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Review

Cheese ripening: A review on modern technologies towards flavor enhancement, process acceleration and improved quality assessment

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ABSTRACT

Background: Cheese is one of the fermented milk-based foods characterized by its many different flavor, texture and aroma. Ripening is the most crucial technological step in cheese manufacturing, constituting a cascade of biochemical events, mediated by a diverse array of microbial flora that confer the perceived sensory attributes. These sensory attributes are evaluated by various descriptive, instrumental and computational methods.

Scope and approach: The recent biotechnological advancements for accelerating the ripening process and the production of its associated flavor compounds are reviewed herein. The different assessment methodologies, both sensorial descriptive and modern analytical profiling platforms are outlined with their respective applications for either monitoring the ripening process or predicting the different cheese quality attributes. Finally, computational tools employed for rapid detection of cheese artifacts are reviewed.

Key findings and conclusions: The assessment of cheese ripening is such a challenging but imperative process, which warrants the use of methods to effectively study the multitude biochemical changes that occur during this process. Some practices are posed in this review for more future applications to include exploration of a wider range of encapsulated enzyme cocktails and mixed attenuated adjunct cultures, design of intelligent packaging and utilization of IR technology, E-nose, optical techniques to control quality and estimate shelf life of cheeses. The main technological challenge in this reviewed processes for flavor enhancement and ripening acceleration is how applicable to be implemented in the cheese industry.

1. Introduction

Cheese is a type of fermented milk-based foods, with a myriad of cheese types in a wide variety of flavor and forms all over the world, with each region shaping its products according to culture and resources. Cheese can be regarded as a biocomplex ecosystem colonized by a diverse group of microorganisms, known as cheese flora, provided by raw milk, starter and adjunct cultures. These flora are the major contributors to the perceived sensory attributes of the different cheese types owing to their complex interaction with milk proteins, carbohydrates and fats that mainly occur in an important technological process in cheese manufacturing, known as ''ripening'' (Forde & Fitzgerald, 2000).

Ripening is a crucial technological process in cheese manufacturing constituting a cascade of biochemical and microbiological events, mediated by the metabolic flux of primary and adjunct cultures (P. F. Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015). This process needs to be thoroughly investigated to produce cheese products with improved and consistent quality with minimal cost and highest consumer acceptance. The organoleptic quality of cheese is determined by complex changes that occur during ripening. Degree of ripening plays a crucial role in the development of the cheese characteristics aroma and flavor due to its effect on the chemical composition (Bart Weimer, Seefeldt, & Dias, 1999).

Hence, the cheese quality can be evaluated by determining the degree of ripening and its associated flavor compounds (Forde & Fitzgerald, 2000). There are technological incentives to enhance the rate of cheese ripening and to reduce the costs which might substantially affect the sensory attributes of cheese and hence, its quality. Variability of cheese properties can also arise from the uncontrollable growth and interaction of cheese flora (Cocolin et al., 2018).

The increased consumer awareness in food quality and safety

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obliges the food industrial sector to establish and maintain the product identity and characteristics (Mary A Drake & Delahunty, 2017). However, high degree of variation is usually encountered in biological processes and matrices such as cheeses which cannot be readily addressed by traditional methods. Instrumental techniques and sensory panels can be expensive and require trained personnel warranting for more innovative, rapid-detection systems for ripening monitoring and evaluation of cheese quality such as infrared (IR) spectroscopy, electronic nose and optical techniques (Ampuero & Bosset, 2003; González-Martín et al., 2011).

Cheese manufacturing process has been indeed discussed extensively in the literature. As such, this review delves into the ripening process with its associated biochemical changes that lead to the production of several flavor compounds. The different assessment methodologies, both sensorial descriptive and modern analytical profiling platforms are outlined with their respective applications. In addition to the computational tools employed to spot some common cheese defects are revisited. The cheese ripening process and the different technological practices employed to increase the ripening rate and flavor development under review, along with the assessment tools of the different cheese quality attributes are sketched in Fig. 1.

2. Chemical perplexity behind sensorial changes upon cheese ripening

Several biochemical events occur during ripening that alter the physical and chemical properties of the manufactured cheese, which include three main reactions mostly: metabolism of the residual lactose, lactate and citrate, proteolysis and lipolysis (McSweeney, 2011).

Enzymes that participate in the ripening process come from various sources. From the milk, lipoprotein lipase survives pasteurization to participate in lipolysis. From coagulation, some of the rennet enzyme "chymosin" is retained to participate in proteolysis. The starter bacteria, in addition to their primary function of fermentation, provide proteinase and esterase enzymes, among other enzymes. NSLAB and secondary or adjunct cultures are among the major contributors in the ripening process owing to their strong metabolic activity (Clark, Costello, Drake, & Bodyfelt, 2009).

2.1. Metabolism of residual lactose, lactate and citrate

Milk, whether from goat, sheep or cow, is pasteurized to remove pathogens, then fermented by starter bacteria. The resultant is coagulated, or turned into a solid-gel product by the clumping of casein proteins as induced by chymosin. After coagulation, the product is pressed to expel moisture (P. F. Fox et al., 2015). The role of probiotics in the cheese-making process, especially flavor development, is vital, for two reasons. First, the starter bacteria from milk, also referred to as lactic acid bacteria (LAB) metabolize the principal milk carbohydrate "lactose", to lactate during fermentation *via* the process of glycolysis. Lactate is then oxidized into acetate and CO_2 by non-tarter lactic acid bacteria (NSLAB). 1–2% of the lactose remains unmetabolized and is dealt with during the ripening process. Fig. 2 depicts the basic glycolytic and proteolytic biochemical reactions that occur during cheese manufacturing. There are five pathways by which lactate is metabolized. It could be racemised into DL-lactate by NSLAB or converted to butyrate and H_2 by *Clostridium* sp. which leads to the formation of cracks and off-flavors. Pyruvate, an intermediate in lactose metabolism, is the precursor for the production of several short-chain flavor compounds *i.e.* acetate, acetoin, diacetyl, ethanol and acetaldehyde (Melchiorsen, Jokumsen, Villadsen, Israelsen, & Arnau, 2002).

Lactate could also be metabolized into formate, acetate and CO_2 by NSLAB, converted into propionate, acetate, H_2O and CO_2 by *Propionibacterium* sp. as in Swiss cheeses, or simply converted into CO_2 and H_2O by *Penicillium* sp. (Hassan, El-Gawad, & Enab, 2013). This process is responsible for cheese acidification, subsequently lactate gives cheese a distinct acidic flavor and is one of the reasons behind the sourness of some cheese types (Iwasawa, Suzuki-Iwashima, Iida, & Shiota, 2014). Examples of commonly used starter bacteria are *Lactobacillus* spp. which are used in most cases as they have the ability to produce acid, *Lactococcus lactis, L. cremoris* both used to make Feta, Dutch and Blue cheese, *Streptococcus thermophilus*, which is used in

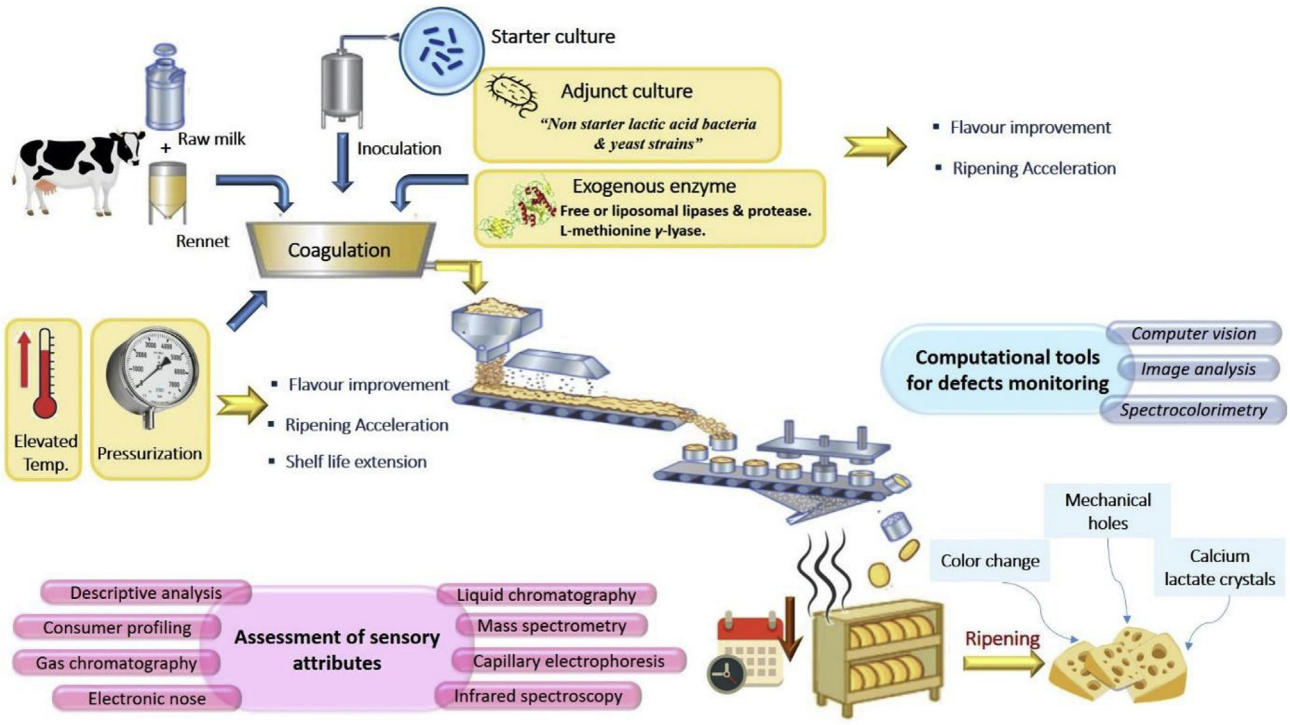

Fig. 1. Illustrative sketch of the cheese ripening process and the points of technological interventions for accelerating the process and enhancing flavor, in addition to the different quality assessment tools discussed all over the review.

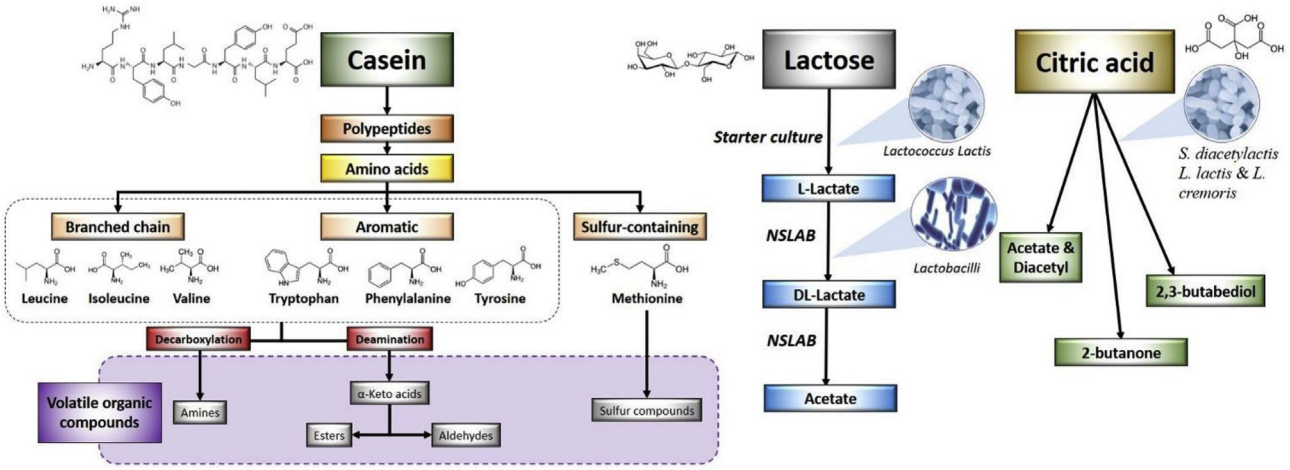

Fig. 2. Glycolytic and proteolytic biochemical reactions involved in the development of cheese flavor.
The dotted box outlines the produced volatile organic compounds; NSLAB "Non-starter lactic acid bacteria".

