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A review on technological parameters and recent advances in the fortification of processed cheese

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Abstract

Background

Although the consumption of processed foods is growing in overseas markets, the increased awareness of consumers to health and wellbeing in recent years has led to a decline in the growth of processed food sales in the Western market. The added pressure on the food manufacturing industry to increase the perceived healthiness of processed foods has opened up new market potential in the area of fortified processed foods, such as processed cheeses.

Scope and Approach

This review paper provides an overview of the current methodologies into the production of a processed cheese with added health benefits, including the use of probiotics and prebiotics, vitamin and mineral fortification and the addition of plant macromolecules.

Key Findings and Conclusions

Processed cheeses with increased health benefits have been of great interest to manufacturers, with reduced salt and reduced fat options commercially available. Although processed cheeses fortified with vitamins, mineral, probiotics and prebiotics are not as widespread, further work in these areas has been identified as a way to produce high value processed cheese products with added health benefits.

Keywords: Processed Cheese, Fortification, Probiotics, Vitamins

1.0 Introduction to Processed Cheese

Cheese has been present in the human diet for many years, with recent literature suggesting early cheese-making practices date as far back as 5,200 BC (Salque et al., 2013). Although the high protein and calcium content of natural cheese is a proven nutritional energy source, issues arising over its low stability lead to the initial production of processed cheese (PC), a natural cheese derivative with higher stability and reduced need for refrigeration (Guinee, Caric, & Kalab, 2004). Since processed cheese was first manufactured in the early 20th century, many different types of processed cheese have been manufactured around the world. In the United States, processed cheese can be sub-divided into four main cheese groups depending on the relative amount and type of ingredients used in its manufacture, namely pasteurized blended cheese (PBC), processed cheese blocks (PCB), processed cheese foods (PCF), and processed cheese spreads (PCS) (Carić & Kaláb, 1993), and legally has to contain more than 51% natural cheese. As well as these major groups, processed cheese analogues (PCAs) can also be manufactured to reduce the amount of natural cheese in the raw material. These analogues can be produced from either dairy, part-dairy, or non-dairy derivatives (Guinee et al., 2004; Mounsey & O’Riordan, 2007), with or without the inclusion of natural cheese. In the US, analogue cheese is further split
into two categories, with legislation stating any analogue cheese with the same nutritional value as processed cheese can be recognised as a substitute analogue, and analogues with a lower nutritional value are known as imitation analogues (Guinee & Kilcawley, 2004; O'Riordan, 2011).

PCs can be manufactured to have a variety of characteristics when compared to natural cheese, for instance increased meltability, longer shelf-life stability, high diversity, and reduced refrigeration requirements, which make it a suitable and reliable product for the hospitality, bakery and fast food sectors. Recent studies have found that in 2015, 70% of all American households purchased processed cheese for every-day consumption (IDDBA, 2016), which is attributed to the large variety of targeted processed cheese products now available on the market. With such a large existing consumer base, and projected market growth in developing countries around the world (Tamime, 2011), it has been predicted that the dairy sector will experience continued demand of processed cheese, with a high potential for market growth in the future.

The objective of this paper is to provide an overview on the recent advances into processed cheese fortification to provide increased health benefits to the consumer, and to discuss the benefits and challenges associated with the development of each fortification method.

2.0 Common ingredients and their characteristic properties in Processed Cheese

2.1 Natural Cheese

Most PC, with the exception of PC analogues, are manufactured from natural cheese, with the type and amount of cheese used varying between different PC products (Guinee et al., 2004). In Australia and the US, cheddar cheese is the most widely used natural cheese base, however gouda, mozzarella and emmental are extensively used in other countries (Carić & Kaláb, 1993). Often, multiple types of cheese can be used in one PC to promote a specific product flavour (Guinee et al., 2004). The amount, age, and composition of natural cheese can affect the texture and taste of the manufactured processed cheese, and as such needs to be chosen carefully with respect to the required properties of the product.

The age of the natural cheese added to the PC mixture is an important factor, with multiple research efforts directed at the effects of cheese maturity on the textural and rheological properties of the product (Brickley, Auty, Piraino, & McSweeney, 2007; Guinee et al., 2004). The hydrolysis of protein, primarily casein, in natural cheese increases during storage time, which leads to the reduction of protein-protein interactions in aged cheese, and an overall softer product (Purna, Pollard, & Metzger, 2006). These textural properties of the natural cheese used are still apparent in the processed cheese product, with the cheese also having a much softer texture when made with mature cheeses comparative to less mature cheeses due to the extensive casein hydrolysis (Mulsow, Jaros, & Rohm, 2007). Conversely, young cheeses have a higher percentage of intact casein arising from less extensive proteolysis, and are used to give processed cheese a firmer, more elastic texture (Mulsow et al., 2007). Although the use of young cheeses is more cost effective as the maturation process does not need to occur, high amounts in a product can lead to a tasteless, rubbery cheese. On
the other hand, while mature cheese produce a full-bodied taste, the product cheeses often have poorer emulsion stability (Carić & Kaláb, 1993). To combat this, the enhancement of the flavour profile when using young cheeses can be achieved through the use of enzyme-modified cheeses (EMCs) (Osthoff, Slabber, Kneifel, & Durrschmid, 2011), which are ripened with enzymes instead of through natural proteolysis. This allows for a high flavour intensity of 15-30 times that of natural cheese to be developed in just 1-3 days, compared to traditional methods which can take months (Kilcawley, Wilkinson, & Fox, 1998). The increased flavour intensity of EMC’s allows for small amounts of 0.1% w/w EMC to be used in a PC (Kilcawley et al., 1998).

