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The cheese matrix: Understanding the impact of cheese structure on aspects of cardiovascular health – A food science and a human nutrition perspective

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Health guidelines recommend limiting saturated fat consumption due to adverse associations with low-density lipoprotein cholesterol, a marker for cardiovascular disease risk. Recently, this advice is being questioned, since it does not account for the diversity of fatty acids present in different foods and may be overly simplistic. Current research suggests that for dairy foods and cheese in particular, a matrix effect exists, whereby the other components present interact with the overall structure, leading to health benefits. This review examines how factors in cheese production and processing impact this matrix, and considers how they affect biological function, and the potential impact on human health.

Keywords Cheese matrix, Structure, Composition, Saturated fat, Cardiovascular disease, Human health.

INTRODUCTION

The current global cheese market is valued at ~ $100 bn, and global cheese consumption is expected to increase by ~13.8% between 2019 and 2029 (OECD/FAO, 2020). Although cheese has been part of the European diet for 6000–8000 years (Salque *et al*. 2013), it is now also gaining popularity in countries where it was not traditionally part of the national diet, likely due to the Westernisation of the diet (OECD/FAO 2020). Dairy consumption has increased significantly in Asia in the last two decades (Lipoeto *et al*. 2012), and although milk still accounts for much of the consumption (de Goede *et al*. 2016), it is estimated that the retail value of cheese in China will increase at an annual rate of 12% during the forecast period of 2019–2024 (Euromonitor 2019).

Approximately 2000 cheese varieties are reported, mostly prepared by coagulation of milk by chymosin, and matured for between 2 weeks and 2 years (McSweeney 2004). During maturation numerous physico-chemical, microbiological and biochemical changes occur within the cheese matrices resulting in development of the characteristic texture, aroma and flavour. Other cheeses are prepared by acid coagulation (e.g. Cottage, Quarg) or by a combination of heat and acid (e.g. Queso blanco, Paneer), and are normally consumed fresh. All of the above categories of cheese are referred to as natural cheeses, alternatively other cheese types may be categorised as 'processed' or 'analogue' cheese (Guinee 2016). Processed cheeses are prepared by blending shredded natural cheese with other dairy and nondairy ingredients, including emulsifying salts, which act as calcium chelating agents promoting disaggregation of the protein in the cheese matrix, and where the fat is contained as an emulsion within the matrix (Kapoor and Metzger, 2008; Guinee 2016). Cheese analogues are cheese-like products in which milk fat, milk protein or both are partially or wholly replaced by non-milk-based components and prepared by blending various edible fats/oils, proteins, other ingredients and water into a smooth homogenous blend with the aid of heat, mechanical shear and emulsifying salts (Cunha *et al*. 2010; Guinee 2016). Such

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diversity in manufacture procedures results in cheeses as complex dairy products, having broad ranges in composition and structural characteristics.

Cheeses are generally nutrient-dense foods and are a valuable source of high-quality proteins, lipids, vitamins (e.g. vitamin A, B2 and B12) and minerals (particularly calcium and phosphorus). In the Irish diet, as well as in the UK and the United States, they make a significant contribution to nutrient intake and diet quality (Feeney *et al*. 2016). In addition to macro and micronutrients, some matured cheeses contain bioactive components (e.g. bioactive peptides), which have health benefits, while beneficial bacteria present in the cheese matrix can potentially improve human gut health by producing short-chain fatty acids (Santiago-López *et al*. 2018). However, cheese also contains relatively high levels of saturated fatty acids (SFAs), which are commonly perceived as negatively impacting the healthfulness of the diet, and have been associated with increased blood LDL-cholesterol levels, often considered a marker for cardiovascular disease (CVD) risk (Ference *et al*. 2019).

Current dietary guidelines recommend that the daily intakes of saturated fat (SFA) should be as low as possible (EFSA, 2010), and should exceed no more than 10% of total energy intake (USDA, 2015). Population intakes of SFA are considerably higher than this, 13%–14% in Ireland (Tierney *et al*. 2011) and 12.6% in the UK (PHE, 2014). Recent draft guidelines published by the WHO (2018) recommend a reduction in population SFA intakes, and replacement with poly- and monounsaturated fatty acids in order to reduce rates of chronic disease and related deaths.

Dairy foods are a major contributor to population SFA intakes, as circa 60% of the fats in dairy fat are in the saturated form, contributing approximately 1/5 of SFA on average, according to data from UK, Irish and US food consumption databases (Feeney *et al*. 2016). Therefore, dairy foods, and cheese in particular, are a considerable target for reduction on a population intake level. However, the supporting evidence for a link between SFA intake and health is more nuanced and indicates that the food source of the saturated fatty acids may be of particular importance. A number of recent meta-analyses suggest that while SFA from meat and processed meat sources are associated with detrimental health effects (de Oliveira Otto *et al*. 2012), SFA intake from dairy sources on the other hand has contrasting health outcomes and is associated generally with either neutral health effects (Benatar *et al*. 2013) or with beneficial health associations (Elwood *et al*. 2010; Chen *et al*. 2012; de Oliveira Otto *et al*. 2012; Kratz *et al*. 2013). For a recent review of these effects from association studies and randomised controlled trials, examining the individual products milk, cheese and yoghurt, please see Timon *et al*. (2020).

Lawrence (2013) concluded that the scientific evidence did not support a blanket minimisation of SFA intakes, and called for a revisit of the guidelines. This debate continues, with a recent group noting that the 2018 draft WHO guidelines could even inadvertently promote a *less* healthful diet through unnecessary reduction of nutrient-dense foods, such as cheese (Astrup *et al*. 2019). Saturated fats are not a homogenous group, but rather they vary considerably, from the type and length of the SFA, to the nature of the food in which they are contained, including other nutrients, as well as the overall food structure (Thorning *et al*. 2017), and specific fatty acids have different effects on health outcomes. This demonstrates a need to distinguish the individual food source of the saturated fat in dietary guidelines (Astrup *et al*. 2019; Wu *et al*. 2019). Further, Astrup *et al*. (2020) note that while SFA intake is associated with total LDL-c levels, LDL-c particle size is a much greater indicator of CVD risk and the small dense LDL particles are considered more atherogenic and thus confer a greater CVD risk than large LDL-c particles. Specifically, they note these smaller LDL-c particles are *not* reduced via dietary SFA restriction in most individuals. Therefore, SFA restriction can result in a disproportionately greater reduction of the large LCL-c particles, which are much less associated with CVD risk. For this reason, they highlight the pressing need to develop more reliable markers to monitor the effects on CVD risk from dietary SFA.

