of Central Lancashire

Researchers contact details

Miss Suruchi Pradhan: 01772 894914 Professor Nicola Lowe: 01772 893599

Email ID – spradhan@uclan.ac.uk Email ID – nmlowe@uclan.ac.uk

Please read the following statements and initial the boxes to indicate your agreement

I confirm that I have read and understand the information sheet, dated …………. for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, up to submission of the completed survey to the researcher. ☐

I agree that my data gathered in this study may be stored (after it has been anonymised) at UCLan and may be used for future research. ☐

I understand that it will not be possible to withdraw my data from the study after completion of the sensory survey. ☐

I understand that all the data collected will be completely anonymous ☐

I agree to take part in the above study. ☐

Name of Participant	Date	Signature
_______________________	____________________________	_________________
Name of Researcher	Date	Signature
_______________________	____________________________	_________________

2.12 Iodine information leaflet by dairy council

2.13 Campden Overall Eating Recommended Scoring scale

Table 2.13.1 Campden Overall Eating Recommended Scoring Scale

Score	Product Quality band	Description
10	Excellent	For commercial and technical reasons, manufactured food products in this zone are very rare.
9	Very Good	Products in this band are of high achievement, reflecting special attention to raw materials and technology and usually commanding a price premium
8	Good	
7	Fairly Good	Products that fall into this zone are considered to offer marketplace quality. Within this broad band, it is likely that specific products will have been positioned for a particular market. Historically, majority of new products have fallen in this band
6	Satisfactory	
5	Acceptable	
4	Not quite acceptable	Products in this band are considered to be disappointing and failing to meet their description
3	Poor	It is inconceivable that any manufacturer would aim to market product in this zone. We would assume technical failure or product abuse
2	Bad	
1	Inedible	

2.14 Extensive sensory report by sensory experts for newly developed cheese samples

Appendix 3

3.1 Trials for method development for iodine analysis

3.2 Details of the method used for microbial analysis of newly developed cheese samples

3.3 Results of statistical analysis for sensory evaluation by non-expert consumers and sensory experts

3.3.1 Tabular format of Chi square test values and p values for all the attributes

3.3.2 Detailed information about the statistical tests using SPSS software

3.4 Tabular format of cheese varieties in different supermarkets

3.5 Photograph of cheese varieties analysed for iodine content

3.1 Trials of method development for iodine analysis

3.1.1 Determination of iodine content using the Sandell-Kolthoff reaction (method 1)

This method for iodine analysis was based on the original method developed by Zimmermann et al., (2005) using Sandell-Kolthoff reaction which consists of both the reaction and the digestion process into a microplate format.

For the purpose of the present research, the standard operating procedure (SOP) was requested from this original group of researchers and was adapted to determine the iodine content of the commonly consumed varieties of cheese in the UK.

Principle - the iodine content was determined by using the ability of iodide to catalyse the reduction of yellow Ce(IV) to colourless Ce(III) in the presence of arsenious acid. The rate of colour disappearance is directly proportional to the iodide concentration:

$$2\,Ce^{4+} + As^{3+} \xrightarrow{I^-} 2Ce^{3+} + As^{5+}$$

Sample preparation – Certified reference material (CRM) powder was accurately weighed in triplicate and this was digested overnight (o/n) at room temperature by adding 20ml of nitric acid (70% ACS reagent, Sigma Aldrich, Product of Germany, Sample code- 438073-2.5L) in a closed 50-mL graduated polypropylene tube. Following the o/n incubation, this tube was incubated in an oven (VWR – VENTI-LINE Oven, Model number – VL112) at 60-70°C for 1 hour. At the end of the incubation period these were cooled to room temperature before analysis (Kim and Song 2014)

Results

Along with sample analysis, the values obtained for the standards were graphically plotted to obtain an accurate standard curve.

Plotting of Standard curve - Iodine standards (potassium iodate 99.5%, 215929-100G Sigma Aldrich, Dorset, UK) were prepared with iodine concentration ranging from 50µg/l to 400µg/l using nanopure water (18.2MΏ cm – Thermo Scientific) as a diluent. Results were read using a microplate reader (Thermo Scientific Multiskan® FC microplate photometer, Thermo Fisher Scientific, 96 well plate model with wavelength range 340 to 850 nm) and the absorbance was measured at 405nm. A standard curve was plotted by converting absorbance values into log values for achieving maximum accuracy. A number of experimental runs were conducted to achieve optimal standard curve and the standard curves from 3 experiments are presented below (figures 1, 2, and 3). These figures illustrate the between run variation in the standard curves obtained.

