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Encapsulation of fatty acids in ruminant nutrition for improved meat and milk quality: A review

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Abstract

In recent years, the methods of producing protected fat supplements for feed have greatly developed. As a means of preserving unsaturated fats from oxidation, encapsulation has been used by food industry researchers to reduce unpleasant odor and taste, and as an effective method of protecting unsaturated fats. The process of encapsulating involves covering or trapping the target substance(s) in another substance or system. Similarly, vitamins and micronutrient compounds in food do not remain stable for long and are subject to decomposition, which depends on chemical structure, food matrix characteristics, handling parameters, and storage conditions. Consequently, encapsulation can prevent these compounds from being destroyed until they are transferred to the right location or slow down decomposition processes (such as oxidation or hydrolysis). That concept can be expanded to lipids (oils and fats). Currently, emulsion spray drying is the most common method of fine oil microcoating. The mass formation method produces more stable microcoatings with higher oil content than spray drying, as recently discovered. Biodegradable polymers have gained much attention as encapsulation materials. Microencapsulated lipids can increase the meat and milk quality of ruminants.

Keywords: encapsulation; coating; fatty acids; ruminants; milk

Introduction

The significance of incorporating fats as an energy source in the diets of high-producing ruminants, particularly dairy cows during the early stages of lactation, has been well-documented. Furthermore, the physiological roles of certain unsaturated fatty acids have established a foundation for the strategic utilization of various fatty acids, independent of their energy contribution, to improve the quality of farmed animal products (Reynolds et al., 2003; Santos et al., 2008). The inclusion of dietary fats is essential for the preservation of animals' health. In contemporary research, the incorporation of oils and oilseeds into animal feed has garnered significant interest due to their various biological functions. Reducing the energy demand in livestock through the reduction of milk fat at the beginning of lactation, by some specific fatty acids (Bauman and Griinari, 2003), increasing the feeding of carbohydrates and fats to meet the metabolic needs of livestock (Overton and Waldron, 2004), and the tendency to increase the conjugated linoleic acid concentration in livestock products due to its effects on human health (Pariza et al., 2001; Privé et al., 2010a,b) can be considered as the reasons for this approach. Humans, like other vertebrates, cannot synthesize the unsaturated fatty acids linoleic (ω-6 fatty acid, LA (18:2n-6) and α-linolenic acid (ω-3 fatty acid, ALA (18:3n-3)) and need to obtain them through food (Simopoulos, 1991). Red meat is one of the principal sources of saturated fats in our nutrition. Attempts were made to control the level of unsaturated fats in meat and milk (Ladeira et al., 2014). There is a higher concentration of unsaturated fats in ruminants' tissue fat than in that of non-ruminants, due to the fat digestion mechanisms in the rumen, including biohydrogenation and lipolysis (Jenkins, 2002). Oilseeds contain high levels of unsaturated lipids. Several studies have shown that the utilization of oilseeds

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in the eating routine influences the presence of unsaturated fats in various body tissues of livestock (Peng et al., 2010). Also, unsaturated fatty acids may prevent triglyceride accumulation in the liver by changing the metabolism of fats (Yoshikawa et al., 2002). Therefore, oil seeds can be effective as rich sources of unsaturated fatty acids in fat metabolism and even in preventing metabolic disorders, including fatty liver disease. On the one hand, oil seeds, which have high levels of energy, show more resistance to microbial digestion in the rumen. This leads to the passage of these feed ingredients through the rumen and their digestion and absorption in the small intestine, and as a result, improved livestock performance. On the other hand, dietary fats can affect rumen digestibility. The primary effects of this intervention encompass the following: a decrease in fiber digestion, a reduction in methane emissions, and a diminished ratio of acetate to propionate. These changes subsequently lead to a reduction in protozoan populations as well as a decrease in the growth and metabolic activity of bacteria, particularly those strains that are responsible for cellulose degradation. These effects are mainly caused by unsaturated fatty acids. Predicting the amount of biohydrogenation of unsaturated fatty acids and the effect of dietary fat on feed digestibility in the rumen is very difficult and variable. One of the effective factors in this field is the amount of fodder and ration concentrate (Chilliard et al., 2009). Enjalbert et al. (2003) reported that the presence of fiber in the diet increases the biohydrogenation rate of feed fatty acids in the rumen. An increase in fiber content leads to the increase of cellulolytic bacteria such as *Butyrivibrio fibrisolvens*, which play a vital role in the biohydrogenation of fatty acids in the rumen (Maia et al., 2010).

One of the most important plant sources of unsaturated fatty acids with several double bonds is flaxseed (*Linum usitatissimum*), which is characterized by its high content in alpha-linoleic acid (approximately 40% by weight) and more than 50% of fatty acids in total mass (Khattab and Zeitoun, 2013). Due to its low cost and advantageous composition, soybean oil is known as a main component of feed, since it provides oil containing omega-3 fatty acids. In fact, consuming seeds has been linked to several potential health benefits in ruminants. Due to the fact that the consumption of oilseeds such as flaxseed is not common in human nutrition and milk is easily and abundantly used as a consumable feed in human nutrition, it is possible to use oil-rich seeds in feeding dairy cows in order to increase compounds that improve human health in milk.

Biohydrogenation refers to a metabolic process occurring in the rumen, wherein hydrogen is incorporated into the double bonds of unsaturated fatty acids. This process is primarily facilitated by rumen microorganisms, with protozoa playing a relatively minor role in the overall mechanism (Harfoot and Hazlewood, 1997). The predominant portion of unsaturated fats found in the feed of ruminants undergoes biohydrogenation within the rumen, resulting in their transformation into saturated fats, which have lesser nutritional value. Consequently, the regulation of ruminal biohydrogenation of unsaturated fats represents a critical challenge for food supplement manufacturers and nutritionists, as it is essential to maintain the integrity of unsaturated fats within the digestive system (Vasta et al., 2009). There are various techniques to shield different fats and oils from rumen biohydrogenation. Utilizing new strategies, for example, microencapsulation of unsaturated fats, recently emerged as a successful strategy for saving unsaturated fats from oxidation, and decreasing the smell while simultaneously improving the taste (Carvalho da Silva et al., 2022). Encapsulation is a process in which a target substance is covered with another substance or system or trapped inside it (Madene et al., 2006). A majority of vitamins and micronutrient compounds are unstable and decompose over time, depending on a number of factors, including their chemical structure, the matrix of the food, the processing conditions, and the storage environment (Farhang, 2013). Consequently, it may be feasible to decelerate decomposition processes, including oxidation and hydrolysis, or to safeguard these compounds from degradation during transit to their intended location through the use of encapsulation techniques. Currently, emulsion spray drying is the predominant method employed for the micro-coating of fine oils (Berraquero-García et al., 2023; El-Messery et al., 2020). This technique yields more stable micro-coatings with higher oil content compared to spray drying, which has recently received attention (Tamjidi et al., 2012). Natural polymers have garnered significant interest as materials for coating and encapsulation, primarily owing to their biodegradability (Lawrie et al., 2007). The key polysaccharides employed for controlled release within the body include: 1- Chitosan, 2- Alginate, 3- Pectin, 4- Dextran, 5- Starch, and 6- Inulin (Guo et al., 2024; Lawrie et al., 2007).

