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COORDINATOR: PROF. MATTEO ALESSANDRO DEL NOBILE

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FOODS ENRICHED WITH PROBIOTICS AND PREBIOTICS

PhD Candidate: Luisa Angiolillo

Tutor: Dott.ssa Amalia Conte
Co-tutor: Dott. Michele Faccia

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1. Introduction

1.1 Innovation in the food industry: The case of functional foods

The food industry is one of the most important branches of the national economy in Italy and in the European Union in general, playing a central role for the processing of agricultural raw materials and food supply. As a consequence, many authors stressed its relevance for employment and economic output (Menrad, 2004). In innovation literature, the food industry is traditionally regarded as a sector with low research intensity (Garcia Martinez and Briz, 2000). Notwithstanding, innovations understood as new products, processes or services are recognized as an important instrument for companies belonging to the food industry to stand out from competitors and to satisfy consumer expectations (Menrad, 2004). In particular during the last decade, consumer requirements in the field of food production have changed considerably: in fact, consumers increasingly believe that food contribute directly to their health (Mollet & Rowland, 2002). Thus, foods are no more intended to only satisfy hunger and to provide the necessary nutrients, but also and especially to prevent nutrition-related diseases and to improve physical and mental well-being (Menrad, 2003; Robertfroid, 2000b).

Innovations introduced in the food industry in recent years mainly refer to new scientific and technical approaches in food processing, and to the introduction of novel foods. In this regard, functional foods play an outstanding role, as demonstrated by their increasing demand derived from the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of life in their later years (Kotilainen, Rajalahti, Ragasa, & Pehu, 2006; Robertfroid, 2000a and Robertfroid, 2000b). As such, researchers agree in stating that functional foods represent one of the most interesting areas of research and innovation in the food industry (Annunziata & Vecchio, 2011; Sirò, Kàpolna, Kàpolna, & Lugasi, 2008).

Their relevance is related to the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of life in their later years

(Kotilainen *et al.*,2006; Robertfroid, 2000a and Robertfroid, 2000b). The term "functional food" was first used in 1984 in Japan as a result of a study on the relationships between nutrition, sensory satisfaction, fortification and modulation of physiological systems in order to define those food products fortified with special constituents that possess advantageous physiological effects (Hardy, 2000; Kwak & Jukes, 2001). Functional foods' objectives are manifold: they improve the general conditions of the body (e.g., pre- and probiotics), decrease the risk of some diseases (e.g., cholesterol-lowering products), and could be used for curing some illnesses (Mark-Herbert, 2004; Side, 2006). Notwithstanding the increasing interest from both researchers and the food industry toward functional foods, it is not still clearly defined which foods are considered as functional. As a consequence, it is difficult to estimate the market of these products (Kotilainen *et al.*, 2006). Despite the mismatch of information on this market, in particular in terms of total turnover and volume of functional foods sold, it emerges as a business in rapid growth. The rise of functional foods market is mainly due to a series of critical awareness of personal health. According to a Euromonitor survey, Japan is the world's largest market, followed by the US, while the European market still appears to be less developed. These three dominant markets contribute to over 90% of the total sales (Benkouider, 2005). Functional foods have been developed almost in all food categories, even if they are not homogeneously distributed over all segments of the food industry. As a consequence, consumer preferences may vary between markets. Among all the food markets, functional foods have been mainly launched in the dairy, confectionery, soft-drinks, bakery and baby-food market (Kotilainen *et al.*, 2006; Menrad, 2003). The extant literature proposes different classification of functional foods. From a product point of view, Kotilainen *et al.*(2006), Sloan (2000) and Spence (2006), have proposed the following classification:

- food fortified with additional nutrients (labeled *fortified products*), such as fruit juices fortified with vitamin C, vitamin E, folic acid, zinc and calcium;
- food with additional new nutrients or components not normally found in a particular food (labeled *enriched products*), like probiotics or prebiotics;

• food from which a deleterious component has been removed, reduced or replaced by another with beneficial effects (labeled *altered products*), for example fibers as fat replacer in meat or ice cream;

• food in which one of the components have been naturally enhanced (labeled *enhanced commodities*), e.g., eggs with increased omega-3 content.

According to alternative classification based on the aim of functional foods, they can be classified as follows (e.g., Makinen-Aakula, 2006):

• functional foods that add good to life or improve children's life, like prebiotics and probiotics;

• functional foods that reduce an existing health risk problem such as high cholesterol or high blood pressure;

• functional foods which makes life easier, such as lactose-free or gluten-free products.