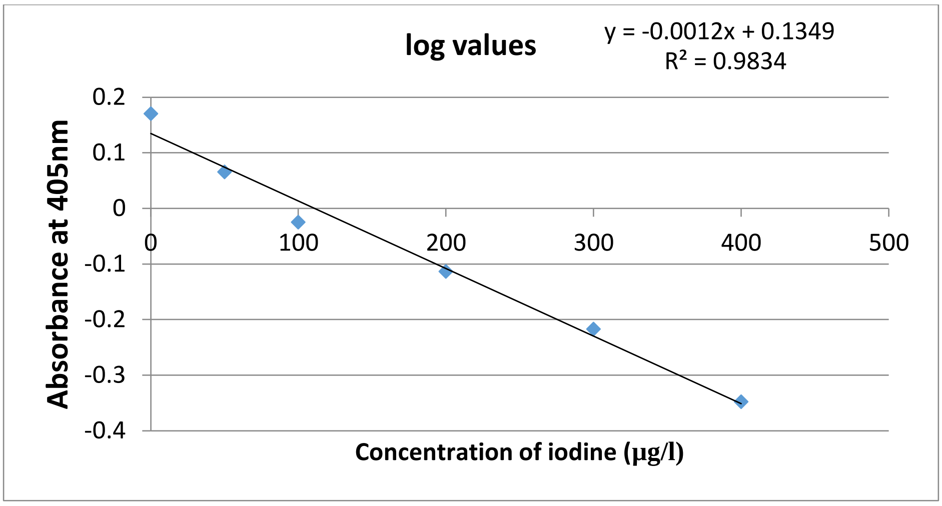

Figure 1. 1st Example of a Standard curve for Iodine by using method 1

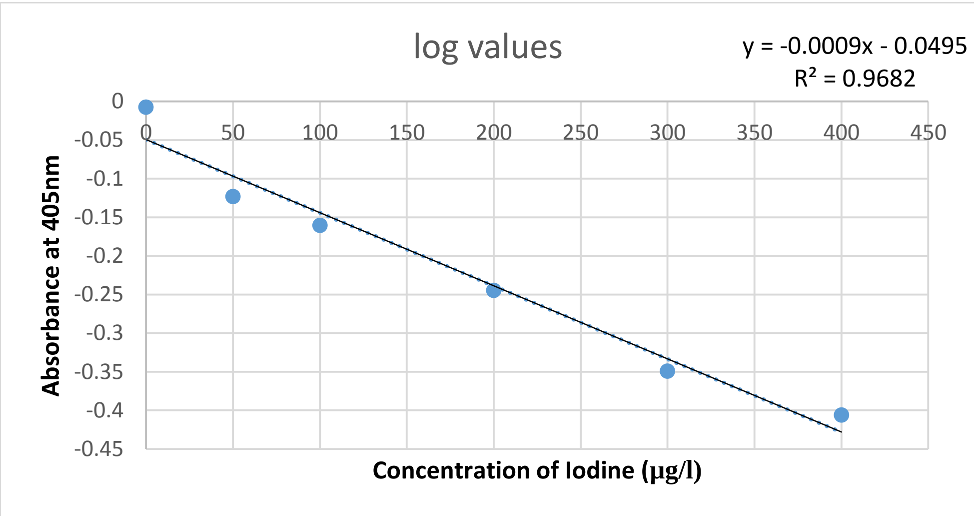

Figure 2. 2nd Example of a Standard curve for Iodine by using method 1.