Lipids

Lipids constitute a category of organic compounds characterized by their solubility in organic solvents, including chloroform, hexane, alcohol, and ether, while exhibiting insolubility in water (Mayes, 1996). This group of nutrients is recognized as a significant component of a balanced diet, owing to its provision of essential fatty acids, fat-soluble vitamins, and substantial energy content (McDonald et al., 1997; Ahmed et al., 2023; National Research Council, 1989). Oil and fat are both lipids. They have a similar general structure and chemical properties but are physically distinct from each other. Oils are liquid at room temperature, while fats are solid at room temperature Catsberg et al., 1990). Lipids can regulate the digestion and absorption of various nutrients in diets. For example, the inclusion of fat in diets can help prevent rumen acidosis and reduce milk fat loss (Palmquist, 1984). Also, lipids can be added to the diet to alter the proportion of specific fatty acids in milk and meat, such as conjugated linoleic acid and fatty acids with multiple double bonds (polyunsaturated fatty acids), in order to improve their suitability for human consumption (Grummer and Carroll, 1991).

Lipids Classification

Structural lipids are present in the foliage of grasses and in plants that accumulate energy as carbohydrates, such as cereal grains. Typically, these structural lipids manifest themselves as phospholipids and glycolipids (Ahmed et al., 2023; Church, 1988; Bauman et al., 1999). Lipids present in forage account for 6-7% of the dry matter content of leaves. Fatty acids within these lipids comprise 43% of the ether extract, predominantly consisting of linolic acid, followed by linoleic acid, which are the primary unsaturated fatty acids. The remainder of the ether extract is composed of waxes, chlorophyll, galactose, and various other unsaponifiable substances (Church, 1988; Harfoot and Hazlewood, 1997; Mir et al., 2006; Finley and deMan, 2018). The second major group of lipids are storage lipids, which are found in plants that store energy in the form of oil. Oilseeds are a good example that lipids are mainly found in the form of triglycerides. Usually, oilseeds have a high level of unsaturated fatty acids, and their fatty acid profile differs among various oilseeds (Murphy, 2016).

Table 1. Lipid classification (Source: Pérez-Barbería, 2020)

Function	Location	Chemical compound
Energy storage	Cell	Triglycerides (fatty acids)
Structural	Cell membranes	Phospholipids, Glycolipids,
		Chlorophylls, Carotenoids,
		Sterols, Acylated sterol
		glycosides
	Epidermis waxy cuticle	n-alkanes, (long-chain carbohydrates)
		Very long chain fatty acids
		Long-chain alcohols (fatty alcohols)
		Long-chain aldehydes (fatty aldehydes)
		Ketones
		Esters
		Polyester polymers (cutin)

Digestion of lipids in the rumen

Ordinarily, ruminants eat under 3% fat (in terms of dry matter) (Palmquist and Jenkins, 1980). Lipids in the eating regimen of dairy cows can be of plant origin (like seeds, oils, and palmoil-derived unsaturated fats) or animal origin (e.g., fish oil) (Chilliard, 1993). In the rumen, feed lipids are presented to the action of rumen microorganisms, where two significant changes happen. In the primary structure change, all ester bonds in feed lipids are hydrolyzed by microbial lipases (Harfoot and Hazlewood, 1997; Bauman et al., 1999). Because of this hydrolysis, free unsaturated fats are delivered into the rumen (Bauman et al., 1999). It is followed by the biohydrogenation of unsaturated fats to saturated ones (Bauman et al., 1999). Under ordinary circumstances, rumen microorganisms almost completely convert the unsaturated fatty acids to saturated ones (Figure 1) (Harfoot and Hazlewood, 1997; Bauman et al., 1999). The 2 steps are lipolysis and biohydrogenation (Figure 2).

Lipolysis in the rumen

Lipolysis is a very fast and intense process that is carried out by microbial lipolytic enzymes. However, this can be a rate-limiting step. Excessive lipolysis may lead to the accumulation of large amounts of free unsaturated fatty acids, which can affect fiber digestion and prevent biohydrogenation. Usually, post-ruminal digesta are rich in stearic acid, while the amounts of linoleic and linolenic acids decrease compared to the initial diet. This decrease in linoleic and linolenic acids is attributed to the effect of rumen hydrolysis and biohydrogenation (Harfoot and Hazlewood, 1997; Gunthier et al., 2004). Hydrolysis of feed lipids progresses rapidly and intensively in the rumen, resulting in various compounds such as free fatty acids, glycerol and galactose (Harfoot and Hazlewood, 1997; Bauman et al., 1999). Free unsaturated fatty acids released due to hydrolysis undergo biohydrogenation (Van Soest, 1994; Bauman et al., 1999).

Biohydrogenation of fatty acids in the rumen

Biohydrogenation is essentially performed by rumen microorganisms, while protozoa assume a minor part (Harfoot and Hazlewood, 1997). The species and number of bacteria present in the rumen determine their ability to biohydrogenate fatty acids (Harfoot and Hazlewood, 1997). Unsaturated fatty acids that exist freely in the rumen are biohydrogenated quickly. These fatty acids are toxic to rumen microorganisms, and the hydrogenation process reduces their toxicity (Maia et al., 2010). The exact role of biohydrogenation in lipid metabolism is not clearly defined. However, it has been suggested that certain intermediate fatty acids produced during rumen biohydrogenation are required for building microbial membranes (Harfoot and Hazlewood, 1997). In addition, rumen microorganisms benefit from the biohydrogenation