Natural cheese pH also affects the characteristics of the product, even if pH adjustment is undertaken during production (Kapoor, Metzger, Biswas, & Muthukummarappan, 2007; Olson, Vakaleris, Price, & Knight, 1958). Acidic cheeses like feta cheese have pH values between 4.6 - 4.9 and exhibit dry, inelastic and crumbly properties (Lucey, 2013), as the casein is close to its isoelectric point of approximately 4.6 (Sádlíková et al., 2010). The reduction of charge on the protein at its isoelectric point causes protein aggregation and reduction of water affinity (Lee, Klostermeyer, & Anema, 2015; Sádlíková et al., 2010), leading to crumbly and dry cheeses being formed cheese exhibiting these characteristics (Brickley et al., 2008; Marchesseau, Gastaldi, & Cuq, 1995). This aggregation also accounts for the characteristic white appearance of acidic cheeses, as larger protein agglomerates enhance light scattering (Brickley et al., 2008). Conversely, processed cheese with high pH values of 6 and over are generally very soft and elastic, as the proteins are highly charged, and are able to bind more water, leading to a higher moisture content in the product (Brickley et al., 2008; Marchesseau et al., 1995; Sádlíková et al., 2010).

2.2 Emulsifying Salts

With the exception of PBCs, all processed cheeses are made using emulsifying salt (ES). ES aids in physico-chemical changes in the cheese during processing, through the promotion of calcium sequestration from casein, pH control, and by altering the structural and physical properties of the cheese including fat globule dispersion (Carić & Kaláb, 1993; Guinee et al., 2004). Despite the name, the salts are not direct emulsifiers as they do not themselves aid in fat homogenisation in the cheese matrix (Acharya & Mistry, 2005; Carić & Kaláb, 1993). Instead, fat dispersion and homogenisation occurs through the enhancement of the emulsification ability of the casein present in the cheese as insoluble calcium caseinate (Olson et al., 1958; Zehren & Nusbaum, 2000). Addition of ES at around 2-3 w/w % of the total product helps removes the calcium bound to the casein molecules, allowing for the casein to directly interact with and emulsify the fat present in the matrix, leading to the formation of a homogenous, stable product, with highly uniform fat droplet dispersion (Weiserová et al., 2011). The most commonly used emulsifying salts in industry are trisodium citrate and disodium phosphate (Purna et al., 2006), however other salts including basic sodium aluminium phosphates, sodium potassium tartrate, sodium orthophosphates and sodium pyrophosphates are also used (Guinee et al., 2004). These salts consist of a monovalent cation, typically sodium, attached to a charged polyvalent
anionic tail, which aids with ES dissolution in the hydrophilic portion of the cheese matrix (Mulsow et al., 2007). Different ESs generally have varying effects on the product cheese, as summarised in Table 1. To ensure that the buffering, sequestration and emulsification capacities of the added ES are all maximised, it is common practice in industry to use multiple types of ES with different emulsification capabilities in a single product (Buňka et al., 2012).

Table 1: Summary of different capacities of different types of emulsifying salts

<table>
<thead>
<tr>
<th>ES Type</th>
<th>ES Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrates</td>
<td>High buffering capacity</td>
</tr>
<tr>
<td>Phosphates</td>
<td>High buffering capacity</td>
</tr>
<tr>
<td>Polyphosphates</td>
<td>Very high calcium sequestration rates, but very low buffering capacity</td>
</tr>
<tr>
<td>Pyrophosphates</td>
<td>Very good emulsification capacity and para-casein hydration</td>
</tr>
</tbody>
</table>

In general, the degree of casein dissociation, pH, and hardness of cheese product all increase with increasing concentrations of ES used while the meltability of the product decreases (Acharya & Mistry, 2005; Purna et al., 2006; Shirashoji, Jaeggi, & Lucey, 2006), although the rate of increase is highly dependent on the specific salt used. Conflicting studies, have shown an increase in processed cheese meltability with increasing ES addition (Brickley et al., 2008; Purna et al., 2006), although it was acknowledged that these conflicting results may be due to the difference of pH of the products, which is known to alter protein interactions in the cheese, and may have resulted in the observed increased meltability (Brickley et al., 2008).

Sodium based salts are widely used in industry as they generate a strong salty taste without imparting bitterness like other monovalent cationic salts (El-Bakry, 2012). However, the recent link of high-sodium diets with coronary diseases and high blood pressure (El-Bakry, 2012; Grummer, Karalus, Zhang, Vickers, & Schoenfuss, 2012; Li, Xia, Zhou, & Xie, 2013) has prompted extensive research into producing a stable processed cheese product with reduced salt content. As sodium emulsifying salts are added at 2-3% of the final product,
the reduction of ES in the blend would be an ideal method to reduce the overall sodium concentration in the cheese. Aside from adding permeate, which has a high mineral content and thus a salty taste (Lucey, 2013), the reduction of the sodium chloride added to the processed cheese is not a valid method for sodium reduction, as it negatively affects the taste of the product. Sodium chloride also affects the water activity of the cheese (Guinee & Fox, 2004), and may result in a less stable product. Another method of sodium reduction is to simply reduce the amount of emulsifying salt in cheese, however this has been found to negatively affect fat homogenisation and product stability, leading to decreased customer satisfaction (El-Bakry, 2012; McIntyre, O’Sullivan, & O’Riordan, 2017).

Variation of the monovalent cation in the salt is the most common method of sodium reduction, with the use of potassium based emulsifying salts widely reported in the literature (Acharya & Mistry, 2005; Buňka et al., 2012; El-Bakry, 2012; Mulso et al., 2007; Nogueira et al., 2018; Shirashoji et al., 2006; Tamime, 2011). Although these salts tend to produce cheese with comparable textures to sodium based ES (Gupta, Karahadian, & Lindsay, 1984), they increase the bitterness of the product, which limits the degree of substitution of sodium with potassium whilst retaining customer satisfaction (Nogueira et al., 2018; Tamime, 2011). Recent work into the incorporation of ‘bitter-blockers’ in processed cheeses with high potassium content has been undertaken, with arginine, yeast extract and hydrolysed vegetable proteins found to mask the bitter taste of high-potassium cheeses (Ferrão et al., 2018; Khetra, Kanawjia, & Puri, 2016). Another method of ES reduction is to substitute the ES with traditional emulsifiers for instance Tween 80, which directly interact with the fat. Although several research groups have studied this particular field of research (Bunka et al., 2007; Holtorff, 1951; Lee, Klostermeyer, Schrader, & Buchheim, 1996), extensive research into this area of study has not been undertaken.