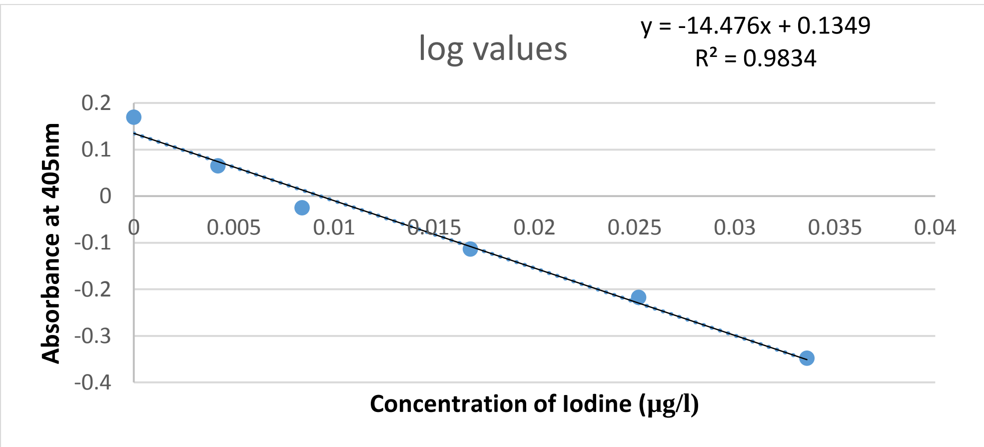

Figure 3. 3rd Example of a Standard curve for Iodine by using method 1.

Validation of the method

In order to ensure that the results obtained by the method used for iodine analysis were valid a certified reference material (CRM) with a known iodine content was run simultaneously. The CRM used in this study was skimmed milk powder (European Reference materials, ERM – BD150, Sample No. 0406) with a known iodine content of 1.73mg/kg.

Results for CRM– Following the experimental runs described above the standard curves where used to estimate the iodine concentration in the CRM and these calculations typically yielded iodine concentrations of 13.04 mg/kg. This value is significantly higher than the expected value of 1.73 mg/kg.

Challenges in the trials during development of the method

Many experimental runs over a period of 4 months were conducted to achieve an appropriate standard curve, which could subsequently form the basis for determination of iodine content of cheese samples.

1. Even after these trials it was not possible to obtain a consistent standard curve.
2. The speed of the reaction taking place during the final stage of the analysis (Sandell-Kolthoff reaction) from yellow to colourless was faster than expected (28minutes). So the change in colour reaction in the microplate was monitored and the plate was read at different time intervals (3minutes, 5minutes, and 10 minutes).
3. In depth discussions were conducted and the procedure for analysis was reviewed critically with the supervisory team as well as with one of the researcher contributing to developing the original SOP.

Modifications made to overcome the challenges

1. Efforts were made to eliminate all possible sources of contamination.
2. A separate trial was conducted to test the purity of water to ensure that the nanopure water (18.2MΩ cm) was free of any traces of iodine.
3. All the glassware was acid washed o/n and air dried and stored in airtight plastic container before use.
4. All the chemicals were freshly made before the analysis.
5. The template for pipetting standards and samples on to the well-plate given in the original method was modified.
6. The shaking step (28 minutes) before the analysis was eliminated.

Conclusions from various trials with modifications

1. The standard curve obtained was satisfactory for the individual run but was not reproducible as the range varied considerably with every run (9 trials).
2. The results obtained for iodine content for the certified reference material were higher than the expected value even after modifying method to reduce manual errors and eliminating different sources of contaminations.
3. The method is extremely sensitive and so is not reproducible in wide range of settings.

For the reasons outlined above, it was concluded that this was not a suitable method for analysis of iodine in food samples

3.1.2 Determination of iodine content using Inductively Coupled Plasma optical emission spectrometry (ICP-OES) (method 2)

Principle : The liquid sample is converted to an aerosol through a process called nebulization. The sample aerosol is then transported to the RF generated plasma where it is desolvated, vaporised, atomised and excited and/or ionised. The excited atoms and ions emit their characteristic radiation which is diffracted by spectrometer (echelle optical design) into wavelength (166-847 nm). The radiation (UV and Vis) is detected by the detector and turned into electronic signals that are processed by Qtegra software. The measured intensity of wavelength corresponds to the concentration of the element present in the original sample. The sample's intensities are compared to the intensities of standards of known concentration to obtain elemental concentration in unknown sample. The intensities for the standard or sample are expressed as counts (YCPS Counts in the machine), so the graphs are plotted as counts vs element concentration (ThermoScientific, 2013)