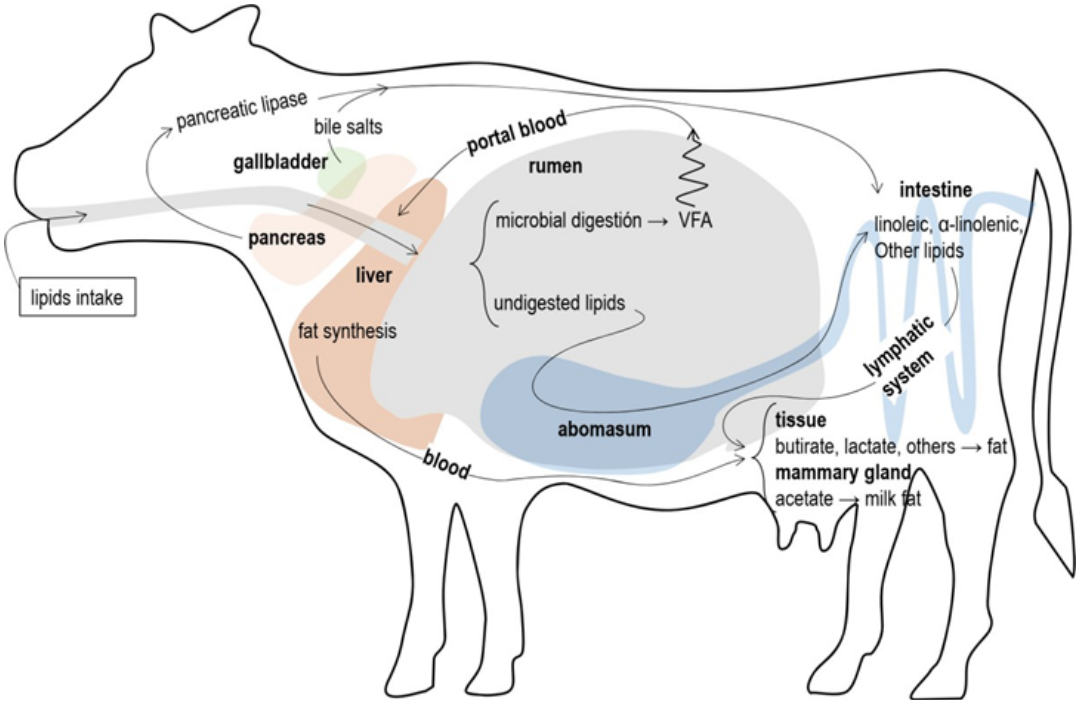

Figure 1. Lipid use in ruminants (Source: Pérez-Barbería, 2020).

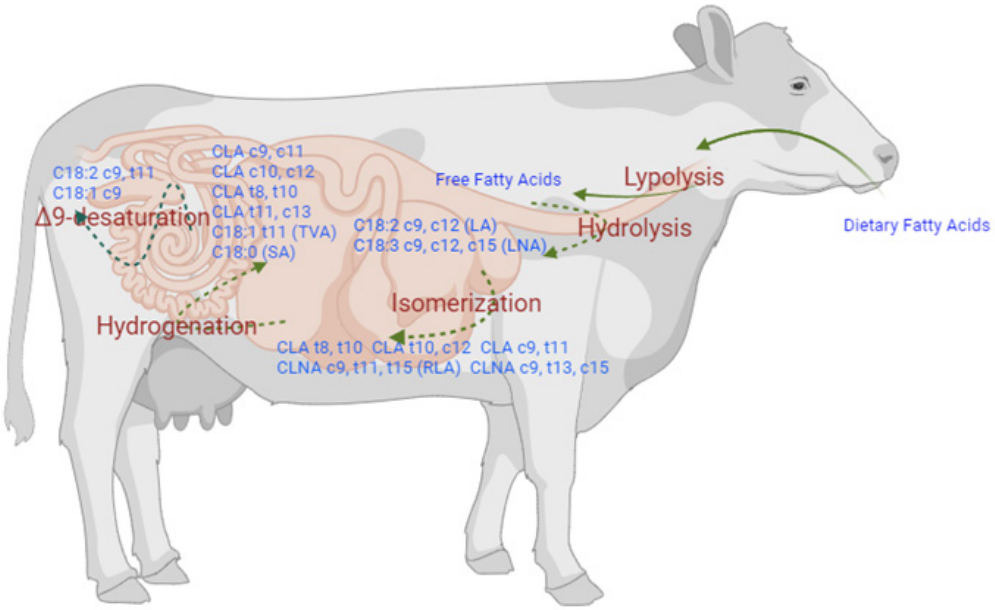

Figure 2. Mechanism of linoleic acid and linolenic acid biohydrogenation. (Source: Aziz et al., 2022)

process as a site for hydrogen removal (Church, 1988; Harfoot and Hazlewood, 1997). The most common hypothesis is that long-chain unsaturated fatty acids can damage the cell wall, and biohydrogenation acts as a detoxification mechanism (Jenkins, 1993; Harfoot and Hazlewood, 1997; Garnsworthy, 1996). Most of the unsaturated fatty acids present in the feed of ruminants are biohydrogenated in their rumen, so that the input composition of fatty acids in the rumen and their output composition are significantly different. For this reason, manufacturers of feed supplements and animal nutritionists consider minimizing the process of biohydrogenation of these fatty acids in the rumen to increase the unsaturated fatty acids

in the intestine (Figure 2) (Vasta et al., 2009).

The steps of fatty acids biohydrogenation in the rumen

The biohydrogenation of unsaturated fatty acids is a complex, multi-step process that cannot be accomplished by a single species of rumen bacteria. Rumen bacteria can be categorized into two primary groups based on the specific reactions they facilitate and the resultant end products of the biohydrogenation process (Dewanckele et al., 2020).

Group "B" microorganisms consume trans-11 linoleic unsaturated fat as one of the fundamental substrates, and the end result is stearic acid (Bauman et al., 1999).

The initial phase of the biohydrogenation process involves the isomerization of the 12-cis double bonds present in linoleic acid. This isomerization results in the production of the cis-9, trans-11 conjugated isomer, commonly referred to as conjugated linoleic acid (CLA) (Jenkins, 1993; Harfoot and Hazlewood, 1997; Bauman et al., 1999; Gómez-Cortés et al., 2009). Linoleate isomerase is the enzyme that catalyzes the preceding reaction, facilitating the formation of conjugated double bonds in both alpha and gamma linolenic acid (Jenkins, 1993; Bauman et al., 1999). With the formation of the trans-11 bond, a reduction reaction (hydrogenation of the cis-9 bond in C18:2) is continued, and the isomer of cis-9, trans-11, is converted to trans-11-octadecanoic acid (Bauman et al., 1999). This reduction reaction is carried out by microbial reductases to finally obtain saturated fatty acids (e.g., stearic acid) (Jenkins, 1993; Harfoot and Hazlewood, 1997; Bauman et al., 1999). Some *in vitro* studies have shown rapid conversion of cis-9, trans-11 (CLA) to trans-11 octadecanoic fatty acid after isomerization of the cis-12 double bond (Harfoot and Hazlewood, 1997; Bauman et al., 1999; Dewanckele et al., 2020). The biohydrogenation process of linolenic acid closely resembles that of linoleic acid; however, it involves an additional step for hydrogenation of the cis-15 double bond. This is subsequently followed by isomerization and reduction reactions, ultimately leading to the production of stearic acid (Harfoot and Hazlewood, 1997; Bauman et al., 1999; Dewanckele et al., 2020).