Recently, both in Japan and Europe the market of functional foods is mainly dominated by probiotics with more than 370 products launched worldwide in 2005 (Ouwehand, 2007). Within the probiotic field, Lactic acid bacteria (LAB) and bifidobacteria are the most studied and widely used ones (Kociubinski & Salminen, 2006). Researchers agree in stating that their success among functional foods is mainly due to their general positive image among consumers (Makinen-Aakula, 2006), but also due to their intrinsic characteristics (the products kept at cold temperature, they have relatively short shelf life, etc.). Their success is confirmed by the increasingly extensive research and development concerning probiotics aiming to introduce new dairy products (e.g., probiotic drinking yogurt like Actimel and Activia, dairy products containing Lactobacillus fermentum ME-3 like Hellus, etc.) (Sirò *et al.*, 2008; Szakàly, 2007). As for the prebiotics category, inulin and oligofructose are amongst the most studied and well established (Gibson, 2004). Bosscher, Van Loo, and Franck (2006) have shown that prebiotics increase calcium absorption, thus improving bone mineral content and density. According to Lòpez-Molina *et al.* (2005), they also influence the formation of blood glucose, thus reducing the levels of cholesterol and serum lipids. Moreover, prebiotics might enhance the growth and survival of the probiotic cultures by influencing the growth and metabolites of both the probiotic and the starter. In most countries there is no legislative

definition of the term and drawing a border line between conventional and functional foods is challenging even for nutritionists and food experts (Mark-Herbert, 2004; Niva, 2007). Moreover, the European legislation does not consider functional food as specific food categories, but rather as a concept (Coppens, Fernandes Da Silva, & Pettman, 2006; Stanton, Ross, Fitzgerald, & Van Sinderen, 2005). To date, a number of national authorities, academic bodies and the industry have proposed definitions for functional food. While some definitions simply suggest that any food, if marketed with the appropriate positioning, is a functional food (Hollingsworth, 1999), others are more complex and maintain that only fortified, enriched, or enhanced food with a component having a health benefit beyond basic nutrition can be considered functional foods (Kleinschmidt, 2003). Doyon and Labrecque (2008), helps to simplify the complex framework of functional foods definitions. Through an analysis of all the existing definitions in the literature and discussions with various experts, the authors aimed to arrive at an operational definition of functional foods that would also take cultural and temporal aspects into account, in order to be able to then identify the key concepts and the boundaries of the functional foods universe. Beginning from an analysis of over one hundred definitions, they selected the twenty-six definitions they considered the most representative. From these, the authors went on to identify four key concepts that characterize all the definitions of functional foods:

- Health benefits. However, it is not indicated whether these positive effects should be scientifically proven, nor is the type of proof required stated (among the numerous definitions that convey this concept we can mention: Health Canada, 2006; FOSHU Japan, 1991 - quoted in Anon, 2003; The European Food Information Council – quoted in Anon, 2003; National Institute of Nutrition, 2000; Center for Science in the Public Interest, 1999; International Life Science Institute – quoted in Milner, 2002; Adelajia and Schilling, 1999; Riemersma, 1996; Diplock et al. 1999; Hasler, 2000; Roberfroid, 2002; Smith et al. – quoted in Roberfroid, 2002; Kleinschmidt, 2003)

- The nature of the food. To be functional, it should maintain traditional food characteristics; in some cases it is specified that the food must be enriched, fortified or have had an ingredient added, while others stress the elimination of components considered harmful to the health (Health Canada, 2006; FOSHU Japan, 1991; National Institute of Nutrition, 2000; Center for Science in the Public Interest, 1999; Adelajia and Schilling, 1999; Diplock et al., 1999; Roberfroid, 2002; Kleinschmidt, 2003, etc.).
- Level of function. More than half of the selected definitions state that the crucial feature of a functional food is its ability to produce benefits over and above its basic nutritional functions. This type of food is not categorized as a particular product, but according to its function (Health Canada, 2006; FOSHU Japan, 1991; The European Food Information Council – quoted in Anon, 2003; Center for Science in the Public Interest, 1999; International Life Science Institute – quoted in Milner, 2002; Diplock et al., 1999; Adelajia and Schilling, 1999; Hasler, 2000; Kleinschmidt, 2003).
- Consumption pattern. According to many definitions these foods should be part of a normal diet, in relation to the typical consumption pattern in a specific cultural and geographical context (Health Canada, 2006; Jansen and Krijger, 2003; Diplock et al., 1999; Smith et al. – quoted in Roberfroid, 2002).

Using these elements as their starting-point, the authors outline the following definition:

"A functional food is, or appears similar to, a conventional food. It is part of a standard diet and is consumed on a regular basis, in normal quantities. It has proven health benefits that reduce the risk of specific chronic diseases or beneficially affect target functions beyond its basic nutritional functions". (Doydon e Labrecque, 2008: 1144).