Although one initial study undertaken by Holtorff showed that the use of low molecular weight surface active emulsifiers showed reduced emulsification ability (Holtorff, 1951), Bunka et al reported that the amount of ES used could be reduced by 50% with the addition of 1% of the monoglyceride emulsifier into the PC mixture without compromising product texture (Bunka et al., 2007). Other emulsifying agents have also been trialled in processed cheese (Euston, 2008), with Lee et al trialling Sodium dodecyl sulfate (SDS), Cetrimonium bromide (CTAB) and Glycerol monostearate (GMS), which were all seen to increase the emulsification in the product (Lee et al., 1996). Milk protein co-precipitates also been shown to have a high emulsification capacity, and can be added at amounts up to 5% of the total cheese composition making it possible to further reduce the amount of emulsifying salt used whilst retaining the desired fat emulsification in the product (Carić & Kaláb, 1993).

Although such techniques could be used in industry, substituting the low-cost emulsifying salts for more expensive emulsifiers reduces the profit margin on the product, and can also lead to unclean package labelling as more ingredients have to be declared.

In 2009, Kraft Food Holdings released a patent for a processed cheese containing 0% emulsifying salts, which does not exhibit separation and forms a stable product (Smith, 2009). As emulsifying salts primarily sequestrate calcium from the Ca-casein substrates,
Smith developed a processed cheese using a low-calcium casein derivative (Smith, 2009). This low calcium content reduces the need for emulsifying salts to remove the casein-bound calcium, and allows for casein activation at regular cook temperatures using no emulsifying salts (Smith, 2009), however the cost of this pre-treated casein is higher than Ca-casein and increases manufacturing costs.

2.3 Fats

Although approximately 90% of the fat in processed cheese is sourced from the natural cheese base (M. E. Johnson, Kapoor, McMahon, McCoy, & Narasimon, 2009), PCs require supplementary fat addition to the cheese mixture to ensure satisfactory texture and sensory characteristics. PCs primarily use dairy-based fats including butter and cream as this additional source, however the cost reduction through the use of vegetable-derived fats is also a common practise in industry (Carić & Kaláb, 1993; Cunha, Dias, & Viotto, 2010; Rinaldoni, Palatnik, Zaritzky, & Campderrós, 2014). The extent of fat homogenisation is important in the production of a product, as the size, distribution and amount of fat globules directly affects the physical characteristics of the product. Fat globules present in the product have been found to act as weak points in the structure, allowing for easy fracture under large scale deformations such as chewing (Marshall, 1990). Cheeses with a higher fat homogenisation have been found to have reduced fracturability, giving rise to harder cheeses (Duggan, Noronha, O’Riordan, & O’Sullivan, 2008; Guinee & O’Callaghan, 2013).

Increasing health concerns from consumers regarding high fat contents in foods increase the need for industry to provide reduced-fat alternatives. In compliance with FDA requirements, ‘Reduced-Fat’ cheeses need to have a 25% reduction of fat when compared to a reference cheese, and approximately an 82% reduction of fat before it can be legally labelled as ‘Low-Fat’ (M. E. Johnson et al., 2009). However, it has been found that a simple reduction of fat by more than 50% adversely affects the taste and texture of the product (M. E. Johnson et al., 2009). Although fat reduction can reduce customer satisfaction, it is of interest to industry as the use of dairy fat contributes significantly to manufacturing costs. One method to reduce costs associated with high fat content in processed cheese is to substitute dairy-based fats with vegetable-derived fats (Cunha, Grimaldi, Alcântara, & Viotto, 2013; Rinaldoni et al., 2014). Although the total fat in the product is not decreased through this substitution, vegetable fats provide reduced cholesterol content compared to dairy fats (Rinaldoni et al., 2014). As such, this substitution can also be utilised to increase health benefits to the consumer.

Research into how the substitution of dairy fats for vegetable fats impacted the sensory characteristics of requieija cremoso cheese samples was undertaken by Cunha et al (Cunha et al., 2010). In this study, 25% and 50% of the dairy fat was replaced by vegetable oil, and the analogues analysed for changes in microstructure and sensory characteristics with respect to the traditional processed cheese. Scanning Electron Microscopy (SEM) results of these cheeses showed that fat globule size increased with increasing substitution, which
was attributed to the presence of more long chain fatty acids in the vegetable oil, reducing its inclusion into the protein network (Cunha et al., 2010). However, the results were not consistent with their later findings, which concluded that the control cheese gave rise to the largest fat globules of all samples (Cunha et al., 2013). Although they stated similar manufacturing procedures and cheese compositions (Cunha et al., 2010; Cunha et al., 2013), the difference highlighted the need for consistency in the cheese making approach in order to obtain reproducible data. One potential factor to explain the different results may have been the variance of pH between the two data sets. In their earlier work, the three cheese samples had similar pH readings of approximately 6.5, however the analogues produced in their later paper had much lower pH values of 5.55 (Cunha et al., 2010; Cunha et al., 2013). As previously mentioned, the cheese pH is a major factor in determining cheese characteristics, as it directly affects the charge of the casein molecules and can cause changes to protein-protein interactions and thus in the hardness of the cheese (Brickley et al., 2008; Lee et al., 2015; Marchesseau et al., 1995; Sádlíková et al., 2010). In a recent study, 100% substitution of milkfat for canola oil in a model cheese system was seen to greatly affect the physical properties of the cheese by reducing hardness and meltability, however physical characteristics were retained in a system with 49% substitution of milkfat with canola oil using oat fibre at 0.05 volume fraction to aid in matrix stabilisation (Ramel & Marangoni, 2018). Although this study was undertaken solely on model cheese, it shows the potential for the substitution of saturated milkfat for canola oil without compromising the properties of the product, when coupled with a filler such as dietary fibre to help with matrix reinforcement (Ramel & Marangoni, 2018).

