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Review

Probiotics and their potential applications in active edible films and coatings

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ABSTRACT

The global market for probiotics has been increasingly growing in recent years guided by the rising consumers' demand for healthy diets and wellness. This has caused food industries to develop new probiotic-containing food products as well as researchers to study specific characteristics of probiotics as well as their effects on human health. Probiotics are defined as live microorganisms that confer a health benefit to the host when administered in adequate quantities. Probiotics have been added to several food products as well as incorporated into biopolymeric matrices to develop active food packaging as an alternative method for controlling foodborne microorganisms, improving food safety, and providing health benefits. This review includes definition of probiotics, description of their effects on human health, discussion on their applications in edible biopolymeric matrices to develop active edible films and coatings, as well as the probiotics-related legislation.

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Abbreviations: CFU, colony-forming units; CMC, carboxymethyl cellulose; COS, chitosan oligosaccharide; EFSA, European Food Safety Authority; EU, European Union; FAO, Food and Agriculture Organization; FOS, fructo-oligosaccharides; GRAS, generally regarded as safe; GOS, gluco-oligosaccharides; GTOS, galacto-oligosaccharide; HPMC, hydroxypropyl methylcellulose; MC, methylcellulose; TSA, tryptose soy agar; WHO, World Health Organization; WPC, whey protein concentrate.

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1. Introduction

Consuming foods with probiotics has increased because of consumer concerns regarding healthy diets and wellness. The global market for probiotics – including their use as ingredients, supplements, and incorporation in food products – accounted for 14.9 and 16.0 billion US dollars in 2007 and 2008, respectively (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). In 2010 and 2011, the global sales of probiotics increased to 21.6 and 24.23 billion dollars, respectively. According to the Transparency Market Research, disclosed in 2015, the global market for probiotics was valued at 62.6 billion dollars in 2014, and is estimated to reach 96.0 billion dollars by 2020. This has aroused the attention of food industries to produce new food products containing probiotics as well as researchers who have studied specific characteristics of probiotics and their effects on human health.

The term probiotic is a relatively new word. It means "for life" and describes bacteria with beneficial effects on humans and animals (FAO, 2001). Indeed, probiotics were originally defined as a "mono- or mixed culture of live micro-organisms which, when applied to man or animal, affects beneficially the host by improving the properties of indigenous microflora" (Huis Veld & Havenaar, 1991). Probiotics are defined by FAO/WHO as "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host" (FAO, 2002). The Japanese definition of probiotics includes cells of nonviable microorganisms that provide health benefits in addition to live microorganisms (Salminen, Ouwehand, Benno, & Lee, 1999). The concept of viability should be used with care as it is defined by most regulatory authorities as culturability, which in turn is highly depended upon culture conditions and media.

Reviews have shown positive effects of probiotics at *in vivo* studies, as well as on human health (Aureli et al., 2011; Clarke, Cryan, Dinan, & Quigley, 2012; Hempel et al., 2012; Mattila-Sandholm et al., 1999; Ooi & Liong, 2010; Singh, Kallali, Kumar, & Thaker, 2011; Satish Kumar & Arul, 2015). Probiotics have been incorporated into several food products and supplements, most of them dairy products, such as cheeses, dairy desserts, ice-cream, although fermented milks such as yogurts are the most popular matrices, which can be obtained from bovine (Batista et al., 2015), caprine (Ranadheera, Evans, Adams & Baines, 2012a, b; Ranadheera, Evans, Adams & Baines, 2016a, b) and ovine (Balthazar et al., 2016) milk. Recent studies regarding probiotic microorganisms and their applications in food matrices are presented in Table 1.

The most frequently commercially used bacteria belong to the genera *Lactobacillus* and *Bifidobacterium*, although *Streptococcus thermophilus* and *Saccharomyces boulardii* are available in some dairy products (Rastall, Fuller, Gaskins, & Gibson, 2000). Moreover, non-dairy probiotic products have drawn attention due to the growing interest in veganism, as well as to the higher number of consumers with diet restrictions such as lactose intolerance, allergies to milk proteins, and even cholesterol restriction. Hence, non-dairy products (e.g. fruit juices, minimally processed fruits, and fermented vegetables) allow the development of probiotic foods free of cholesterol, lactose and allergens usually found in dairy products (Martins et al., 2013).

