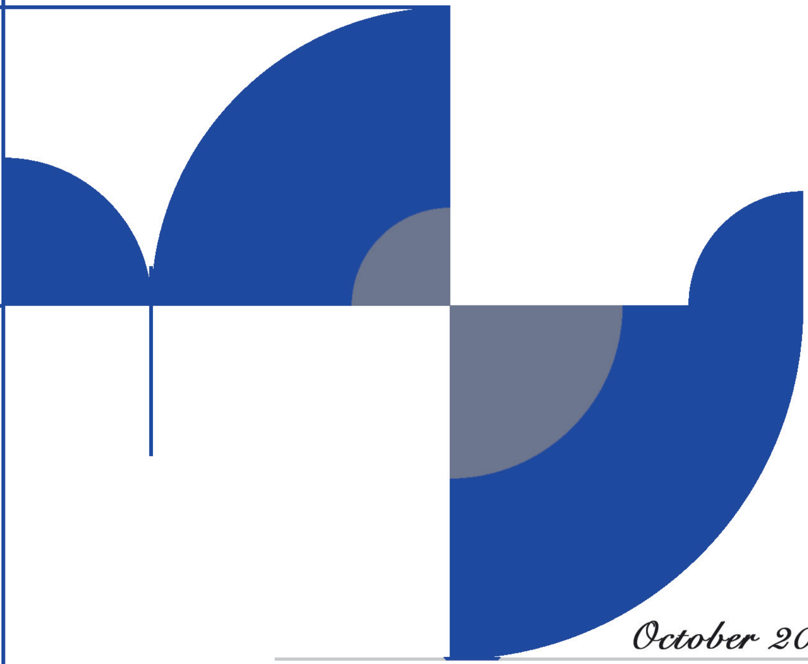

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INTERNATIONAL STUDIES AND EVALUATIONS IN THE FIELD OF

FOOD ENGINEERING

EDITOR

PROF. DR. ARZU KAVAZ YÜKSEL

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Chapter 1

PROBIOTIC FOODS AND THEIR EFFECTS ON HEALTH

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Introduction

The word 'probiotic' comes from the Greek term (pro bios) and mean 'life' (Fuller, 1989). The probiotics are referred to as a single or combination of living microorganisms which are used on both human beings and animals (e.g. fermented products) for having the benefit for the host as the characteristics of indigenous microflora are improved (Huis Veld & Havenaar, 1991). When the microbiologist examined the gut flora of a healthy person vs a sick individual, it was shown to be significantly different. Nobel Prize winner Elie Metchnikoff noted that microbiota which choose particular useful ones can affect gut health. The bacteria appear to strengthen its body natural defenses (Getahun et al., 2016). Fuller, (1989) was the one who provided the first definition "Probiotics are live microorganisms that enhance the microbial equilibrium of the host, resulting in beneficial effects". "Probiotics are the live microorganisms that, when consumed in proper quantities, benefit the host's health". According to the FAO official definition, probiotics are living microorganisms that upon ingestion in certain amounts, become beneficial for the health of the host (FAO/WHO, 2002). A consultative committee was established by the Association of International Scientists of symbiosis's, in Oct 2013 to modernize the concept of probiotics through the process of development and the amendment of the definition provided by FAO and WHO (Rashmi & Gayathri, 2017). Food products labeled as 'probiotic' need to maintain a viable microbial count of ≥6 log CFU per milliliter or gram throughout their shelf life in order to deliver the health benefits they promise (Ganguly S & Sabikhi L, 2012). The popularity of utilizing probiotics for bio-therapeutic use has increased resulting from the arousement of consumers' awareness. Wide studies on these bacteria developed the belief that it could be consumed on regular basis to add several health benefits. These probiotic products find their way into many other food products hence helping to create a new segment which is referred to as 'probiotic foods' in the course of their inclusion into different food products. Lactobacillus and Bifidobacterium bacteria is the most commonly known cell that is known as probiotic (Doron & Gorbach, 2006).

Microorganism as Probiotics

It was shown that *Lactic acid bacteria* (LAB), *bifidobacteria*, as well as *Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium lactis, Escherichia coli, Saccharomyces cerevisiae, Saccharomyces boulardii,* and *Streptococcus lactis*, etc also are the most common probiotics as seen on the (Table 1). Most of the work concentrated on the in vitro isolate culture and very few have carried it till the animal and/or human model. In spite of that however, the strainspecific activity is the agent with most of the important probiotics. Lactic acid bacteria such as Lactobacillus, Streptococcus, Enterococcus, Lactococcus, and Leuconostoc do not form spores and are not capable of movement. These species receive nutrients by fermentation of sugars. They are usually facultative anaerobes (Das et al., 2022; Khaneghah & Fakhri, 2019). As reported by Zheng et al., (2020) Scientists reclassified the Lactobacillus genus into a more specific grouping. They divided it into 25 different categories, with two familiar names: *Lactobacillus delbrueckii* and *Paralactobacillus.* These two belong to a group adapted to living on or in hosts. The remaining 23 new genera have names like Holzapfelia, Amylolactobacillus, and Schleiferilactobacillus.

Table 1 Probiotics Microorganisms

Genus	**Species**
Lactobacillus	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus salivarius, Lactobacillus johnsonii, Lactobacillus gallinarum, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus brevis, Lactobacillus murinus, Lactobacillus crispatus, Lactobacillus amylovorus*
Bifidobacterium	*Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium thermophilum, Bifidobacterium pseudolongum*
Yeast	*Saccharomyces boulardii, Saccharomyces lactis, Saccharomyces carlsbergensis, Kluyveromyces marxianus, Saccharomyces cerevisiae*
Other Microorganisms	*Bacillus licheniformis, Bacillus subtilis, Enterococcus faecium, Enterococcus faecalis, Leuconostoc mesenteroides, Lactococcus lactis, Lactococcus citreum, Streptococcus salivarius subsp., Propionibacterium freudenreichii, Pediococcus pentosaceus, Pediococcus acidilactici. thermophilus, Streptococcus infantarius*

LAB is frequently found in the intestinal tracts as well as in various fermented foods like pickled cucumbers, fermented milk product, alcoholic beverage, fermented grape drink, fruit drinks, and sausage. Lactic acid bacteria can offer various health benefits in addition to their nutritional advantages. Probiotics enhance the nutritional content of food, regulate intestinal infections, improve lactose digestion by increasing lactase production, influence certain varieties of tumors, and reduced levels of cholesterol in the blood (Table 2).