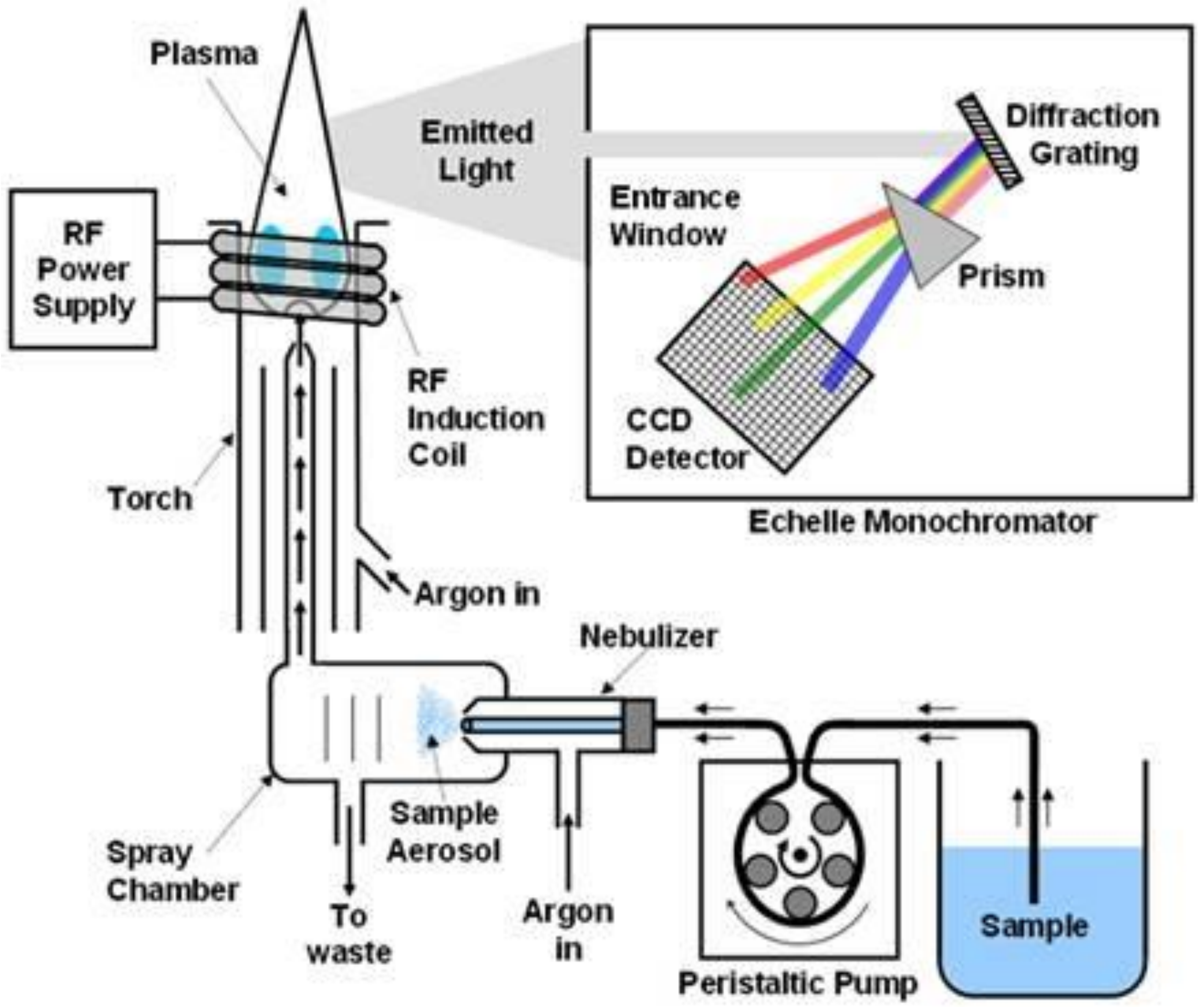

Fig no 4. A simplified linear schematic of an ICP-OES instrument
Source: website : https://blogimagesxyz.blogspot.com/2016/11/design-of-experiments-format.html (accessed on : 11 May 2018)

Preparation of standard curve for iodine – Potassium iodide solution was used to prepare dilutions for plotting standard curve. From this solution, five standard dilutions were made with concentrations from 100ppb to 500ppb.

Sample preparation and analysis

Procedure – In order to obtain the known concentration of iodine in the CRM (European Reference materials, ERM – BD150, Sample No. 0406), different quantities of the CRM were accurately weighed (0.01g, 0.0173g, 0.025g, 0.05g, 0.075g and 0.1g) in a microwave digester tubes. Subsequently 10ml of nitric acid (70% ACS reagent, Sigma Aldrich, Product of Germany, Sample code- 438073-2.5L) was pipetted accurately in each of the samples. These samples were digested in a microwave digester (Milestone Ethos EZ Microwave digest system from Analytix) for 1 hour. At the end of the digestion period, the digested solutions were transferred quantitatively into a 10ml graduated tubes. A blank sample (10ml of nitric acid) was also subjected to microwave digestion along with other samples in order to identify any kind of contamination in the entire process.