There has been particular interest in dairy foods as a source of saturated fat in recent times, and specifically in cheese. In one cross-sectional study of over 18 000 subjects, cheese consumption was correlated with beneficial metabolic health markers, including higher circulating HDL-c levels (Hostmark *et al*. 2009), while a later meta-analysis of 15 prospective cohort studies found a (nonlinear) reduced risk of CVD risk with increasing cheese consumption (Chen *et al*. 2016). Further, several randomised controlled trials have demonstrated that the consumption of fat from cheese specifically, does not increase LDL-c compared to the same amount of fat from some other dairy sources (Biong *et al*. 2004; Nestel *et al*. 2005; Hjerpsted *et al*. 2011; Hjerpsted and Tholstrup, 2011; de Goede *et al*. 2015; Feeney *et al*. 2018).

Since the fat contained in different dairy foods share a similar fatty acid profile, this further highlights the importance of the food source and structure, and the interaction with the other nutrients contained within that food (i.e. the food matrix; Thorning *et al*. 2017) in the study of saturated fat consumption, and health effects. Although equally the validity of LDL-c as a marker of CVD risk is under debate (Astrup *et al*. 2020), the existence of a food matrix effect is now well accepted.

The aim of this review is to examine the cheese matrix from a both a physico-chemical and biological perspective, and to determine how interactions between both phases may exert beneficial effects arising from cheese consumption on aspects of cardiovascular health.

CHEESE COMPOSITION AND STRUCTURE

Cheese compositions vary according to variety (Table 1) including for the major components: moisture, protein (mainly casein) and fat. For example, Cottage cheese contains high levels of moisture and low levels of protein and fat, whereas Parmesan contains low level of moisture and high levels of protein and fat (Table 1). Additionally, cheeses also contain varying quantities of other numerous minor components, including calcium (calcium phosphate), and salt (sodium chloride).

The cheese matrix itself is a complex assembly of the individual components/nutrients. Protein, especially casein, hydrated with water forms networks (within the cheese matrix) in which fat globules/pools, minerals, bacteria and dissolved solutes such as lactic acid, potentially residual lactose, soluble salts and peptides are all interspersed (Hickey *et al*. 2015; Lamichhane *et al*. 2018b). The manner in which the individual components/nutrients assemble within the cheese matrix and their interactions determines the structure of cheese. The structural organisation of the major constituents of various cheeses (which can be observed under various types of microscopy) differs markedly (Figure 1) due to a number of factors, such as composition and pretreatment of cheese milk (e.g. homogenisation and heat treatment), cheese manufacturing conditions (e.g. method of coagulation), maturation conditions (e.g. duration and maturation temperatures) and preparation before consumption (e.g. cooking, baking, grilling). For example, cream cheese microstructure is composed of compact fat and protein aggregates with large spaces filled with whey (Figure 1a), which is mainly due to its composition (fat: protein ratio, moisture content) as well as the prior homogenisation of cheese milk. The 'cheddaring' steps during Cheddar cheese manufacture and cooking of curds to high temperatures (50–55 °C) during Emmental manufacture contributes to the coalescence of fat globules and formation of large irregular fat pools within the cheese matrix (Figure 1b,e). Similarly, the stretching of curd in hot water or cooling in brine during the production of Mozzarella cheese results in the orientation of protein stands and aggregated fat globules/pools in the direction of stretching (Figure 1d). The specific manufacturing steps of process cheese results in entrapment of spherical fat globules within the homogenous protein matrix

Table 1 Approximate composition of selected cheese varieties.[a]

Cheese type	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	MNFS (%)	FDM (%)	Calcium (mg/100 g)	Calcium (mg/g protein)	pH	Salt (g/100 g)	S/M (%)	Cholesterol (mg/100 g)
Extra hard											
Parmesan	30.6	34.9	29	43	41.79	1200	34.38	5.2–5.3	1.89	6.18	88
Hard											
Cheddar	34.5–37.5	24.7–25.1	32.8–33.9	51–56	51.5–52.5	780–830	28.24	5.2–5.3	1.6–2.2	4.5–5.8	100
Emmental	35.7	28.7	29.7	51	46.19	970	33.80	5.5	0.70	1.96	90
Gruyère	35	27.2	33.3	52	51.23	950	34.93	5.5	1.48	4.23	100
Semihard											
Mozzarella	49.8	25.1	21	63	41.83	590	23.51	5.1–5.3	1.40	2.81	65
Gouda	40.1	24	31	58	51.75	740	30.83	5.2–5.4	2.30	5.74	100
Edam	43.8	26	25.4	59	45.20	770	29.62	5.2–5.4	1.45	3.31	80
Semisoft											
Danish blue	41.3	19.7	32.9	62	56.05	530	26.90	5.3	4.10	9.93	90
Stilton	45.3	20.1	29.6	64	54.11	500	24.88	6.6	3.30	7.28	75
Roquefort	38.6	22.7	35.5	60	57.82	320	14.10	6.0–6.50	2.20–2.70	5.70–6.99	105
Soft											
Cottage	79.1	13.8	3.9	82	18.66	73	5.29	4.8	0.50–0.70	0.63–0.88	13
Brie	48.6	19.3	26.9	66	52.33	540	27.98	7.5	1.40–2.10	2.88–4.32	100
Fromage frais	77.9	6.8	7.1	84	32.13	89	13.09	4.5–4.8	0.15	0.19	25

MNFS, moisture-in-non-fat substance; FDM, fat in dry matter; and S/M, salt-in-moisture.

[a]Data compiled from multiple sources: Lawlor *et al.* (2002), Lawlor *et al.* (2003), Govindasamy-Lucey *et al*. (2004), Guinee (2007), Heino *et al.* (2010), Calzada *et al*. (2014), Guinee (2016), McCarthy *et al.* (2017), and O'Brien and O'Connor (2017).