Table 2 Common Fermented Foods and Their Lactic Acid Bacteria (LAB) Strains

Fermented Food	LAB Strains	Reference
Yogurt	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus*	(Castellone et al., 2021)
Cheddar Cheese	*Lactobacillus lactis subsp. Lactis, Lactobacillus lactis subsp. Cremoris, Streptococcus thermophilus*	(Oberg et al., 2022)
Italian Cheese (Mozzarella)	*Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus helveticus, Lactococcus lactis, Streptococcus thermophilus*	(Guidone et al., 2016)
Swiss Cheese	*Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus lactis, Propionibacterium shermanii, Lactobacillus lactis subsp. biovar diacetylactis, Leuconostoc mesenteroides subsp. cremoris, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Streptococcus thermophilus*	(Wiegmann et al., 2022)
Goat and Sheep Cheese	*Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. biovar diacetylactis, Leuconostoc mesenteroides subsp. cremoris*	(Tilocca et al., 2022)
Butter and Buttermilk	*Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. lactis biovar diacetylactis, Lactococcus lactis subsp. cremoris, Leuconostoc mesenteroides subsp. cremoris*	(Ogrodowczyk et al., 2021)
Kefir	*Lactobacillus kefir, Lactobacillus kefiranofacies, Lactobacillus brevis*	(Azizi et al., 2021)
Fermented, Probiotic Milk	*Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus johnsonii, Bifidobacterium lactis, Bifidobacterium bifidum, Bifidobacterium breve*	(Castellone et al., 2021)
Fermented Sausages	*Lactobacillus sakei, Lactobacillus curvatus, Pediococcus acidilactici, Pediococcus pentosaceus*	(Agüero et al., 2020)

Probiotic Foods

Probiotics basically refers to live microorganisms which are used to activate, counteract, and maintain health in the human body. They produce enzymes that help with digestion. (Amara & Shibl, 2015). Furthermore, they contribute to the production of B complex vitamins and help in controlling intestinal infections from harmful microorganisms (Saad et al., 2013; Sabo et al., 2020; M. P. Silva et al., 2016). In the creation of probiotic foods, the food matrix is artificially infused with an adequate amount of live probiotic strains (Terpou et al., 2019; Wilkinson, 2018). Consuming 100 grams of probiotics per day can help improve human health, although the specific benefits might differ based on the strain used (Derrien & van Hylckama Vlieg, 2015; Rezac et al., 2018). The quantity of microorganisms in the included food can range anywhere from one to seven species. The majority of probiotic foods on the market are dairy products, making them an ideal source for beneficial bacteria like *Lactobacillus casei, Lactobacillus acidophilus*, and certain *Bifidobacterium* species (Vlasova et al., 2016). Fermented dairy products with probiotics naturally provide a supportive environment, while non-dairy probiotic foods face more challenges. Every type of food has its own specific structure, requiring more effort from food experts to create a dependable and affordable non-dairy probiotic product that is rich in nutrients and appealing to the senses. Many non-dairy probiotic foods have been featured on shelves of supermarkets worldwide, despite facing numerous technical challenges (Dey, 2018). For a probiotic product to be successful, the viability of the microbial cultures needs to be preserved during the production process and storage. This is mostly done by direct vat set method (DVS).

For a probiotic product to be successful, the viability of the microbial cultures needs to be preserved during the production process and storage (Sanz, 2007). This is mostly done by method of setting vat directly (Kailasapathy, 2013). Freeze-dried powders or highly concentrated deep-frozen cultures are employed for culturing purposes. The most common DVS mass cultures are regarded with favor as only a few of the probiotic cells can be cultured in an industrial production plant. Frozen culture with a content of microbial population higher than 1010 CFU/g compared to freeze-dried culture holds a content of at least 1011 CFU/g (Høier et al., 2010).

The first steps for the launch of probioticic food products are to isolate strains that are able to resist the gastric conditions like digestive enzymes in the stomach and bile (Saha, 2017). Additionally, it enhances the duration of food on its shelf products and survives passing through the intestine (Singh et al., 2011). Another critical trait needed to be considered are the ability to stick to the intestinal mucosal cells for colonization, ability to release antibacterial substances against various pathogenic bacteria and long-term survival adequacy(Fooks et al., 1999). Additionally, the chosen strains must have the capability to suppress the virulence gene's expression, including toxin production (Manzoni et al., 2008; Singh et al., 2011).

Probiotic Yogurt

Yogurt has been a common fermented food in many societies' diets for millennia, coming from the Turkish word 'yogurmak' meaning to thicken, clot, or curdle (Rul F, 2017). Yogurt is made by causing milk to acidify, resulting in the formation of curd. Furthermore, that processes with acidification in which there is availability of and multiplication of natural lactic acid bacteria (LAB) namely *Lactobacillus delbrueckii, Streptococccus thermophilus*, as well as *L. acidophilus*, *Lactobacillus casei* and *Lactobacillus paracasei, Bifidobacterium* (Schillinger et al., 2005). Several research have shown that yogurt with probiotics containing specific strains of probiotics offers benefits for health like reducing blood cholesterol levels, decreasing blood pressure and heart rate, as well as providing antihypertensive effects (Nadelman et al., 2019; Sahu & Panda, 2018; Sarfraz et al., 2019; Shafi et al., 2019).

Probiotic Milk

Ethnic communities across South Asia prepare a variety of cultured milk with varying local names. The area of the Himalayas (Dewan & Tamang, 2007). The main probiotic microorganisms present in fermented milk include *Lactobacillus bifermentans, Lactobacillus paracasei, Lactobacillus kefir, Lactobacillus hilgardii, Lactobacillus alimentarius, Lactobacillus plantarum, Lactococcus lactis, Lactococcus cremoris, Enterococcus faecium,* and *Bifidobacterium longum.* Probiotic fermented milk may improve plasma cholesterol levels and aid in lowering Levels of LDL in individuals with high cholesterol and reducing hypertension (Sahu & Panda, 2018). Dong et al., (2013) found that probiotic milk has a beneficial effect in lowering blood pressure, people who have high blood pressure or are at risk of developing high blood pressure. Certain researchers carried out significant research on the cultured milk from sheeps and goats, uncovering the probiotic survival and bioactivity of the products (Balthazar et al., 2019; Mituniewicz–Małek et al., 2019).

Another Dairy Drink Containing Probiotics

Cheese, a fermented milk-based food, is a common element in the traditional eating habits of various societies. According to archaeological findings, the making of cheese was seen to be carried out in Poland, Europe around 5500BC. Eventually, the practice of cheese production expanded to various regions including Europe, the Middle East, and other places. The whey and buttermilk that comes from making cheese create suitable physicochemical environments for probiotic microbes to grow and thrive, leading to the use of probiotic cultures in the cheese fermentation process (Sahu & Panda, 2018). A group of researchers created methods to develop new types of cheese and products similar to cheese that make specific claims related to health and enhance sensory qualities. For example, they produced probiotic prato cheese, which was discovered to decrease oxidative stress in the lungs, liver, and intestines, as well as tulum cheese with *L. acidophilus* (Soares et al., 2019; Tomar, 2019; Vasconcelos et al., 2019).