Table 1
Summary of cheese types and their associated microbial flora.

	Primary starters	Adjunct starters	Sensory attributes
Mold Ripened			
Brie Camembert	*Lactobacillus spp.* *Streptococcus cremoris*	*Penicillium camemberti* *Geotrichum candidum*	Mushroomy, soft
Roqueforti	*Streptococcus cremoris*	*Penicillium roqueforti*	Pepery
Surface Ripened			
Limburger Tilsit	*Streptococcus cremoris* *Arthrobacter, Corynebacterium, Brevibacterium linens*		Strong odor
Internally Ripened			
Pasta filata			
Mozzarella	*Streptococcus cremoris*	–	delicate
Provolone	*Streptococcus thermophilus*	–	Smoky
High Salt			
Feta Cheese with Eyes	*Lactococcus lactis*	*Enterococcus faecium*	Light color
Dutch (Gouda, Edam)	*Lactococcus lactis* *Lactoccous cremoris*	*Clostridium tyrobutyricum*	
Swiss (Emmental, Gruyere)	*Streptococcus thermophilus*	*Propionibacterium freudenreuchii* *Lactobacilluscasei*	Nutty
Semi-hard			
Monterey Jack	–	–	–
Hard			
Cheddar	*Streptococcus cremoris* *Streptococcus thermophilus*	*Clostridium tyrobutyricum*	Sharp
Extra hard			
Asiago	*Streptococcus thermophilus*		Fruity (melon)

Swiss, Mozzarella, Cheddar and Emmental cheeses and finally *S. cremoris* which can be used to make Cream, Brie, Camembert, Limburger, Blue and Cheddar cheeses (Clark et al., 2009). Table 1 lists the different cheese types and their associated microflora. Other strains were disregarded as they may produce an intensely bitter taste. It has also been reported that certain strains of *Latococcus* can absorb glutathione, which contains sulphur; an important aspect of cheese flavor (Patrick Fox Fox, Guinee, Cogan, & McSweeney, 2017).

A study by Menéndez, Centeno, Godínez, & Rodríguez-Otero, 2000 reported the effect of some *Lactobacillii* and *Lactococci* strains inclusion isolated from raw-milk Arzúa-Ulloa cheeses in starter cultures of Arzua-Ulloa on improving acidification during ripening, increasing diacetyl–acetoin levels responsible for buttery aroma and enhancing the desirable spicy aroma (Menéndez et al., 2000).

Secondly, adjunct bacterial cultures and NSLAB are the main contributors to the ripening process by which most of the cheese flavor develops. The variety in cheese flavor and aroma across the different types arises from the incorporation of different secondary probiotics in the production process (Andiç, Tunçtürk, & Boran, 2014). Cheese types are actually classified according to their ripening method (Fig. 3). Mould-ripened cheese includes Brie, Camembert and Roqueforti and surface-ripened cheese include Limburger and Tilsit. Internally-ripened cheeses are split into six categories: pasta filata that include Mozzarella and Provolone, high salt cheese like Feta, cheese with eyes like Dutch types (Gouda and Edam) and Swiss types (Emmental and Gruyere), semi-hard cheese like Monterey Jack, hard cheese like Cheddar and extra-hard cheese like Parmesan and Asiago (McSweeney, 2011).

The residual citrate in the curd is metabolized by some citrate-positive LAB *i.e. S. diacetylactis* and *L. lactis* and *L. cremoris* into several flavor compounds *viz.* acetoin, acetate, diacetyl, 2-butanone and 2,3-butanediol, to which eye formation in Dutch cheeses (Table 1) and Cottage cheese is attributed (Hassan et al., 2013).

2.2. Proteolysis and metabolism of amino acids and sulfur compounds

Proteolysis is the process responsible for metabolism of different caseins, *i.e.* αs1, αs2-, β- and κ-casein, present in bovine milk into smaller peptides and free amino acids by the milk endogenous proteinases and other proteolytic enzymes of LAB (starter cultures) (Gan, Yan, Linforth, & Fisk, 2016). Chymosin in residual coagulant initiates milk clotting by cleaving casein into short hydrophobic bitter peptides. The process of metabolizing casein induces textural changes of the cheese from rubbery tough curd to a creamy smooth surface (Clark et al., 2009). Proteinases and peptidases catalyze the cleavage of the polypeptides chains to produce free amino acids which undergo several biochemical reactions that result in flavor compounds (McSweeney, Ottogalli, & Fox, 2004).

Aminotransferases convert free amino acids into their corresponding *α*-keto acids that undergo further degradation *via* different pathways to produce volatile organic compounds (VOCs). These different pathways and the resultant compounds are displayed in Fig. 2. Branched chain amino acids such as leucine (Leu), isoleucine (Ile) and valine (Val) can be decarboxylated into amines, *i.e.* ketoisocaproate, *α*-keto-L-methyl valerate and *α*-keto isovalerate, respectively, which possess strong unpleasant aromas (McSweeney et al., 2004). Microflora

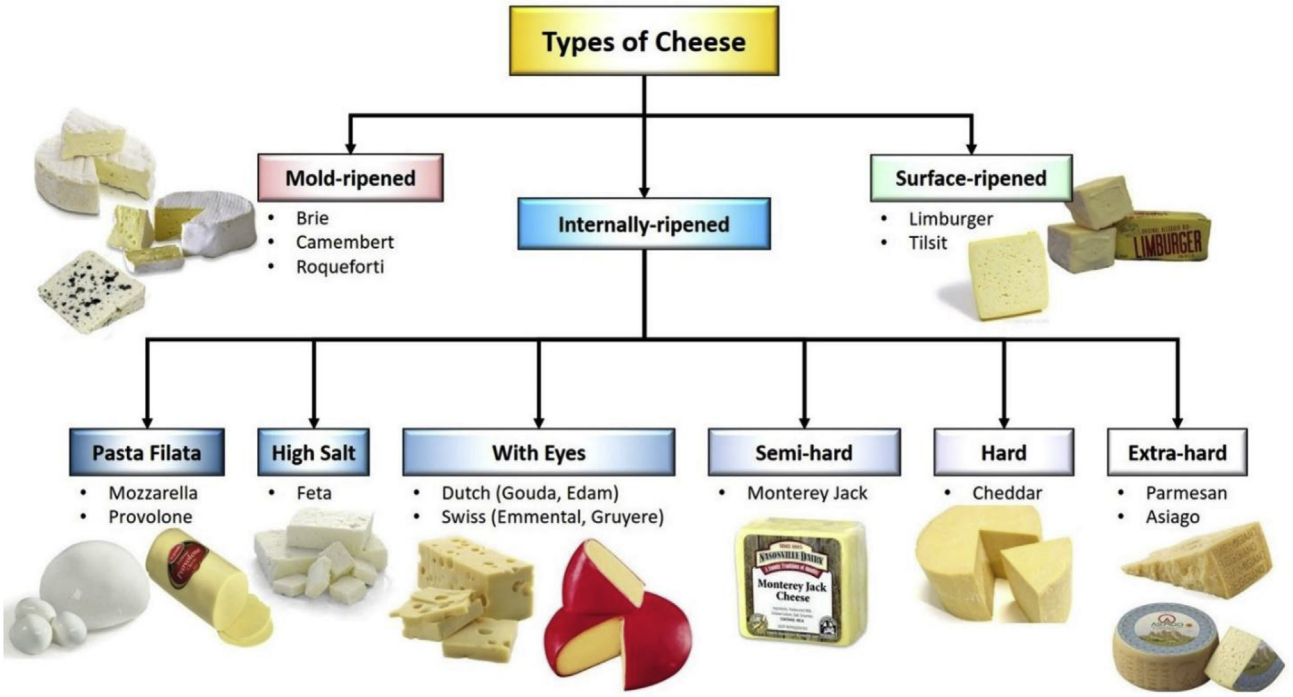

Fig. 3. Cheese classification based on ripening process.

that have decarboxylation activity are *Enterobacteria*, *Pseudomonas* spp. *Enterococci* and lactic acid bacteria (Stratton, Hutkins, & Taylor, 1991). Branched chain amino acids could also undergo deamination and result in carboxylic acids and ammonia. The deamination could be catalyzed by dehydrogenases and NAD^+ would be the electron acceptor, as in Swiss cheese. In Camembert and surface ripened cheeses, oxidases catalyze deamination and oxygen acts as the electron acceptor (McSweeney, 2011). Carboxylic acids produced include butyric acid, which has a rancid flavor and pentanoic acid which has a fatty flavor (Gan et al., 2016).

Aromatic amino acids are also converted into their respective α-keto acids and are further degraded. For example, tryptophan (Trp) becomes indole-3-pyruvate which is converted into acetate. Tyrosine (Tyr) is converted to hydroxybenzaldehyde and phenylalanine (Phe) is converted to benzaldehyde which exhibits a bitter almond flavor (McSweeney et al., 2004; Smit, Smit, & Engels, 2005).

Casein contains small amounts of sulfur containing amino acids such as methionine (Met) which is employed for the catabolism in milk and cheese ripening (Burbank & Qian, 2008). Met acts as a precursor for other potent odorants *viz.* sulfur-containing compounds like methional (cooked potato/meat like aroma), methanethiol (rancidity) and dimethyl sulfide (garlic) (Smit et al., 2005). On another note, when amino acids are converted into α-keto acids, the amine groups are converted into α-ketoglutarate and a new amino acid is produced, customarily glutamic acid (McSweeney et al., 2004), the later is responsible for the umami flavor in cheese (Iwasawa et al., 2014).

In addition to producing flavor compounds, proteolysis results in the formation of low molecular weight nitrogenous compounds called biogenic amines (BA) produced *via* amino acid decarboxylation. Examples of BA are histamine, tyramine and putrescine. From the names, it can be deduced that they are responsible for histamine poisoning and tyramine toxicity that may occur after cheese ingestion. Ingestion of cheese with high levels of tyramine leads to hypertension that is referred to as the "cheese reaction" (Stratton et al., 1991). Monitoring levels of these biogenic amines is warranted to ensure cheese safety (Staruszkiewicz & Bond, 1981).

Several factors affect proteolysis and the flavor compounds generation as outlined which would account for difference in cheese aroma from different manufacturers.

a) Ripening period affects the degree of proteolysis. As storage time of cheese increases, so does the degree of proteolysis. Texture of cheese becomes brittle rather than ductile over time (Joyner (Melito), Francis, Luzzi, & Johnson, 2018). An increased ripening period is also associated with development of bitter taste due to an increase in the formation of bitter peptides, which are mostly derived from 84–89 and 193–209 of the C-terminal region of β-casein (Karametsi, Kokkinidou, Ronningen, & Peterson, 2014) (Lemieux & Simard, 2007). The degree of bitterness varies according to the ripening period. For example, white-brined cheese is preferred to ripen for less than 90 days to prevent bitterness (Sahingil, Hayaloglu, Simsek, & Ozer, 2014).
b) The degree of proteolysis also varies according to cheese types. Mozzarella cheese undergoes limited proteolysis, Cheddar and Gouda undergo moderate proteolysis whereas, Blue cheeses need to undergo extensive proteolysis (Clark et al., 2009). Such proteolysis degree differences are reflected in cheese bitterness level. Since Blue cheeses undergo extensive proteolysis, they have sharp, bitter and moldy flavors, and pungent smells. The addition of adjunct cultures increases proteolysis and the amounts of free amino acids in cheese (Andiç et al., 2014).
c) Packaging also affects volatile compound formation. If vacuum packaging is utilized, there will be less production of ammonia and decreasing degrees of proteolysis in general (Duval et al., 2018).