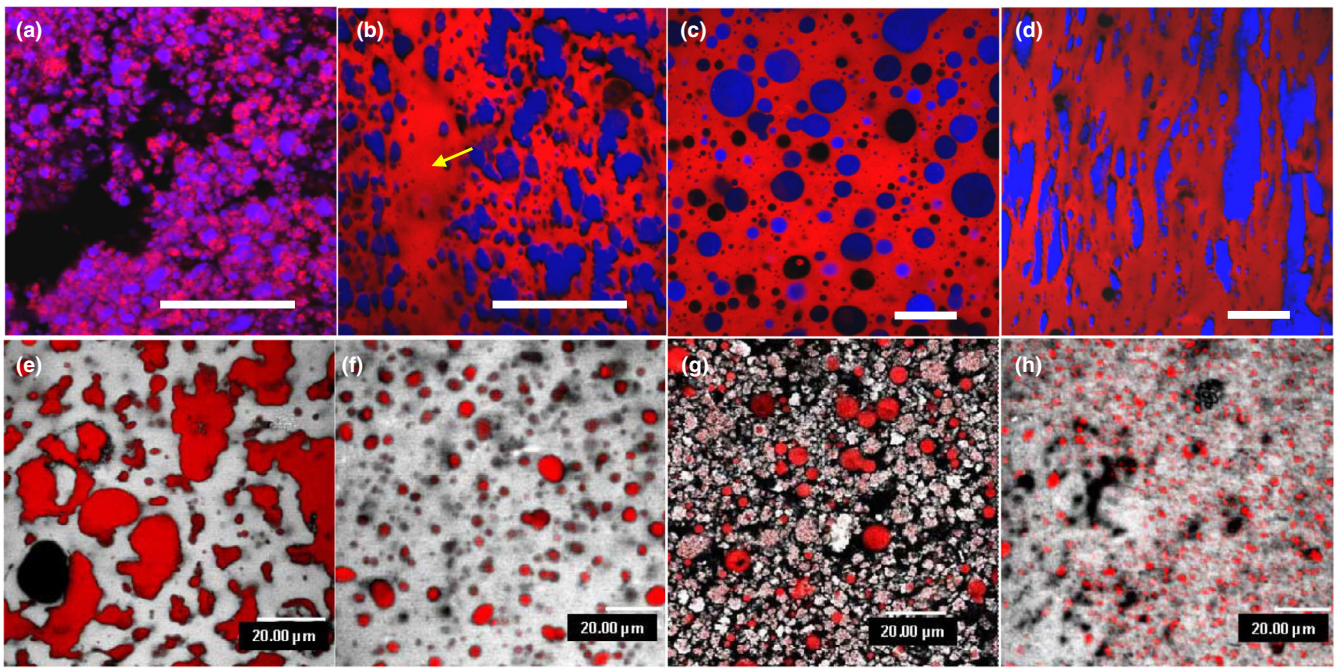

Figure 1 Microstructure of various cheese types: (a) Cream cheese, (b) Cheddar cheese; arrow shows curd granule junction, (c) Processed Cheese, (d) Mozzarella cheese, (e) Emmental cheese, (f) Camembert cheese, (g) whipped cream cheese and (h) soft cheese made with ultrafiltration technology. Micrographs (a) to (d) are adapted from Auty et al. (2001); the protein phase appears red while the fat phase appears blue; scale bar 25 µm. Micrographs (e) to (h) are adapted from Lopez (2005); fat is coloured in red, proteins are in grey levels. Black areas correspond to serum or gas holes.

(Figure 1c). In the following sections, factors influencing the cheese matrix as well as their nutritional importance will be discussed.

Milk pretreatment and coagulation of milk

Caseins and whey proteins are the two major groups of protein found in milk, comprising ~80% and ~20% of total milk protein, respectively. In milk, the caseins exist as large colloidal aggregates, known as casein micelles, where the κ-casein present on the surface of casein micelles stabilises against aggregation in milk (Dalgleish and Corredig, 2012). During cheese manufacture, casein micelles are destabilised intentionally, which is achieved through chymosin addition, acidification, heat or a combination of acidification and heat. The structure of casein micelles is altered differently depending on the coagulation method used (Dalgleish and Corredig, 2012). For example, in chymosin-induced gelation, destabilisation of casein micelles occurs due to selective hydrolysis of κ-casein (present on the surfaces of casein micelles) at the Phe_{105}-Met_{106} peptide bond by enzymes present in chymosin, whereas in acid-induced gelation, destabilisation of casein micelles occurs due to acidification (using starter cultures or food grade acids and/or acidulants), which decrease the negative charge of the micelle surface (Lucey 2002; Dalgleish and Corredig, 2012; Guinee 2016). Detailed mechanisms of destabilisation of casein micelles by different means have previously been reviewed extensively (please see review by Dalgleish and Corredig, 2012). The destabilised casein micelles aggregate into chains and clusters, leading to formation of a 3-dimensional gel.

It is expected that coagulation methods also alter the structure of casein networks, as well as the molecular interactions between the caseins within the cheese matrices (Lucey 2002; Lamichhane *et al*. 2018b). Such different structures within casein networks will also behave differently during digestion, affecting the bioavailability of the nutrients contained within. For example, Floury *et al*. (2018) showed that milk-based chymosin gels, but not acid gels formed compact protein aggregates under acidic conditions of the stomach. Moreover, the kinetics of protein hydrolysis was slow in milk-based chymosin gels as compared to acid gels. Similarly, Barbé *et al*. (2014) in *in vivo* studies, using mini pigs, showed that the digestion of milk proteins in chymosin gels was delayed as compared to acid gels.

Milk used for the manufacture of most cheese varieties is generally pasteurised, most commonly at 72 °C for 15 s, to eliminate pathogenic bacteria (Kelly *et al*. 2008); such a mild heat treatment has minimal impact on the structure of milk components. However, more severe heat treatments (e.g. 85 °C for 5 min for the production of Queso blanco;

Farkye 2004) are applied in the manufacture of several acid-heat coagulated cheeses (e.g. Queso blanco and Paneer) and may also be used in the manufacture of reduced or low-fat cheeses. High heat treatment of milk (greater than pasteurisation) results in high levels of whey protein denaturation. For example, ~34 % of total whey protein has been reported to denature in milk heated at 87 °C for 26 s (Rynne *et al*. 2004). The denatured whey protein interacts with caseins, MFGM proteins or both *via* a number of molecular forces, including disulphide bonds (Kelly *et al*. 2008; Michalski 2009), and there is current interest in understanding how such process-induced modifications of structures or nutrients influence their disintegration or release patterns in the gastrointestinal tract under digestion.

To date, there is little published data on the digestion behaviour of cheese made from high heat-treated milk (e.g. Queso blanco and Paneer). In cheese made from unheated (or minimally heated) milk, caseins are responsible for network formation, whereas in cheese made from high heat-treated milk, both denatured whey proteins and caseins are responsible for network formation. Moreover, denatured whey protein associated with the surface of casein micelles may restrict the rearrangements and/or fusion of casein particles in cheese made from high heat-treated milk (Lucey *et al*. 2001). Therefore, cheeses made from high heat-treated milk are more likely to show different digestion behaviours, and further research on this is recommended.

A number of *in vitro* studies however investigated the impact of heat treatment of milk on its digestion behaviour. For example, Ye *et al*. (2016a; 2016b) observed a faster rate of proteolysis by pepsin in high heat-treated (90 °C for 20 min) milk samples as compared to unheated milk. Similarly, Mulet-Cabero *et al*. (2019) observed an accelerated protein hydrolysis in ultra-high-temperature (140 °C for 3 s) processed milk. Moreover, apart from protein hydrolysis, studies have also reported that high temperature processing of milk increased the free fatty acid release during *in vitro* digestion (Tunick *et al*. 2016; Ye *et al*. 2016a). In addition, during *in vitro* digestion high heat-treated milk formed fragmented and soft coagula, whereas unheated milk formed dense-structured coagula (Ye *et al*. 2016a; 2016b; Mulet-Cabero *et al*. 2019), which may potentially be the reason for different rates of protein hydrolysis as well as different rates of fat globule release from the coagula (Ye *et al*. 2016a).