From a more practical point of view, Diplock et al. (1999) and Ashwell (2003) – authors of the two key works about the definition of these products – say that a functional food can be:

▪ a natural food in which specific components have been introduced or improved through particular farming techniques (for example, whole foods);

▪ a food where there has been the addition of a particular component with the aim of producing a benefit (for example, foods with the addition of probiotics or prebiotics);

▪ a food in which one component has been removed in order to reduce or eliminate possible adverse health effects (for example, the reduction of saturated fat);

▪ a food in which the structure of a nutrient has been chemically modified in order to improve health (for example, hydrolyzed proteins used to reduce the risk of allergies in infant formulas);

▪ a food where the nutrient bioavailability has been increased or reduced, in order to enhance the assimilation of a beneficial component (such as lycopene in tomatoes);

▪ a food that is the result of any combination of the possibilities mentioned above.

A functional food, in order to be defined as such, should maintain the appearance of food (otherwise it would fall into the category of nutritional supplements, in the form of capsules, tablets or sachets), but should also possess a number of components (usually artificially added or modified) capable of improving certain features of our body beyond the effects of basic nutrition (that is to say, beyond the simple amount of vitamins, minerals and energy necessary to the body's wellness resulting from normal intake of proteins, carbohydrates and fats). This is why a product that improves nutritional balance should not be considered functional solely for that reason and should be so, only if it reduces the risk of disease and also helps to improve health. In this sense, the universe of functional foods includes the following: products that improve nutritional balance, products that reduce the risk of certain diseases related to diet, and those that enhance some functions improving general health, each one with a minimum level of intensity. A purely functional food should have a minimum level of such intensity (generally higher than normal) measured through its physiological effects and starting from the concentration of bioactive components. Pharmaceuticals, however, are outside the boundaries of functional foods, which in fact follow a statute governed by completely different laws. As can be seen in Figure 1.1, Doydon and Labrecque (2008) provide an interesting graphic summary of what we have stated above.

Figure 1.1 Frontiers of the functional food universe (Source: DOYON e LABRECQUE, 2008: 1143)

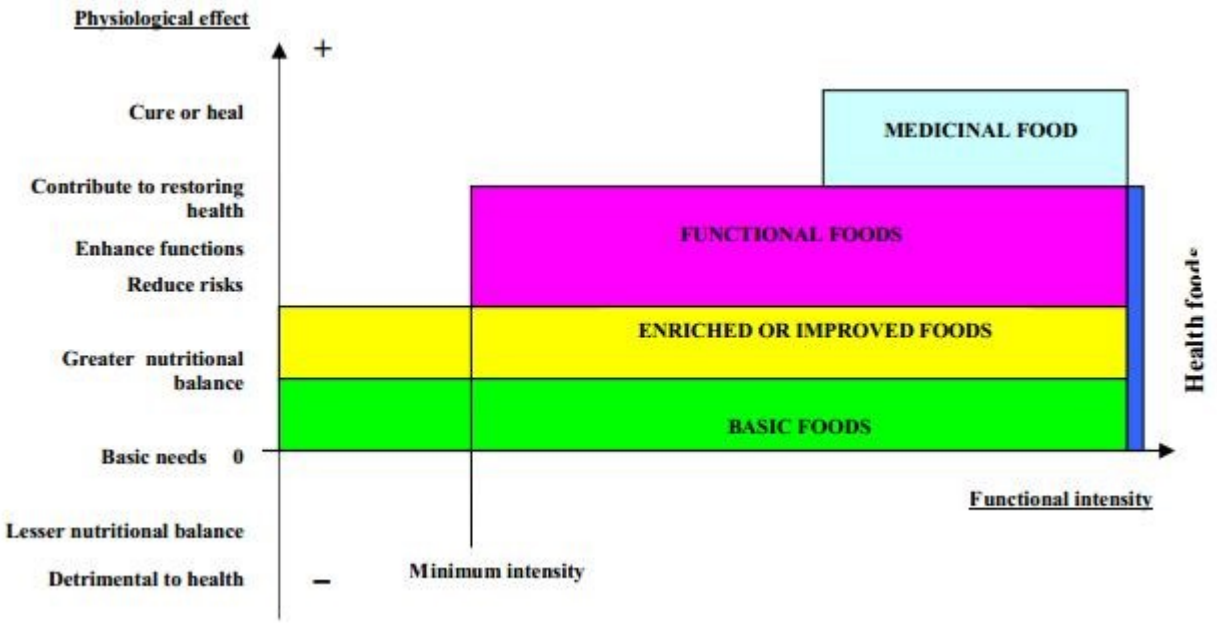


Table 1.1 Some differences between functional foods and medicines. (Source: HOWLETT, 2008:23)

	Functional Food	Medicine
Mode of Action	Modulation of physiological process within the normal range	Intervention in a disturbed physiological process or modulation of a physiological process outside the normal range
Purpose	To restore or enhance normal functions in order to optimize health, wellbeing and performance. To reduce risk factors for disease.	To treat or prevent disease. To enhance performance beyond normal range
Form	Food consumed as a part of a normal diet.	Pill, tablet, capsule, or syrup taken in controlled dose according to a timetable.