2.3. Lipolysis and metabolism of fatty acids

Milk triglycerides are hydrolyzed by bacterial and endogenous milk enzymes into fatty acids with short- and intermediate-chains and free fatty acids *via* lipolysis (Fig. 4) (Collins, McSweeney, & Wilkinson, 2003). Short-chain fatty acids play a significant role in flavor development, but intensive lipolysis might be undesirable in some cheese varieties because of the rancidity development as observed in Cheddar, Gouda and Swiss cheese (Forde & Fitzgerald, 2000). However, the

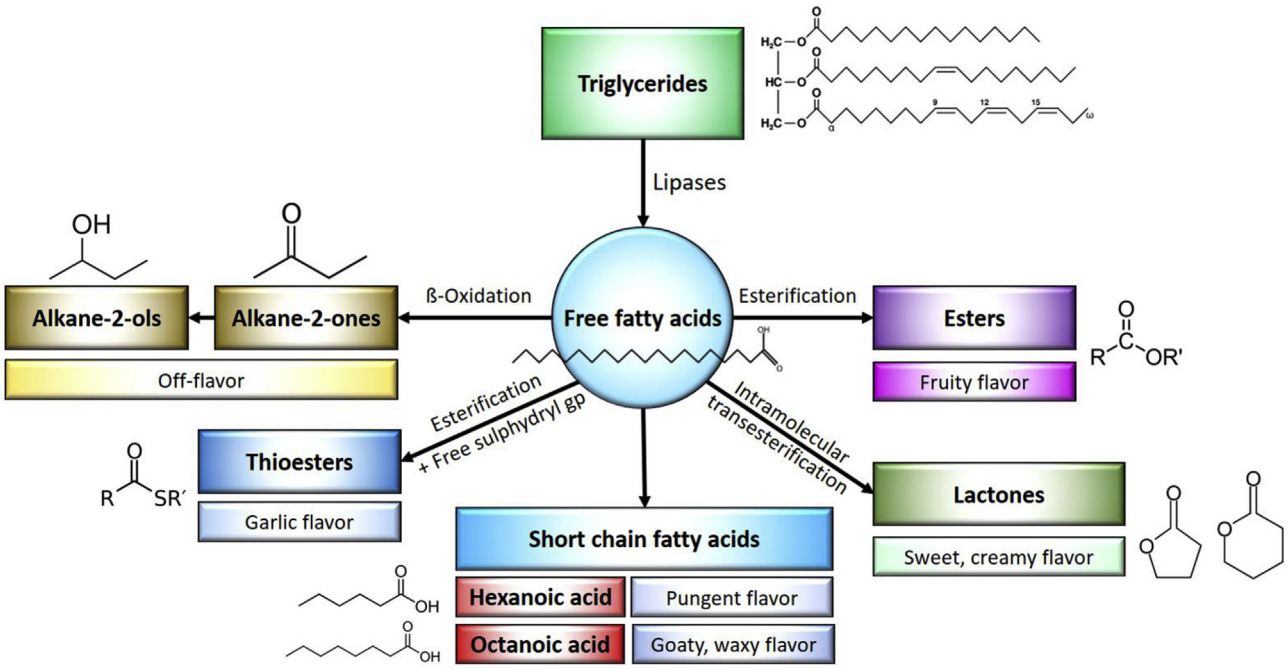

Fig. 4. Lipolytic biochemical changes leading to the development of cheese flavor and off-flavor.

extensive lipolysis is favored in Blue cheese, Emmental, Parmesan cheeses and Italian cheeses such as Romano and Provolone (Clark et al., 2009).

Microflora that provide lipolytic enzymes are *Propionibacterium freudenreichii, Geotrichum candidum* and *Pencillium* spp. which are most common in mold-ripened cheese (Table 1) for which extensive lipolysis is desired (McSweeney, 2011).

In lipolysis, enzymes cleave the ester linkages between fatty acids and glycerol in the triacylglycerides. Esterases hydrolyze short acyl ester chains (C_2-C_8), while lipases hydrolyze the longer acyl ester chains which are comprised of more than 10 carbons (Collins et al., 2003).

Several short chain fatty acids *i.e.* hexanoic, octanoic and decanoic acids are reported to contribute to cheese flavor and aroma. Hexanoic acid is responsible for sweaty, pungent and rancid flavors (Clark et al., 2009). Octanoic acid has a goaty waxy flavor and decanoic acid imparts fatty, citrus odors (Gan et al., 2016), whereas, dodecanoic acid imparts fatty odors (Clark et al., 2009).

Free fatty acids are the end products of the second pathway in lipolysis, which are found to be precursors to a plethora of VOCs that directly contribute to cheese flavor due to their low flavor threshold. The reaction of free fatty acids with alcohols yields esters. The most prevalent alcohol is usually ethanol, and thus ethyl esters, *i.e.* ethyl butanoate, ethyl hexanoate, ethyl acetate, ethyl octanoate, ethyl decanoate, and methyl hexanoate, are dominant in cheese. Ethanol sources are amino acid catabolism or lactose fermentation (McSweeney, 2011). The microflora responsible for ester formation are *G. candidum* and *Pseudomas fragi*, which provides a distinct melon odor (Collins et al., 2003). The reaction of free fatty acids with sulfhydryl groups like methional produces thioesters *i.e.* S-methyl thioacetate, thioethyl-2-methylpropanoate and S-methyl thiobutyrate which impart garlic and sulfur or eggy flavors (Iwasawa et al., 2014). This reaction is enhanced by *Brevibacterium linens* and *Micrococaceae* (Collins et al., 2003). Intramolecular trans-esterification of hydroxyacids yields lactones, *i.e.* nonalactone, which are responsible for sweet, creamy or buttery flavors (Gan et al., 2016; McSweeney et al., 2004). Lastly, β-oxidation and subsequent decarboxylation of free fatty acids produces methyl ketones or alkan-2-ones, especially heptanone and nonanone. This reaction is caused by *G. candidum, P. camemberti* and *P. roqueforti* (McSweeney et al., 2004). The mouldy flavor of blue cheese is due to alkan-2-ones (Hassan et al., 2013). It should be noted that these esters can also be reduced to their respective secondary alcohols causing off-flavors (Clark et al., 2009). One factor that could affect the volatile compounds produced is the milk fat levels. Low and full fat Cheddar showed a differential flavor pattern. It was reported that Feta cheese made with low-fat milk exhibited a higher concentration of aldehydes than Feta with full fat milk. Conversely, full fat Feta encompasses a higher concentration of alcohols, ketones and esters (Andiç et al., 2014). It was also reported that low-fat Feta had less flavor intensity than full fat Feta. Katsari et al. attempted to improve the sensory attributes of low-fat Feta by adding commercial adjunct lactose negative culture CR-213 which consists of *L. lactis* and *L. cremoris* (Table 1). The results revealed that low-fat Feta made with the adjunct culture exhibited similar flavors to full fat Feta but it had a significantly lower overall quality (Katsiari, Voutsinas, Kondyli, & Alichanidis, 2002). Lipolysis plays an important role in cheese ripening, especially in blue cheese varieties as it results in the formation of free fatty acids (FFA), which acts as precursors of flavor compounds such as methylketones, alcohols and lactones (Smit, Johan, Smit, Ayad, & Engels, 2002). In addition, additives can also affect lipolysis, the levels of free fatty acids produced and the subsequent resulting compounds. Buttermilk is a low value side product of butter production that has comparative composition with skim milk. Buttermilk powder (BMP) is essentially dehydrated buttermilk. Both were investigated as additives to Cheddar cheese. When buttermilk was added, the produced cheese was softer and had higher free fatty acid levels. It also had negative sensory attributes like sweaty, sour, oxidized and overall off-flavors. Analysis of the volatile compounds revealed that the cheese was composed of several compounds that are associated with rancid and sweaty aromas. When BMP was used as an additive, the produced cheese had similar texture to control cheese and a lower concentration of free fatty acids. That study revealed that BMP is a more favorable additive than just buttermilk (Hickey et al., 2018). Lastly, packaging could also affect texture and fatty acid content of cheese. A study was conducted to compare aluminum foil and vacuum packaging. Vacuum packaging, which prevented oxidation and release of CO_2 and H_2O, led to the development of more fatty esters as a result of the availability of alcohols, fatty acid substrates and a moist environment (Duval et al., 2018). The influence on packaging material on cheese aroma is a topic that has yet to be thoroughly reviewed.

3. Novel biotechnological practices for ripening acceleration and flavor improvement

Cheese ripening is a long intricate process which may be ongoing for over two years. The duration and conditions of this process has a significant impact on the development and improvement of cheese flavor. The characteristic flavor to each cheese variety is expressed by a balanced complex blend of several compounds that are mainly produced through protein and lipid metabolism, in addition to numerous volatile compounds formed as a result of amino acid and fatty acid degradation.

A lot of advancements have been witnessed in the biotechnological practices employed for modifying cheese flavor as well as accelerating the ripening process (Bart Weimer et al., 1999). These practices include co-inclusion of adjunct cultures or exogenous enzymes, and elevated temperature and high pressurization of cheese milk (depicted in Fig. 1) to be discussed over the next sections.

3.1. Inoculation of adjunct cultures

3.1.1. Lactococci and Lactobacillii strains

Flavor improvement was classically improved by inoculating the primary starter culture with bacteria found in high-quality aged cheese (Forde & Fitzgerald, 2000). Lynch, McSweeney, Fox, Cogan, and Drinan (1997) showed that the co-addition of adjunct *Lactobacilli* in cheese manufacturing resulted in a much better flavor intensity and higher free amino acid content in comparison to control cheeses, which is attributed to the increased proteolysis caused by the nonstarter cultures growth (Lynch et al., 1997). Both *Lactobacillii* and *Lactococci* strains are employed in accelerating cheese ripening and improving the organoleptic characteristics of cheese (Lee et al., 1990).

Flavor imparting potential of wild *Lactococci* strains was assessed against their counterparts commonly used as industrial cultures with the wild strains proved to produce unique flavor profile with abundant primary alcohols and branched aldehydes (Ayad, Verheul, De Jong, Wouters, & Smit, 1999). This was attributed to their unique endogenous amino acid convertase enzymes which made them more tolerable to grow on media supplemented with less amino acid content than those supplied to other industrially used strains. Mixed adjunct culture containing *Lc. lactis* subsp. *lactis*, *Lb. casei* subsp. *casei*, *Lb. plantarum*, *Leuconostoc. mesenteroides* subsp. *dextranicum* and *Ln. paramesenteroides* provided semi-hard goat's milk cheese with better aroma and flavor (Rodríguez, Requena, Goudédranche, Maubois, & Juárez, 1996). Consequently, these strains provide good technological opportunity to develop new cheeses and/or flavors type (Ayad et al., 1999).

Adjunct culture was employed as a promising approach to improve the flavor and texture of low-fat cheeses. The sensory quality of low-fat Kefalograviera-type cheese was improved and showed similarity to that of full-fat cheese upon the addition of commercial adjunct cultures namely LBC 80 (*Lactobacillus casei* subsp. *Rhamnosus*) and CR-213 (*Lactococcus lactic* subsp. *Cremoris* and *L. lactis* subsp. *Lactis*). However, the low-fat cheese recorded significantly lower body and texture scores than those of the full-fat cheese (Katsiari, Voutsinas, & Kondyli, 2002).