Fermented Meat Products

Kitoza plays a crucial role in Malagasy cuisine as a diverse selection of smoked, salted, and dried pork or beef products (Possas et al., 2019). Beef, or pork can be used to make fermented sausages, a combination of both, they are known for their strong and pungent scent and flavor. The live good bacteria bacteria found in fermented sausage include *Lactobacillus gasseri, Lactobacillus fermentum, Lactobacillus casei/paracasei,* and *Lactobacillus rhamnosus* (Bis-Souza et al., 2019). Currently, there is a growing fascination with probiotic bacteria due to the increasing amount of evidence showing the significant role the human gut microbiota plays in overall health, especially in its interactions with other organs like the skin and brain. Fermented sausages can serve as an effective vehicle for probiotic lactic acid bacteria, creating a functional food while utilizing the metabolic traits of the added strains, which then function as starter cultures (Carballo, 2021).

Kefir

Kefir has gained recognition as an innovative probiotic food worldwide. The drink is made at home, thick, slightly bubbly, and acidic (Karaçalı et al., 2018; Plessas et al., 2016). The consortium of bacteria and yeast in kefir grains work together in symbiotic associations to ferment and produce kefir. In general, kefir grains contain a fairly solid and unique combination of microorganisms surrounded by a complicated mixture of polysaccharides and proteins (Yao et al., 2017). Homofermentative Streptococci, Lactic acid bacteria and acetic acid bacteria are the primary microbes present in kefir culture (Leite et al., 2013). Assist in rebalancing the ratio of beneficial and harmful microbes in your gut, which may result in enhanced digestion, a more robust immune system, and an improved mood (Kechagia et al., 2013). Research indicates that kefir might help decrease inflammation by blocking inflammatory proteins (Culpepper, 2022). Although initial findings show kefir's possible benefits in managing blood sugar, cholesterol, and specific cancers, further research is necessary for confirmation (Azizi et al., 2021).

The impact of probiotics on health

Numerous positive impacts of probiotics on maintaining intestinal balance have been observed (Maldonado Galdeano et al., 2019). Probiotics offer health benefits that are linked to the avoidance and reduction of various health problems, including allergies, The tumor, high cholesterol, diseases that cause inflammation in the intestines, symptoms of diarrhea, and a condition known as irritable bowel syndrome (Grom et al., 2020). As in the (figure 1). The microorganisms in the gut represents a promising treatment goal for treating diseases caused by allergies, as it plays a role in regulating immune and inflammatory responses, which, in turn, influence the onset and progression of sensitization and allergies (Fiocchi et al., 2015; Harata et al., 2016). Research by Yang et al., (2021) found that certain strains of Lactobacillus might have anti-allergic properties. In a study involving mice with soybean sensitization, daily administration of these bacteria for 28 days increased levels of immune-boosting factors like interferon-γ and IL-2, while reducing allergy-associated markers such as IL-4 and IL-6. The treatment also promoted regulatory T cells, known to suppress inflammation. Although these findings are from studies on mice, they suggest that certain Lactobacillus strains could potentially be used as a dietary intervention to alleviate allergic reactions by shifting the immune system to a less inflammatory state.

Probiotics have the potential to adjust gut bacteria and boost immunity, making them a possible additional treatment for different cancer types. They stop the development, advancement, and spread of tumors that can be transplanted or induced chemically (Samanta, 2022). Lee et al., (2019) investigated the anti-tumor effects of the beneficial bacteria strain known as *Lactobacillus fermentum* and found that it may induce intrinsic apoptosis in human colon cancer cell lines. research showed that *Lactobacillus fermentum* could trigger the intrinsic apoptosis pathway, which is a form of programmed cell death activated by internal signals. The study's findings suggest that *Lactobacillus fermentum* may have potential as a therapeutic agent or dietary supplement in cancer treatment, specifically by activating apoptosis in cancer cells.

Probiotics are a helpful method for reducing blood cholesterol levels. They have the ability to lower cholesterol levels in the body either directly or indirectly (Thakkar et al., 2016). A Research done by Palaniyandi et al., (2020) demonstrated that *Lactobacillus fermentum* could be beneficial in treating hypercholesterolemia. The researchers administered 5×10^{10} CFU of this probiotic to male mice and observed a substantial decrease in overall cholesterol and low-density lipoprotein (LDL) cholesterol levels. Additionally, the treatment led to an increase in the expression of the LDL receptor (LDLR) gene, which plays a crucial role in regulating cholesterol levels in the body. These findings suggest that *Lactobacillus fermentum* might be a potential dietary supplement for managing high cholesterol and LDL levels, though further studies are needed to explore its efficacy and safety in humans.

The gut is essential for breaking down and absorbing nutrients while also preserving the integrity of the mucosal barrier. Many beneficial bacteria live in the human gastrointestinal system, forming a dynamic community that significantly impacts human health (Shehata et al., 2022). Tamaki et al., (2016) explored the effects of *Bifidobacterium longum* 536 (BB536) on ulcerative colitis (UC) in a clinical trial involving 56 patients with mild to moderate UC. Participants took $2–3 \times 10^{11}$ CFU of BB536 three times daily for eight weeks. The results showed a decrease in the Mayo subscore and the Rachmilewitz endoscopic index (EI), indicating an improvement in UC symptoms and endoscopic findings. This suggests that BB536 could be a beneficial probiotic treatment to help manage ulcerative colitis.