1.2 Probiotic Concept

The term »probiotics« was introduced by Lilly and Stillwell in 1965 for growth promoting factors produced by microorganisms. The word »probiotic« is derived from Greek and means »pro life«. In 1974, Parker used the term for »organisms and substances« which influenced the intestinal microflora and had beneficial effects on animals. The term »substances« is imprecise and would include even antibiotics. Therefore, in 1989 Fuller defined »probiotics« as »a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance«. However, according to this definition probiotics were restricted to feed supplements, animals and the intestinal tract, and the term »probiotic« thus could not be used for living microorganisms administered in any other way than in food or feed, or for locations other than the gastrointestinal tract. Consequently, in 1992 Havenaar and Huis in't Veld proposed to broaden Fuller's definition into »a probiotic is a mono- or mixed culture of live microorganisms which, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora«. Probiotic strains can be used only, and are active only, on/or in the body of the host if they fulfil a large number of criteria. The criteria for the selection and assessment of probiotic microorganisms were the result of the collaboration of research institutions and universities with food industries. The list of properties expected from potential probiotic strains of lactic acid bacteria, compiled by several authors (Kullen, et al., 1999) are:

- accurate taxonomic identification.
- normal inhabitant of the species targeted: human origin for human probiotics.
- nontoxic and nonpathogenic.
- genetically stable.
- capable of survival, proliferation, and metabolic activity at the target site.
- adherance and colonization potential preferred.

- stability of desired characteristics during culture preparation, storage, and delivery viability at high populations preferred at 10^6– 10^8
- production of antimicrobial substances, including bacteriocins, hydrogen peroxide, and organic acids.
- antagonistic toward pathogenic/cariogenic bacteria.
- able to compete with the normal microflora, including the same or closely related species; potentially resistant to bacteriocins, acid, and other antimicrobials produced by residing microflora.
- resistant to bile.
- resistant to acid conditions.
- immunostimulatory activity.
- able to exert one or more clinically documented health benefits.
- stable during the production process: adequate growth, recovery, concentration, freezing, dehydration, storage, and distribution.
- able to impart desirable organoleptic qualities (or no undesirable qualities) when included in fermented products.

The importance of an indigenous microflora in the gastrointestinal tract as a natural resistance factor against potential pathogenic microorganisms, was already recognised in the 19th century by Metchnikoff during his research on cholera. It is now recognised that the indigenous microflora of humans and animals provide protection against infections with pathogenic microorganisms. This phenomenon is often called »bacterial antagonism« »barrier effect«, »competitive exclusion« or widely used term »colonization resistance«. The ability to compete for limiting nutrients and possibly for the adhesion sites on food particles or on the colonic mucosa, is likely to be the most important factor that determines the composition of intestinal microflora. Species that are unable to compete successfully are rapidly eliminated from the intestinal ecosystem (Macfalrane et al., 1994).

These findings lead to consider the possibility to choose substrates (prebiotics) that can improve the survival of the indigenous microflora of the colon.

Although the composition of the intestinal microflora is rather stable in healthy individuals, it can be altered by many endogenous and exogenous factors (Table 1.2).

Table 1.2 Factors that affects intestinal balance.

Exogenous	Endogenous
antibiotic therapy	nutrient availability
excessive hygiene	types of diet
emotional stress	pH value of intestinal lumen
ageing	redox potential
travelling	diarrhoea
peristaltic disorders	bacterial antagonism
surgical operations	bacterial co-operation
liver or kidney diseases	mucin
radiation therapy	lysozyme
chemotherapy	defensins
pernicious anemy	
disorders of immune system	

Disturbances in intestinal ecosystem are generally characterised by a remarkable increase in bacterial counts in the small intestine, by an increase of aerobes, mostly Enterobacteriaceae and streptococci, by the reduction or disappearance of bifidobacteria and/or often by the incidence of Clostridium perfringens. These evidences would suggest that the loss of indigenous microorganisms implies deregulation of autogenic factors and vacated habitats. Consequently, commensal or transient microorganisms have chance to take possession of these vacant niches. If these microorganisms are potentially pathogenic, the outbreak of an opportunistic infectious disease is quite possible. Disturbance of intestinal microflora can also be due to stress. During stress conditions the number of lactobacilli decreases and the number of enterotoxigenic strains of E. coli increases. Salminen et al. (1998) published the results confirming that some strains of lactic acid bacteria may provide protection against traveller's diarrhoea. All these conditions, where the

balance of the gut microflora was disturbed, are situations in which probiotic microorganisms can have significant effect on its re-establishment.