Alternatively, probiotics may be carried within edible polymer matrices used in the food packaging industry. In this way, probiotics – as well as many other active compounds (Otoni, Espitia, Avena-Bustillos, & McHugh, 2016) – have been incorporated into biopolymeric matrices to develop active/bioactive food packaging materials as an alternative method for controlling pathogenic microorganisms and improving food safety, besides having the potential to favor consumer health. An overview of the chronological scenario concerning the investigations on probiotics and on food packaging demonstrates that the number of publications on these topics independently has been increasing remarkably throughout the past couple of decades (Fig. Fig. 1). However, to the best of our knowledge, literature on the applications of probiotics in active food packaging is scarce, and thus far there is no review article focused solely on this subject. This review highlights the nature of probiotics and their incorporation into biopolymer materials intended for active food packaging applications as well as legislation related to probiotics.

2. Probiotics: history, definition, and effect on human health

Ancient civilizations, such as the Greeks and Romans, used fermented dairy foods to maintain health. However, research on microorganisms in fermented food products and their effects on human health have only been studied recently. The history of probiotics started in 1908 when Élie Metchnikoff, Nobel Laureate at the Pasteur Institute, established the relationship between health and longevity with the ingestion of bacteria from yogurt. Dr. Metchnikoff proposed that the bacteria helped control infections caused by enteric pathogens and regulated toxaemia, both of which playing major roles in aging and mortality. This observation resulted in increased yogurt production and consumption (Shah, 2007).

The term probiotic has been widely used. According to Hamilton-Miller, Gibson, and Bruck (2003), this term was first used by Lilly and Stillwell in 1965 and referred to observations of *in vitro* protozoa growth stimulated by other protozoa. During the following decade, the term probiotic was used by Fujii and Cook in 1973 and denoted synthetic chemicals in mice that conferred protection against *Staphylococcus aureus* infection. In 1974, the term was used by Parker in a wider sense to refer to microorganism interactions with the animal or human host, i.e. "organisms and substances, which contribute to intestinal microbial balance". Several works concerning probiotics have been published since then.

In 2002, FAO/WHO held an expert consultation to evaluate health and nutritional properties of probiotics and establish a definition for probiotics (FAO, 2001). Recently, Wassenaar and Klein (2008) slightly modified the definition to "food or food supplements containing defined microorganisms in sufficient numbers to reach the gut in viable status resulting in positive health effects after consumption". The authors claim this definition does not contradict the internationally and scientifically accepted definition, although they added qualitative (defined microorganisms) and quantitative (sufficient numbers) requirements to the presumed positive health effects.

Probiotic effects are strain specific, thus knowledge of the probiotic genus and species is necessary to obtain the desired effects in the host. The main characteristics of probiotic strains in their relationship with the host are resistance to gastric and bile acid, adherence to mucus or human epithelial cells, antimicrobial activity against pathogenic bacteria, and the ability to reduce pathogen adhesion to surfaces and bile salt hydrolase activity (FAO, 2002).

There are several mechanisms by which probiotics may benefit human, including production of antimicrobial substances, strengthening of intestinal barrier, modulation of immune response, and antagonism of pathogenic microorganisms either by production of antimicrobial agents or by competition for binding sites, nutrients, and growth factors (FAO, 2001; Marco, Pavan, & Kleerebezem, 2006; Parvez, Malik, Ah Kang, & Kim, 2006).

When probiotic microorganisms are incorporated into foods, they must be able to survive through the digestive tract and successfully proliferate in the gut. Thus, they must be resistant to gastric juices and be able to grow in the conditions of the intestine. An interesting option is to use a food matrix that protects them and favors their survival.

Several factors affect the survival of ingested probiotics in the gastrointestinal tract, including stomach acid, bile salt concentrations, time of exposure, and probiotic species and strains. However, many probiotics are able to pass through the gastrointestinal tract and enter the colon in viable numbers in order to impart beneficial effects. In this regard, recent studies have explored the effect of the food matrix in the survival of probiotic to the conditions of the gastrointestinal tract and their adhesion to intestinal cells. Ranadheera, Baines & Adams (2010) have deeply