Whey is also used as a starting material for manufacture of some cheese varieties (also called whey-based cheeses), such as Ricotta, Anari and Mysost (Phelan *et al*. 1993; Jelen 2002). Ricotta and Anari are manufactured by coagulation of whey proteins, which is achieved by high heat treatment of whey (~90 °C for few minutes) and often addition of organic acids (e.g. acetic, citric) and/or mineral salts (e.g. calcium), to the whey (Smithers 2008). For manufacture of the Norwegian-style whey cheese, Mysost, whey with some other added components (such as milk fat or cream) is concentrated by evaporation to achieve the final product (Jelen 2002).

In vitro and *in vivo* studies have reported that the whey-based cheese showed different digestion and absorption behaviour as compared to casein-based cheese (Lorieau *et al*. 2018; Lorieau *et al*. 2019). For example, in an *in vivo* study using pigs, Lorieau *et al*. (2019) observed a higher amino acid bioavailability in whey-based cheeses as compared to casein-based cheeses, and those authors suggested that cheese based on whey proteins rather than those based on caseins are potentially more suitable for muscle synthesis in elderly people.

Cheese manufacture processes

Although acid or acid-heat coagulated cheese types undergo minimal curd handling processes after curd formation, chymosin-coagulated curd is subjected to several post-curd formation processes, such as cutting, cooking, 'cheddaring' (in Cheddar cheese) and hot-water stretching (in pasta-filata type cheeses), and moulding and pressing. Such processes contribute to the characteristic structure, texture and rheological properties of the final cheese. For example, draining of whey at higher curd pH, such as 6.4–6.5 in Emmental and Maasdam cheeses, results in higher levels of micellar/insoluble calcium (i.e. calcium associated with casein), which is responsible for their rubbery or elastic texture. Levels of insoluble calcium in ripened Gouda, Emmental and Maasdam cheese are ~24 mg/g protein (Lamichhane *et al*. 2018b; Lamichhane *et al*. 2019), higher than those in Cheddar cheese (~18 mg/g protein), as whey is drained at lower pH (i.e. 6.15–6.30) during Cheddar cheese manufacture. In acid coagulated cheeses, such as Cottage cheese, the level of insoluble calcium is very low (i.e. <5 mg/g protein), most probably due to solubilisation of the colloidal calcium phosphate associated with the caseins at low pH (i.e. ~4.6). During Cheddar cheese manufacture, the curd granules are allowed to fuse by stacking 'loaves' of curd on top of one another, called 'cheddaring', which results in coalescence of fat globules into large fat pools and development of the characteristic fibrous structure (Lucey *et al*. 2003). Similarly, the stretching of curd in hot water or brine during the production of Mozzarella cheese results in orientation of protein stands in the direction of stretching, resulting in fibrous structure and anisotropy, that is physical and mechanical properties dependent on the direction of examination (Bast *et al*. 2015; Sharma *et al*. 2018). The specific manufacturing conditions of Parmesan-type cheeses, such as cutting of coagula into small curd granules, high scalding temperatures (55 °C for 30 min) and long brining times, is responsible for a very dry cheese with a very hard and brittle texture and evinced by its low moisture and also moisture-in-non-fat substance (MNFS) levels, 43 % in comparison with 82 % in Cottage cheese. Levels of curd hydration are also influenced by curd

pH, such as the swelling of the outer layers of the para-casein matrix in Brie and Camembert as pH increases during maturation and by salt-in-moisture levels. Such initial cheese characteristics may have an impact on the breakdown/disintegration behaviour in the mouth during mastication and in the gastrointestinal tract during digestion, which may affect the kinetics of release of nutrients. Matrix disintegration is one of the important events for digestion of solid food matrix, such as cheese, which facilitates release of nutrients to the digestion medium.

The first step of the food digestion process occurs in the mouth, where food is subjected to a complex series of oral manipulations, including ingestion, size reduction and mixing with saliva, to form a bolus for safe swallowing. Solid foods, including cheese, are broken down into number of pieces into the mouth during oral manipulation. Breakdown processes (i.e. number of fragments and their size) are largely dependent on the mechanical properties of food and their interaction with individual oral processing parameters such as level of mastication (Chen 2009). For example, Guo *et al*. (2013) reported that hard whey protein gel with an inhomogeneous microstructure had a fast-crack propagation pattern and a high degree of fragmentation as compared to soft gels of homogeneous microstructure. The mechanical properties of cheese may vary between cheese types, such as the elastic and rubbery texture of Emmental cheese, and the firm and brittle texture of Parmesan (Guinee 2016). Moreover, the mechanical properties of cheese also largely depend on the level of maturation; cheese usually becomes softer as maturation increases (Lamichhane *et al*. 2019). Apart from mechanical processes, cheese is exposed to biochemical processes in the oral cavity, such as interactions between components of cheese and the active components of saliva (e.g. enzymes and glycoprotein; Norton *et al*. 2014). Fat globules within the natural cheese matrices have been reported to be held weakly within the structure (Lamichhane *et al*. 2020); therefore, it is expected that the same fat globules may be released and subsequently mixed with saliva. Moreover, the level of water-soluble nitrogen (due to hydrolysis of caseins) within the cheese matrices increases with the level of maturation, and is likely to be solubilised in the saliva during oral manipulation. Thus, it can be assumed that the bolus characteristics may depend on the cheese composition, structure, mechanical properties and their level of maturation. Bolui with different characteristics are most likely to behave differently in the following digestion steps (Singh *et al*. 2015).

The bolus formed in mouth passes to the stomach through the oesophagus (Kong and Singh, 2008). In the stomach, the bolus is further disintegrated due to diffusion of gastric juice which is a complex mixture of gastric acid, bile salts and digestive enzymes secreted by the gastric glands (Kong and Singh, 2008). In addition, mechanical force generated by contraction of the stomach also play a significant role on the disintegration of the food bolus (Kong and Singh, 2008; Singh *et al*. 2015).

Disintegrated and partially digested food from the stomach is then passed to the small intestine, where many of the digestive reactions occur, such as hydrolysis of protein and fat into amino acids and free fatty acids, and absorption of most of the nutrients occurs (Kong and Singh, 2008; Norton *et al*. 2014). Insoluble and indigested food materials from the small intestine then pass to the large intestine, where microorganisms perform fermentation producing gas and other compounds, such as short-chain fatty acids (Norton *et al*. 2014). A schematic overview of physico-chemical, structural and biochemical transformations of cheese as it passes through the oral cavity, the stomach, and the small and large intestines is shown in Figure 2. More detailed information regarding transformation of food in the human digestive systems has been reviewed by Kong and Singh (2008), Norton *et al*. (2014), Singh *et al*. (2015) and Somaratne *et al*. (2020).