1.3 Lactic acid bacteria as probiotics

1.3.1 Physiological and taxonomical characteristics of lactic acid bacteria

Traditionally, the lactic acid bacteria are defined by formation of lactic acid as a sole or main end-product from carbohydrate metabolism. Lactic acid bacteria comprise a diverse group of Gram-positive, non-spore forming bacteria. They occur as cocci or rods and are generally lacking catalase, although pseudo-catalase can be found in rare cases. They are chemoorganotrophic and grow only in complex media. Fermentable carbohydrates are used as energy source. Hexoses are degraded mainly to lactate (homofermentatives) or to lactate and additional products such as acetate, ethanol, CO_2 , formate or succinate (heterofermentatives). Lactic acid bacteria are found in foods (dairy products, fermented meat, sour dough, fermented vegetables, silage, beverages), on plants, in sewage, but also in the genital, intestinal and respiratory tracts of humans and animals. Modern classification mainly based upon comparative sequence analysis of 16S ribosomal ribonucleic acid (16S rRNA), determined phylogenetic relationships of lactic acid bacteria and bifidobacteria. Based on 16S and 23S rRNA sequence data, Gram-positive bacteria form two lines of descent. One phylum consists of Gram-positive bacteria with a DNA base composition of less than 50 mol % guanine plus cytosine (G+C), the socalled Clostridium branch, whereas the other branch (Actinomyces) comprises organisms with a G+C content that is higher than 50 mol %. The typical lactic acid bacteria such as genera Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Pediococcus, Streptococcus and Carnobacterium have a G+C content of less than 50 mol % and belong to the Clostridium branch.

L. rhamnosus is homofermentative and mostly used in yogurts as a natural preservative. It attaches to the epithelial cell wall lining of the stomach and encourages the growth of useful organisms. It can survive in extreme acidic and bile conditions. Eczema affected children fed with L. rhamnosus

diets showed a significant decrease in gastro intestinal activity by reinforcing gut defense through immunomodulation and immunoregulation (Isolauri, 2001; Rosenfeldt et al., 2004). It is also proven very effective against Clostridium difficile associated diarrhea and traveller's diarrhea, suppressing the side effects of H. pylori antibiotics, alleviating symptoms of Crohn's disease, preventing dental caries, atopic infections, and increasing the non-specific antibody secreting cells against rotavirus-induced diarrhea (Jankovic et al., 2010). L. rhamnosus can also interrupt the transportation of enterococcus in kidney-related infections. It aids postmenopausal women in fighting against chronic urinary tract infections. Studies have proven that even dead L. rhamnosus was able to trigger an inflammatory response, but slightly lesser than live cells. This could be an advantage when there might be possible risks associated with the live cells, though a high dosage is recommended (Kataria et al., 2009). Elevation of serum concentration of interleukin-10 (a better immune response) was reported in children fed four weeks with L. rhamnosus strains and L. acidophilus (Rosenfeldt et al., 2004).

1.3.2 Probiotic activity mechanism of lactic acid bacteria

The scientific basis for the development of probiotics is in their protective role in the host (humans and animals) against colonisation of intestinal tract by non-indigenous microorganisms. The mechanism of probiotic action is still unknown but different approaches could be developed. According to Fuller (1992) and Huis in't Veld and Havenaar (1993) probiotic effect of lactic acid bacteria may be expressed by three main mechanisms of action:

1. Suppression of pathogenic microorganisms in intestinal tract by:

a) production of antibacterial substances including primary metabolites, such as lactic acid, acetic acid, carbon dioxide, diacetyl, acetaldehyde, hydrogen peroxide (Yoshimura et al., 2000) and bacteriocins which are proteinaceous compounds that manifest antimicrobial activities against other closely related bacteria. In vitro-studies showed inhibition of pathogen replication mediated by low-molecular-weight substances. Top of this list are short chain fatty acids e.g. lactic acid. A similar