Sample analysis – 0.1ml of these digested solutions were pipetted in 10 ml of graduated tubes. To this 9.9ml of nano-pure water (18.2MΩ cm) was added making the entire volume of solution for analysis to 10ml. These samples and standards were then analysed for their iodine content using ICP-OES (ICAP 7000 Series, ICP- Spectrometer, Thermo Scientific UK)

Results

An example of the standard curve obtained using ICPOES is presented in Figure 4.

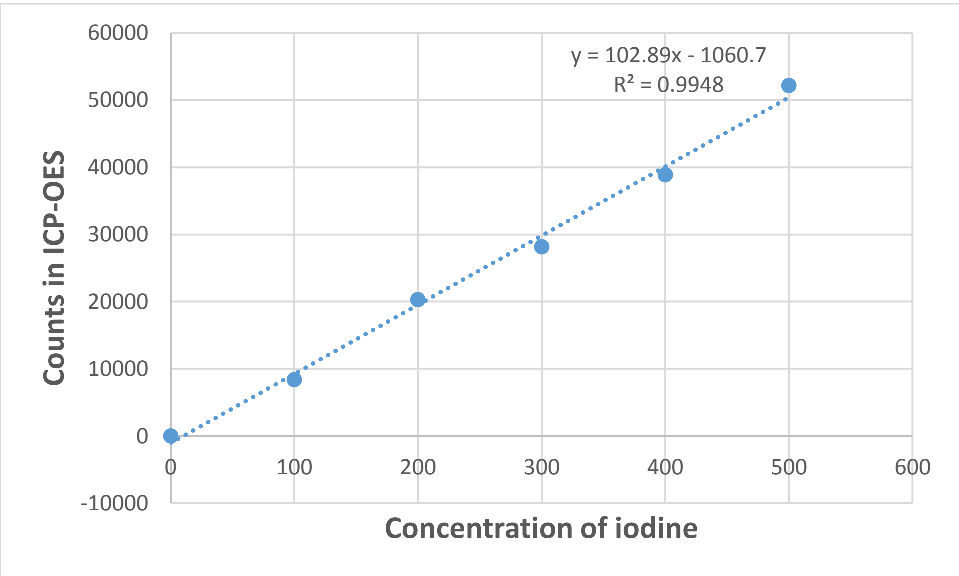

Figure 5. – An Example of a Standard curve for Iodine by using method 2.

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Challenges in sample analysis

The standard curve was satisfactory but when certified reference material was run along with standards, the calculated values were lower (0.08mg/kg) than expected result (1.73mg/kg).

So considering these results, it was decided to trial a third method for iodine analysis

3.1.3 Determination of iodine content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (method 3)

Preparation of standard curve for iodine–Potassium iodide solution was used to prepare dilutions for plotting standard curve. From this solution, five standard dilutions were made with concentrations from 100ppb to 500ppb, as described for method 2.

Sample preparation

These standards were analysed using ICP-MS (Thermo Electron Corporation, X-Series 1 and then this was followed by sample solution analysis using auto sampler (ASX-510, Cetac) for iodine. The standard curve for iodine is presented as follows