Factors affecting biohydrogenation

Assessing the extent of biohydrogenation of unsaturated fatty acids in the rumen as well as the influence of dietary fat on feed digestibility within this environment presents a complex challenge. One critical factor that significantly affects these processes is the proportion of forage and concentrate in the diet (Goiri et al., 2010). The hydrogenation of fatty acids within the rumen is influenced by several factors, including the specific characteristics of the fat, the duration of retention in the rumen, and the composition of the microbial population present. Additional determinants encompass the concentration of fats in the diet, the ratio of forage to concentrate, the pH level of the rumen, and the metabolic activity of the microorganisms. When the fat content in the rumen is low, hydrogenation occurs nearly completely; however, at elevated concentrations, only approximately 80-90% of the fatty acids undergo hydrogenation. Furthermore, the inclusion of diets with high concentrate content results in a reduction of biohydrogenation within the rumen (Qiu et al., 2021; Enjalbert et al., 2003). This phenomenon is primarily attributed to the acidification of the rumen environment, which subsequently diminishes the activity of the rumen microorganisms responsible for biohydrogenation (Enjalbert et al., 2003). Enjalbert et al. (2003) reported that the presence of fiber in the diet increases the biohydrogenation rate of feed fatty acids in the rumen, because the increase of fibers leads to the increase of cellulolytic bacteria such as *Butyrivibrio fibrisolvens*, which play an important role in the biohydrogenation of fatty acids in the rumen (Maia et al., 2010). Baumgard et al. (2000) reported that biohydrogenation is affected by various factors, including the type and length of fatty acid chains, dietary nitrogen content, and the proportion of forage in the diet. A notable adverse consequence of the biohydrogenation of fatty acids within the rumen is the accumulation of fatty acids resulting from incomplete biohydrogenation. This accumulation includes unprotected fatty acids, which may significantly contribute to the reduction of milk fat (Hawkins et al., 2013) and the elevation of cardiovascular risks in consumers (Talaei et al., 2013).

In ruminants, fat encapsulated in a matrix of protein treated with aldehyde has shown significant increases in unsaturated fatty acid absorption (Jenkins, 1993; Doreau and Chillard, 1997). Mineral salts of long-chain fatty acids, such as calcium salts of some oils, have a small inhibitory effect on rumen fermentation and lead to biohydrogenation resistance in the rumen (Jenkins, 1993; Doreau and Chilliard, 1997; NRC, 2001). The reduction in rumen biohydrogenation can be ascribed to certain constraints regarding the availability of free carboxyl groups when interacting with calcium salts. Furthermore, these salts exhibit diminished solubility in aqueous environments (Jenkins, 1993; NRC, 2001). Alternative strategies to mitigate the impact of rumen biohydrogenation on unsaturated fatty acids include the conversion of free fatty acids into fatty acyl amides. Additionally, the supplementation of oilseeds can enhance the flow of unsaturated fatty acids to the small intestine, thereby facilitating the absorption of advantageous fatty acids by the mammary glands (Jenkins, 1993; Maia et al., 2010). The administration of unsaturated fatty acids containing multiple double bonds to ruminants, delivered in the form of whole oil seeds, exerts a negligible impact on rumen fermentation. This phenomenon can be attributed to the liberation of oil from the seeds into the rumen fluid (NRC, 2001).

There are three categories of fat sources based on their resistance to biohydrogenation in the rumen:

1. Neutral fats (inactive fats)

These lipids typically encompass saturated fatty acids and hydrogenated fats, commonly referred to as inactive fats, which are utilized in limited amounts within ruminant diets. The protective mechanism of neutral fats is contingent upon

the melting point of the overall fat or fat mixture. This melting point is influenced by the melting points of the individual fatty acids that comprise the mixture. Furthermore, the melting point of a specific fatty acid is determined by both the length of its carbon chain and the quantity of double bonds present (Dhaka et al., 2011).

2. Active fats

It has been shown that these types of fats strongly inhibit microbial fermentation in the rumen and reduce food digestibility to varying degrees, especially fibrous carbohydrates (Ferreira et al., 2016). A higher percentage of unsaturated fatty acids is found in most of these fats. It is possible to include fish oil and flax oil among these sources of fat (Ferreira et al., 2016).

3. Protected fats

These types of fats are widely considered as sources of fats resistant to biohydrogenation by ruminal microorganisms, which are used in ruminant diets and change the pattern of fatty acids in body tissues and milk. Large amounts of fats that contain unsaturated fatty acids are protected by formaldehyde capsules and are spared from the biohydrogenation process. Also, the amides of oilseeds also act as protected fats to some extent (Behan et al., 2019).

Saturated and unsaturated fatty acids

Fatty acids can be categorized into two primary classifications: saturated fatty acids, which lack a single or multiple double bonds in their molecular structure, and unsaturated fatty acids, which contain one or more double bonds. Unsaturated fatty acids are further subdivided based on the quantity of double bonds present; specifically, they are classified into monounsaturated fatty acids, which possess a single double bond, and polyunsaturated fatty acids, which contain multiple double bonds (Chilliard et al., 2000; Jenkins, 1993). In recent decades, there has been a heightened focus on polyunsaturated fatty acids (PUFAs), specifically omega-3 and omega-6 fatty acids (Williams et al., 2011). Omega-3 fatty acids include alpha- linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and omega-6 fatty acids include arachidonic acid and linoleic acid (Williams et al., 2011). Unsaturated fatty acids are crucial for human health, as they contribute to the prevention of cardiovascular diseases, autoimmune disorders, and certain types of cancer (Connor, 2000). Due to the inability of humans, similar to other mammals, to synthesize linoleic and linolenic fatty acids endogenously, it is essential to obtain these fatty acids through dietary consumption (Simopoulos, 1991). Given that red meat serves as a primary source of fatty acids for humans, researchers are actively seeking to enhance human health by altering the fatty acid composition in meat and dairy products (Ladeira et al., 2014). During the process of ruminal fermentation, a significant portion of the unsaturated fats present in the diet is transformed into saturated fats. This biochemical conversion accounts for the elevated levels of saturated fats found in the milk and meat of ruminant animals (Michel and Ferlay, 2015). Various studies have shown that feeding fatty acids with several double bonds to dairy cows at different times leads to improved innate immunity in the postpartum period, reduction of fat accumulation in the liver and prevention of fatty liver in the transfer period (Petit et al., 2004), reduction of milk fat percentage (Dirandeh et al., 2015), improvement of livestock health (Dirandeh et al., 2015) and increased milk production (Jehani Moghadam et al., 2015).