The addition of *Lb. reuteri* and *Lb. helveticus* as adjunct cultures to reduced fat Edam cheese has enhanced texture quality of cheeses. However, the addition of *Lb. helveticus* and *Lc. Lactis* ssp. *diacetylactis* increased proteolysis (*i.e.* possessed the highest aminopeptidase activity) leading to flavor improvement between 3 and 6 months ripening period. Reduced fat cheeses containing *Lb. helveticus* exhibited the highest free fatty acids content (Tungjaroenchai, Drake, & White, 2001). Cheeses containing *Lb. helveticus* and *Lb. reuteri* exhibited increased texture quality compared with full fat control cheese. No synergistic effect of using multiple adjuncts in reduced fat cheese was observed. The use of mixed adjuncts resulted in a decreased texture quality and did not enhance the proteolytic activity compared with the use of single adjunct. During cheese ripening, numbers of starter cells decreases due to cell death and hence, autolysis occur releasing intracellular peptidases that is responsible for peptides hydrolysis. It is quite evident that adjunct cultures have an inconsistent effect on cheese flavor during ripening. Accordingly, autolysis has a key role in cheese ripening that led to flavor enhancement to occur (Tungjaroenchai et al., 2001).

Attenuated adjunct cultures have received a considerable interest in their ability to accelerate and improve flavor development in a controlled manner in particularly low fat cheeses. Attenuation of bacterial strains causes autolysis leading to the release of bacterial intracellular enzymes into the cheese matrix leading to considerable flavor development. Attenuated *Lactococcus* and *Lactobacillus* genera were reported to be co-added with the primary starter culture in cheese manufacturing, found effective in enhancing proteolysis and lipolysis, as well as accelerating ripening time and improving flavor (Klein & Lortal, 1999).

In another study, three different attenuation procedures, *i.e.* freeze shocking, heat shocking and spray drying, of two Lactobacilli strains *i.e. Lactobacillus helveticus* and *Lactobacillus casei* were experimented as adjunct cultures in Cheddar cheese curd with *Lactococcus* starter (Madkor, Tong, & El Soda, 2000). The greatest flavor enhancement was observed with freeze shocked *Lb. helveticus,* among other cultures or attenuated treatments, that is attributed to its higher rate of cell autolysis that lead to considerable level of proteolytic activity (Madkor et al., 2000).

3.1.2. Brevibacterium strain

Another strain, *Brevibacterium linens* BL2 was successfully employed as an adjunct culture to improve the flavor of 60% reduced fat Cheddar, due to its inherent catalytic potential of methionine-γ-lyase which is more efficient than cystathionine enzymes. The *B. linens* BL2 enzymatic system was capable of performing direct deamination and decarboxylation of methionine producing methanethiol (B. Weimer et al., 1997).

The inclusion of *Brevibacterium linens* BL2 to aseptic cheddar cheese slurry system containing *Lactococcus lactis* ssp. *cremoris* S2 (starter culture) resulted in an enhanced production of the volatile sulfur compound “methanethiol” which is responsible for the distinctive flavor of good quality Cheddar (Dias & Weimer, 1999).

3.1.3. Yeast

Manufacturing of hard cheese varieties such as Cheddar is a costly process as it involves a long ripening that is essential to develop products with optimum flavor and texture. The rate and extent of inherent proteolysis has a significant contribution to flavor development (Raksakulthai, Rosenberg, & Haard, 2002). Accordingly, many endeavors in shortening ripening period were reported to minimize manufacturing costs (Garde, Tomillo, Gaya, Medina, & Nunez, 2002). Co-addition of certain yeast species as adjunct culture contributed to both flavor and texture development during the ripening of some cheese types because of their lipolytic and proteolytic activity that leads to shortening the ripening time and thus providing an economic value in cheese manufacturing (Roostita & Fleet, 1996).

Debaryomyces hansenii and *Yarrowia lipolytica* are well known for their proteolytic and lipolytic activity (Roostita & Fleet, 1996) (Fleet & Mian, 1987)as well as being biocompatible with lactic acid starter cultures (Laubsher & Viljoen, 1999). *D. hansenii* and *Y. lipolytica* have been used in accelerating the ripening process of Cheddar cheese and enhancing its flavor characteristics (Ferreira & Viljoen, 2003) (De Wit, Osthoff, Viljoen, & Hugo, 2005).

The mould *Penicillium roqueforti* promotes ripening and contributes to the aroma development in blue cheese (Table 1), which has *Yarrowia lipolytica* and *Kluyveromyces lactis* as uncontrolled secondary flora growing during production of blue cheese, leading to variable aroma profiles (Viljoen, Knox, De Jager, & Lourens-Hattingh, 2003; Cantor, van den Tempel, Hansen, & Ardö, 2017). That is why, the effect of different inoculum concentrations of both strains on aroma production of Stilton cheese, British Protected Designation of Origin (PDO) Blue

cheese, containing *P. roqueforti* was evaluated using solid-phase microextraction (SPME GC–MS) and sensory analysis. In this study, low *K. lactis* inoculum concentrations proved to produce blue cheese-related attributes with an increased ketone production (Price et al., 2014).

Controlling *Y. lipolytica* and *K. lactis* culture count during cheese production and ripening provide the cheese-makers with a cost effective approach to produce Stilton cheese with consistent sensory attributes (Price et al., 2014). *Y. lipolytica* strains were proposed to be more favored as an adjunct yeast due to its ability to overcome the other naturally occurring yeasts and compatibility with lactic acid bacteria (LAB). Increased production of different free fatty acids and enhanced lipolysis was revealed using fourier transform infrared (FTIR) profiles and fatty acid analysis.

These yeast strains are more advantageous in their tolerance to high salt concentration and low temperature, lactate and citrate assimilation and production of extracellular proteolytic and lipolytic enzymes. As concluded from this study, FTIR offered a non-destructive tool to monitor compositional and biochemical changes during cheese ripening (Lanciotti, Vannini, Chaves Lopez, Gobbetti, & Guerzoni, 2005). Non-destructive techniques are much favored in food analysis considering the possible recovery of specimens for further analysis.

3.2. Exogenous enzymes

Some starter strains are not able to produce the optimal flavor and taste in the ripened cheese. Thus, exogenous enzymes were thought to speed up cheese ripening and aid in producing characteristic flavors in some cheese varieties. Few examples of different enzymes or their mixtures were reported in that respect and are presented herein for each type.

3.2.1. Lipases

Lipases are among the experimented enzymes mostly used for the production of cheese with enhanced flavor profiles. They are capable of hydrolyzing triglycerides to mono and diglycerides, glycerol and free fatty acids (Fig. 4). They are obtained from various resources such as plants, animals, and microbes. Among the different sources, only the microbial sources (yeast, *Micrococci* and *Lactobacilli*) are of commercial significance owing to its ease in production (Sharma, Chisti, & Banerjee, 2001). They are especially employed in manufacturing cheeses from cows' milk rather than ewe or goat milk. The addition of commercial microbial lipase (Piccantase A) to Tulum cheese has resulted in the increase in volatile free fatty acids concurrent with an increased microbial lipase level. This has led to the shortening of ripening time without any flavor loss (Yilmaz, Ayar, & Akin, 2005). Microbial lipase at a concentration of 200U reduced the ripening time of Swiss cheese by 1 month with maintaining its genuine quality. Above such level, no significant change in quality and sensory properties was achieved (Rani, Sapna, 2018).

Addition of lipases to cow milk during ripening of white pickled cheese led to the production of increased levels of fatty acids with short chains (C_4 to C_8) from triglycerides, which are partially esterified by ethanol and improved the cheese sensory properties *i.e.* appearance, flavor and texture (Akın, Aydemir, Koçak, & Yıldız, 2003). The use of other lipase products was reported in Feta cheese production to improve the desired mild rancid flavor of Feta cheese due to the increased production of free C_2 fatty acids *i.e.* butyric, caproic and caprylic acid, whereas, free fatty acids above C_{10} having rancid flavor were minimally produced (Efthymiou & Mattick, 1964).

As different types of enzymes (lipases and proteases) normally develop the characteristic aroma, flavor and texture of a cheese, it is advisable to use mixture of both enzymes in order to accelerate ripening and not to disturb the flavor component equilibrium that might cause flavor defects. Asides, the direct addition of free proteolytic and lipolytic enzymes to the cheese milk was reported to suffer from some drawbacks *viz.* premature attack leading to excessive lipolysis accompanied by texture and flavor defects, enzymes loss in the whey or poor enzyme distribution (El Soda, 1993).

3.2.1.1. Liposome encapsulated lipase and/or protease. The use of encapsulated enzymes has been proposed to regulate enzyme/substrate reactions and thereby avoiding the drawbacks of free enzymes. Enzyme microcapsules physically separate the enzyme from the substrate in the curd, with the enzyme being only released into the curd upon capsule breakdown during ripening. And thus, the enzyme cocktail generates a well-balanced equilibrium between casein and milk fat hydrolysis products without producing bitter, rancid or texture defects (El Soda, 1993). Addition of liposome encapsulated lipases (Palatase M and Lipase 50) at levels up to 0.5 unit/g milk fat to the cheese milk accelerated the lipolysis in cheddar cheese without facing texture or flavor defects. However, a pronounced soapy off-flavor was produced after 2 and 3 months of ripening upon increasing the level of Palatase M and Lipase 50, respectively (E. E. Kheadr, Vuillemard, & El-Deeb, 2002).

Accelerated cheddar cheese ripening was also reported by the addition of liposome-encapsulated enzymatic cocktails to cheese milk, *i.e.* flavorzyme, neutral bacterial protease, acid fungal protease and lipase (Palatase M), each was individually entrapped in liposomes and added prior to renneting. Among the experimented cocktails, a higher flavor intensity was attained in a shorter time in cheese fortified with a mixture of lipase and bacterial protease. Cheeses with added lipase and bacterial protease has the superior cheddar flavor characteristics after 2 months of ripening and with no off-flavor when extending the ripening for a third month (Ehab E. Kheadr, Vuillemard, & El-Deeb, 2003).

It is well known that hard cheeses require longer period of ripening than cheeses with higher moisture content that is why most of the endeavors to accelerate the ripening were conducted on hard cheeses. The ripening of Feta, a soft white brine cheese, takes 3 months, however, consumers prefer older Feta cheese, hence, decreasing its ripening time was sought. The best organoleptic properties were obtained in heat-shocked culture treated cheeses followed by those treated with neutral or acid proteinase in almost halved the normal ripening time. However, adding a minute amount of lamb lipase (0-1-0-2 g/100 kg milk) to the cheese milk was found necessary to keep a balanced flavor (Vafopoulou, Alichanidis, & Zerfiridis, 1989).

3.2.1.2. Design of enzyme-modified cheese flavors. Another economic tools employed for preparing cheese with adjusted as well as constant flavor is the production of enzyme-modified cheese (EMC). The EMC flavors are designed to be a complementary natural combination of proteolytic and lipolytic enzymes added to cheese emulsions as natural substrates. These flavors are prepared not only to be used in cheese but also to be incorporated in any food to which cheesy note is required to be imparted (Dirinck & De Winne, 1999; Wilkinson, Doolan, & Kilcawley, 2011).

The desired overall flavor and quality attributes of different cheese varieties are specifically dependent on its types, for which GC–MS analyses was used to decipher and monitor the flavor profiles characteristic to each cheese variety (Dirinck & De Winne, 1999).

3.2.2. L-methionine γ-lyase

Other than lipases, the addition of the purified *B. Linens* BL2 L-methionine γ-lyase (MGL) to cheddar cheese slurries made with *L. lactis* ssp. *cremoris* S2 with added methionine was reported to significantly increase the production of the flavor compound "methanethiol" (Dias & Weimer, 1999).

3.3. Elevated temperature and high pressurization

The growing demand for safe fresh products with a longer shelf-life, is influencing the development of industries that process food. This can be physically attained by manipulating temperature and/or pressure.