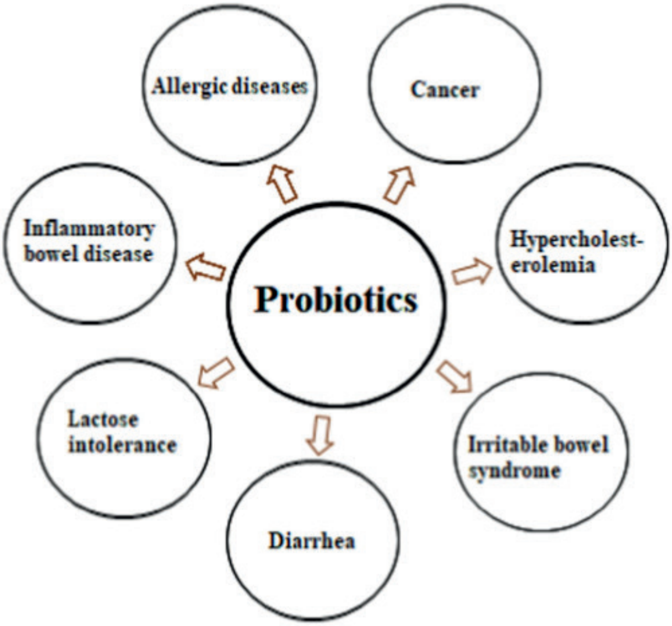

Figure 1 Benefits of probiotics for health (Latif et al., 2023)

Conclusion

Probiotic foods offer a range of health benefits, from improving gut health to reducing cholesterol and blood pressure. These foods, including yogurt, milk, cheese, fermented sausages, and kefir, contain live microorganisms that can positively impact human health. While there is significant evidence supporting their benefits, further research is required to establish specific mechanisms and long-term effects. Given the increasing interest in probiotics, future studies should focus on identifying strains with the most substantial health benefits and developing effective probiotic products.

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Chapter 2

THE PROBLEM OF MICROPLASTIC IN FOOD AND ITS EFFECTS ON HUMAN HEALTH

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Introduction

Although the use of plastic in every field today has created convenience in modern life, it causes environmental pollution globally. Today, world plastic consumption has reached 370 million tons annually (Eryilmaz and Demirarslan 2022). Since plastic products last for a long time (400-1000 years), their damage to the environment continues long years (Worm et al., 2017). Big plastic materials can break down in nature and turn into small particles under the influence of various factors such as ultraviolet radiation and weather conditions (Cooper and Corcoran, 2010; Santos et al., 2009;). Particles smaller than 5 mm in size are called microplastic (MP) (Thompson et al., 2004). In addition to this resource, microplastics consist of plastics produced primarily in small sizes (Gregory, 2009; Cole et al., 2011). Latest studies show that plastic materials and particles (micro/nano size) and their related chemicals may harm to human health (Rist et al., 2018). Plastic particles deliberately produced for consumer care products or industrial uses are called primary microplastics, while products of degradation of large volumes of plastic materials in the environment under natural processes are called secondary microplastics. Nanoplastics are plastic particles smaller than 1 µm(Arthur et al., 2009; GESAMP, 2015; Andrady, 2017; Fonseca et al., 2017; Aslan, 2018; Welle & Franz, 2018; ECHA, 2019). Microplastics, which are fragments in the range of 0.1-5000 µm, are a heterogeneous mixture of materials of different shapes called fibers, spheres, granules, lumps, flakes or beads. (CONTAM 2016).

Transmission Ways of Microplastics

With daily consumption, water represents the most common vehicle for chronic exposure to microplastics and is the most significant source of microplastics in the diet. Additionally, large amounts of water are consumed in the production stages of food, in the sanitation and cleaning of food processing facilities, in its use as a food ingredient and in many various machining operations. Microplastics can interfere drinking water supplies in a variety of ways: surface runoff (e.g. after rain), wastewater effluents (treated and untreated), combined sewer system overflows, industrial sewage, decomposed plastic waste and atmospheric deposition (Rist et al. al., 2018; WHO 2019; Hayoglu et al., 2022). Plastic bottles and caps in packaged water can also be a source of microplastics ingested through drinking water. The primary cause of the global problem of contamination of drinking water with microplastics is the relatively higher amount of microplastics in bottled water. Contamination of microplastic has been found in studies of packaged drinking water, as well as various other types of alcoholic and non-alcoholic beverages. For some products of other beverage types, such as beer, the source of microplastic contamination is presumed to be water, but for other foods, the source of microplastic contamination is not only water but also the environment, the materials used, the production processes or the packaging materials (Udovicki et al., 2022).

Plastics can enter both groundwater and surface water bodies in different ways. It is also thought that 80% of plastic litter in marine habitats originates from terrestrial sources such as garbage, inland waterways, wastewater discharges, industrial waste and various natural phenomena (Jambeck et al., 2015). These plastic wastes have the possibility of being ingested by some organisms (invertebrates and vertebrates) depending on their size and various characteristics (physiological and behavioral) of some organisms. This type of behavior has been commonly observed in a number of marine organisms (Horton et al. 2017). The best indicator of microplastic pollution in aquatic environments is the microplastic levels in marine organisms, most commonly fish and shellfish. The risk of large fish species ingesting microplastics is thought to be much higher than the risk of humans ingesting microplastics. This is because microplastics are partially removed by the gastrointestinal tract of organisms that consume them. On the other hand, people who consume smaller fish, such as sardines, anchovies and many types of shellfish, rather than larger fish, are at higher risk of consuming more microplastics. Furthermore, the possibility of trophic transfer of microplastics in pelagic, benthic and aquatic food chains is of increasing concern, as predators feed on prey that consume microplastics and may accumulate microplastics, although not directly (Lusher et al. 2017). When it comes to the role of microplastics in the daily human diet, fish and seafood have been the most studied and understood sources, and many studies have shown that microplastics are abundant and occurring in these sources. Over the last decade, researchers have found that microplastics are extremely common not only in fish and shellfish from aquaculture farms or markets, but also in wild-caught fish and shellfish from the sea (Kwon et al. 2020).

Another common vehicle for exposure to microplastics is salts and sugars, as these foodstuffs are frequently consumed both alone and as part of a variety of food products. Salts and sugars are also frequently used as additives, stabilizers and thickeners in the cosmetic and pharmaceutical industries. Salt is classified into the following categories depending on its source: sea and lake salt from evaporation, river or well salt from wells located away from the coast, and rock salt from mining (Iñiguez et al., 2017). Researchers investigating microplastic particles using a spectroscopic identification method revealed that candies contain high levels of microplastic (Udovicki et al., 2022).

Terrestrial plants are in direct contact with plastic contamination from multiple sources, such as sewage sludge and implementation of organic fertilizers, agricultural plastic films or deposition of atmospheric (Tympa et al. 2021). While washing or cleaning is effective in eliminating the presence of microplastics on plant surfaces and the resulting human exposure to microplastics, whether microplastics can contaminate the edible tissues of plants is also an important issue. Mateos-Cárdenas et al. (2021) investigated the effects of microplastics on terrestrial plants and aquatic macrophytes in a review. According to this research, the ability of microplastics to be adsorbed and/or internalized to a certain extent by plants or macrophytes has been proven and supported by many similar studies.