effect was observed for hydrogen peroxide. Also low-molecular-weight bacteriocins (LMWB) and high-molecular-weight bacteriocins (class III) are produced by lactobacilli. The LMBW are antimicrobial peptides. LMWB can be grouped into three classes: (class I) lantibiotics, posttranslationally modified peptides harbouring unusual amino acids such as lanthionin, (class II) heat stable non-lantibiotics, (class IV) cyclic antimicrobial peptides (Maqueda et al., 2008). Besides LMWB probiotics produce also certain antibiotics. The production of the antibiotic reuterin (3-hydroxypropionaldehyde) by Lactobacillus reuteri strain ATCC55730 has been reported. Reuterin is a broadspectrum antibiotic active not only against Gram-positive and Gram-negative bacteria but also against yeast, fungi, protozoa and viruses (Cleusix et al., 2008). Finally microcines, which are peptides with a narrow window of activity, have to be mentioned, because they are also synthesised by many probiotics. A specific antimicrobial function is the property of proteins (molecular weight 420 kDa) belonging to the family of bacteriocins. These proteins are sometimes termed group III bacteriocins of Gram-positive bacteria. However, such proteins (bacteriocins) are also produced by Gram-negative bacteria.

Probiotic bacteria are able to produce so-called deconjugated bile acids which are derivatives of bile salts. Deconjugated bile acids show a stronger antimicrobial activity compared to the bile salts synthesized by the host organism. How the probiotics protect themselves from these ‘‘selfmade’’ metabolites or if they are resistant to deconjugated bile acids at all remains to be elucidated.

b) competition for nutrients. Freter et al. (1983) have stated that competition for limiting nutrients (specific carbohydrates) is one of the determining factors that has received the greatest scientific support. An important example for a limited substance in the host is iron. But for almost all bacteria iron is an essential element with the exception of lactobacilli. They do not need iron in their natural habitat. This might be a crucial advantage in competition with other microorganisms which depend on iron. Nevertheless L. acidophilus and L. delbrueckii are able to bind ferric hydroxide at their cell surface, rendering it unavailable to pathogenic microorganisms.

c) competition for adhesion receptors on the gut epithelium. Probiotic strains can adhere specifically ornon-specifically. Specific adhesion occurs when an adhesin on the bacterial cell binds to a receptor on the epithelial cell, which is often defined as a lock and key function. Non-specific adhesion is a more general phenomenon mediated by hydrophobic or electrostatic interaction. Non-specific adhesion may not have any significance in the colonization of epithelia in vivo, but may possibly be important in the colonisation of luminal contents. For example, non-specific adhesion may enhance substrate uptake and thus enforces growth. Because probiotic bacteria are able to adhere to epithelial cells in cell culture assays, thereby blocking adherence of pathogens, it is extrapolated that this mechanism is important for the probiotic effect in the host. The anti-adhesive effect might be the result of competition between probiotic and pathogen for the same receptor or the induction by probiotics of (increased) mucin production. Mack et al. (2003) reported indeed induction of MUC3 mucin in HT20-MTX cells when co-cultured with L. plantarum 299v or L. rhamnosus GG. MUC3 mucin inhibited subsequently the adhesion of enteropathogenic E. coli strain E2348/69. Even adhesion of pathogenic Salmonella, Clostridium and E. coli strains to pig intestinal mucus could be reduced in the presence of probiotic Bifidobacterium lactis Bb12 and/or Lactobacillus rhamnosus LGG (Collado et al., 2007a). However, the ability to inhibit the adhesion of pathogens to immobilized human mucus appears to depend on both the specific probiotic strains and the pathogens. The most prominent adhesins of probiotics are surface proteins of Lactobacillus (e.g. Mub: mucus-binding protein of L. reuteri 1063; Roos and Jonsson, 2002) which share common characteristics such as the presence of a signal peptide, a C-terminal cell wall-anchoring motif (LPXTG) and several repeated domains with putative adhesion function (Sanchez et al., 2008). Besides competitive exclusion, i.e. competition for the same receptor by probiotics and pathogens as described above, other modes of anti-adhesiveness expressed by probiotics could be degradation of carbohydrate receptors by secreted proteins, establishing a biofilm, production of receptor analogues and the induction of biosurfactants.

d) the mediation with the pathogen invasion. Not only adhesion to but also invasion of epithelial cells is an important property for full pathogenicity of many gut pathogens. This fact led to investigations for probable anti-invasive effects of probiotics. The standard assay for quantification of invasiveness is the gentamicin protection assay (Hess et al., 2004). In this cell culture assay, differentiation between intracellular and extracellular bacteria is achieved by gentamicin, which kills the extracellular bacteria. The number of intracellular bacteria is enumerated after release of the bacteria by lysis of the epithelial cells and determination of the colony-forming units. All antimicrobial substances produced by probiotics result in reduced numbers of intracellular bacteria although these substances do not directly inhibit invasion but just kill the pathogens. However, there are probiotics able to specifically interfere with bacterial host cell invasion.