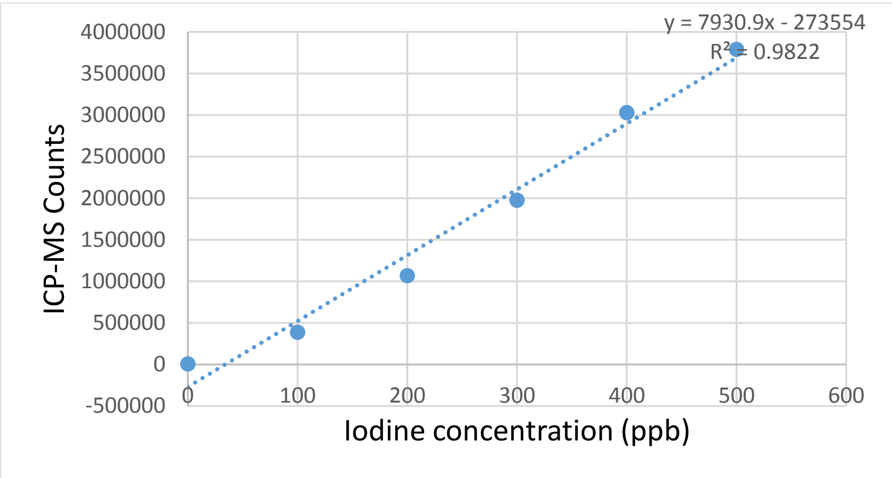

Figure 6. An Example of a Standard curve for iodine using method 3

Challenges in sample analysis

1. The standard curve was satisfactory but when certified reference material was run along with standards, the calculated values were lower than expected result.
2. Due to the sensitivity of the ICP-MS machine major variations were observed in the readings of same samples in 2 different runs.
3. The expected iodine concentrations in the samples were lower and so if sample is diluted to suit the ICP-MS machine sensitivity, then the amount obtained was found to be negligible.

Modified method for sample analysis- Since the concentration of iodine in the samples was expected to be low, it was essential to increase the amount of sample in order to obtain adequate levels of iodine.

1. The microwave digestion procedure used for samples was changed to wet ashing method as used in method 1
2. The ratio of amount of sample to amount of acid was modified and it was decided that 5g of sample will be digested in 10ml of acid and this will be made up to 100ml instead of 0.1ml of digested sample made up to 100ml as in the method 3.

Modified sample preparation – 5g of cheese was digested in 10ml of concentrated nitric acid (70%) and then following procedure was same as used in method 1. These digested samples were diluted by making up the volume to 100ml and then analysed using ICP-MS. The standard range was also modified and changed to 0-100ppb instead of 100-500ppb

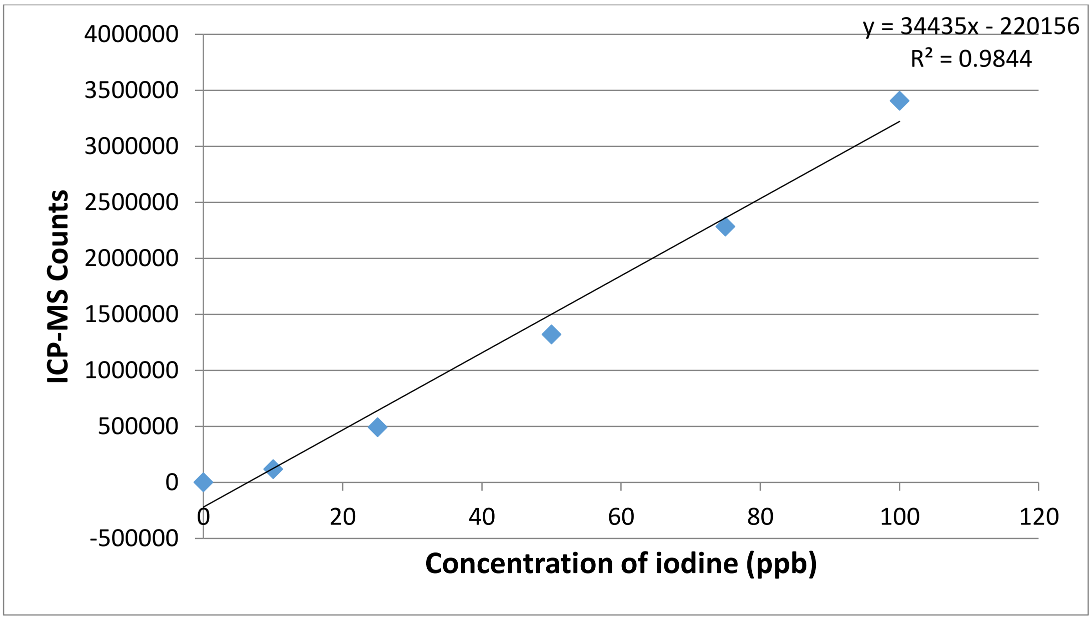

Figure 7. – An Example of a Standard curve for iodine using the modified ICPMS method

Challenges identified – the standard curve plotted for the modified ICP-MS method is similar to the one obtained in method 3. Changing the standard range from 0-100ppb instead of 100-500ppb did not sufficiently improve the sensitivity, with the samples remaining below the concentration of the lowest standard, thus reducing the reliability of the values obtained. Due to this, the values obtained for iodine content of cheese samples could not be considered as reliable.