Studies show that humans consume approximately 39,000 to 52,000 microplastic particles per year through food alone (Schmaltz et al., 2020). For example, packaging has been proven to preserve the shelf life and nutritional value of fruits and vegetables after harvest (Kızıldeniz et al., 2023). Materials in contact with food, ingredients and articles that are likely to come into contact with food at any stage of the food chain. These stages are food processing, handling, preparation, storage, and service. Therefore, any of these stages can be a potential source of various physical, biological and chemical hazards. The general safety principles of EU Directives (EC) No 1935/2004 and (EC) No 2023/2006 apply to food contact materials(European Commission 2004, 2006). In addition to general legislation, there is also specific legislation for plastic materials (recycled), active and smart materials and certain food contaminated materials such as BPA, ceramics, nitrosamines, regenerated cellulose films, and epoxy derivatives (EFSA 2020). However, the recent presence of microplastics in mandatory food safety has revealed that there are new dangers and that precautions must be taken. (Udovicki et al., 2022).

The presence of microplastics is well known as a result of contamination by materials in some packaged products, such as bottled water (Mason et al. 2018; Schymanski et al. 2018). It is known that baby bottles made of polypropylene raw material are likely to disperse up to 16,200,000 microplastic particles per liter (Li et al. 2020). Kedzierski et al. (2020) reported polystyrene food containers as a source of microplastic contamination in meat. Microplastics can also be produced during people's daily activities. Examples of these include opening the plastic packaging of chocolate, tearing or cutting the packaging tape when opening the package, bending or opening the bottle when consuming water or another beverage. When such simple daily activities were examined, it was stated that approximately 0.46–250 particles/cm of microplastic could be produced (Sobhani et al. 2020). Even brewing a single plastic tea bag at an appropriate brewing temperature can release large quantities of microplastic particles into the environment (Hernandez et al. 2019).

The effect of microplastics on human health

Microplastics usually enter the human food chain through contaminated food and pose a potential health risk. Microplastics can also get into the human body through inhalation. (Karbalaei et al., 2018). Skin and dermal contact are among the causes of microplastic intake into the human body, although at low rates (Campanale et al., 2020).

When Wu et al. (2019) examined the health impacts of microplastics, they stated that it has an effect on the colon efflux pump inhibition ability and causes and initiates cytotoxicity in human intestinal cells (Barboza et al., 2018 ; Pitt et al., 2018 ; Brandts et al., 2018). Oxidative stress through the production of free radicals from reactive oxygen stress species (ROS) can occur due to cytotoxicity caused by microplastics (Qu et al., 2018 , Tang et al., 2018; Liu et al., 2019). Various studies have found this connection in the monogonont rotifer living in aquatic areas (Jeong et al., 2016), the zebrafish Danio rerio (Lu et al., 2016), the flatworm Caenorhabditis elegans (Lei et al., 2018), and the mouse liver (Yang et al., 2019) and human intestinal cells (Wu B., et al., 2019). Overproduced ROS may have an effect on cell homeostasis via antioxidant systems. ROS suppress antioxidants produced in response to destruction that affects cellular ingredients, including carbohydrates, lipids, proteins and DNA. Gene imbalance, physiological changes and carcinogenesis can be given as examples of these damages (Birben et al., 2012 ; Nita et al., 2016). It is stated that microplastics cause toxicity in these organisms mainly by triggering oxidative stress. Microplastics can also impact macronutrient metabolism and digestion, as well as bile acid metabolism. They have also been shown to affects lipid metabolism in the liver by altering total cholesterol, triglyceride, and pyruvate levels. A study in a mouse model found that microplastics increased acetylcholinesterase (AChE) activity and related neurotransmitters such as aspartate. threonine and taurine (Wu B. et al., 2019).

Stock et al. (2019) stated that microplastic ingestion into the human body leads to a significant decrease in cell viability in human in vitro systems. A study by Hwang et al. (2019) found that high concentrations of small-sized microplastic particles increased cytokine and histamine levels, indicating that these small microplastic particles pose a potential risk to the immune system.

Problems observed in the gastrointestinal system have emerged as an important health problem related to exposed to microplastics. Research shows that microplastic particles ingested through contact with contaminated food or water can cause varied gastrointestinal problems (Zhao et al., 2023). Examples of these problems include digestive system inflammation, irritable bowel syndrome, constipation, impairment of intestinal microbiota and changes in intestinal permeability (Zhao et al., 2023; Qiao et al., 2019). Additionally, studies have discovered that microplastics can accumulate and cause physical irritation and blockages in the digestive system (Wright and Kelly, 2017Researchers speculate that the cellular effects of microplastics in the gastrointestinal tract may be due to their adjuvant activity, thereby enhancing the immune response to biomolecules adsorbed on their surface (Powell et al., 2010)

In a recent study demonstrating the first possible polymer degradation during an active human digestive tract, Tamargo et al. (2022) simulated the passage of polyethylene terephthalate (PET) microplastics through the digestive tract and observed the transformation of microplastics in the gastrointestinal tract and their effects on human gut microbiota. Researchers have found that PET microplastics cause structural changes in the digestive tract, particularly in the colon, and that microplastics alter the gut microbiota. These findings suggest that microplastics have a potentially harmful effect on the digestive system. On the other hand, when individuals with inflammatory bowel disease were compared with healthy individuals, lower concentrations of microplastics were detected in the feces of healthy individuals, suggesting a possible link between the development or progression of inflammatory bowel disease and ingested microplastics (Yan et al., 2021).

Endocrine disruption is also recognized as a potential impact of microplastics. In addition to endocrine disrupting compounds (EDCs), microplastics can contain and take up various chemicals from the environment. EDCs are substances or combinations of substances from environmental sources that have the potential to influence and alter the normal functioning of the endocrine system and may lead to harmful health effects in these organisms. (Surana et al., 2022). EDCs such as octylphenol, bisphenol A (BPA), phthalate esters, and nonylphenol are highly used in the production of plastics. These substances are found in microplastics produced as reaction reagents or additives. (Domenech et al., 2021; Wee et al., 2022). EDCs can be released when microplastics are ingested (oral) or come into contact with organisms that can affect and harm the endocrine system in any way. This disruption can have negative effects on some systems, such as reproductive function, hormonal and endocrine balance, growth and development, and general health. The small size and wide distribution of microplastics increases the likelihood of human exposure to EDCs (Kontrick, 2018; Gallo et al., 2018).

When microplastics enter the body via the oral route, both microplastics and related pathogenic bacteria can accumulate in the gastrointestinal system, resulting in inflammatory responses and potential infections. Studies have reported that some pathogenic bacteria in microplastics are associated with respiratory tract infections, gastrointestinal system diseases and dermatological diseases in humans. (Hu et al., 2021, Galafassi et al., 2021).