The absorption of unsaturated fatty acids in the small intestine of ruminants exhibits similarities to that observed in monogastric animals; however, it differs from the absorption of saturated fatty acids (Bauchart, 1993). The coefficient of intestinal absorption of fatty acids in ruminants is notably greater than that observed in monogastric animals. This enhanced absorption efficiency of saturated fatty acids in ruminants can be attributed to the superior capacity of bile salts to solubilize fatty acids under the acidic conditions present in the duodenum and jejunum. In ruminants, long-chain unsaturated fatty acids are incorporated into triacylglycerols in relatively small quantities; instead, they are predominantly found in membrane phospholipids and are stored in significant amounts within intramuscular tissue. This represents a key distinction between ruminants and monogastrics. The composition of intramuscular fatty acids in monogastric animals exhibits considerable variability, reflecting the fatty acid profile of their diet. Conversely, in ruminants, the process of rumen biohydrogenation results in a reduced variability in the composition of intramuscular fatty acids. Given that phospholipids are integral components of cell membranes, their composition is less susceptible to dietary influences. Omega-6 and omega-3 fatty acids compete for incorporation into phospholipids, with the relative proportions of these fatty acids being contingent upon their dietary intake. In comparison to ruminants, the meat of monogastric animals typically exhibits a higher omega-6 to omega-3 ratio, largely due to diets that are rich in linoleic acid. Additionally, both genetic and environmental factors significantly influence the fatty acid composition in muscle tissue, with dietary fatty acids playing a crucial role among these factors. The impact of genetic determinants, such as gender, species, and breed, on the variations in fatty acid composition of meat has also been the subject of discussion and investigation (De Smet et al., 2004).

Utilization of fat in ruminant diets

The significance of incorporating fats as an energy source in the diets of high-producing ruminants, particularly dairy cows, during the early stages of lactation is common practice due to the high energy demand in that phase. Furthermore, the physiological relevance of certain unsaturated fatty acids has established a foundation for the strategic application of various fatty acids, independent of their energy contribution (Reynolds et al., 2003; Santos et al., 2008). The inclusion of dietary fat is essential for the maintenance of health. In ruminant nutrition, fat supplements are incorporated to enhance energy density,

promote food intake, augment milk and meat production, and influence the fatty acid composition (Bauman and Peterson, 2003). Dietary fat has the potential to influence digestibility within the rumen. The primary impacts of dietary fat include a decrease in fiber digestion, a reduction in methane production, and a diminished ratio of acetate to propionate, which subsequently leads to a decrease in protozoan populations as well as the growth and metabolic activity of bacteria, particularly those involved in cellulose decomposition. When acetate is low, the formation of methane is at the lowest level, and energy efficiency becomes higher (Figure 1). These effects are predominantly attributed to unsaturated fatty acids. Furthermore, the manner in which animals digest fat significantly affects the transfer of fatty acids from the diet to animal-derived products (Patra, 2014; Rasmussen and Harrison, 2011; Sun et al., 2021).

In monogastric animals, the primary site for the digestion of dietary fats is the small intestine. In contrast to ruminants, monogastrics absorb dietary fatty acids in their unchanged form, subsequently transferring them to tissue fat. Consequently, the type of dietary fat directly influences the fatty acid composition of poultry and pork products. An increase in unsaturated fatty acids within the diet serves as a straightforward method to enhance their proportion in tissue. Additionally, dietary fat can impact ruminal digestion and metabolic activity of bacteria and protozoa (Nguyen et al., 2018; Polidori et al., 2019). The precise mechanisms through which fat supplementation influences the reduction of dry matter intake remain inadequately elucidated. However, several factors can be identified: adverse impacts on rumen fermentation and gastrointestinal motility, the secretion of intestinal hormones, hepatic fat oxidation leading to decreased feed intake, and the accumulation of feed within the rumen due to impaired fiber digestion. Additionally, metabolic regulation mediated by the hormone cholecystokinin, which acts on the satiety center in the brain, may also play a role. Furthermore, the presence of polyunsaturated fatty acids in the small intestine may diminish intestinal motility, thereby contributing to a decrease in dry matter consumption (Fiorentini et al., 2015; Gouvêa et al., 2022).

Protected fats in ruminant diets

Fats in feed are easily attacked by rumen microorganisms, especially when they are not protected against microbes. At this stage, fats are hydrolyzed in the rumen, which is a very important process related to the digestion and metabolism of fats in the following parts of the digestive tract (Gouin, 2018). Inactivated or protected fats represent a category of fat supplements that have undergone various processing methods. A portion of these supplements is able to reach the intestine without being altered

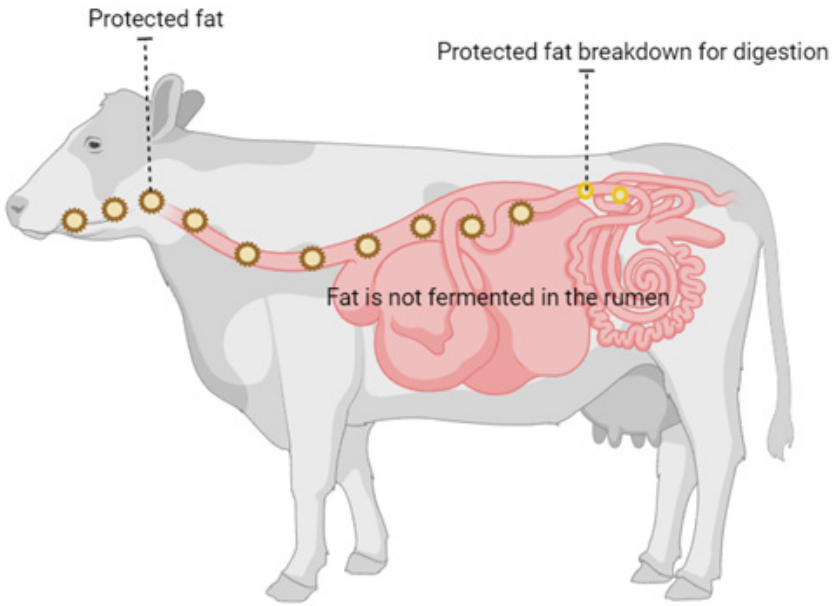

Figure 3. Schematic of stability and breakdown of protected lipids in ruminants. Source: IMPORTANCE OF BYPASS FAT – IN DAIRY ANIMALS DIET | Pashudhan praharee (https://www.pashudhanpraharee.com/importance-of-bypass-fat-in-dairy-animals-diet/)

by diminishing fiber digestion, reducing methane production, and altering the acetate-to-propionate ratio. These effects are attributed to the inhibitory influence of unsaturated fatty acids, particularly those with multiple double bonds, on the growth by rumen microorganisms, which is why they are often referred to as transitory fats. These fat supplements are characterized by their solid form and ease of application (Jenkins and McGuire, 2006). Various techniques have been developed to protected

fats and oils from rumen biohydrogenation. Notably, there has been significant advancement in the production of protected fat supplements in recent years. These techniques encompass processing with formaldehyde, coating, calciumization, amidation, and the creation of fat supplements that are sensitive to melting point, which are inactive in the rumen (Elnashar et al., 2015, Besharati et al., 2022).