3.2 Details of the method used for microbial analysis of newly developed cheese samples

3.3 Results of statistical analysis for sensory evaluation by non-expert consumers and sensory experts

3.4 Tabular format of cheese varieties in different supermarkets

Table 3.4.1 Basic information about cheese varieties in Sainsbury's

Name of cheese	Country of origin
Anchor Cheddar Grated	UK
Anchor Cheddar Mature	
Anchor Mature Lighter Cheddar	
Applewood cheese snacking	
Butlers Blacksticks Smooth Blue Cheese (Full fat soft coloured blue veined cow's milk cheese)	Preston, UK
Cathedral City Caramelised Onion Cheddar	Great Britain with British milk
Cathedral City Extra Mature Cheddar	Great Britain with British milk
Chevington Grated Light Cheese	Traditionally made KEDASSIA Conforming to the requirements of the Rabbinate of the Union of Orthodox Hebrew Congregations - London
Davidstow Cornish Classic Mature Cheddar	Telford, England, UK
Mature Blue Stilton (Full fat blue veined hard cheese)	Made at Tuxford and Tebbutt dairy in Leicestershire
Blue Stilton	UK
Shropshire Blue	
Stilton Organic	
Beacon Fell Creamy Lancashire	
Butlers Farmhouse Gloucester, Red Leicester, Lancashire – 1.25g	
Caerphilly Cheese (Cows' Milk)	
Gevrick Goat cheese	
Kidderton Ash Goat cheese	
Parlick Fell Hard sheep cheese	

Somerset Goat cheese	Produced in United Kingdom, Packed in United Kingdom. Produced using English Goats milk
Sainsbury UK cheese county specific varieties Lake district vanilla quark	
Castello Danish Blue cheese (Full fat blue veined cheese)	Denmark
Castello Pineapple Halo (Soft cheese with pineapple and almonds)	
Lactofree Soft White Cheese	
Savera Paneer	
Apetina Mediterranean style cheese cubes and its varieties	
Light Cypriot Halloumi	Cyprus
Creme de Saint Agur (Blue cheese spread made with pasteurised cows' milk)	France
Bleu D'Auvergne	
Roquefort blue cheese	
President Brie	
French Goat cheese (medium fat soft cheese)	
Coeur De Lion Pie D'Angloys Cheese, Coeur De Lion La Buche Goats Cheese	
Camembert Goat cheese	
Colliers cheese	
Cracker Barrel Cheddar (Smooth mature Irish cheese)	Produced in Ireland Packed in Belgium
Galbani Dolcelatte (Soft blue veined cheese)	Italy
Gorgonzola Dolce (Semi-soft full fat blue cheese)	

Piccante Gorgonzola (Pasteurised Semi-soft full fat blue cheese)	
Galbani Mascarpone Galbani Mozarella – 0.7g Galbani Ricotta -0.4g Dried grated hard cheese – 2.62g	
Cambozola (Full fat soft mould ripened blue vein cheese)	Produced in Germany and Packed in the UK
Philadelphia	
Leerdammer	
Norwegian Jalsberg cheese slices	Produced in Norway and Packed in the UK
Greek style salad cheese	Greece
Greek Feta Cheese	Greece
President Emmental slices	Czech Republic
Austrian Oak Smoked cheese	Austria
Bavarian slices (Smoked Processed cheese with butter)	Germany
Emmental cheese	
German Smoked cheese	
Sainsbury Dutch Edam and varieties	Netherlands
Gouda cheese	
Maasdam cheese	
Vintage Gouda Cheese	Produced in Netherlands, Packed in the UK
Swiss Emmental cheese	Switzerland
Swiss Mature Reserve Gruyere	
Swiss Gruyere	Produced in Switzerland using Swiss milk, Packed in Austria
Sainsbury Iberico (Full fat hard cheese made with pasteurised cows', sheep and goats' milk)	Spain

Table 3.4.2 Basic information about cheese varieties available in TESCO

Type of cheese	Name of cheese	Manufacturer and country of origin
Cheddar	Strong cheddar white (Scottish cheddar cheese)	Cheshunt , **UK**
	Cornish Classic Mature Cheddar	
	Pilgrims Choice Mature Cheddar	Produced in the **UK**, Ireland, New Zealand and Australia, using milk from the UK, Ireland, New Zealand and Australia. Packed in the UK
	Canadian Vintage Cheddar (Full fat hard Canadian Cheddar cheese)	Produced in **Canada**. using milk from Canada. Contains Milk. Made from unpasteurised milk.
	Mild White Cheddar (Full fat hard white Cheddar cheese)	
	Maryland Farmhouse Vintage Cheddar (West Country Farmhouse mature Cheddar full fat hard cheese)	Produced in the **UK** using milk from the UK. Contains Milk.
	Medium British Cheddar (Full fat hard white Cheddar cheese)	
	Mature British Cheddar (Full fat hard mature white Cheddar cheese)	
	Cave Aged Cheddar (West Country Farmhouse mature Cheddar full fat hard cheese. Traditional Farmhouse Cheddar)	
Continental	Ilchester Applewood Smoked Flavour Cheddar	Produced in the **UK** using milk from the UK Contains Milk.
	Blue Stilton (Full fat blue veined hard cheese)	