Research in the synthetic textile and husbandry industries has shown that workers exposed to airborne microplastics are more likely to experience respiratory symptoms related to the onset of respiratory and pulmonary interstitial lung diseases (Atis et al., 2015, Ahmad et al., 2023). Additionally, previous studies investigating lung tissue exposure to microplastics in textile industry workers have observed respiratory irritation and the presence of synthetic fibers (Eschenbacher et al., 1999).

Other studies have shown that exposure to microplastics can cause or worsen cardiovascular disorders and diseases such as atherosclerosis, hypertension, and heart rhythm disorders (Zhao et al., 2021; Persiani et al., 2023). The potential for allergic reactions and dermal irritation should also be considered, although there are insufficient studies showing adverse effects of microplastics through direct or indirect skin contact.

Conclusion and suggestions

Microplastics can enter the human body in many ways; examples include food, drink, skin and breathing. Almost all of the plastic products that are produced and used are still in our environment. It is clear that these plastic products will break down into microplastics as they deteriorate and that human exposure to microplastics will increase in the coming years. There are numerous negative health risks arising from microplastics themselves or hazards associated with them. There are many issues that require further investigation, such as the interplay of toxicity and chemical loading of polymers, the potential pathogenicity of plastics- associated microorganisms and their toxins, and the impact of microplastics on increasing antibiotic resistance. While the research to be conducted will contribute to a better understanding of the fate of microplastics taken into the human body through food and other sources and the actual consequences of consumed microplastics, it will also provide guidance on how to minimize the transmission routes of microplastics or how to protect people from microplastics.

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Chapter 3

RECENT TREND IN FOOD-GRADE EMULSION TECHNOLOGY: EMULSION GELS; FUNDAMENTALS, FABRICATION METHODS AND USES

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1. Introduction: emulsion gels

Colloidal dispersions can be used as a vehicle for delivery system of nutrients and nutraceuticals (Zhang, Murray, Holmes, Ettelaie, & Sarkar, 2023). Specifically, oil-in-water (O/W) emulsions have been generally used in food, pharmaceutical, cosmetic, and paint industries (Farjami & Madadlou, 2019). Nevertheless, emulsions are not thermodynamically stable and try to fall apart and return to the water and oil phases over time due to coalescence, gravitational separation, flocculation, Ostwald ripening and phase separation, which limits their convectional application (Farjami & Madadlou, 2019). In this context, there is a growing need in the literature to design improved and/or more stable emulsion systems (Zhang et al., 2023). Accordingly, increasing the stability of emulsions by converting them into emulsion gels is a recent trend (Wan, Cheng, Zeng, & Huang, 2023).

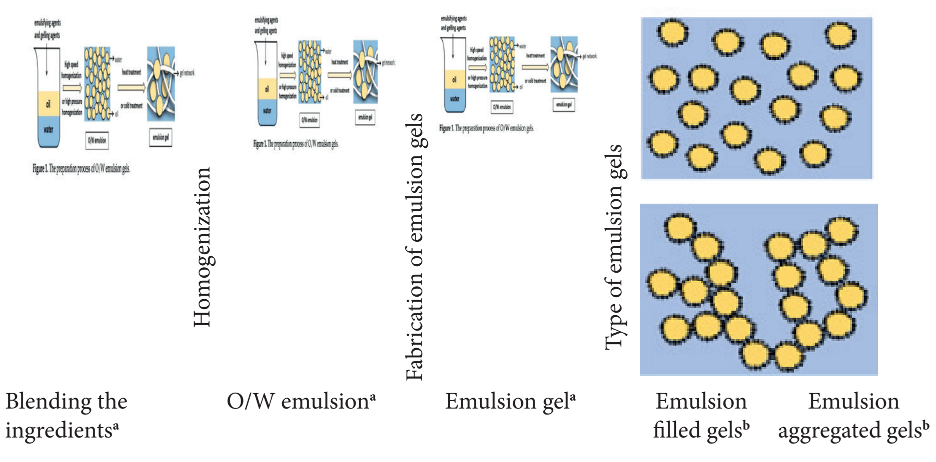
Emulsion gel (EG) is soft-solid-like substances composing of emulsified droplets entrapped in a three-dimensional cross-linked polymer network (i.e., emulsion-filled gels) or a network of aggregated emulsified droplets (i.e., emulsion particulate gels) (Wan et al., 2023). EGs come together the useful characteristics of emulsions and gels (Tan, Zhang, & McClements, 2023). Further, the physico-chemical and functional attributes of EGs can be tailored by varying emulsion droplet properties (composition, size, interfacial characteristics and etc.) and/or protein properties (type, concentration, gelling mechanism and etc.) (Tan et al., 2023).

Moreover, structure of EG is suitable for transporting and preserving oxidative lipids and sensible flavorings (Ren et al., 2022), they can be a vehicle for encapsulation of hydrophobic nutraceuticals and improvement their stability (Cui, Guo, & Meng, 2023), as well as, improving nutritional profile of the food (Ren et al., 2022). EGs with alike rheological attributes of solid/semi solid fats are a novel way of manufacturing non-hydrogenated, zero-trans, and low-saturated fatty acid solid fats to take place of usual fats (Cui et al., 2023). Since the EG can decrease the amount of fat within meat analogues, it ables product's being labelled as reduced fat content (Ren et al., 2022) Hence, it is a novel approach for the fabrication of nutritive and healthy foods (Zhi et al., 2023).

2. Fabrication methods of EGs

EG properties, preparation mechanism and preparation methods overviewed comprehensively by Ren et al. (2022), Farjami & Madadlou (2019), Zhi et al. (2023), Yiu et al. (2023) and Wan et al. (2023). In the following part, it is goaled to draw brief summary of these subjects.

The EG fabrication procedure principally composing of two stages: stable emulsion preparation and gel formation (Ren et al., 2022). The basic fabrication schema of O/W EGs is given in Figure 1. After introduction of an emulsifier, water and oil are blended by high-pressure homogenizer or high-speed mixer to fabricate stable emulsions (Ren et al., 2022). Gel formation stage includes transformation of the stable emulsion to an EG, i.e., continuous phase gelation (emulsion filled gels) or droplet aggregation (emulsion aggregated gels) (Ren et al., 2022). There are many ways of fabrication of EGs. EG fabrication methods are summarized in Figure 2.