Studies have reported that the cheese matrix disintegration was correlated with textural properties of cheese (Sharma Khanal *et al*. 2020). For example, texture parameters springiness, cohesiveness, chewiness and hardness were negatively correlated to the rate of cheese disintegration during *in vitro* gastric digestion (Fang *et al*. 2016a; Guinot *et al*. 2019). Moreover, the rate of protein/peptide release was influenced by the textural properties of cheese (Fang *et al*. 2016a; Sharma Khanal *et al*. 2020).

Textural properties of cheese largely depend on its composition and the structural organisation of its structural components and their interactions. Casein network within cheese matrix contribute for the rigidity of cheese matrix, whereas moisture contribute for softening of cheese texture (Guinee 2016; Lamichhane *et al*. 2018b). Solid milk fat in cheese is known to act as reinforcing fillers, contributing to elastic properties of unheated cheese, whereas liquid fat acts as a plasticiser between casein strands, making cheese more soft and flexible (Rogers *et al*. 2010; Shima and Tanimoto, 2016). Age-related changes, such as proteolysis and solubilisation of micellar calcium, as well as hydration of the casein strand reduce the rigidity of casein network.

Ripening

The length of the maturation period can vary between cheese types, such as instant freezing for certain Pizza/Mozzarella cheese, ~2–4 weeks for some surface-ripened mould cheese, 2–3 months for Maasdam and more than 1–2 years for mature Cheddar or Parmigiano-Reggiano (McSweeney 2004). Cheese maturation is a complex process and various physico-chemical, microbiological and biochemical changes (such as proteolysis, lipolysis and the metabolism of residual lactose and of lactate and citrate) occur within the cheese matrices resulting in the texture and flavour characteristic of a particular variety (McSweeney 2004).

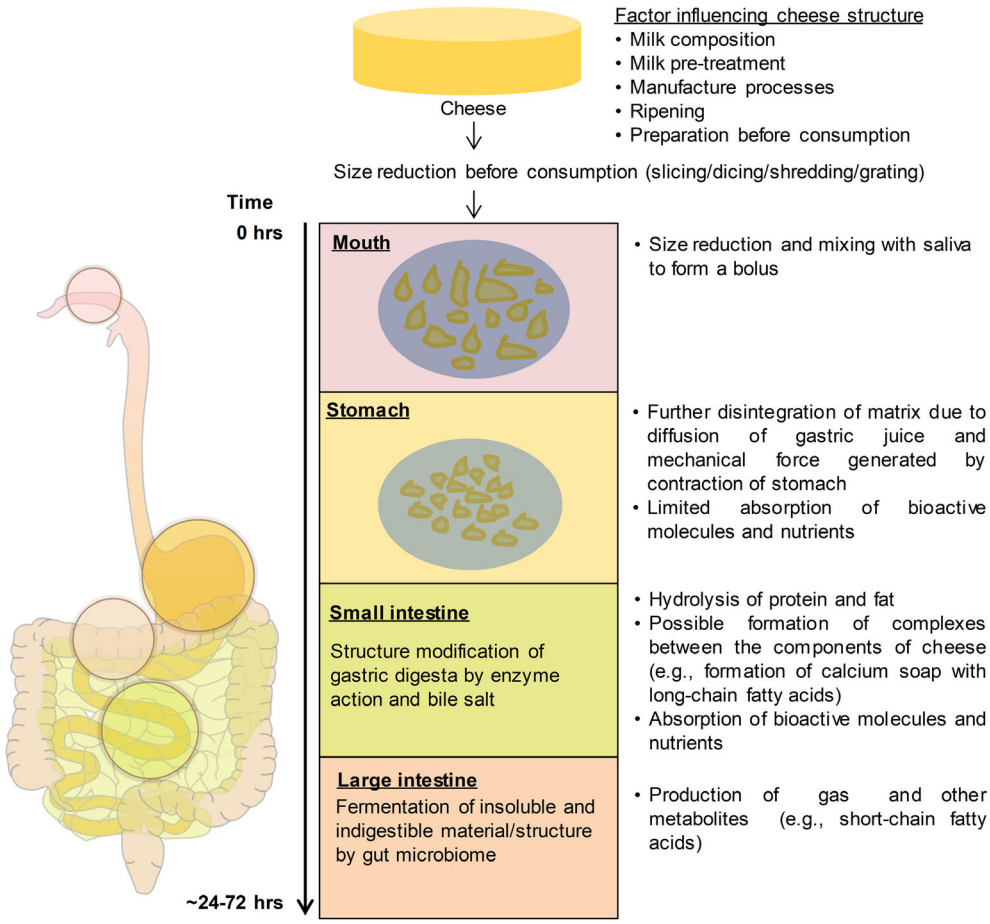

Figure 2 A schematic overview of physico-chemical, structural and biochemical transformations of cheese as it passes through the oral cavity, the stomach, and the small and large intestines. Abbreviation: LCFA, long-chain fatty acids. Adapted from Norton *et al*. (2014) and Singh (*et al*. 2015).

Hydrolysis of intact caseins into small and medium size peptides and free amino acids is one of the important biochemical events, which contribute to weakening of protein network. α_{S1}-casein and β-casein are the two important caseins within the cheese matrix, and these undergo varying degree of hydrolysis during maturation in different cheese varieties through the action of various proteolytic agents, such as residual coagulant and plasmin (Sheehan 2013; Lamichhane *et al*. 2018b; Lamichhane *et al*. 2019). For example, the extent of α_{S1}-casein hydrolysis in Cheddar and Maasdam cheeses is considerably higher than β-casein (O'Mahony *et al*. 2005; Lamichhane *et al*. 2019), whereas in blue-vined cheeses both caseins are completely hydrolysed at the end of maturation (McSweeney 2004). Some studies have indicated that the specific hydrolysis patterns of casein can influence melting and fracture properties of cheese (Bogenrief and Olson, 1995; Lamichhane *et al*. 2019). For example, Lamichhane *et al*. (2019) observed that the rigidity or strength of the Maasdam cheese matrix was negatively correlated with the hydrolysis of α_{S1}-casein, whereas the brittleness or shortness of the cheese matrix was positively correlated with the hydrolysis of β-casein. Moreover, rapid solubilisation of calcium associated with casein at early stages of maturation is another important age-related change within the cheese matrix, which also contributes to weakening of cheese structure as the calcium associated with casein enhances the cross-linking of casein within the cheese matrix (O'Mahony *et al*. 2005; Lamichhane *et al*. 2019).

Other numerous changes occur within the cheese matrix during maturation. For example, changes in the composition of serum phase due to solubilisation of minerals associated with caseins (primarily calcium and phosphate) and accumulation of breakdown products of caseins and lipids, such as peptides, free amino acids and free fatty acids. In addition, the pH of the cheese matrix changes due to metabolism of residual lactose and lactic acid, and proteolytic liberation of basic compounds. For example, in Swiss, Dutch and related eye-type cheeses pH increase from 5.2–5.3 at first week of maturation to 5.8–6.0 at the end of maturation (Govindasamy-Lucey *et al*. 2011; Lamichhane *et al*. 2018a), and in Camembert-type cheeses, pH increase dramatically from

4.5–4.6 at 1 day to 7.0–8.0 after 3 months of maturation (Sousa and McSweeney, 2001).