2.4 Protein

One of the main components of processed cheese is protein, usually derived from the natural cheese base used in blend formulation (Carić & Kaláb, 1993; Guinee et al., 2004; Tamime, 2011). The protein in the processed cheese forms a continuous network, which provides backbone of the cheese matrix in which the fat phase is dispersed through. With natural cheese being the most high cost raw ingredient in processed cheese manufacture (Guinee et al., 2004), other dairy and non-dairy proteins can be used to decrease natural cheese use. The most commonly used dairy protein is rennet casein, although other milk powders like skim milk powder and whey proteins also extensively used (Kapoor et al., 2007; Lee & Anema, 2009; Lee, Huss, Klostermeyer, & Anema, 2013; Savello, Ernstrom, & Kalab, 1989). These dairy-proteins can again be substituted for vegetable proteins, with soy protein being extensively researched in several recent publications (Li et al., 2013; Rinaldoni et al., 2014). Not only is soy protein less expensive than rennet casein, but consumption of soy has been linked with many health benefits, including prevention of cancer, diabetes, osteoporosis, and obesity (Li et al., 2013). However, sensory analysis of soy products indicate that the characteristic soy taste is often exhibited in the final cheese product, which is generally not appealing to consumers (Li et al., 2013; Rinaldoni et al., 2014). Pea protein is also of interest to industry due to its high protein content, low risk of initiating an allergic response compared to soy and nut proteins, and high solubility which allows for easier handling and incorporation into high moisture foods (Swanson, 1990).
Another benefit of pea protein is its naturally high lysine content (Arntfield & Maskus, 2011). When mixed with rice protein, which is naturally high in the sulphur-based amino acids cysteine and methionine (Swanson, 1990), a superior amino acid composition can be produced which is deemed comparable to dairy derived proteins (Arntfield & Maskus, 2011). New technologies in the manufacturing of pea proteins have also lead to the alleviation of the strong pea flavour that is often imparted in the cheese when using regular pea protein (Schindler et al., 2012).

2.5 Optional Fillers

2.5.1 Dairy By-Products

By products from the dairy industry are often used as fillers in processed cheese to reduce the cost of the raw materials and provide a source of dairy protein. The two most common fillers are whey and skim milk powders, both of which are derived from wastes associated with other dairy processes. Whey powders can be used as a source of protein as a substitute for rennet casein and natural cheese, however its use greatly reduces flowability and meltability of the product cheese, which is not desirable for most processed cheese products (Guinee et al., 2004; Kapoor, 2007; Lee & Anema, 2009; Lee et al., 2013; Savello et al., 1989). This decrease in cheese meltability may be avoided through pH modification, as whey denaturation occurs at a slower rate at low pH, potentially allowing for more extensive incorporation into the matrix before agglomeration (Lee & Anema, 2009). As well as the use of powdered whey in processed cheese manufacture, liquid whey can also be utilised. Salty whey remains one of the largest waste streams in cheese manufacture (Kapoor & Metzger, 2004), and has been incorporated into processed cheese without affecting the final characteristics of the product (Kapoor & Metzger, 2004). The reduced need for spray drying the salty whey stream before use also leads to increased savings during manufacturing.

The addition of whey also increases the concentration of lactose in the cheese. Although increasing levels of lactose have been found to have limited effect on meltability and cheese hardness (Biswas, Muthukumarappan, Marella, & Metzger, 2015; Kapoor, 2007), the crystallisation of lactose can occur at high concentrations (Kapoor, 2007). Lactose solubility in water is approximately 17% at 20°C (Kapoor, 2007), which allows for a maximum total lactose concentration of approximately 7% in processed cheeses with an average moisture content of between 40-50% before crystallisation occurs (Kapoor, 2007). In practice, the lactose content of cheese is generally lower than this theoretical maximum, as high lactose concentrations can increase the rate of Maillard browning and adversely affect the product cheese (Kapoor, 2007).

2.5.2 Starch-Substituted Products

The reduction of dairy ingredients including natural cheese from PCs reduces the overall cost of manufacture, with further cost-cutting reductions through the addition of polysaccharides such as starch being widely adopted. Starches tend to form a gel-like
structure upon cooling, offering matrix stabilisation to the food product, and has the potential to form a low-protein PC with no discernible physical or sensorial differences when compared to a control. Starch is commonly used in many different areas of food production, however the type of starch used differs depending on the specific application. The main components of starch are the two types of carbohydrates, amylose and amylopectin, with different starches containing different ratios of these components. Much research has been undertaken into incorporating different starches into analogue cheese to determine which starches give rise to cheeses with the required characteristics. Although many patents have been released claiming up to 100% substitution of casein with starch (Atapattu & Fannon, 2013; Carpenter, Finnie, & Olsen, 1998), all such patents require extensive starch pre-treatment such as hydrolysis, gelatinisation and retrogradation prior to use, which increases the capital and running costs of the plant and negates any cost savings of using starch as a substitute (Mounsey & O’Riordan, 2008a).

Recent literature has since managed to incorporate high levels of starch into cheese without requiring pre-treatment (Gampala & Brennan, 2008; Montesinos-Herrero, Cottell, O’Riordan, & O’Sullivan, 2006; Mounsey & O’Riordan, 2007, 2008a; Noronha, Duggan, Ziegler, O’Riordan, & O’Sullivan, 2008; Trivedi et al., 2008a, 2008b; Ye & Hewitt, 2009; Ye, Hewitt, & Taylor, 2009). High amylose starches have been found to give rise to harder, less meltable cheeses (Gampala & Brennan, 2008; Mounsey & O’Riordan, 2008a; Trivedi et al., 2008a, 2008b; Ye & Hewitt, 2009; Ye et al., 2009), whereas cheese produced with low amylose starches are generally much softer, even when compared to control analogue cheeses (Mounsey & O’Riordan, 2007; O’Riordan, 2011; Trivedi et al., 2008a; Ye & Hewitt, 2009; Ye et al., 2009). The increased hardness of cheeses made with high-amylose starch is attributed to the leaching of amylose from the starch granules and into the cheese matrix, increasing the hydrogen bonding between the carbohydrate and protein molecules, in turn increasing of cheese hardness (Mounsey & O’Riordan, 2001).