[a] Schematic images are taken from Ren et al. (2022)
[b] Schematic images are taken from Zhi et al. (2023)

Figure 1. *The basic fabrication schema of O/W emulsion gels (Ren et al., 2022; Zhi et al., 2023).*

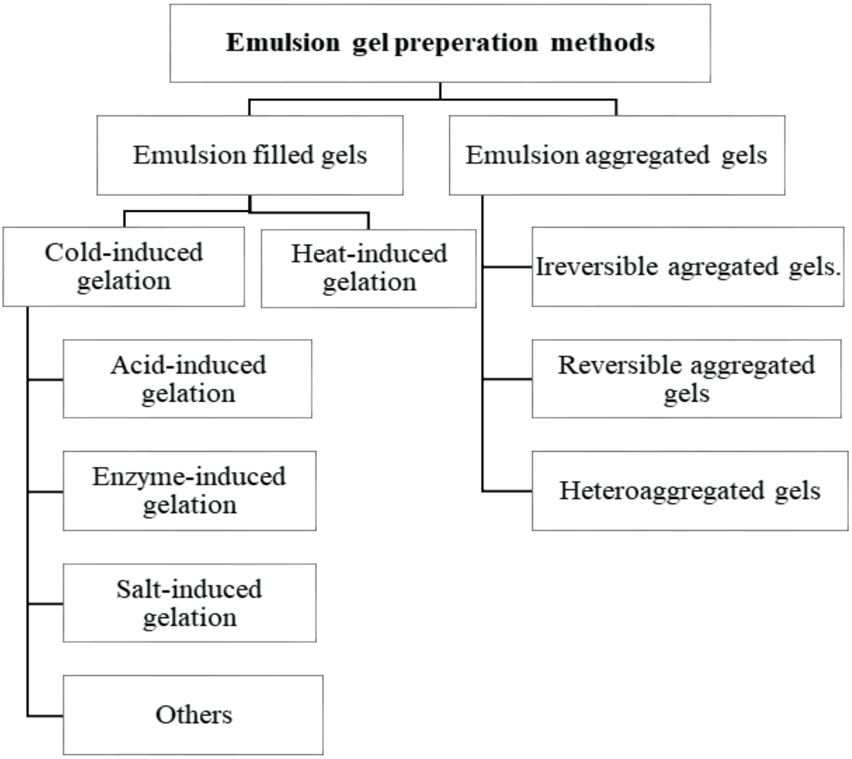


Figure 2. *Emulsion gel preparation methods.*

2.1. Emulsion filled gels

Emulsion-filled gels are fabricated by placing an emulsion inside either a gel matrix or a pre-gel polymer solution continued with gelation in place (Wan et al., 2023). That is, in emulsion filled gels, the continuous phase of the stable emulsion constructs a gel network structure, while the dispersed phase fills inside the gel network to produce a soft solid EG (Ren et al., 2022). Methods for preparing emulsion-filled gels can be divided into two groups: heat induced gelation using traditional heat treatment methods and cold-set gelation (enzyme-induced, acid-induced, salt induced, and etc.) by using appropriate gelation agents (Ren et al., 2022; Wan et al., 2023). Suitable fabrication method bases on the form and attributes of the gel matrix and the prerequisites of the final EGs (Wan et al., 2023).

Heat-induced gelation: Convectional heat treatment (>65°C) is a plain, quick method ordinarily utilized for transformation of protein-stabilized emulsions into gels (Ren et al., 2022). To produce heat-induced gel, the emulsion is heated and then quickly cooled to fabricate gel structure (Wan et al., 2023). The heat assists protein unfolding and denaturation and extra sulfhydryl and hydrophobic groups disclosure, which may also construct three-dimensional network arrangements on account of chemical forces (i.e., intermolecular disulfide bonds, electrostatic interactions, hydrophobic interactions, and hydrogen bonds), accompanied with the fixed emulsified droplets within the construction (Ren et al., 2022; Wan et al., 2023).

Cold-induced gelation: Cold-induced gelation can be further classified as acid-, salt-, and enzyme- induced methods due to the nature of gelling agent (Ren et al., 2022). Cold-set gel fabrication involves preheating step of emulsion followed by addition of gelling agents (such as salt, acid, enzyme and etc.) to an emulsion to form gel matrix (Ren et al., 2022). The emulsion is first preheated to lead both still being denatured and soluble (Ren et al., 2022). Unlike heat-induced gelation methods, the gel formation does not take place during the preheating step but just after introduction of gelling agent (Ren et al., 2022).

Acid-induced gelation generally consist of two stages: protein unfolding and denaturation via heat treatment and gel matrix development by intermolecular cross-linking by Van der Waals forces and hydrophobic interaction of proteins on account of reducing pH under the isoelectric point of the proteins and decreasing the electrostatic repulsive forces among proteins (Ren et al., 2022; Wan et al., 2023). Acid-induced gelation is extensively used in food manufacturing, especially for production of fermented dairy products such as yogurt (Wan et al., 2023).

Enzyme-inducted gelation is a soft and governable method to produce stiffened and flexible emulsion-filled gels with no by-products (Ren et al., 2022; Wan et al., 2023). Enzyme-induction includes the introduction of enzymes to promote covalent cross-linking of proteins (Ren et al., 2022). Microbial transglutaminase, a group of transferase which able to catalyze the crosslinking between glutamine and lysine residues and facilitate cross-links among proteins to build protein network, is usually utilized for this manner (Wan et al., 2023). Moreover, the method not need heating step, vary in pH, and/or ionic strength and it does not cause unpleasant odors and preserves essential nutrients (Guo, Cui, & Meng, 2023).

Salt-induced gelation usually comprises the addition of metallic ions (mostly calcium Ca^{2+} from $CaCl_2$), which varies the pH and ionic strength to decrease the electrostatic repulsion among the proteins. The fabrication mechanism generally consists of electrostatic repulsion followed by hydrophobic or disulphide interactions and calcium-bridging interactions among proteins (Wan et al., 2023). This system boosts ionic crosslinking, letting protein aggregates to form gel structures, rising ionic strength, and speeding up protein aggregation to fabricate bigger aggregates (Ren et al., 2022).

Furthermore, research has shown that there are also novel gelling mechanisms such as using ethanol (Ren et al., 2022) and synergistic effect of functionalized polymers (Wan et al., 2023) to form a gel structure.