In some studies, the use of non-protected fat sources caused a decrease in fiber digestibility and milk fat production (Grommer, 1984). Consequently, protected fats are currently utilized in the nutrition of ruminant animals. Novel protected fats, such as calcium salts derived from palm oil fatty acids, are commercially employed in various regions globally. These fats are purported to be insoluble at the natural pH and temperature of the rumen, and they reportedly do not adversely affect rumen fermentation (Behan et al., 2019; Satir et al., 2023). Several studies have indicated that incorporating protected fats into the diets of dairy cows results in enhanced milk production without negatively impacting the fat content of the milk (Palmquist and Jenkins, 1980). Also, these fat sources have been able to improve energy conversion efficiency in lactating cows (Figure 3) (Chalupa et al., 1986).

Oil seeds

A significant determinant of the concentration of unsaturated fatty acids in ruminant milk is nutrition. According to the researchers, incorporating oilseeds such as canola, safflower, soybean, cottonseed, and other similar sources into the diet of dairy cows enhances the levels of omega-3 conjugated linoleic acid in the milk produced (Glasser et al., 2008). Flaxseed serves as a significant and valuable source of omega-3 fatty acids, particularly alpha-linolenic acid (Wright et al., 1998). Nowadays, the use of oils and oilseeds in animal feed has attracted the attention of researchers due to their multiple biological roles. Decreasing the energy demand in animals through the decrease of milk fat toward the start of lactation, by a few explicit unsaturated fats (Bauman and Griinari, 2003), increasing the feeding of carbohydrates and fats to meet the metabolic needs of livestock (Overton and Waldron, 2004), and the tendency to increase the concentration of conjugated linoleic acid in livestock products due to its effects on human health (Pariza et al., 2001; Privé et al., 2010a,b) can be counted among the reasons for this approach. Oilseeds are characterized by their high fat content, and the proportion of unsaturated fatty acids present in these seeds has gained significant importance. Research indicates that the incorporation of oilseeds into the diet of farm animals influences the fatty acid composition found in various tissues throughout their bodies (Peng et al., 2010).

Various studies in poultry (Ponnampalam et al., 2001), pigs (Morgan et al., 1992), and cattle (Mandell et al., 1997) have proven the change in the amount of omega-3 fatty acids in meat due to feeding with natural oils or oilseeds. Additionally, unsaturated fatty acids may prevent the accumulation of triglycerides in the liver by changing fat metabolism (Yoshikawa et al., 2002). Therefore, oilseeds, as rich sources of unsaturated fatty acids, can affect fat metabolism and even metabolic disorders such as fatty liver disease. On the other hand, these protein sources, which also have high energy levels, show great resistance against microbial digestion in the rumen. This causes these proteins to pass through the rumen and be digested and absorbed in the small intestine, which results in improved animal performance (Bourre, 2005). Flaxseed is one of the oilseeds and a rich source of omega-3 fatty acids, the linolenic acid contained in it is biohydrogenated by rumen microorganisms, and as a result, the amount of this fatty acid is less than 1% of the total fatty acids of milk. When this oil seed is placed in the intestine by canola, the amount of linolenic acid increases to 14% of the total fatty acids (Petit et al., 2002). A significant aspect of oilseed nutrition is the detrimental impact of unsaturated fatty acids found in these seeds on rumen fermentation. An increase in the concentration of these fatty acids within the rumen can lead to disruptions in the fermentation process, inhibition of microbial growth, and, ultimately, a decrease in digestibility within the rumen (Jenkins, 1993).

Manipulation of ruminal lipid metabolism

Initiatives have been undertaken to enhance the delivery of unsaturated fatty acids to the mammary glands in ruminants, in light of the numerous health implications associated with unsaturated fatty acids in dairy products (Jenkins, 1993). Researchers have focused on increasing the absorption and storage of desirable fatty acids in the mammary glands, which can be achieved by controlling rumen biohydrogenation (Chilliard, 1993; Jenkins, 1993; Palmquist, 1994). Doreau and Chillard (1997) showed that rumen biohydration can be manipulated by restricting biohydrogenation and managing disturbances in rumen carbohydrate digestion. The main goals of manipulating rumen lipid metabolism are to protect feed lipids from the biohydrogenation process and minimize the interference of dietary fat with rumen fermentation parameters (Doreau and Chilliard, 1997). As a result, this will increase the level of unsaturated fatty acids passed from rumen to mammary glands (Jenkins, 1993). A range of methodologies has been established to modulate lipid metabolism within the rumen, thereby facilitating the transfer of unsaturated fatty acids to the mammary glands. These methodologies encompass both physical and chemical treatments of consumable lipids. In recent decades, the introduction of innovative techniques, such as the microencapsulation of fatty acids, has garnered significant interest among researchers in the food industry. This approach is recognized as an effective strategy for safeguarding unsaturated fatty acids from oxidation while simultaneously mitigating undesirable odors and flavors (Shahidi and Han, 1993; Buccioni et al., 2012; Toral et al., 2018).

Preparation of nanoemulsions

An important step before microcoating is preparing a stable emulsion with the right properties (Hosseini et al., 2013). In general, oily nanocapsules and aqueous nanocapsules are made by using an oil-in-water or water-in-oil emulsion. Capsules can

be used in different fields depending on the type of emulsion used. As an example, aqueous nanocapsules are required for intravenous injection, so water-in- oil emulsion should be used to create them. However, the nature of the encapsulated materials (i.e., whether they are hydrophilic or hydrophobic) additionally determines the kind of nanocapsules required, which may not be viable with the expected application. Covering the droplets or particles with different layers can help. Proteins and different polymers can be used as coatings, which can be chosen for different properties other than hydrophilicity or hydrophobicity (Besharati et al., 2022).