Although starch can be added to the processed cheese as an additional ingredient, it can also be added as a direct replacement for the protein and fat components in the cheese. As dairy protein is one of the most expensive raw ingredients (Sarker et al., 2013) , much work has been done on the inclusion of starch as the main matrix backbone in the cheese. In 2001, Mounsey and O’Riordan researched the effect of different starches on imitation cheese characteristics through replacing up to 15% of the casein matrix with starch (Mounsey & O’Riordan, 2001). These 3% starch cheeses were seen to contain irregularly-shaped particles within the cheese matrix when analysed by SEM, which lead them to conclude that starch was not fully incorporated into the cheese matrix (Mounsey & O’Riordan, 2001). Fat globule size was also seen to be reduced with increased substitution of the protein content with starch, indicating more extensive emulsification. In contrast to previous studies, it was shown that rice starch, which promoted the largest degree of emulsification, gave rise to the highest melting
characteristics, leading the authors to conclude that there was no direct relationship
between fat globule size and degree of melting as previously thought (Mounsey &
O’Riordan, 2001; Savello et al., 1989).

Subsequently, the same researchers later found that increased starch substitution lead
to larger fat globules than the control cheese, with rice starch giving rise to the largest
fat globules of all 5 starches tested (Mounsey & O’Riordan, 2007). This finding (Mounsey
& O’Riordan, 2007), also supported by (Ye & Hewitt, 2009), did not impact cheese
meltability scores, with rice starch again being found to exhibit the best meltability of all
cheeses when compared to the control. Other research does not conclusively link the
addition of starch with changing fat globule size, although a slight fat globule increase
with protein reduction has been observed (Trivedi et al., 2008a).

The increased hardness of products containing starch can be offset through increasing
the moisture content of the product, as water can act as a plasticiser in cheese and
result in reduced hardness (Hennelly, Dunne, O’Sullivan, & O’Riordan, 2006; Mounsey &
O’Riordan, 2001). The moisture saturation point of processed cheese is 54% (Duggan et
al., 2008; Hennelly et al., 2006), however increased moisture contents of up to 60% can
be achieved in processed cheeses containing starch (Noronha, O’Riordan, & O’Sullivan,
2007). This is attributed to the formation of pockets around the starch globules,
reducing the free-movement of water in the cheese matrix (Duggan et al., 2008). In the
same year, Montesinos-Herrero et al studied the effects of different starches on the
characteristics of fat-replaced imitation cheeses using Novolose 240 and 330 (N240 and
N330) (Montesinos-Herrero et al., 2006). In this novel approach, the starch was added
at the end of the processing stage rather than during initial formulation, in order to
minimise the dehydration of the protein matrix as suggested by Mounsey and O’Riordan
(Mounsey & O’Riordan, 2001). This addition did result in the expected reduction of
cheese hardness, with N240 especially having comparable hardness values to the
control cheese.

Table 2 summarises the known effects to date that the main factors that can affect a processed
cheese product. Physical parameters affecting the product including pH and cheese maturity
have been well researched, however work into starch incorporation into processed cheese is has
not yet been conclusively studied. One such area of research could include the effect of amylose
content on the final product, as although it is well reported that an increase in amylose can lead
to reduced sensory acceptance due to increased starch-starch bonding, the intermolecular
bonding mechanisms between the three-phase starch, protein and fat system in the cheese are
not yet understood. In addition, although some researchers have identified that the adverse
effects of starch addition can be negated by changing the moisture content, cook time or pH of
the cheese (Duggan et al., 2008; Mounsey & O’Riordan, 2008b), the shelf-life and sensory studies
into the production of a high-starch containing cheese with adapted pH and moisture content
have not been undertaken.
3.0 Processed Cheese Fortification

In order to increase consumer acceptance of processed cheese products, manufacturers are often seeking new ways to increase their functionality. A functional food is a food that has been given increased functionality through ingredient incorporation, and adequately provides increased health benefits to the consumer when compared to regular foods (Karimi, Mortazavian, & Gomes Da Cruz, 2011; Ouwehand, Ibrahim, & Forssten, 2010). The main cheese fortification methods are through the incorporation of probiotics and prebiotics, vitamin enhancement and fortification of the PC with other macronutrients.

Table 2: Summary of the main factors affecting a processed cheese

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect on Cheese product</th>
<th>Relevant Literature</th>
</tr>
</thead>
</table>
| Cheese as a raw ingredient | - Mature cheeses impart high levels of cheese flavour to the product PC, while relatively little flavour profile associated with the use of young cheeses  
- High intact casein in young cheese imparts good texture and body into the product, giving rise to firmer processed cheeses. The high degree of proteolysis in mature cheeses leads to decreased fat emulsification in the product processed cheese, and therefore reduces product stability | (Brickley et al., 2007; Carić & Kaláb, 1993; Guinee et al., 2004; Guinee & Kilcawley, 2004; Olson et al., 1958; Purna et al., 2006; Tamime, 2011) |
| pH                | - pH affects the charge on the protein, and can cause changes in intramolecular bonding.  
- At a pH of 4.6 the protein has an overall net charge of zero, leading to increased protein agglomeration and increased product hardness | (Kapoor, 2007; Lee & Anema, 2009; Lee & Klostermeyer, 2001; Marchesseau et al., 1995) |
| Moisture Content  | - Increased water content of PC can lead to an increase in free water (water activity), which can increase the likelihood of bacterial blooming and product spoilage | (Carić & Kaláb, 1993; El-Bakry, 2012) |
| Dairy Fillers     | - Rennet casein, whey, and skim milk powder are now being used as a substitute to the natural cheese base as a cost reduction method.  
- Whey In particular has a salty taste due to its high mineral content, and is often used to reduce the amount of sodium-based salt added to the PC mixture without adversely affecting the taste of the product. | (Carić & Kaláb, 1993; Carpenter et al., 1998; Guinee, 2011; Lee & Anema, 2009; Savello et al., 1989) |
| Starch            | - Starch is often used as a filler in processed cheese, as when heated it can crosslink and add to the body texture of the product cheese.  
- Starch can affect the final product in various ways depending on the type of starch, but it generally increases product hardiness and decreases meltability, which can lower sensory acceptance of the product.  
- Starches with a higher amylose content, such as maize and potato starches generally lead to increased hardness and decreased meltability more so than lower amylose starches, due to amylose leaching out of the starch granules in the final product, an increasing protein-carbohydrate bonding.  
- Generally starch forms its own continuous network separate from both the protein and fat portions of the cheese matrix. | (Benaouadj, Ziane-Zafour, & Rebiha, 2017; Bennett, Trivedi, Hemar, & Reid, 2006; Carpenter et al., 1998; Considine et al., 2011; Duggan et al., 2008; Gampala & Brennan, 2008; Mounsey & O’Riordan, 2007; Noronha et al., 2008; Sołowiej et al., 2015) |
3.1 Probiotic Inclusion