Thermal treatment was traditionally employed for its potential to deactivate microorganisms but it causes a loss in nutrients and flavor. That is why a considerable interest was shifted to high pressurization (HP) treatment that offers a competitive advantage over thermal treatment in extending the shelf-life of food without altering its nutritional and sensory quality. HP is reported to accelerate cheese ripening by controlling enzymatic activities, improving rennet or acid coagulation of milk and increasing cheese yield. Combining both treatments offers a synergistic effect on acceleration of cheese ripening and decrease in microbial spoilage (Trujillo et al., 2000).

Elevated temperature (16 °C) was reported to enhance the ripening of Dutch-type cheese, however, strict hygienic measures have to be followed in order to counteract the quick growth of LAB, NSLAB and other contaminating microorganisms that might deteriorate the cheese quality or cause intoxication or infection (Sihufe, Zorrilla, Perotti, et al., 2010). Increasing ripening temperature of Reggianito Argentino cheese to 6 °C resulted in doubling the ripening rate and free amino acid contents with no off-flavor (Sihufe, Zorrilla, Sabbag, Costa, & Rubiolo, 2010).

The elevated ripening temperature increases decarboxylases activity of LAB leading to higher yield of biogenic amines, such as tyramine, putrescine and cadaverine. Production of these compounds may pose a negative health impact on cheese consumers sensitive to them (Stratton et al., 1991).

Several studies have reported the use of HP as a technological tool for moisture homogenization in cheese blocks, inactivation of pathogenic microbes in ripened cheese and acceleration of ripening (Patterson, 2005; Trujillo et al., 2000).

Cheese production is initiated *via* milk coagulation by chymosin action on the stabilizing layer of the casein micelle (Everett & Auty, 2008). The indigenous milk microbiota was reported to be modified upon applying high pressure to cheese milk leading to alteration in cheese matrix, yield and sensory attributes (M. A. Drake, Harrison, Asplund, Barbosa-Canovas, & Swanson, 1997). HP milk processing at room temperature causes several protein modifications, such as whey protein denaturation, fragmentation of casein micelles, which are also affected by the temperature, pH, and processing time, and storage conditions (Huppertz, Fox, & Kelly, 2004). These changes were reported to improve the rennet coagulation and cheese yield which became more pronounced at pressure values between 300 and 800 MPa, accompanied by an improved growth and survival of probiotic bacteria in the cheese (Huppertz et al., 2004). The viability of these bacteria was reported to be negatively affected by the stressful ripening environmental conditions *i.e.* decreased water activity and pH which does not occur when HP is applied to cheese milk (O'Reilly, Kelly, Murphy, & Beresford, 2001). Hence, HP contributes, together with the adjunct probiotic cultures, to the modification of the products sensory characters (Trujillo, Royo, Guamis, & Ferragut, 1999).

High pressure treatment (100 MPa) of milk used for the manufacture of Crescenza, very popular soft Italian cheese, with commercial probiotic *Lactobacilli* (*Lactobacillus paracasei* A13 and *L. acidophilus* H5) resulted in a 1% cheese yield increase and with a positive influence on the viability of the probiotic culture (Burns et al., 2008). This hyperbaric treatment had a significant positive effect on free fatty acids release and cheese proteolysis but with no significant changes recorded in its sensory characters and overall acceptance than those of conventional Crescenza cheese (Burns et al., 2008).

In case of Cheddar cheese ripening, a ripening period from 4 to 12 months or even more, depending on its grade, is necessary, and this shall substantially increase its cost because of the required storage facilities under controlled conditions (P. F. Fox et al., 1996). Application of high pressure 50 MPa for 3 days at 25 °C to cheese milk was reported to enhance the proteolysis which is attributed to the potential of high pressure treatment to increase the activity of intracellular peptidases of starter culture and thus accelerating cheese ripening (O'Reilly, O'Connor, Murphy, Kelly, & Beresford, 2000).

The highest degree of proteolysis in Camembert cheese was attained by pressurization of Camembert and Gouda cheese at 50 MPa, however no pronounced effect observed in Gouda cheese treated similarly (Kolakowski et al., 1998).

Since the primary step in casein (CN) proteolysis is the cleavage of α_{s1}-CN and its conversion to α_{s1}-I-CN by chymosin, the ratio of α_{s1}-CN/α_{s1}-I-CN was considered a good ripening indicator of young Gouda-type cheeses. The decrease in that ration indicates cheese ripening and that could be attained by many cheese treatments such as employing temperature 25 °C and pressure 50 MPa (Haasnoot, Stouten, & Venema, 1989). Elevated ripening temperatures was reported to accelerate the Cheddar cheese ripening (Folkertsma, Fox, & McSweeney, 1996).

Gram-positive bacteria are more resistant to HP effect than Gram-negative, however, molds and yeasts are very sensitive. The most resistant microbial form is the spores that can survive at pressures above 1000 MPa (Smelt, 1998). The utility of HP in extending the shelf life of cheese has been explored but in a limited number of publications, for examples, the shelf life Mató cheese was found to be longer when HP is applied due to its ability to inactivate the pathogenic microorganisms such as *Escherichia coli* (Marta Capellas, Mor-Mur, Sendra, Pla, & Guamis, 1996). HP was used to explore the optimal shelf life required for decreasing microbial counts in fresh goat's milk cheese (Mató) without affecting its organoleptic or physical features or consumer preference (M. Capellas, Mor-Mur, Sendra, & Guamis, 2001). In another study a higher pressure HP (300–600 MPa for 5 min) was observed to increase the shelf life of fresh lactic curd cheeses up to 8 weeks without detrimental effects to the product quality (Daryaei, Coventry, Versteeg, & Sherkat, 2006).

Levels of proteolysis in a hard caprine milk cheese were increased when subjected to HP treatment at 400 MPa for 5 min which was persistent throughout the ripening. The produced cheese encompassed a larger content of free amino acids due to the higher peptidase activity, which is an index of cheese maturity (Saldo, McSweeney, Sendra, Kelly, & Guamis, 2002).

4. Sensory analyses methodologies for the assessment of ripened cheese sensory attributes

Sensory characters undeniably influence food consumption worldwide. Urala & Leahteenmeaki posed the idea that flavor and texture affect the likelihood of a product's consumption (Urala & Lähteenmäki, 2004). Classified according to smell, taste and texture, cheese was also known to be a type of functional food. Widely used to exhibit positive effects on consumers, functional foods were tested on participants in an attempt to determine their relative likeability, thereby highlighting the importance of sensory characters on food intake. A study was conducted on many products, among which the low fat cheese, which showed a high willingness of consumption and was on the higher side of evaluated healthiness, confirming the assumption that sensory characters are also huge factors of overall acceptance of food products (Urala & Lähteenmäki, 2004). Several methods of analysis of cheese are reported to identify qualitative and quantitative properties of numerous cheeses and understanding how sensory characters play a huge role in the cheese quality (Foegeding, Brown, Drake, & Daubert, 2003). Of the many methodologies to evaluate sensory attributes of cheese, we can classify them into two broad categories; conventional sensory methodologies such as descriptive profiling analysis which is carried out by trained panelists, and other methodologies which are executed by untrained consumers namely check all that apply, acceptability testing, triangle test, projective mapping and flash profiling or napping.

4.1. Conventional sensory methodologies

Cheese sensory attributes, flavor, texture and appearance are indeed of critical significance in product acceptability and product success in the marketplace (Mary A Drake & Delahunty, 2017). Accordingly,

quantitative measurements reliably evaluating sensory attributes is important in food industry to rapidly respond to the changing demands of both consumers and the market (De Belie et al., 2003).

4.1.1. Quantitative descriptive analysis (QDA): panel testing

Descriptive analysis is a classical methodology applied to evaluate sensory attributes of the products, which are rated by trained panelists based on a consensual vocabulary known as lexicon. Quantitative Descriptive Analysis (QDA) has been applied to evaluate cheese sensory characteristics and their correlation with the volatile profile and the physico-chemical properties (Kraggerud, Skeie, Høy, Røkke, & Abrahamsen, 2008).

4.1.1.1. Flavor lexicon. A comprehensive sensory language or flavor lexicon was established for the descriptive analysis of each cheese type. The flavor lexicon represents a technical dictionary that is developed to describe the flavor attributes characteristic to each cheese type (M. A. Drake & Civille, 2003). The trained panelist uses this language to record product flavor or compare different products as well as extrapolate to consumer acceptability and chemical data of the product.

The following studies revealed the significant contribution of descriptive analysis in providing characteristic sensory descriptors for cheeses produced in different geographical localities, prepared with different ripening time or treated with different processing techniques or ripening conditions. A descriptive language for hard cheeses with variable ages obtained from different georegions and maturity levels was generated (D. D. Muir, Hunter, Banks, & Horne, 1995), found successful to differentiate between Comte cheese produced from different georegions was carried out using their respective flavor lexicon (Monnet, Berodier, & Badot, 2000).

A study by Piggott and Mowat (1991) on Cheddar cheese maturation has developed 23 descriptive flavor and aroma (Piggott & Mowat, 1991). Several studies reported characteristics flavor cheddar lexicons to study cheese aging (D Donald Muir & Hunter, 1992) (HEISSERER & IV, 1993), in addition to determining the effect of starter and adjunct cultures (D. D. Muir, Banks, & Hunter, 1996; M. A. Drake, Boylston, Spence, & Swanson, 1997).

The main sensory characteristics of ewes cheeses were evaluated using an established sensory lexicon. The development and selection of their sensory attributes was aided by two chemometrics models *viz.* stepwise discriminant analysis (SDA) and principal component analysis (PCA). The developed lexicon can be used for the characterization of the ewes milk and the standardization of its sensory analysis (Bárcenas, Pérez Elortondo, Salmerón, Albisu, & Bárcenas Eguia, 1999).

Owing to the absence of standard terminology in the literature that fully describes the flavor of French cheeses, a study by Rétiveau, Chambers, & Esteve, 2005, has developed a general lexicon containing 31 descriptive sensory attributes for a wide variety of French cheeses comprising terms, definitions, and references. These sensory descriptors facilitated both the definition and protection of the reputation of high quality traditional products from inferior ones, as well as encouraged the successful promotion of typical French cheeses based on their unique sensory attributes (Rétiveau et al., 2005).

Cross-cultural differences were observed in their sensory language and perception. That is why standardized, descriptive languages should be now established to provide comparable and consistent results. Cheddar cheeses selected from three different countries (Ireland, USA and New Zealand) were incorporated in a study by Drake et al., 2005, for standardizing the descriptive sensory analysis of cheese flavors to allow for the universal interpretation or reproduction of the analysis (M. A. Drake et al., 2005).

These methods nevertheless suffered from some limitations in being expensive, time-consuming and costly, requiring very well trained panelists to provide reproducible and consistent rating for the product attributes. There are some alternative rapid techniques developed which operate without trained panels such as projective mapping (napping) and flash profiling that will be discussed in details in the next section (Perrin et al., 2008).

4.1.2. Consumer profiling techniques

The limiting issue in food industries is the time required to train several and different panels, in accordance with the products to be analyzed. Therefore, another alternative methodology to the use of trained panelists, which is both time consuming and cost intensive is to obtain the sensory evaluation from typical consumers of the product (Worch, Lê, & Punter, 2010). Two common techniques were extensively employed for analysing consumer responses towards the sensory attributes of food products which are:

4.1.2.1. Check all that apply (CATA). In a study on goat cheese produced with *Lb. mucosae* using the CATA method consumers were told to check from a list of 14 attributes the ones that they believed to have the greatest influence on the quality of cheese (de Moraes, dos Santos, de Barcelos, Lopes, & do Egito, 2018). The results from the QDA by trained panelist were in agreement with the results of the CATA study on consumers, rendering saltiness an essential factor in the consumption of cheese. The produced cheese showed closest sensory characteristics to an ideal goat cheese as considered by the consumers, thus highlighting the strain capability to be further employed in Coalho goat cheese production (de Moraes et al., 2018).