Encapsulation

Microencapsulation was first utilized commercially in 1954 (Shahidi and Hahn, 1993). This innovation has been utilized in the drug business for the controlled delivery of medications in the body for a very long time (Rosinski et al., 2002). However, in the food industry, it is a new method that helps to increase the duration of drug storage during the process and to store and deliver the same drugs to target sites in the body (Korhonen, 2002). Encapsulation is a process of covering or enclosing one or more substances with a substance or system (Madene et al., 2006). Encapsulation ?an likewise be characterized as the innovation by which strong, fluid or vaporous materials are stuffed into little shut containers and the items are delivered at a controlled rate and under unambiguous circumstances. Microcapsules can safeguard a substance in a finely partitioned state and deliver it when required. These microcapsules commonly range in width from a couple of nanometers to a couple of millimeters and have various shapes relying upon the materials and techniques utilized in their production (Desai and Park, 2005). Most of the vitamins and micronutrient compounds in food are not stable and are subject to decomposition, the speed and amount of which depends on the chemical structure of micronutrients, the characteristics of the food matrix, the process conditions and the storage environment (Farhang, 2013). Therefore, encapsulation can slow down decomposition processes (such as oxidation or hydrolysis) or prevent the destruction of their compounds until the product is transferred to the right place. The microcapsule consists of a core and a shell. The center might comprise of crystalline material, retentive particles, emulsion, suspension of solids or a suspension of microcapsules. By and large, the hydrophobic center is safeguarded by the hydrophilic shell, while the hydrophilic material is encircled by the hydrophobic shell. The plan of the container shell is made to forestall the entrance and arrival of substances into the food until the ideal time. The job of the shell incorporates safeguarding delicate food parts like flavors, nutrients, or salts from water and oxygen, as well as protecting them from light. Contingent upon the physical and substance properties of the center, the synthesis of the wall, and the microencapsulation technique utilized, different sorts of fine particles can be acquired. These incorporate a basic circle with a covering of uniform thickness, a center with a sporadic shape, and center particles implanted in a grid of wall materials. There are many approved shell materials that can be used to produce a variety of microcapsules. All shell materials do not meet all the required properties and are often used together with other coating materials or modifying compounds such as oxygen absorbers, antioxidants, forking agents and surface-active materials. The approved ingredients for the capsule shell are: protein, gelatin, whey protein, sodium caseinate, fat and wax, hydrogenated vegetable oil, beeswax, soy proteins, polysaccharide, gum arabic, modified starch, maltodextrin, alginate, pectin and carrageenan (Shahidi and Han, 1993).

Compounds used in encapsulation

Carbohydrates such as starch and maltodextrin are used for food microencapsulation (Kenyon, 1995). These materials have low viscosity and good solubility in high content of solid materials, but they have weak interfacial properties, which is required for high efficiency of microencapsulation, and materials such as proteins and gums have this property (Dalgleish, 2006). An interaction to further develop the epitome properties of wall materials incorporates the synthetic change of sugars. For instance, certain adjusted starches have surface movement properties and are fundamentally used in microencapsulation through the shower drying technique (Sheik et al., 2006). Starch and cyclodextrin can actually retain unpredictable substances from the climate. This issue has made them phenomenal materials for flavors. Arabic gum is a generally utilized covering material due to its consistency, dissolvability, and emulsifying properties. However, because of its excessive cost, it has been utilized less regularly (Shahidi and Han, 1993). Arabic gum is a polymer comprising of diglucuronic corrosive, L-rhamnose, digalactose, and L-arabinose, and it contains roughly 2% protein. The emulsifying properties of this gum are credited to the presence of its protein part. This gum is a suitable wall material for microencapsulation of oleoresins compared to maltodextrin and modified starch. The resulting microcapsule exhibits free-flow properties (Krishnan et al., 2005; Dickinson, 2003). Maltodextrins provide good stability for encapsulated oils, but they have poor emulsifying ability and emulsion stability. Maltodextrins with a dextrose equivalent (DE) between 10 and 20 are suitable for use as wall materials. These samples are suitable for preserving flavors because they can be dissolved in water up to 35.5% without forming turbidity (Raja et al., 1989). Alginate, which is extracted from brown seaweed and reacts with calcium ions to form a stable gel, is utilized to encapsulate essential oils at room temperature (Rosenberg and Sheu, 1996). Whey proteins have been utilized as wall materials for encapsulating dehydrated milk fat through spray drying, achieving an efficiency rate of over 90% (Young et al., 1993). The addition of lactose to the whey protein-based wall system can restrict the movement of polar substances across the wall. Microencapsulation efficiency is improved by replacing 50% of whey protein with lactose. Lactose, in its amorphous state, acts as a hydrophilic sealant, thereby increasing the efficiency of microencapsulation (Bylaite et al., 2001).

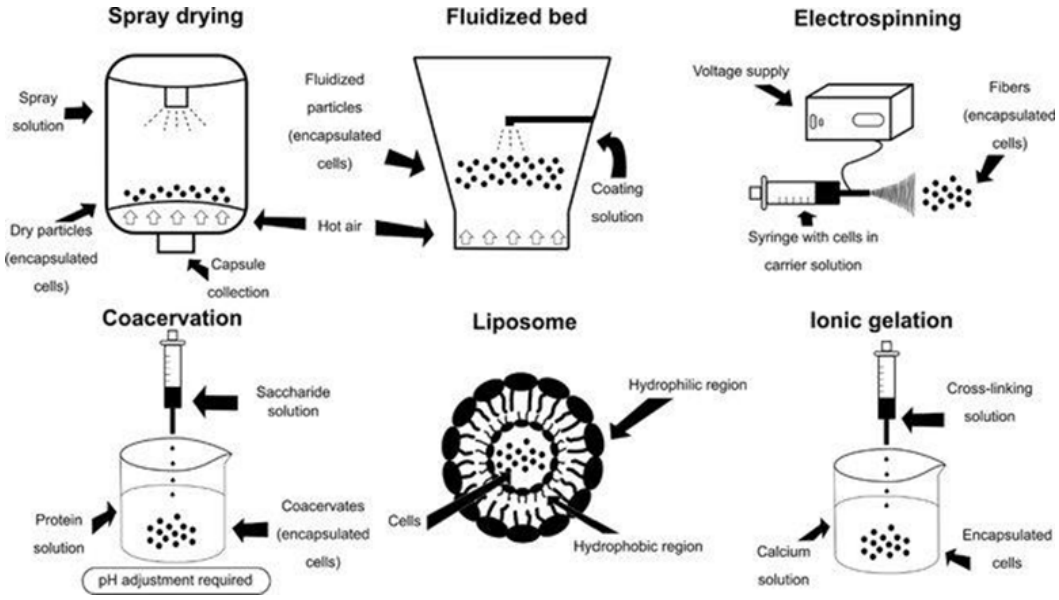

Figure 4. Examples of encapsulation techniques: Spray drying, fluidized bed, electrospinning, liposome, coacervation and ionic gelation. (Source: Valdivia- Rivera et al., 2022)

Encapsulation methods

The variety of products used, as well as the different chemical structures, makes it impossible to develop a comprehensive method for microencapsulating bioactive molecules. The process needs to be tailored to the materials (Figure 4).