One way to increase the functionality of cheese is to incorporate probiotics into the final product. Probiotic dairy-based foods have been extensively researched in the last few years, with short shelf life products including fermented milk drinks and yoghurts being the most common vectors (Cruz et al., 2011; Karimi et al., 2011). Probiotic bacteria are ‘live microorganisms, which when consumed in adequate amounts, confer a health effect on the host’ (FAO & WHO, 2001; Guarner & Schaafsma, 1998), with lactobacillus and bifidobacteria strains being the most commonly used in the production of probiotic foods. In order for a food product to be considered probiotic, a minimum of $10^6$ colony forming units (CFU) viable probiotic bacteria is to be present in 100 g of product (Ehsannia & Sanjabi, 2016a; Gomes et al., 2011), with some countries enforcing a higher probiotic concentration of $10^7$ CFU before a product can be advertised as being probiotic (Cruz et al., 2011; Karimi et al., 2011). Although short shelf life products are primarily used as probiotic vectors, several characteristics of cheese including its high fat content, high solids content and good buffering capability, make it a good candidate as a probiotic vector. Although much work has been done into the field of probiotic natural cheese (Gomes et al., 2011; Karimi et al., 2011; Ong & Shah, 2009; Ouwehand et al., 2010) little research has been undertaken into the incorporation of probiotics in processed cheese, namely due to probiotic intolerance to high temperatures. As most processed cheeses are manufactured at approximately 90 °C, most probiotic bacteria are denatured at these high temperatures and thus do not contribute to an overall increase in gut health upon consumption. To overcome the issue of heat stability, addition of probiotics to the processed cheese upon cooling has been reported (Sadek, Refaat, Abd El-Shakour, Mehanna, & Hassan, 2017). The mixed lactobacillus culture showed enhanced antimicrobial activity against undesirable microorganisms in the cheese (Sadek et al., 2017), however product inoculation after manufacture may not be viable during large-scale manufacturing. Other issues posed are the lengthy storage times and high salt concentrations often found in processed cheese products. In order for products to classify as probiotic, the probiotic viability needs to be maintained over the entire shelf life of the product, which is much longer for processed cheese foods than for fresh cheeses. Research has also indicated that a salt concentration of over 4% is detrimental to the probiotic bacteria, and can result in total reduction of cell viability (Cruz et al., 2011; Karimi et al., 2011).

The first reported incorporation of probiotic cultures into processed cheese during manufacture used bacillus spores as the probiotic culture (Ehsannia & Sanjabi, 2016a) instead of the lactobacillus and bifidobacterium probiotics commonly used in probiotic food manufacture (Wells, 2011). Bacillus spores are known for their resistance to high temperatures, and can thus survive the harsh processing conditions required for processed cheeses. At the end of the 60 day storage period, the probiotic cheeses were found to contain over $10^6$ CFU of viable probiotic culture, which is in excess of the minimum viable probiotic concentration required to be legally classified as a probiotic cheese. Sensory analysis was also undertaken on the probiotic cheeses, and showed a reduction in the presence of ‘off’ aromas and tastes in the probiotic cheeses compared to the control. This
enhanced sensory appeal was attributed to the anti-microbial compounds produced by the bacillus, which are known to inhibit the growth of other bacteria present in the cheese (Ehsannia & Sanjabi, 2016a). This shows that the inclusion of bacillus probiotic into the cheese also reduces the amount of contaminant bacteria in the cheese, which could potentially lead to increased shelf life for the product. The same research group further studied the effect of bacillus spores inoculation in processed cheese on the shelf life of the product over a 30 day period (Ehsannia & Sanjabi, 2016b). During storage, it was shown that the pH of the cheeses containing the probiotic bacteria decreased in pH at a faster rate than the control cheese, due to the high production of lactic acid from lactose. As bacillus bacteria are sensitive to pH below 4.6, the production of lactic acid in the cheese would negatively affect cell viability during shelf life (Ehsannia & Sanjabi, 2016b). It was also reported that the sensory attributes of the cheese were negatively affected when the cheese was inoculated with bacillus spores when compared to the control. Although both papers recorded high bacillus viability after 60 days of storage, no work has been done to analyse the viable probiotic cell concentration of processed cheese with increased storage times of 1 – 2 years. In addition, the large scale production of probiotic processed cheeses would have to account for the changing pH of the cheese during shelf life due to the production of lactic acid from bacterial respiration, as it would greatly reduce the shelf life of the product.

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Addition to blend</th>
<th>Key Findings</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Bacillus</td>
<td>During cook</td>
<td>Bacillus spores are tolerant of high temperatures during processed cheese manufacture</td>
<td>(Ehsannia &amp; Sanjabi, 2016a)</td>
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<tr>
<td></td>
<td></td>
<td>Increased resistance of cheese to bacterial spoilage during storage</td>
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<tr>
<td></td>
<td></td>
<td>Adequate probiotic CFU level after 60 days storage</td>
<td>(Ehsannia &amp; Sanjabi, 2016b)</td>
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<tr>
<td></td>
<td></td>
<td>Increase in lactic acid production can affect pH of final product</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Sensory attributes may be affected by probiotic incorporation into processed cheese</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>After cook</td>
<td>Probiotic inoculation after manufacture can provide antimicrobial activity in cheese, and increase storage life</td>
<td>(Sadek et al., 2017)</td>
</tr>
</tbody>
</table>