Studies have reported that the rate of component release and cheese matrix disintegration behaviour during *in vitro* digestion is significantly influenced by the level of maturation of cheese. Fang *et al*. (2016b) found a positive effect of proteolysis on disintegration of cheese during *in vitro* digestion. Similarly, Lamothe *et al*. (2012) found a rapid disintegration of aged Cheddar cheese in the gastric phase as compared to mild Cheddar cheese; moreover, the free oil released from aged Cheddar was higher than the mild Cheddar cheese at the end of gastric phase. In another study, Asensio-Grau *et al*. (2019) observed a higher extent of lipolysis in matured cheeses (ripened for 240 days) as compared to mild cheeses (ripened for 30 days) during *in vitro* intestinal digestion. Rapid disintegration, and higher extent of free oil release and lipolysis of matured cheese have been attributed to weakening of protein network due to age-related changes within the cheese matrix, primarily proteolysis and solubilisation of colloidal calcium, which facilitates the release of fat globules/pools (entrapped within the protein network) to the digestion medium and subsequently increase their accessibility to lipases (Lamothe *et al*. 2012; Asensio-Grau *et al*. 2019).

Overall, age-related changes within the cheese matrix seem to have a great influence on the digestion behaviour of cheese. However, to date, little information is available regarding the impact of maturation on the digestion behaviour of different cheese varieties. A better understanding of the roles of maturation on digestion behaviour of cheese may contribute to establish dietary recommendations for general population or specific population groups. For example, easily digestible matured cheeses may be more suitable for those individuals who have diseases, such as cystic fibrosis, and other pancreatic deficiencies (Asensio-Grau *et al*. 2019).

Maturation may also impact the biological activity of cheeses. The proteolytic changes that occur with ageing can result in the release of latent bioactivity of the cheese proteins. Bioactive peptides, typically 2–20 amino acids in length, have been identified from a wide range of cheeses including Mozzarella, Parmesan, goat's cheese, Gouda and Cheddar (for an extensive review please refer to the review by Santiago-López *et al*. 2018). Antioxidant, anti-microbial and angiotensin converting enzyme (ACE)-inhibitory activity (antihypertensive) are the most commonly reported functionalities in cheese (Santiago-López *et al*. 2018). Antihypertensive peptides in particular have been observed in many cheese varieties and have been used from food sources, to successfully lower blood pressure (Martinez-Maqueda *et al*. 2012). Gamalost, a Norwegian cheese, has also shown some potential for lowering of blood pressure in human feeding studies when consumed simply as cheese (Nilsen *et al*. 2016). Two well-recognised lactotripeptides with ACE-inhibitory properties are Valine-Proline-Proline and Isoleucine-Proline-Proline (VPP and IPP), first isolated from fermented dairy products from *Lactobacillus helveticus* (Nakamura *et al*. 1995). In cheeses, when *L. helveticus* was included in starter culture (Meyer *et al*. 2009) VPP and IPP levels were notably high. While levels of VPP and IPP differ across cheese varieties and starter cultures and peak at different maturation points in some cheeses compared to others, generally the peptide levels increase with maturation, with peak activity in cheese observed at 4–7 months in age (Meyer *et al*. 2009).

LIPIDS

In milk, lipid fractions are dispersed in the form of fat globules ranging from 0.2 to 15 μm with an average diameter of 4 μm (Lopez 2005). The milk lipid fraction is mainly composed of triacylglycerols (representing ~98 % of total lipids), with the remainder comprising di- and mono-acylglycerols, phospholipids, cholesterol, free fatty acids and other lipophilic molecules (e.g. carotenoids and vitamins) (Alothman *et al*. 2019). Milk fat contains more than 400 different fatty acids (FA), the levels and composition of which are influenced by a number of factors, such as stage of lactation, breed of cow, genetics and diet composition (Lindmark Månsson 2008). As stated above, the major proportion of FA found in milk are saturated fatty acids (SFAs), accounting for 60%–70% of the total milk FA, followed by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Jensen 2002; Alothman *et al*. 2019). The fat globules are surrounded by a thin membrane, called the milk fat globule membrane (MFGM), which prevents the globules from coalescence and enzymatic degradation (Dewettinck *et al*. 2008).

A number of studies have investigated the *in vitro* digestion behaviour of fat in different cheese varieties. In cheese, fat globules/pools are entrapped within the protein network which has to be disintegrated to release fat globules/pools from the cheese matrix to the digestion medium, where the lipolytic enzymes carry out lipolysis. *In vitro* studies have reported that the release of fat from the cheese matrix and the extent of lipolysis are closely related to the extent of the cheese matrix disintegration (Lamothe *et al*. 2012; Guinot *et al*. 2019; Calvo-Lerma *et al*. 2020).

Moreover, cheese manufacturing processes (e.g. scalding, cheddaring, hot water stretching and pressing) greatly affect the structure of the native milk fat globules and their membrane. In cheese, milk fat can exist as individual fat globules, aggregated, coalesced (spherical but larger than typical milk fat globules), elongated (especially in pasta-filata cheese types) or even nonglobular forms (Lamichhane *et al*. 2018b). Such cheese manufacture induced modifications of the fat globules/pools are likely to influence digestion behaviour.

Although homogenisation of milk is not common for the manufacture of most natural cheeses, homogenisation of milk and cream is practised for cream cheese manufacture and for some blue mould (to increase lipolysis) and of a portion of the milk for reduced-fat cheeses (to improve textural properties). In raw milk, fat globule size commonly ranges from ~0.2–15 μm, and homogenisation decreases fat globule size (0.2–0.5 μm of volume mean diameter; Lopez 2005) and greatly increases the surface area of fat globules, but also changes the surface composition of the fat globules, through adsorption of milk casein micelles and whey proteins (Kelly *et al*. 2008). For this purpose, two-stage valve homogenisers are commonly used, which operate at pressure of ~20 MPa (Kelly *et al*. 2008). Moreover, microstructural analysis of the milk-based chymosin gels revealed that the milk fat globules are embedded in the casein matrix of the gel made from homogenised milk, suggesting that the homogenised fat globules interact with the casein matrix of the gel, whereas the fat globules in gel from nonhomogenised milk were weakly held or entrapped within the casein matrix of the gel (Figure 3; Ong *et al*. 2010; Lamichhane *et al*. 2020).

Cheeses made from homogenised milk showed different fat digestion behaviour as compared to cheeses made from nonhomogenised milk. For example, Lamothe *et al*. (2017) observed a faster release of free fatty acids from cheese made from homogenised milk than from nonhomogenised milk during *in vitro* intestinal digestion and suggested that the degree of lipid distribution within the cheese matrix is a more important factor than the breakdown of the matrix for modulation of lipid digestion kinetics.