Spray drying

This is the most common and oldest method for food microencapsulation. It is also used for active food molecules and live probiotics. Spray drying is a cheap and quick process (Shahidi and Hahn, 1993). The steps of this method are preparing the emulsion or dispersion for the process, homogenizing the dispersion and then atomizing it in the drying chamber (Gibbs et al., 1999). This assists with eliminating the dissolvable (water) rapidly. The powder particles are isolated from the drying air at the power source and at a lower temperature. This technique is utilized for typifying dynamic food mixtures like nutrients, minerals, flavors, unsaturated oils, and proteins. Just water-based scatterings are utilized in the splash drying process. Thusly, the lattice should be profoundly dissolvable in water. The principal impediment of this strategy is the limited scope of wall materials, which should have great dissolvability in water. Another inconvenience is that the subsequent microcapsule powder is extremely fine and requires an interaction like agglomeration (Rosenberg et al., 1990).

Spray cooling & Spray chilling

The procedure is similar to spray drying. The materials to be enclosed are dispersed in a liquid coating and then differentiated, with the difference that the temperature used in this method is cooler than spray drying, and in spray cooling it is from the ambient temperature and in spray cooling, it uses refrigerator. The wall materials used in these methods are melted fat and wax, and the melting point of fat is usually 32-42°C (Risch and Reineccius, 1995). Due to the lipid coating, the produced microcapsules are insoluble in water. Materials that are encapsulated by spray cooling include frozen liquids, materials that are sensitive to heat, and those materials that are insoluble in common solvents. This method is also used for foods with high-fat content, dry products such as dried soup mix and baking products and in order to protect flavors, enzymes, proteins and minerals (Gibbs et al., 1999).

Coacervation

The process involved in this technique is known as phase separation, which is considered a genuine encapsulation method as it fully encloses the core material within the matrix. This method includes the precipitation or partition of a colloidal stage from a fluid stage (Dziezak, 1998). Simple and complex methods of protection can be used in both cases. In a simple coating, an insoluble or mostly water-soluble polymer is applied. This polymer competes with gelatin protein solution for solubility through hydrophobic interactions. In

complex protection, the capsule is formed through the ionic interaction between two polymers with opposite charges. Typically, the positive charges of protein molecules interact with anionic molecules like gelatin and gum arabic. The capsule is produced when these opposite charges neutralize each other. Coacervation consists of separating the liquid phase of the coating material from the polymer solution and then coating that phase as a uniform layer around the suspended particles. By altering the ion concentration, adjusting the ratio of the protective material to the core material, and selecting different background materials, the characteristics and size of the capsules can be modified. The produced microcapsules are stabilized by cross-linking, heating and redissolving processes. These microcapsules are separated by centrifugation and dried by spray drying or fluid bed. Hydrophilic protective materials such as gelatin are used for microencapsulation of hydrophobic materials such as vitamin A, vegetable oils and citrus oils.

Liposome Entrapment

Liposomes are single or multi-layered vesicles with an aqueous core and a phospholipid membrane. The outer layer of the liposome is formed by phospholipids. The hydrophilic part connects with the aqueous phase, while the hydrophobic parts are in contact with the hydrophobic parts and other lipids, forming a sheet. When this sheet is folded into a spherical shape, it creates a highly stable capsule. The size of liposomes can vary from a few nanometers to a few microns (Risch and Reineccius, 1995). One of its important applications can be mentioned in the dairy industry. Proteolytic enzymes encapsulated in liposomes are used to speed up cheese ripening. These liposomes containing proteolytic enzymes have the ability to improve the taste and texture of low-fat cheese. Increased proteolysis leads to a softer texture and distinct flavor and aroma in low-fat cheeses. To use the enzyme encapsulated in the liposome, the following conditions must be present (Reineccius, 1994):

- The ability of the enzyme to withstand the encapsulation process without losing its activity.

High efficiency of encapsulation, which allows the accumulation of a suitable amount of enzyme in cheese.

Enzymes release at the right time.

High retention of liposomes in cheese.

Fluidized bed coating

A high-speed chamber is used for atomizing coating materials of solid particles suspended from the air at a high temperature and humidity. A particle size of 50 to 500 microns produces optimal results. Limited distribution of particle sizes is also necessary. In the chamber, the amount of material that covers particles depends on how long the particles have been there. In this method, hot melt coatings such as starch, gums, maltodextrins, emulsifiers, and waxes are mixed with hydrogenated vegetable oil, stearin, fatty acids, emulsifiers, or waxes are mixed with solvents. The carrier for hot-melt materials is hardened by cool air, while the solvent is evaporated by hot air for solvent-based coatings. As temperatures increase or physical fracture occurs, hot melt components release their contents, while water-soluble coatings release their contents when water is added. In multivitamins and small tablets such as children's vitamins, the fluid bed capsule can separate iron from ascorbic acid. It is common to find fluid bed encapsulated components in fortified foods, nutrient mixtures, and dry mixes. It is recommended that you avoid eating products that contain citric acid, lactic acid, sorbic acid, vitamin C, sodium bicarbonate, and sodium chloride (Risch and Reineccius, 1995).

Extrusion

The capsule material is produced by passing through the nozzle of the equipment with the pressure of the solution to produce small drops. A smaller inner diameter of the nozzle results in a smaller capsule. As well as being nontoxic and applicable to aerobic as well as anaerobic conditions, this method has many advantages for encapsulating microbes. For substances that are unstable in the presence of heat, such as flavors, enzymes, proteins, colors, and vitamins, this process is used. By using this method, encapsulated flavors are produced for drinks, desserts, and mixes (Besharati et al., 2022; Shahidi and Han, 1993; Dziezak, 1988).

Conclusions

Encapsulation can prevent sensitive compounds from being destroyed until they are transferred to the right location or slow down decomposition processes (such as oxidation or hydrolysis). Currently, emulsion spray drying is the most common method of fine oil microcoating. The mass formation method produces more stable microcoatings with higher oil content than spray drying, as recently discovered. Biodegradable polymers have gained much attention as encapsulation materials. Unsaturated lipids (fats and oils) that have been encapsulated can be added to ruminants' feed and will be protected from ruminal hydrogenation, thereby yielding meat and milk with higher levels of these healthy compounds. The method is a simple means of improving product quality.

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