3.2 Prebiotic Inclusion

An alternative to the addition of live probiotic bacteria to food samples is the addition of prebiotic compounds. Prebiotic compounds are short-chained carbohydrates that are not enzymatically metabolised in the human gut, which provide nutrients for the gut probiotic flora and allow for favourable growth conditions for selective gut probiotics such as lactobacilli and bifidobacteria (Ferrão et al., 2016). The most commonly used prebiotic compounds are hydrocolloids. Hennelly et al incorporated up to 3.44% inulin into the processed cheese product as a partial fat replacement, resulting in a prebiotic cheese with 63% fat reduction when compared to a control (Hennelly et al., 2006). Although the
reduced fat content leads to the reduction of fat globule size, the meltability and hardness of the cheese were not affected. It was also found that a slight increase of water content in the cheese matrix could be used to counteract any increase in hardness seen in the low-fat cheeses (Hennelly et al., 2006). However, no work was undertaken as to customer acceptance of the prebiotic cheese, nor on the shelf life stability of the high-moisture product. Another study also used inulin as a partial replacement for fat, and studied the rheological effects of this substitution on processed cheese spreads (Punit, 2012). It was found that the incorporation of 7% inulin as a fat replacement yielded similar rheological properties to the control cheese, however again no sensory analysis on the inulin-containing product was undertaken (Punit, 2012). Further work into the incorporation of inulin into processed cheese lead to the finding that inulin incorporation into cheese leads to a decrease in water activity, moisture, pH and titratable acidity, which is in accordance to previous literature (Karimi, Azizi, Ghasemlou, & Vaziri, 2015). The decrease in moisture and water activity was attributed to the increase of total solids in the cheese, which may account for other research papers reporting inulin incorporation having no effect on the water activity of the cheese (Sołowiej et al., 2015). In this study, the cheeses were also subjected to sensory analysis, with the results showing a reduction in sensory appeal in processed cheese substituted with insulin at concentrations of 8%. Spreadability of the processed cheese spread was also seen to decrease at 8% inulin incorporation, however no detectable difference was seen between the control cheese and the cheese with only 6% inulin incorporation (Giri, Kanawjia, & Singh, 2017). Konjac glucomannan, the polysaccharide derived from the konjac tuber (Teste r & Al-Ghazzewi, 2017) has also been used as a fat replacement in processed cheese (Felix da Silva, Barbosa de Souza Ferreira, Bruschi, Britten, & Matumoto-Pintro, 2016). Although its use as a fat substitute was shown to enhance the rheological properties of a low-fat processed cheese, the substituted product still showed increased hardness and reduced meltability to the control with a fat substitution rate of 50% (Felix da Silva et al., 2016).

Recent work has also been carried out into the inclusion of galactooligosaccharide (GOS) and xylooligosaccharide (XOS) incorporation into processed cheese (Belsito et al., 2017; Ferrão et al., 2018). Substitution of both these prebiotics at a rate of 3-4% produced a denser and more compact cheese structure, with similar characteristics to the control cheese (Belsito et al., 2017; Ferrão et al., 2018). The inclusion of 3-4% GOS in the requeijão cremoso was seen to increase the aroma and taste acceptability of the product whilst compared to the control, indicating that prebiotic inclusion can be undertaken without negatively affecting the final product (Belsito et al., 2017). Inclusion of XOS into the processed cheese at 3.3% as an 80% fat replacer with similar physicochemical and rheological characteristics to the control cheese, however with reduced sensory appeal due to the lack of ‘buttery aroma’ associated with requeijão cremoso (Ferrão et al., 2018).

Table 4: Summary of research into prebiotic incorporation in processed cheese
3.3 Vitamin Fortification

The most common method to enhance processed cheese functionality is through the incorporation of vitamins into the cheese blend. The fat-soluble vitamins A, D, and E exhibit good heat stability (Hrnčirík, 2010), and do not degrade over time, allowing for a high-vitamin product to be produced with comparable ease when compared to probiotic incorporation. However, some water-soluble vitamins including vitamin C, thiamine, and folate degrade on heating, with vitamin C degradation being found to be one of the least stable vitamin upon heating (Coultate, 2009). Vitamin addition to foods can be undertaken for a variety of reasons; to restore the levels back to natural levels before losses during processing occurred, to enrich vitamin levels at higher concentrations than the natural vitamin content of the foods, and to fortify foods which do not have any natural vitamin content.

Fortification of cheese with vitamin D has been extensively researched around the world. Although vitamin D is synthesised naturally in the skin when exposed to sunlight, it is estimated that 50-70% of Americans alone do not ingest the recommended daily requirement of vitamin D (Ganesan, Brothersen, & McMahon, 2011; Jarvis, 2010). Deficiency in vitamin D can lead to a range of health problems, including rickets in young children and osteoporosis in adults (Banville, Vuillemaud, & Lacroix, 2000). Recent research also indicates that adequate levels of this vitamin in the bloodstream are also linked to the reduced risk of several cancers, multiple sclerosis, cardiovascular disease, hypertension, arthritis (Ganesan et al., 2011; Jarvis, 2010; Kazmi, Vieth, & Rousseau, 2007). Most published work defines a vitamin D deficiency as less than 20 ng/ml (50nmol/L) of serum-25(OH)D (Jarvis, 2010; Zhu et al., 2012), with the optimum serum level defined as 30-50 ng/ml (75-125 nmol/L) (Pludowski et al., 2018). In Canada, mandatory fortification of margarine and fluid milk with vitamin D is required, whereas such fortification is optional in