The health benefits of milk constituents, including some fatty acids and components of MFGM, have been extensively reviewed (Spitsberg 2005; Dewettinck *et al*. 2008; Gómez-Cortés *et al*. 2018). Some fatty acids found in milk, including butyric acid (4:0), conjugated linoleic acid (CLA) as well as branched-chain FA, have been reported to have positive health outcomes, such as maintenance of gut microbiota, weight control, gut health at birth and the prevention of chronic inflammatory diseases (Bruen *et al*. 2017; Gómez-Cortés *et al*. 2018). Moreover, the components of MFGM are reported to have several health promoting effects, including reducing the risk of cardiovascular disease (CVD), anti-inflammatory activity, cholesterol-lowering and anticarcinogenic activity (Spitsberg 2005; Dewettinck *et al*. 2008; Lordan *et al*. 2017).

Dairy fat also contains significant levels of lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0). Intake of these saturated fatty acids in isolation has been associated in the past with increased blood LDL-cholesterol levels, increasing CVD risk. However, recent studies have suggested that food/dairy matrix in which these fatty acids are contained may influence health outcomes (Thorning *et al*. 2017; Feeney *et al*. 2018), with strong evidence from dietary intervention trials for cheese as having either neutral or beneficial CVD impact (Timon *et al*. 2020). It is hypothesised that other components/nutrients present in the cheese matrix, such as protein, peptides, phospholipids derived from milk fat globule membrane, calcium and phosphorous, are likely to influence the absorption of SFAs in the human body (Thorning *et al*. 2017).

In addition to the impact on texture and rheological properties of the cheese matrix, high levels of calcium also appear to impact the absorption of the fat following consumption. A 2009 meta-analysis of RCTs examining calcium intake (from dairy and nondairy sources) and fat excretion observed that higher dairy calcium intakes specifically were associated with higher fat excretion, and

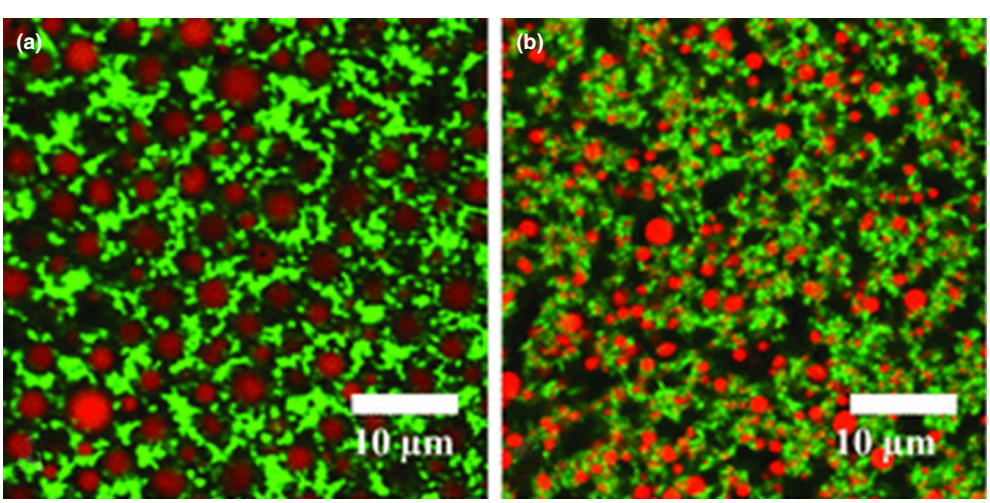

Figure 3 Microstructure of rennet induced gel made from (a) non-homogenized and (b) homogenized milk. Reprint from Ong *et al*. (2010). The protein phase appears green while the fat phase appears red.

calculated that 1241 mg of dairy Ca resulted in an average additional excretion of 5.2 g of fat per day (Christensen *et al*. 2009). Hjerpsted *et al*. (2011) in a crossover intervention study using dairy fat from cheese vs butter where 13% of energy was replaced noted that in the cheese group (with a higher Ca intake vs the butter group), the LDL-c levels were reduced vs the butter group after 6 weeks. They observed that the faecal fat excretion was 11.6% higher in the cheese period, but this was not statistically significant. The study assigned volunteers to low, medium and high energy diets depending on their individual requirements, with the medium diet providing approx. 1200 mg of dairy Ca per day in the cheese period, and 0 mg in the butter period. A later study by Soerensen *et al*. (2014) using milk and cheese delivering similar amounts of dairy Ca (1700 mg per day) vs the control (500 mg per day) day each resulted in a significant increase in the faecal fat compared to the control (Soerensen *et al*. 2014). It is reported that calcium in cheese can produce insoluble calcium soaps with long-chain fatty acids at intestinal pH conditions (Jenkins and Palmquist, 1982; Ayala-Bribiesca *et al*. 2017), limiting its absorption in the human body, and this could be one of the possible reasons for higher faecal excretions of fatty acids in humans who consumed a high-calcium diet than a low-calcium diet (Thorning *et al*. 2017). More research is needed to better understand the impact of calcium consumed in different dairy matrices on faecal fat and blood lipid profiles.

As mentioned earlier, the fat globules are surrounded by a thin membrane, called the MFGM. Buttermilk (BM) is a particularly rich source of MFGM, being the serum fraction released during the churning of cream in the butter-making process. Buttermilk powder (BMP) is the dehydrated form, obtained from BM (Vanderghem *et al*. 2010; Hickey *et al*. 2017), and both these BM and BMP are used in the industry to add MGFM-derived phospholipids to various dairy products, such as cheese, yoghurt and infant milk formulas, enhancing both the sensory and nutritional properties (Romeih *et al*. 2014; Hickey *et al*. 2017; Lopez *et al*. 2017; Hickey *et al*. 2018).

The content of the plasma phospholipids within MFGM, and particularly sphingomyelin, may have health implications, specifically on aspects of cholesterol metabolism. Rosqvist *et al*. (2015) tested the impact of dairy fat consumption with and without MGFM on these aspects, with scones made using dairy fat in the form of cream (i.e. with MFGM; Figure 4a), or with butteroil (no MFGM; Figure 4b). Participants consumed 40 g of dairy fat in either of these two forms, in a randomised, parallel-arm study design. Following 8 weeks of daily consumption, the butteroil group (no MGFM), differences were observed in both LDL- and total cholesterol, both being significantly higher in the butteroil group compared to the cream. Additionally, there were differences observed in expression levels for 19 genes, some of which were associated with cholesterol metabolism and fatty acid synthesis. The expression levels were greater in the butteroil group and reduced in the MGFM group following the 8-week intervention, suggesting a phospholipid-induced mechanism. More recently, Vors *et al*. (2019) added milk polar lipids (PL) to cream cheese in a dose-dependent manner up (0, 3 and 5 g per day) in a study of 58 post menopausal females. The authors observed that the PL increased total lipid beta-oxidation in the study, and reduced a range of markers including the ratios of total-c/HDL-c, and of ApoB /ApoA1. 5 g/day of PL reduced LDL-c levels