4.1.2.2. Acceptability testing. The goat cheese containing *Lactobacillus mucosae* showed a greater sensory acceptability post ripening than the control cheese, which was due to the flavor compound “diacetyl (2,3-butanedione)” that is formed by *Lb. mucosae*. In the evaluation of acceptability, color, texture, aroma, flavor and overall impression were tested. While all of the previously stated factors were positively correlated to acceptability, texture was not. As storage time increased, the adhesiveness of the cheese was altered, though this did not affect product overall acceptance (de Moraes et al., 2018). The addition of *S. thermophilus* co-culture to *Lb. mucosae* produced cheese with greater consumer acceptance than the control cheese. These results confirmed that different types of microorganisms in cheese can notably alter its sensory attributes (de Moraes et al., 2018).

In another study, the acceptance test was done by preselecting consumers according to their interests in cheese to assess and compare the nutritional, textural and sensory characteristics of Coalho cheese made of goat's, cow's milk and their mixtures during 28 days of cold storage. They used a hedonic scale to evaluate the degree of their likeness of the vital sensory aspects of cheese such as odor, taste and texture and overall appreciation. This mixture cheese possessed a sensory acceptance with positive nutritional characteristics of goat's cheese, especially with respect to the fatty acids profile *i.e.* a reduction in short fatty and linoleic acids and a slight increase of palmitoleic acid (Queiroga et al., 2013).

4.1.2.3. Triangle test. In an attempt to point out minor differences in sensory attributes of different cheeses, a triangle test was conducted between full fat cheese, reduced fat cheese and low fat cheese during the ripening process. A large group of consumers were given 3 samples, of which two were identical. The three samples were administered to the consumers in all of the possible orders. They were then asked to identify the odd sample and explain which sensory character imparted for this difference. This test gave rise to the assumption that sensory characters are directly influenced by constituents of cheese; in the above case, they differed in fat content, rendering diverse flavors (Sánchez-Macías et al., 2010).

4.1.2.4. Projective mapping or napping (PM). PM is a sensory analysis that allows consumers to express similarities and differences as well as to group samples by positioning them on a two-dimensional surface using a piece of paper. The objective of this sensory technique is to

provide a way in which consumers can assess samples in a global and simple manner (Valentin, Chollet, Lelièvre, & Abdi, 2012).

The relationship between the consumer acceptability of Cheddar cheese and its descriptive sensory attributes *i.e.,* taste, aroma and textural attributes was determined using PM for three Cheddar cheeses. Aroma and texture of Cheddar cheese also change over ripening time, and although taste changes more readily than other sensory attributes. Neither texture nor aroma could be used to relate descriptive sensory analysis to consumer preference, but that taste attributes relate well to consumer preference. Logistic regression is a unique way to link explanatory variables to the discrete response variables. This regression method resembles multiple linear regression (MLR), however, the response is categorical/discrete rather than being continuous as in MLR (Caspia, Coggins, Schilling, Yoon, & White, 2006).

4.1.2.5. Flash profiling (FP). Flash profile analysis is a simple less time-consuming descriptive method in which relative sensory positioning of a set of products is done based on free-choice profiling of their sensory attributes accompanied by a direct comparison against a sample set, without panelist rating. These characteristics makes the method suitable to be widely applied in food industry. Despite being a new technique, a wide variety of products has been analyzed, among which dairy products (Delarue & Sieffermann, 2004).

The odour perception of microbiological models combining *Penicillium roqueforti* and *Yarrowia lipolytica* blue cheese isolates were compared with the odour of real blue cheeses using FP. Assessors ranked the samples according to their blue cheese odour intensity and discriminated the *P. roqueforti* and *Y. lipolytica* model from the cheese model containing only *P. roqueforti* (control). The synergy between the two strains is responsible for the production of blue cheese odour (Gkatzionis et al., 2013).

The cost and long duration required for panelist training and developing scales for the sensorial evaluation makes this methodology not feasible to be employed in the on-line or at-line analysis of food products in the industry (Data & Analysis, 2005). It is therefore imperative to apply faster and cheaper instrumental metabolites based analyses which is to be discussed in the next section, with emphasis on novel ones yet to be applied at an industrial scale.

5. Metabolites analysis methodologies combined with chemometrics for cheese sensory attributes evaluation during ripening

The study of foods metabolite composition is a multidisciplinary research that embraces analytical, biochemistry and bioinformatics in order to identify all the endogenous small molecules biosynthesized or modified in a particular bioecosystem. The workflow involves an efficient extraction of these endogenous metabolites to be subjected to chromatographic separation and mass spectroscopy for identification and/or quantification purposes. The derived complex metabolite rich dataset is then subjected to multivariate data analysis in order to decipher trends/pattern of metabolic changes. The commonly used multivariate data methods when no prior knowledge of sample groupings are hierarchical cluster analysis (HCA) and principal component analysis (PCA) which are unsupervised pattern recognition methods. However supervised pattern recognition is carried out by soft independent modeling of class analogy (SIMCA) and partial least square regression (OPLS) (Mohamed A. Farag, Khattab, Maamoun, Kropf, & Heiss, 2018).

Being a biocomplex food matrix containing thousands of small molecules (metabolites), cheese was a subject to different metabolite studies to reveal for the chemical diversity of its constituents and their correlation to the cheese quality and sensory attributes conferred from the activity of starter and adjunct cultures, added ingredients or enzymes, ripening conditions or production protocols.

The next section overviews the application of the different analytical and spectroscopic techniques to address issues related to these questions as follows.

5.1. Gas chromatography based metabolites analysis

The biochemical and microbiological actions that occur during ripening yield volatiles (L. Pillonel et al., 2003). The breakdown of amino acids produces short chain amines, aldehydes and alcohols that are volatiles and impacts the flavor of cheese, a crucial sensory attribute (Fig. 2). Gas chromatography is best suited for the analysis of cheese volatile components after their collection by means of distillation, solid-phase microextraction, dynamic headspace or any other technique (Januszkiewicz, Sabik, Azarnia, & Lee, 2008).

GC–MS profiling aided by principal component analysis (PCA) for data analysis as a multivariate analysis tool in an untargeted manner was used for flavor characterization of Gouda and Emmental cheese. The volatile fractions of the different cheeses were composed of fatty acids, methylketones, lactones, aldehydes and alkenes. Principal component analysis coupled to GC-MS allowed for classification of the different cheeses. Gouda cheeses obtained from different producers possessed similar aroma patterns, however Emmental cheeses were observed to produce variable flavor patterns (Dirinck & De Winne, 1999). Aishima and Nakai also used GC patterns for classification of Cheddar, Gouda, Edam, Swiss and Parmesan cheeses (Aishima & Nakai, 1987).

The flavor of commercial Cheddar cheese and enzyme-modified Cheddar cheese (EMCC) was profiled using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC/MS). HS-SPME process was optimized using some of the identified cheese volatiles, *i.e.* dimethyl disulfide, hexanal, hexanol, 2-heptanone, ethyl hexanoate, heptanoic acid, decalactone. The optimization method included other parameters such as the type of HS-SPME fibre that is sought to impact the volatiles extraction efficiency. This method was experimented during the production of EMCC to monitor its profile of flavor compounds as indicators of cheese ripening (Januszkiewicz et al., 2008). GC-MS analysis in relation to microbial assays was used to identify the origin (PDO) status of Italian of Buffalo Mozzarella cheese produced with buffalo and cow milk. The differences in production protocols and also the microbial composition of both cheeses were expressed in their metabolome (Pisano, Scano, Murgia, Cosentino, & Caboni, 2016).

Sensory predictive models for some cheeses such as Cheddar, Gouda, and Parmigiano Reggiano were established *via* GC-TOF-MS. Metabolite profiling of cheeses using both GC-FID and GC-TOF-MS produced comparable efficiencies with the former technique being more advantageous as being inexpensive and easier in its operation (Ochi, Hiroshi, Hiroshige Naito, Keiji Iwatsuki, Takeshi Bamba, 2012).

Volatile profile of various cheeses using GC-MS coupled to olfactometry (GC-O), with a large number of odour-active compounds being identified belonging to different classes *viz.* short-chain carboxylic acids, sulphur compounds, esters, alcohols, ketones and lactones. The volatiles of Tilsit cheeses were profiled using headspace solid-phase microextraction gas chromatography–mass spectrometry/pulsed flame photometric detection and gas chromatography–olfactometry. Buttery-cheesy and sulfury odor notes were perceived due to butanoic acid, 3-methylbutanoic acid, and butane-2,3-dione in addition to sulfur compounds (Fuchsmann, Stern, Brügger, & Breme, 2015).

A study by Kubíčková & Grosch, 1997 reported the key odorants in Camembert cheese using aroma extract dilution and concentration analysis (AEDA & AECA) and headspace gas chromatography-olfactometry (HGCO) to be 2,3-butanedione, 3-methylbutanal, methional, 1-octen-3-ol, 1-octen-3-one, phenethyl acetate, 2-undecanone, δ-decalactone, butyric acid and isovaleric acid. Some volatile sulfur compounds *i.e.,* methanethiol were also detected which are responsible for the garlic note in the Camembert odour profile (Kubíčková & Grosch, 1997).

Aroma of a set of semi-hard cheese was analyzed using GC–O *via* intensity ratings of the significant sensory attributes and odour-active. Partial least squares analysis (PLS) showed a strong correlation between the sensory attributes and the odour-active compounds (Thomsen et al., 2012).

Several volatiles analyses have been directed towards investigation of flavor compounds produced by cheese microbiota to explore their contribution to flavor development. Sgarbi et al. (2013) exploited the aroma profile developed by different *Lb. casei* and *Lb. rhamnosus* strains grown on either starter lab lysed cell medium (LCM) or on cheese based medium (CBM) as a substrate. Differences in volatiles profile between NSLAB grown in LCM and CBM were analyzed by solid-phase microextraction (SPME) gas chromatography mass analysis. The catabolic metabolism of amino acids and fatty acids were required for NSLAB growth on LCM. Conversely, pyruvate metabolism was the main catabolic pathway that supported growth of NSLAB in CBM and thus providing a better understanding of how microbiota contribute to the development of cheese flavor during ripening. It can allow the selection of wild NSLAB that possess specific aromatic profiles to be used as adjunct cultures (Sgarbi et al., 2013).

Heterogeneity in volatile profiles of Grana-Padano cheeses made in two different dairies after a ripening period of 13 months was observed in regards to their microbial composition and starter lysis. Cheese harbored by a complex microbial composition with NSLAB (*Lb. rhamnosus*, *Lb. casei*) as dominant strains were characterized by increased levels of ketones, alcohols, hydrocarbons, acetic acid and propionic acid. However, benzaldehyde, citric and pyroglutamic acid and free fatty acids was observed in cheese with greater lysed starter cells (Lazzi et al., 2016). Metabolomics, defined as the large scale analysis of subset of metabolites at an omic scale is increasingly applied deciphering the contribution of cheese flora producing different metabolites to flavor attributes of cheeses (Pisano et al., 2016). From the reported metabolomics analysis of cheese, the discrimination between closely related cheese varieties, detection of fraudulent PDO cheeses, improvement of cheese quality using different treatments, especially when aided by multivariate data analysis (MVA) for specimens classification (Mohamed A. Farag, Elsebai, & Khattab, 2018).

Metabolomics was employed in differentiating between nonlactic acid bacteria (*Actinobacteria*, *P. freudenreichii* and *Hafnia alvei*) as well as LAB strains based on their ability to produce flavor compounds in a curd-based medium that mimics semi-hard cheese manufacturing conditions (Pogačić et al., 2016).

A similar metabolomics study was performed to explore interspecies and intra-species diversity among 76 dairy *Propionibacteria* strains in relation to their ability to produce different flavor compounds in a curd based medium. *P. freudenreichii* was the superior strain in producing higher amounts of aroma compounds suggesting its potential to modulate cheese flavor, which has yet to be exploited in cheese manufacture (Yee et al., 2014).