For some Lactobacillus kefir strains the component mediating the antiinvasive effect was identified as an S-layer protein which is also shed into the culture medium (Golowczyc et al., 2007). The ability to inhibit bacterial invasion of gut epithelial cells by pathogens is rather wide spread among probiotics. Whether this in vitro property has also in vivo relevance must be validated in animal experiments after identification of the responsible genes with isogenic mutants and their corresponding complemented strains in relation to the parental strain.

e) the inhibition of toxin expression in pathogens. May be the most important group of bacterial virulence factors are toxins. The effectiveness of certain probiotics in diarrhoea is most likely based on their ability to protect the host against toxins. This protection can result from inhibition of toxin expression in pathogens. For Bifidobacterium breve Yakult and Bifidobacterium pseudocatenulatum DSM20439 inhibition of shiga toxin expression in E. coli (STEC) O157:H7 strains in vitro as well as in mice was demonstrated in contrast to other Bifidobacterium isolates. Thus all animals treated with B. breve strain Yakult survived whereas 90% of the mice in the control group died after challenge with STEC. In vitro studies imply the high concentration of acetic acid produced by strain Yakult to be responsible forinhibition of Shiga toxin expression (Asahara et al., 2004).

2. Alteration of microbial metabolism in intestinal tract:

a) increasing the activity of useful enzymes, e.g. galactosidase in the alleviation of lactose maldigestion in lactose-intolerant people (Marteau et al., 1997);

b) decreasing the activity of some colonic enzymes such as glucuronidase, glucosidase, nitroreductase, azoreductase and steroid-7□-dehydroxylase known to have carcinogenic effect (Bengmark et al., 1998; Rowland et al., 1992).

3. Stimulation of immunity

Recent reports have shown that orally administered lactobacilli can improve immune status by increasing circulating and local antibody levels, gamma interferon concentration, macrophage activity and the number of natural killer cells (Fuller et al., 2000). Categories 2 and 3 include such purported health benefits as reductions in large bowel (colon) carcinogens and mutagens, antitumor properties, cholesterol-lowering effects, increased lactose digestion, relief from constipation, stimulation of immunocompetent cells and enhancement of phagocytosis. On the basis of the above-mentioned probiotic activity mechanism of lactic acid bacteria, there is some evidence for their beneficial effect on human health. According to Rowland (1999), beneficial effects claimed for probiotics belong to five areas with varying degrees of experimental support and those are: alleviation of lactose intolerance, preventive and therapeutic effects against diarrhoea, effects on the immune system, plasma cholesterol lowering and prevention of cancer.

1.4 Methods for improving the viability of probiotics in food matrices

Because of the high processing temperature during the manufacture of Fiordilatte cheese it is necessary to find technological solutions to add probiotics to this dairy product. Several methods for improving the viability of probiotics have been proposed with an effect or a modification on/of the process or the organism itself. Inclusion of prebiotics, optimization of the production operation, selection of a cocktail of probiotic organisms, modification (physically or genetically) of probiotics prior to microencapsulation, are some of the possible solutions to ensure the bioactivity of the probiotic products.

1.4.1 Development of edible films and coatings with functional activity

One strategy to functionalize food matrices is the use of edible films as carriers of functional substances. In the last years, consumer demand for more natural foods, and also for environmental protection, contributed to the development of new packaging materials. Edible coatings and films do not pretend to replace traditional packaging materials but to provide an additional stress factor to be applied for food preservation; they can also help to reduce the cost and also the amount of traditional packaging used. They can control moisture, gases, and lipid migration and can be supporters of additives and nutrients. For their formulation, there can be used polysaccharides, proteins, and lipids and they must result neutral with respect to color and flavor. Edible films are intended to lengthen shelf life, to vehicular functional components and also to respond to consumer demand for even more natural products.

Despite the growing interest in incorporating nutraceutical compounds into food products, few studies have suggested their integration into edible films or coatings. In this sense, the concentration of nutrients added to the films/coatings must be carefully studied since it is important to know the effects on their basic functionality, namely on their barrier and mechanical properties. Onother field of research is the biopreservation of food matrices by mens of LAB microorganisms immobilized in alginate beads. Several researchers have observed a reduction of foodborne pathogenic bacteria populations within food samples (or models) wrapped in packagings containing LAB antimicrobial metabolites (mainly bacteriocins) (Cao-Hoang, Chaine, Gregoire, & Waché, 2010; Da Silva Malheiros, Daroit, Da Silveira, & Brandelli, 2010; Ercolini et al., 2010; Iseppi et al., 2011) or, more recently, living LAB cells (Concha-Meyer, Schöbitz, Brito, & Fuentes, 2011; Gialamas et al., 2010; Iseppi et al., 2011). Entrapment of bacteria in calcium alginate beads is the most widely used technique for cells immobilization in probiotic domain or biotechnology (Kim et al., 2008). This new approach to control pathogen growth opens the lines of research on the possibility of using polymers as a support for viable pathogen antagonists and could lead to an alternative method of preservation.