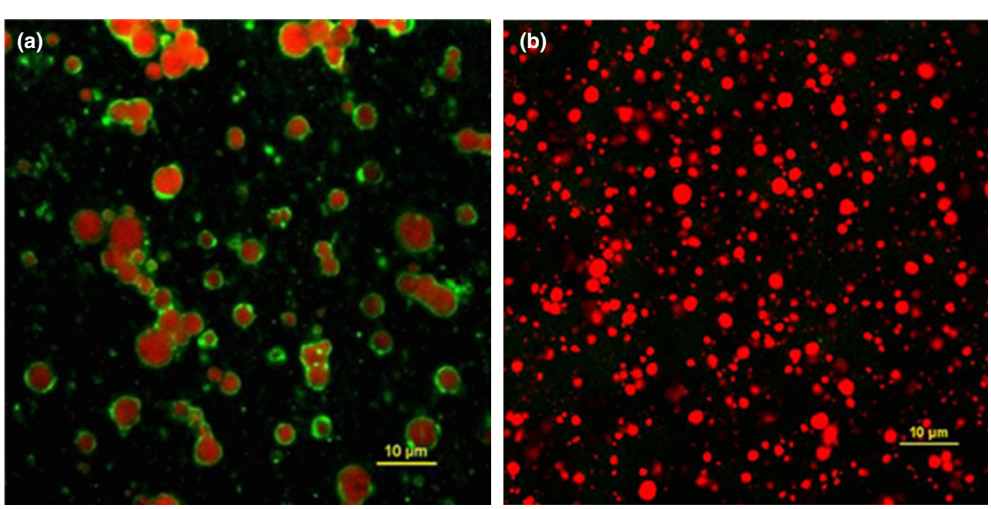

Figure 4 Confocal laser scanning microscopy micrographs of (a) milk fat globules from whipping cream (40% fat) and (b) milk fat globules in an emulsion made from butter oil, purified water, and sodium dodecyl sulfate (15% fat); fat appears red while the milk fat globule membrane appears green. Adapted from Rosqvist *et al*. (2015).

by 8.7%. They also observed reduced intestinal absorption of cholesterol, and greater levels of excreted sphingomyelin, although there was no difference in the profile of excreted short-chain fatty acids.

PREPARATION BEFORE CONSUMPTION (COOKING/BAKING/GRILLING)

Cheese is consumed in both unheated (e.g. mixed with salad) and heated form (e.g. toppings on pizzas and lasagne). The application of heat results in various physico-chemical changes in the structural components of cheese, such as: (i) increase in proportion of liquid fat, aggregation and coalescence of fat and subsequent formation of free oil; (ii) contraction of casein networks due to temperature-induced increases in the strength of hydrophobic interactions and simultaneous release of moisture; and (iii) evaporation of moisture from the cheese matrices (Guinee 2016; Lamichhane *et al*. 2018b). Such heat-induced changes on the structural components of cheese result in melting, flowing and softening of cheese. Although most mature chymosin-coagulated cheeses flow on heating, some cheese types, such as Paneer, Queso blanco and Halloumi, greatly resist flow on heating, frying and baking, due to their specific manufacturing processes (Guinee 2016). In summary, the cheese matrix (structural) properties change substantially on heating.

Although the digestion behaviour of unheated cheese has been studied frequently, surprisingly, no published data are available on the digestion behaviour of heated cheese. It is expected that heat-induced modification of cheese structure may impact its digestion behaviour. Thorning *et al*. (2017) point out that it is not yet known how melting will impact the overall cheese matrix structure, and human intervention studies using cheese have specifically noted that volunteers consumed cheese that has not been melted, due to this unknown factor (e.g. Feeney *et al*. 2018).

Several studies have reported a significant impact of cooking processes on the *in vitro* digestion behaviour of food products. For example, Kong *et al*. (2013) observed a positive impact of different heating/cooking processes (i.e. frying, roasting and boiling) on the gastric disintegration behaviour of peanuts as compared to raw peanuts. Kaur *et al*. (2014) reported that the prolonged cooking of beef meat at 100 °C reduced the protein digestibility. A better understanding of the role of cooking processes on the digestion behaviour of cheese may help to select cooking processes to attain specific digestive outcomes.

CONCLUSIONS

Cheeses are a very diverse category of dairy product, the composition and structure of which can vary greatly dependent on type, milk pretreatment and manufacture process, and maturation regime, among many other factors. As a concentrated source of saturated fats, traditionally cheese has been perceived as an unhealthy food and may be actively avoided in the diet, in the believe that cheese consumption may result in negative health outcomes, particularly on CVD risk. However, recent studies show that foods are more than the sum of their nutrients. This is particularly true for cheese, where growing evidence suggests that the nutrients present appear to work in concert to reduce markers of CVD risk compared to other dairy products when matched for fat content. However, a detailed mechanistic understanding on how the cheese matrix influences health outcomes is still lacking.

In particular, it may be necessary not just to consider cheeses as a distinct product group when considering health impacts, but rather as a diverse group of products with potentially different digestive traits and biological and health properties. For example, whether cheeses are predominantly casein or whey derived, whether a chymosin or acid gelation and/or high heat treatments have been used will impact significantly on the *in vivo* digestion patterns of the protein phase, while factors such as use or otherwise of homogenisation or emulsification will impact on fat digestion. Further research should employ both *in vitro* model digestion systems and *in vivo* clinical trials in this area.

Similarly, there is no consensus on which cheese constituents and interactions between constituents are key to the cheese matrix effect. For example, some studies suggest calcium soap formation for reduced cholesterol absorption, while others have focused on binding of cholesterol with sphingomyelin. These mechanisms in particular require elucidation, again using *in vitro* model digestion systems and/or *in vivo* clinical trials where appropriate.

Finally, other factors meriting further investigation, which to date have received less attention in this context, include the role of herd diet (particularly pasture fed vs total mixed ration) on the cheese fatty acid profile and the mode of cheese consumption. The increasing consumption of cheese in a heated format (food service applications, pizza, toasted sandwiches, etc.) using different heating methods, requires specific research to determine whether the resultant physico-chemical changes to the cheese matrix influence its digestion and ultimately, its health effects.

Overall, an enhanced knowledge of the cheese matrix from both physico-chemical and biological perspectives may contribute to the engineering of ‘healthier’ food structures in the future.

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AUTHOR CONTRIBUTION

Emma Feeney: Conceptualization, Investigation, Methodology, Project administration, Writing-original draft, Writing-review & editing. **Prabin Lamichhane**: Investigation, Writing-original draft, Methodology, Writing-review & editing. **Jeremiah J Sheehan**: Conceptualization, Investigation, Methodology, Project administration, Writing-original draft, Writing-review & editing.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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