In a trial to identify the factors affecting cheese consumer desirability, a study by Shiota, Iwasawa, Suzuki-Iwashima, & Iida, 2015 assessed volatiles profile of some Gouda-type cheese varieties using headspace solid-phase microextraction gas chromatography/mass spectrometry (GC/MS) and steam distillation extraction (SDE)-GC/MS coupled to MVA. Orthogonal partial least squares-discriminant analysis (OPLS-DA) analysis allowed the differentiation between samples having 2 different ripening periods. The model also revealed metabolites that had the highest sensory scores for sweetness, fruity, and sulfurous to be ethanol, ethyl acetate, hexanoic acid, and octanoic acid. A PLS regression model was further applied to predict the desirability of cheese using these parameters, which pointed out to texture and buttery flavors as being responsible for the desirability of Gouda-type cheeses for Japanese consumers. PLS regression is a chemometric projection method that identifies the variables (X matrix) that are linked to the sensory attributes (Y matrix) by establishing a linear multivariate model (Shiota et al., 2015).

5.2. The electronic nose

Electronic noses are introduced as an innovative analytical-sensorial tool to fingerprint food flavor in terms of freshness, different regions and seasoning. It is a biomimetic system that is comprised of complex architecture of electronic chemical sensors with pattern recognition system that can reproduce or mimic the human olfactory functioning system in recognizing simple or complex aromas. The sensors respond to the presence of chemicals, generating electrical signals as a function of their concentration, which are then digitized into multidimensional matrix. Pattern recognition analysis of this matrix result in building an olfactory map or fingerprint in order to allow for the qualitative and quantitative analysis, discriminating a foodstuff simply by its olfactory fingerprint without the need to separate or identify the various components. Correlation between the independent variables present in the map and the dependent variables, characteristics of the sample can then be assessed by different chemometrics tools (Mariaca & Bosset, 1997).

Electronic nose can also be hyphenated with gas chromatography system or mass spectrometer for the analysis of the eluted volatiles in different dairy products (Ampuero & Bosset, 2003). The different odor patterns detected are stored, from which valuable information can be extracted if the electronic nose was trained according to sensory panel classifications.

Discrimination between cheese types with different ages or from different source was achieved by the electronic nose (Gursoy, Somervuo, & Alatossava, 2009). The quality, flavor, and taste of Danish blue cheese are strongly linked to the ripening process, which depends on *Penicillium roqueforti* growth. Thus, E-nose technology can be directly applied in quality assessment of Danish blue cheese products (traditional cheese and from pasteurized milk) and ripening monitoring *via* modelling the e-nose fingerprints. The derived multivariate models allowed prediction of the ripening age of cheese products from the same or different production units. The E-nose technology proved to be easily adapted in monitoring the cheese sensorial attributes without the need of advanced or expensive laboratory equipment enabling quick decisions or actions to be taken during cheese production (And & Nielsen, 2005).

5.3. Liquid chromatography

High-pressure liquid chromatography (HPLC) was used in the study of glycolysis and fermentative pathways, as well as the study of the first intermediates during the amino acids degradation as typical during cheese formation. The production of flavor-producing keto acids *i.e.* ketoisocaproate, 3-methyl- 2-oxovalerate, 3-methyl-2-oxobutyrate, 4-methylthio-2-oxobutyrate, p-phenyl pyruvate, p-hydroxyphenyl pyruvate (Fig. 2), by the aminotransferase action was detected by ion exchange or reverse-phase HPLC (Zeppa, Conterno, & Gerbi, 2001). LC–coupled to mass spectrometry (LC-MS) based metabolite profiling was reported by Le Boucher et al., 2015 for measuring chemical diversity in cheese metabolome resulting from the spatial distribution of *L. lactis* colonies at different stages of cheese ripening (Le Boucher et al., 2015). Monitoring the metabolic changes in Cheddar cheese in response to aging was carried out using HPLC and GC to gain knowledge about the metabolites that can serve as good predictors of the glycolytic, lipolytic, and proteolytic age of the cheese. Ion-exchange-HPLC was used to monitor the changes in pyruvic, lactic, acetic, propionic acids and *O*-phthaldehye derivatives of free amino acids. Propionic and acetic acids were identified as being the best predictors of the glycolytic age; the best predictors of lipolysis were the free fatty acids C_{10}, C_{12}, C_{14}, and C_{16}; and the proteolysis was better predicted by the free amino acids *i.e.* leucine, glutamic and methionine acids. The volatile metabolites detected *via* GC-MS could not provide indication about cheese aging, however, they provided useful information about flavor problems (Marsili, 1981). Combining two different analytical platforms targeting different metabolite classes has proven successful in quality control

evaluation of food products (Mohamed A. Farag, Gad, Heiss, & Wessjohann, 2014; M A Farag, 2014).

5.4. Mass spectroscopy (MS) based metabolomics analysis

The inclusion of chromatographic technique prior to MS spectroscopic detection permits the identification and accurate annotation of metabolites, poses problems with regards to reproducibility and the time consumed during such step (Mohamed A. Farag, Porzel, Schmidt, & Wessjohann, 2012). The direct headspace-mass spectrometry for analysis offers an alternative rapid tool over the expensive and time-consuming chromatographic techniques in the analysis of VOCs. Direct headspace analysis-proton transfer reaction-mass spectrometry (PTR-MS) was used to monitor ageing of Italian hard cheese "Trentingrana" as reflected in its aroma compounds. An increase in the intensity of PTR-MS peaks was observed with ripening and the most significant peaks, mostly different ethyl esters, in differentiating between ripened and young cheese were identified using univariate analysis. Discriminant Partial Least Squares analysis proved to be more efficient than soft class modelling, in classifying the cheese samples relative to their ripening time. Predictive models for ageing time was then established using PLS (Aprea et al., 2007). This technique showed some interesting merits in being fast, the time-dependent variations of the headspace can be monitored, requiring no prior sample treatment and thus reducing the possibility of production of artifacts asides from its high sensitivity suiting it to be applied in industry.

High resolution (HR)-MS-NMR derived metabolite fingerprinting of Mozzarella di Bufala Campana cheese, prepared from buffalo milk was employed to assess the quality and trace the PDO cheese to identify adulterated or false PDO cheeses. The discrimination of Mozzarella di Bufala Campana cheeses according to their geographical regions was accomplished based on the metabolite levels of galactose, lactose, acetic acid and glycerol. However other metabolites *i.e,* isobutyl alcohol, lactic acid and acetic acid were identified as freshness indicators for such cheese type as their levels were reported to increase by cheese aging (Mazzei & Piccolo, 2012). The HRMAS NMR spectroscopy combined with multivariate statistics was used for the discrimination of the Parmigiano Reggiano based on age of ripening (Shintu & Caldarelli, 2005).

PLS-DA modelling of Cheddar cheese (of different maturity, processing and recipes) head space volatile profiles obtained by (GC–MS) was found suited for accurate prediction of cheese age (Gan, Heng Hui, Bingnan Yan, Robert ST Linforth, 2016).

5.5. Capillary electrophoresis

Capillary electrophoresis is a well-recognized technique for monitoring casein hydrolysis in cheese by the action of milk proteinases. The method was used in the analysis of cheeses produced from bovine milk (Recio, Amigo, Ramos, & Lopez-Fandiño, 1997) and ovine milk (Irigoyen, Izco, Ibáñez, & Torre, 2000), providing information on the biochemical proteolysis process occurring during cheese ripening.

The capillary electrophoresis proteolytic profile of casein fractions of Ewe's milk cheese was monitored during a 139-day ripening period to study the impact of the ripening time on the proteolytic process. Predictive models for the ripening time were also established using multivariate methods, *viz*. partial least-squares regression and principal components regression (Albillos, Busto, Perez-Mateos, & Ortega, 2006).

5.6. Near, med infrared (NIR and MIR) and fluorescence spectroscopy

Infrared spectroscopy has proved to be one of the rapid, most efficient non-destructive tool for monitoring and controlling the process and product quality in food industry due to its low cost and the low required sample size. In factories, the cheese-makers usually evaluate the ripening state using traditional physicochemical parameters and by visual and tactile examination, although evaluation of cheese ripening has been extensively studied using spectroscopic methods in literature. IR is more advantageous than conventional methods due to its ability to simultaneously detect and monitor the associated bands of multiple functional groups present *i.e.,* proteins, fats, lactose, and lactic acid in cheese and moreover to determine moisture, fat, protein, phosphorus and calcium contents (Rodriguez-Otero, Hermida, & Cepeda, 1995; Rodriguez-Saona, Koca, Harper, & Alvarez, 2006), determine sensory and texture parameters (Blazquez et al., 2006), geographic origin (L. Pillonel et al., 2003), ripening time (Martín-del-Campo, Picque, Cosío-Ramírez, & Corrieu, 2007) and shelf life (Cattaneo, Giardina, Sinelli, Riva, & Giangiacomo, 2005). Moreover, multi-determination of cheese chemical parameters such as fat, non-protein nitrogen (NPN), pH, water soluble nitrogen (WSN), total nitrogen (TN) and sodium chloride (NaCl) were successfully carried out by IR spectroscopy, surpassing other standard methods due to their limitations regarding analysis time and errors, reagent consumption and operational cost (Etzion, Linker, Cogan, & Shmulevich, 2004). NIR was more efficient in determining fat and TN contents of European Emmental cheese as compared to MIR and the combined NIR-MIR techniques. The best results for the cheese contents of NaCl, NPN and pH were obtained by MIR, however, comparable results were obtained for the WSN content using both techniques (Romdhane Karoui et al., 2006).

Several studies reported the utility of IR for many applications such as the measurements of sensory attributes of cheese (González-Martín et al., 2011), quantification of cheese texture, flavor and appearance, differentiation between different types of cheese based on geographical origin (Woodcock, Fagan, O'Donnell, & Downey, 2008). The best results in many cases are obtained when the whole NIR spectrum (400-2,500 nm) was included in the analysis.

Fats were determined by MIR spectroscopic signals of their respective functional groups *i.e.* acyl-chain C–H (3000–2800 cm^{-1}), peptidic bond C–NH and C=O (1700–1500 cm^{-1}), triacylglycerols ester linkage C–O (~1175 cm^{-1}), C=O (~1750 cm^{-1}) (Romdhane Karoui et al., 2004). However, milk proteins *i.e.* casein, β-lactoglobulin and α-lactalbumin contain at least one tryptophan residue, which shows characteristic fluorescence that allows for better monitoring of protein modification during ripening (Andiç et al., 2014). Concatenation of MIR and fluorescence spectroscopic methods assisted by chemometrics was employed in differentiating between Emmental cheese produced in different European countries and during different seasons (Romdhane Karoui et al., 2004).

5.6.1. IR coupled to chemometrics modeling

Only few chemometric based models based on IR spectroscopic data of cheese were elaborated to predict the color and sensory attributes, ripening time or the effect of addition of adjunct culture during ripening, which are presented as follows. The assessment of sensory properties by NIR has been applied to different batches of semi-hard cheeses with a potential to predict consistency properties of semi-hard cheese *i.e.* springiness, pastiness, coherence, solubility and hardness, and flavor properties, *i.e.* cheesiness and acidity (Sørensen & Jepsen, 1998). Downey et al., 2005 worked out the exploration of sensory attributes of 24 experimentally prepared Cheddar cheese using 5 renneting enzymes under different storage conditions (4 C for up to 9 months). Near-IR spectroscopic data combined with PLS regression enabled the modelling of sensory attributes against the age of Cheddar cheeses with a very low prediction error (Downey et al., 2005).