2.2. Emulsion aggregated gels

Commanded gathering of droplets can be used to fabricate emulsion aggregated gels (Farjami & Madadlou, 2019). In emulsion aggregated gels, the dispersed droplets stabilized by colloidal particles cluster together to fill the available volume, creating gel structure (Wan et al., 2023). Preparation mechanism of an emulsion aggregated gel composes of an assembling of aggregated particles, which fill the enclosing volume, and building a continuous three-dimensional gel network (Farjami & Madadlou, 2019). Depending on the type of the interactions amoung the droplets, aggregation and gelation of emulsion droplets might be strong and irreversible or weak and reversible (Farjami & Madadlou, 2019). Emulsions with great volume amouths of the dispersed phase, like high internal phase emulsions, might fabricate weak and reversible gel structure due to closely associated emulsified droplets (Wan et al., 2023). Emulsions with low or moderate volume amount of dispersed phase, liquid-like emulsions, may be transformed to an emulsion particulate gel by regular physico-chemical processes, like heating, varying pH or ionic strength, since these actions may direct the particle interactions at the oil–water interface to lead droplet aggregation to a great extend (Wan et al., 2023). With respect to the kind and extend of the interactions among the droplets, the emulsion aggregated gels can be grouped as irreversible aggregation (strong and irreversible interaction between the droplets), reversible aggregation (weak and reversible interaction between the droplets), and heteroaggregation (Farjami & Madadlou, 2019; Wan et al., 2023). Heteroaggregation takes place among mixed particle matrix, binary emulsion systems, consisting of different type of materials (Farjami & Madadlou, 2019). In a binary emulsion systems, where each emulsion individually is stabilized electrostatically via biopolymers possessing unlike isoelectric points, heteroaggregation of differently charged particles and gelation mechanism might take place beneath appropriate conditions (Farjami & Madadlou, 2019).

Table 1 summarizes some examples of emulsion gels due to the fabrication mechanism.

Table 1. Examples of emulsion gels due to the fabrication mechanism.

Induction methods	Gel matrix	Fillers	Details/features	References
Acid-induced gelation	Continuous phase with 5% (w/w) heat-treated Whey protein isolate (WPI) solution (80 °C/30 min)	Stock emulsion (30% (w/w) of oil, 5% (w/w) of non-heated WPI)	Gelation occurred by adding glucono-delta-lactone directly to the stock emulsion	(Mantovani, Cavallieri, & Cunha, 2016)
Heat-induced gelation	WPI with xanthan gum (XG)	Curcumin-loaded solid lipid microparticles	Gelation occurred in a thermal bath at 90 C for 30 min.	(Geremias-Andrade, Souki, Moraes, & Pinho, 2017)
Enzyme-inducted gelation	Soy protein isolate and gum arabic under joint induction by ultrasonic pretreatment and transglutaminase (TG)	Allicin loaded oil phase	Gelation occurred through ultrasound treatment and TG addition	(Ma et al., 2022)
Salt-induced gelation	Soy protein isolate (SPI) emulsions (6% (w/v) SPI in deionized water)	Oil phase	Gelation occurred through preheat treatment followed by salt addition	(Yu, Wang, Li, & Wang, 2022)
Particle aggregation induced gelation	Octenylsuccinate quinoa starch granule-based Pickering emulsions	Oil phase	Gelation occurred through the aggregation of oil droplets with increasing in oil volume fraction	(Li et al., 2019)

3. Current uses of EGs

The current uses of EGs can be summarized as follows:

- EGs compose of a three-dimensional construction builded by macromolecular components and oil droplets encapsulated inside them, or they may compose of aggregated oil droplets and solid molecules non-reversible adsorbed at the oil–water interface as well. Both continuous phase and aggregated oil droplets may efficaciously reduce the speed of bioactive materials' release encapsulated within them; hence, EGs are generally utilized in transportation of bioactive substances in the food and pharmaceutical industries (Zhi et al., 2023).

- Since their structure is suitable for transporting and preserving oxidative lipids and sensible flavorings (Ren et al., 2022), they can be used as a vehicle for encapsulation of hydrophobic nutraceuticals and improvement their stability (Cui et al., 2023), as well as, improving nutritional profile of the food (Ren et al., 2022).

- EGs possess an adjustable elasticity and outstanding benefits to be used in upgrading food texture (Zhi et al., 2023).
- EGs with identical rheology as solid/semi solid fats are a novel approach in manufacturing non-hydrogenated, zero-trans, and low-saturated fatty acid solid fats as an alternative to known fats and designing reduced-fat foods (Cui et al., 2023).
- As the EG can decrease the amount of fat in meat analogues, it capables the product's being labelled as reduced fat content (Ren et al., 2022).

Thus, EGs are a rising trend for the manufacturing of nutritive and health promoting food products (Zhi et al., 2023). Table 2 gives the uses of EGs in the food industry.

Table 2. *Uses of emulsion gels in the food industry.*

Target aim	**Emulsion gel composition**	**Target food**	**Details/features**	**Referances**
Fat replacement	Soy protein isolate, inulin, and soybean oil	Bologna sausages	Formulation with EG Reduction of fat from 11% to 34%.	(de Souza Paglarini et al., 2021)
Fat replacement	Chia powder and olive oil	Frankfurters	Fat replacement with EG in formulation resulted in reduced fat level accompanied with improved fat quality and oxidative stability.	(Pintado et al., 2016)
Fat replacement	Alginate, cellulose with collagen and k-carrageenan, echium oil, extra virgin olive oil	Butter	Butter alternative with improved fat quality and reduced fat.	(Gutiérrez-Luna, Ansorena, & Astiasarán, 2022)
Fat replacement	Whey protein, high methoxyl pectin, soybean oil	Mayonnaise	Mayonnaise production with reduced fat (40%) and calorie but similar textural and sensorial properties compared to original formulation.	(Sun et al., 2018)
Functional food design (also encapsulation and delivery of bioactives)	Zein, sodium alginate, corn oil	EG	Model system for polyphenol encapsulation, improved nutraceutical stability and bioavailability	(Yan et al., 2021)

Functional food design	Ethylhexanoate, linalool, sodium caseinate and soy protein isolate	EG	The EGs enhanced the storage stability of flavorings.	(Lee, Choi, & Moon, 2006)
Functional food design (also encapsulation and delivery of bioactives)	Gum Arabic, inulin, flaxseed oil, pectin	Gummy candy	Emulsion filled gels used for enriched food design: vitamin-loaded (vitamin D_3) vegan gummy candy with adequate sensory profile and good stability.	(Ghiraldi, Franco, Moraes, & Pinho, 2021)
Functional food (improved and controlled digestion)	Whey protein isolate concentration, sunflower oil	EG	EGs were more stable during *in vitro* gastric digestion and showed better release of free fatty acids *in vitro* intestinal digestion.	(Torres, Murray, & Sarkar, 2019)

For more examples and detailed information further reading (Ren et al., 2022; Wan et al., 2023; Yiu et al., 2023; Zhi et al., 2023) is recommended.

4. Limitation and further perspectives

EG systems have significant potential for healthy and functional food production. EG systems can enable the production of functional and safe food with long shelf life. Looking at the literature studies, it can be said that there are deficiencies in the application of functional EG applications in real food systems, although their use as an oil alternative has been applied in real food systems. Although the work done to date is new and groundbreaking, it is obvious that more work needs to be done on this subject, and that the studies should be seen as applied in real food systems rather than model systems.

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