<table>
<thead>
<tr>
<th>Prebiotic</th>
<th>Incorporation limit</th>
<th>Key Findings</th>
<th>Reference</th>
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</table>
| Inulin             | 3 - 8%             | - Potential use as a fat replacer  
- 63% fat reduction in cheese at 3.4% inulin addition does not affect product physical characteristics  
- 6% inulin incorporation shows comparable sensory appeal to control cheese, with 8% inulin negatively affecting sensory appeal | (Giri et al., 2017; Hennelly et al., 2006; Punit, 2012)                                      |
| Oligosaccharides   | 3 - 4%             | - Product structure affected, though increased density  
- Taste acceptability of sample similar to control  
- Can be used as a fat replacer, but reduced fat cheeses lead to reduced sensory appeal | (Belsito et al., 2017; Ferrão et al., 2018)                                                   |
| Konjac Glucomannan |                    | - Potential use as a fat replacer in processed cheese  
- Can increase sensory appeal of low-fat processed cheeses                                                                                       | (Felix da Silva et al., 2016)                                                                |
the US (Calvo, Whiting, & Barton, 2004). Whilst the consumption of milk has decreased steadily in America and Canada over the past 20 years (Banville et al., 2000), consumption of cheese has increased by 100% since the 1980’s, and may provide a good alternative to fluid milk for vitamin fortification (Upreti, Mistry, & Warthesen, 2002). As vitamin D is a fat soluble compound with a generally accepted high stability, research has shown it can be readily included into cheese with minimal degradation during storage up to 9 months (Banville et al., 2000; Ganesan et al., 2011; Jarvis, 2010; Kazmi et al., 2007; Upreti et al., 2002). Cheese naturally has a low vitamin D content of around 18 IU/100g (USDA, 2016), with fortification increasing levels to 200-240 IU/100g on average (Dimartino, 2007). Recent literature has shown that fortification up to 400 IU/100g can be undertaken in cheese without affecting the sensory and physical properties of the cheese (Ganesan et al., 2011; Upreti et al., 2002).

Although vitamin D is the most common vitamin with which to fortify cheese, vitamins A C and E are also commonly used in other food industries. Vitamins C and E are known to have good fat stabilisation effects, and may hold promise for incorporation into processed cheese.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fortification level (IU/100g)</th>
<th>Key Findings</th>
<th>Reference</th>
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</table>
| 2002 | 360                         | - No loss of vitamin D during manufacture or 9 months storage  
- Vitamin uniformly distributed  
- Loss of vitamin C (25%) upon heating  
- No affect to final product sensory acceptability | (Upreti et al., 2002) |
| 2005 | 10,000                      | - Vitamin D in processed cheese found to be bioavailable  
- Both fat soluble and water soluble vitamin D can be incorporated into processed cheese | (Johnson et al., 2005) |
| 2011 | 400                         | - Natural cheese  
- 9 months stability of vitamin D in cheese matrix  
- Product taste and flavour unaffected by vitamin D | (Ganesan et al., 2011) |

3.4 Macronutrient incorporation into processed cheeses

There has recently been increased pressure from consumers to use natural raw ingredients and to enhance the health benefits associated with food products, especially processed foods. One of the main ways that this is accomplished is through the addition of macronutrients derived from natural sources, for instance carotenoids, antioxidants, vitamins, and amino acids to name a few. To date, fruit extracts have been incorporated into natural cheese (Monphongchai, 2003) and vegetable extracts including tomato and mushroom has reportedly been used in processed cheese manufacture to provide increased health benefits to the consumer (Khider, Seoudi, & Abdelaliem, 2017; Mehanna,
Hassan, El-Messery, & Mohamed, 2017). Although both the addition of mushroom and
tomato extracts were seen to impart enhanced health benefits to the processed cheese,
the mushroom supplemented cheeses were shown to have increased levels of both
lipolytic and proteolytic bacteria than the control cheese during storage (Khider et al.,
2017) where the tomato enhanced products lead to a higher degree of oil separation in the
product and hence reduced shelf life (Mehanna et al., 2017).

Addition of plant flavonoids to processed cheese including rutin and quercetin has also
been reported to increase the antioxidant capacity of processed cheese (Přikryl et al.,
2018). It was found in this study that the flavonoids were affected by both processing time
and temperature, with the antioxidant activity of the cheese decreasing during processing
(Přikryl et al., 2018). The gel strength of the product was also negatively affected by the
addition of the flavonoids, which was attributed to the antioxidants disrupting the protein
network (Přikryl et al., 2018). Although work in the field of macromolecule incorporation
into processed cheese is in its infancy, future work should be directed at minimising the
effects on the final product with macromolecular addition, so that the mouthfeel of the
product and the bioactivity of the cheese are not negatively affected. Research should also
be done into quantitatively determining the extent of the health claims that can be made
from this fortification method.

Although there are many benefits associated with the incorporation of probiotics, prebiotics,
vitamins and minerals into processed cheese, studies suggest that the shelf life stability and
texture of the product can be compromised, which can reduce customer satisfaction. There is
also much scope for increased research into many of these fortification areas. Although a heat
resistant bacillus spore has been identified as a good candidate for probiotic incorporation into
processed cheese (Ehsannia & Sanjabi, 2016a, 2016b), further work is needed to determine
which species of bacillus has the greater affinity for incorporation into processed cheese.
Extended shelf life studies also need to be undertaken, to ensure that a probiotic processed
cheese inoculated with bacillus spores remains viable for the extended shelf life of the product,
which can be as high as 1-2 years. Future work may also be undertaken into the production of
targeted fortified processed cheeses for individual markets in areas where high vitamin or
mineral deficiencies are prevalent (Ganesan et al., 2011; Upreti et al., 2002; Zhu et al., 2012).

4.0 Summary

The increased pressure from consumers to increase the health benefits associated with
processed cheese has led to multiple developments in the healthy cheese market sector. The
reduction of salt and fat in the processed cheese is one way to increase the perceived healthiness
of the cheese product, however can lead to reduced structural and textural properties when
compared to regular processed cheese which has been shown to reduce customer sensorial
satisfaction. Although research is still being undertaken to determine whether the production of
a low-fat, low-salt processed cheese with high sensory appeal is possible, manufactures are also
giving considerable attention to the incorporation of probiotics and macronutrients as a means to
increase functionality and increase the perceived product healthiness. Although these products have not yet gained considerable market share, such fortification could be seen as a way to increase market competitiveness, as it has the potential to fill the current market gap for a healthy, ready prepared dairy snack. However, fortified processed cheeses to become a major player in the market, work into product fortification while minimizing the adverse effects to cheese properties needs to be undertaken.

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Highlights
- Roles of different components in processed cheese
- Challenges in formulation with alternative ingredients
- Recent advancement in fortification of processed cheeses