Linear discriminant analysis of (FT-IR) data derived from the analysis of Italian Pecorino cheese allowed their classification according to the ripening stages (hard and semi-hard) and manufacturing technique (Lerma-García, Gori, Cerretani, Simó-Alfonso, & Caboni, 2010). PCA assisted IR spectroscopic analysis allowed the discrimination between two different stages of ripening of Camembert (Mould-ripened soft cheeses). However, PLS regressions-based spectral analysis permitted the estimation of the cheese ripening date with only 1 day error

(Martín-del-Campo et al., 2007).

Identification of the ripening degree of Cheddar cheese and its various sensory characteristics constitute an important stage in cheese quality evaluation which is traditionally performed by trained sensory panelists. Predictive models for the sensory attributes and age of Cheddar were developed by modelling NIR reflectance spectroscopy by partial least-squares (PLS) regression (Downey et al., 2005). Prediction of color and sensory attributes of Emmental cheeses originating from different European regions based on their IR spectroscopic data was also reported (R. Karoui, Pillonel, Schaller, Bosset, & De Baerdemaeker, 2006; Laurent Pillonel et al., 2007).

As previously reported in many studies, the season of milk collection affect its fats content and the production of volatile components, hence its sensory characteristics (Abilleira, Schlichtherle-Cerny, Virto, de Renobales, & Barron, 2010; Muñoz, Ortigosa, Torre, & Izco, 2003).

The nonstarter lactic acid bacteria (NSLAB) was believed to be one of the uncontrolled factors in the industrial cheese manufacture till now. The significant compositional changes caused by the addition of *Lactobacillus* spp. as adjunct culture during ripening of Swiss cheese were explored by infrared microspectroscopy and chemometrics (Chen, Kocaoglu-Vurma, Harper, & Rodriguez-Saona, 2009). This tool provided insight into the complex biochemical changes occurring during cheese ripening and the role of adjunct nonstarter lactic acid bacteria on flavor development in cheeses.

FTIR spectroscopy was used to monitor the changes in the levels of amino acids, organic acids during Cheddar cheese ripening and correlating the spectral data to those obtained from chromatographic analysis using chemometric methods. Prediction models are then established to predict the levels of amino acids and organic acids and cheese age, which can be correlated to the differences in spectral region 1800-900 cm^{-1} (Subramanian, Alvarez, Harper, & Rodriguez-Saona, 2011). Such approach can be helpful in understanding the flavor development during cheese ripening and real-time monitoring of the cheese ripening events in order to obtain products with desired sensory attributes and has great potential to be readily applied by manufacturers.

6. Computational tools for monitoring cheese defects during ripening

In addition to flavor changes, visual modifications (color, shape and structure) can influence the choice by consumer. Among the visual modifications of the food products, color provides a clue for many of their qualities such as flavor, sanity, maturity, and drives consumers' choices. Since human inspection of food quality were reported to give inconsistent decisions, instrumental measurements that can imitate human testing methods are more preferred to replace human inspection for evaluating and predicting cheese quality. Both external and internal quality attributes are concerned when searching for automated instrumental techniques for quality evaluation.

Instrumental measurement of the color can be performed either using conventional or digital instruments. Colorimeters and spectrophotometers are used for the conventional color measurement. Automated digital pixel-based color measurements are now increasingly used instead such as computer vision systems (CVS) with online digital cameras/scanners. A variety of non-destructive optical techniques were also experimented among which, light and confocal laser scanning microscopes (CLS), near-infrared (NIR) imaging systems, spectroscopy and hyperspectral imaging systems (HIS), computed tomography and X-ray CT measurements (Lukinac, Jukić, Mastanjević, & Lučan, 2018).

6.1. Computer vision (CV) and digital image analysis

Non-destructive optical techniques such as CV and digital image analysis provide efficient and non-invasive tools that substitute human vision to inspect the cheese physical features, when combined to suitable classification models, such as color, and some cheese defects *i.e.* gas holes, formation of calcium lactate crystals, excessive rind halo and oiling-off. Such tools can also provide information about the compositional and structural attributes of cheese with a better correlation to composition, quality and safety (Lukinac et al., 2018). Image analysis is a non-destructive technique that provides more accurate information than human vision which enables it to be employed for the automated on line analysis within the cheese production unit. This is well suiting the food products that have some characteristics related to their quality difficult to be measured by classical methods of analysis. CV has found many applications in evaluating some cheese quality issues such as browning and melting properties during cooking and shelf life prediction (Yam & Papadakis, 2004).

Internal quality attributes *i.e.* firmness, tenderness, hardness, juiciness and crispness, are difficult to be accessed by visual inspection which necessitates the use of non-destructive optical devices for the sake of routine inspection and quality assurance tasks (Lukinac et al., 2018).

CV includes several operations: image capturing, processing, and image analysis (Bovik, 2005). After image capturing, the images are converted into numerical matrix that is called digitization. As a technique, CVS is able to measure the external features of products, to recognize objects and extract quantitative information from digital images (Gunasekaran, 1996).

It is well known that different cheese varieties normally develop mechanical openings called gas/eyeholes and there is a high demand for a non-destructive tool to monitor eyeholes formation in cheese during ripening. The desirable openings (eyeholes) instead of slit formation (considered a cheese defect) is produced through the ripening process, during which any changes in the mechanical properties of cheese can affect the production of desirables features of eyeholes.

Eyeholes are considered as a sign of good quality in some cheese varieties such as Swiss-type cheese (Emmental), Gouda, Ragusano, and Edam cheese, in which eye holes are formed due to CO_2 and N_2 production. Such gas evolution by Clostridia can have a negative impact on Swiss-type cheese leading to defective eye formation, white spots and a putrid smell during late stage of ripening as reported by Le Bourhis et al. (White et al., 2003).

However, such gases are regarded as defect in other cheese varieties such as Cheddar (Patrick Fox Fox et al., 2017). Slits or cracks can be formed under certain conditions in cheeses where eyes or holes are accepted. Image analysis was employed to measure the surface area of cheese slices occupied by gas holes for Emmental, Ragusano, and Cheddar cheese in which the intensity of the red, green, and blue (RGB) channel of each pixel was measured. The channels that recorded the highest contrast between holes and areas without holes are selected for the quantitative estimation of the total area percentage occupied by gas holes. This method can readily infer amount of gas production with manufacturing conditions or as a quality control tool in cheese manufacturing (Caccamo et al., 2004).

Slits are produced in Cheddar cheese after 90–120 days of aging as a result of gas production by citrate-fermenting *Lactobacilli* (Patrick Fox Fox et al., 2017). The image analysis approach was capable of distinguishing slits from areas with no slits and could provide a quantitative estimate of the percentage of area represented by these slits (Caccamo et al., 2004).

Computed tomography and X-ray CT measurements are also employed as non-destructive techniques for monitoring eye formation and growth during cheeses ripening (Schuetz, Guggisberg, Fröhlich-Wyder, & Wechsler, 2016).

Rind defects such as calcium lactate crystal formation and excessive rind halo which arises from the supersaturation of the cheese serum phase with calcium and lactate ions, which then form crystals producing white hazes on the surface of the cheese (Rajbhandari & Kindstedt, 2008). A study by Rajbhandari and Kindstedt (2008) studied the effects of cheese composition, packaging conditions, and storage temperature

on time and rate of the crystal growth and time of crystal appearance during Cheddar cheese storage using image analysis (Rajbhandari, P., 2005).

6.2. Spectrocolorimetry

Color is considered as one of the important sensory attributes of cheese that provides information about its degree of ripeness and freshness. Milk composition and its different treatments, ripening time and techniques, food additives, manufacturing technology, activity of natural milk microflora have a significant impact on the cheese perceived color (León, Mery, Pedreschi, & León, 2006). Accordingly, the evaluation of cheese color provides a simple fast tool for the detection of anomalies or defects that might be produced during cheese manufacturing. Furthermore, lowering in the three lightness values (L*, a* and b*) was observed during ripening of Asiago cheese because of the significant increase in crude protein and fat content and decreased moisture in a raw milk cheese with a pronounced effect on the formation of mechanical openings in the cheese surface (Marchesini, Balzan, Segato, Novelli, & Andrighetto, 2009).

The orange-brown rind coloration is a sign of good quality red-smear cheeses *viz.* Epoisses, Munster, Maroilles, Livarot, Limburger or Tilsit, which develops due to the formation of carotenoids and other pigments by the action of cheese microflora bacteria, and to interact with deacidifying yeasts. The color development on smeared cheese rinds during ripening of various PDO cheeses and the on shelf cheeses in some PDO areas was evaluated using a spectrocolorimeter (L*a*b* colorimetric system) as a tool to monitor the ripening process and control the quality of this type of cheese (Dufossé, Galaup, Carlet, Flamin, & Valla, 2005).

7. Conclusion and future trends

Ripening plays a major role in the development of cheese flavor, as it involves a series of alterations in the composition of cheese forming fatty acids and also metabolizing lactose. The degree of ripening greatly affects the development of the sensory attributes of the cheese through the different biochemical events (proteolysis, glycolysis, and lipolysis) that occur during this process.

In the first stage of ripening, the αs1-casein is broken down by chymosin; the level of its degradation directly impact the cheese textural properties. Biotechnological trials to hyper express chymosin using strong promoters and protein engineering are also sought to assist in the production of cheese and to maximize its flavor and texture qualities (Smit et al., 2005).

Many factors are found to impact the cheese quality; if discretely managed and checked will lead to indulging flavors, while if not, will result in off-flavors. The fine line between the production of flavor and off-flavor has prompted several studies to be conducted to determine the factors that may alter the chemical and physical composition of cheese. The analysis of flavor, aroma, taste and chemical components has been worked out in identifying and discriminating between different types of cheese. While expert panelists may inevitably not be able to reach a clear consensus, other instruments are employed to produce more concrete results. In this case, not only are the opinions of experts of importance in pinpointing quality attributes of cheese, but also acceptability studies done by consumers have been seen to take place. Both qualitative and quantitative methods of analysis work hand in hand in identifying the key components in what makes a specific type of cheese more tolerable than another, especially when coupled to chemometric models as illustrated in this review. Further, projective mapping and flash profiling can provide a valuable tool to improve or diversify the flavor attributes and select the most appealing products.

The increased consumer awareness in food quality and safety obliges the food industrial sector to establish and maintain the product identity and characteristics. However, high degree of variation is usually encountered in biological processes and matrices such as cheeses which cannot be easily met with traditional methods. Besides, the lack of clear definition for the cheese sensory properties is a major bottleneck in both the flavor characterization and optimization of its development during ripening. Hence, the implementation of a standardized sensory language for the different types of cheeses seems imperative for both research and industry. Future research should continue to address these issues and relate the flavor lexicons with consumer acceptance and instrumental flavor profiles across different cultures and analytical techniques.

The assessment and monitoring of cheese ripening is such a challenging task but also more imperative as the cheese is considered a multifactorial biological system consisting of a heterogenous classes of compounds (fats, proteins and carbohydrates) in a complicated physical matrix. That is why the use of complementary sensorial and analytical methods is greatly demanded to effectively study the multitude biochemical changes during this process. Moreover, IR technology, E-nose, optical techniques such as computer vision and digital image analysis still have much to offer to the analysis and quality control of cheese as well as the estimation of cheese shelf life.

For assuring cheese safety and real time monitoring of freshness, future endeavors should be directed towards the design of intelligent packaging devices such as chemical gas sensors viz. carbon dioxide and oxygen sensors which have the potential to be embedded in agricultural and food packaging for quality indication.

This review provides the most recent state of the art in the biotechnological trends for enhancing cheese sensory attributes and accelerating the ripening process. There appears to be limited studies regarding the utility of encapsulated enzyme cocktails or mixed attenuated adjunct cultures in accelerating cheese ripening, which pose them as future trends that need to be pursued. The main technological challenge appears is to explore the applicability of these trends to be implemented to cheese industry at a large scale.

Conflicts of interest

The authors have declared that there is no conflict of interest.

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