	Finest Mature Blue Stilton (Full fat blue veined hard cheese)	
	Somerset Brie (Brie full fat mould ripened soft cheese)	
	British Blue Shropshire (Full fat blue veined coloured hard cheese)	
	Vintage Applewood Cheddar Cheese	
	Frico Edam Ball (Edam medium fat hard cheese)	Produced in the **Netherlands**. using milk from the Netherlands
	Frico Gouda Wheel (Gouda Holland full fat hard cheese)	
	Finest Parm Reggiano Pre-Cut Portion (medium fat hard Italian cheese, a fruity flavoured and grainy textured Italian cheese)	Produced in **Italy**. using milk from Italy. Made from unpasteurised milk
	Parmesan Gratings	
	Grana Padano Portions (medium fat hard Italian cheese, a fruity and nutty Italian hard cheese with a grainy texture)	
	Parmesan Shavings 80G Pot (medium fat hard Italian cheese)	
	Finest Gorgonzola Piccante (Gorgonzola full fat soft blue veined cheese)	
	Cambozola Blue Brie (Full fat soft mould ripened blue veined cheese. A mild creamy blue vein Brie with subtle blue tones.)	Produced in **Germany**. using milk from Germany.
	Jarlsberg (Medium fat hard cheese)	Preservative (Potassium Sorbate)

	President French Brie (Full Fat Soft Cheese)	
	Swiss Gruyere (Swiss Reserve Gruyere AOP full fat hard cheese)	Produced in **Switzerland** using milk from Switzerland. Made from unpasteurised milk.
	Swiss Emmental (Swiss Emmental AOP full fat hard cheese made with unpasteurised milk. Mild and nutty golden yellow cheese)	
	Dolcelatte (Blue Veined Full Fat Soft Cheese)	
	St Agur (Blue veined full fat soft cheese)	Produced and packed in **France**
	Castello Danish Blue Cheese	
	Goats Cheese Log (Full Fat Soft Cheese)	Produced in **France** with pateurised Goat's Milk
	Normandy Camembert 250G	Produced in **France**
	Finest Spanish Manchego Cheese (Spanish ewes' milk full fat hard cheese with a sweet and nutty flavour)	Produced in **Spain** using milk from Spain. Contains Milk and Egg
	Creamy 60% Brie (Brie mould ripened full fat soft cheese)	Produced in **France** using milk from the **EU**.
	Ripe And Ready Caractere Brie (Brie de caractere mould ripened full fat soft cheese)	
Cheese with Herbs and Spices	Wensleydale And Cranberry (Wensleydale full fat hard cheese with sweetened dried cranberries.)	Creamy Wensleydale from Belton Farm in Shropshire with sweet cranberries Produced in the **UK** using milk from the UK
	Ilchester Mexicana Original Hot Cheddar	
	Five Counties (Layers of Derby, Red Leicester, Cheshire, Double Gloucester and Cheddar)	

	Wensleydale With Mango And Ginger (full fat hard cheese with sweetened dried Mango and crystallised Ginger)	
	Extra Hot Mexicana Cheddar	
	White Stilton And Apricot (Full fat creamy hard cheese with pieces of chopped dried apricot)	
	Cheddar With Garlic And Herb (Cheddar full fat hard cheese with garlic, parsley and chive)	Produced in the **UK** using milk from the UK
	Cheddar With Pickled Onion And Chive	
	Cheddar With Chilli And Lime	
	Cheddar With Caramelised Red Onion Chutney	
	Long Clawson Whirl Herb And Garlic	
	Double Gloucester With Onion And Chives	
	White Stilton And Blueberry (Full fat creamy hard cheese with pieces of chopped sweetened dried blueberries)	

3.5 Photograph of cheese varieties from supermarkets analysed for iodine content at Fera Labs