Some studies have reported the effect of the addition of active compounds in the functionality of edible films. For instance, Mei and Zhao (2003) evaluated the feasibility of milk protein- based edible films to carry high concentrations of calcium (5 or 10% w/v) and vitamin E (0.1% or 0.2% w/v). They concluded that protein-based edible films can carry active compounds, although the film functionality can be compromised. Tapia et al. (2008) reported that the addition of ascorbic (1% w/v) to the alginate and gellan based edible coatings helped to preserve the natural ascorbic acid content in fresh-cut papaya, thus helping to maintain its nutritional quality throughout storage. Han et al. (2004) indicate that chitosan-based coatings had capability to hold high concentrations of calcium or vitamin E, thus significantly increasing their content in fresh and frozen strawberries and red raspberries. For one serving (100 g), coated fruits contained about 34-59 mg of calcium, and 1.7-7.7 mg of vitamin E, depending on the type of fruit and the time of storage, whereas uncoated fruits contained only 19-21 mg of calcium and 0.25-1.15 mg of vitamin E. Similarly, Herna´ndez-Mun˜oz, Almenar, Ocio, and Gavara (2006) observed that chitosan-coated strawberries retained more calcium gluconate (3079 g/kg dry matter) than strawberries dipped into calcium solutions (2340 g/kg). On the other hand, the addition of probiotics to obtain functional edible films and coatings has been scarcely studied. Tapia et al. (2007) developed the first edible films for probiotic coatings on fresh-cut apple and papaya, observing that both fruits were successfully coated with alginate or gellan film-forming solutions containing viable bifidobacteria. In fact, values higher than 10^6 cfu/g Bifidobacterium lactis Bb-12 were maintained for 10 days during refrigerated storage of both papaya and apple pieces, demonstrating the feasibility of these polysaccharide coatings to carry and support viable probiotics on fresh-cut fruit. This work represents a promising advance in the search for new applications of edible films and coatings as carriers of diverse food additives, and opens new possibilities for the development of probiotic products.

Edible films and coatings are usually consumed with the coated products. Therefore, the incorporation of compounds such as antimicrobials, antioxidants and nutraceuticals should not affect consumer acceptance. Some authors have indicated that the incorporation of antimicrobial

agents into edible coatings could impart undesirable sensorial modifications in foods, especially when EOs are used (Burt, 2004). Rojas-Grau¨, Raybaudi-Massilia, et al. (2007) evaluated the sensory quality of fresh-cut apples coated with alginate coatings containing EOs. Coated fresh-cut apples containing vanillin (0.3% w/w) were the most acceptable in terms of flavour quality, whereas coated apple pieces containing 0.1% v/v oregano oil exhibited the lowest overall preference due to a residual aromatic herbal taste detected on cut apples. Good results have also been reported for other antimicrobial compounds. Eswaranandam, Hettiarachchy, and Meullenet (2006) concluded that organic acids (malic and lactic acid) incorporated into soy protein coatings did not adversely impact the sensory properties of fresh-cut cantaloupe melon cubes. Sometimes the incorporation of certain antibrowning agents into edible coatings can yield an unpleasant odour, particularly when high concentrations of sulphur-containing compounds such as N-acetylcysteine and glutathione are used as dipping agents (Iyidogan & Bayindirli, 2004; Rojas-Grau, Sobrino-Lo´pez, Tapia, & Martı´n-Belloso, 2006). Perez-Gago et al. (2006) detected a smell of sulphur compounds in fresh-cut apples coated with whey protein concentrate/beeswax containing cysteine as antioxidant agent into the coating formulation. However, no differences were found between coated and uncoated samples containing ascorbic acid, indicating that this compound can be incorporated in whey protein concentrate coatings without a substantial effect on the organoleptic properties. Recently, Oms-Oliu et al. (2008a) reported that sulphur-containing compounds (N-acetylcysteine and glutathione) incorporated into alginate or pectin coating formulations did not appear to be detected by panelists when applied on fresh-cut pears. It was also reported that these substances are perceived with less intensity when incorporated into an edible coating formulation. Lee et al. (2003) indicated that whey protein concentrate coatings (5% w/v) containing ascorbic acid (1% w/v) and calcium chloride (1% w/v) were the most effective in preserving the sensory quality of cut apples. Not many studies have reported the sensory characteristics of coated dairy products when nutraceutical ingredients are incorporated. The taste of these ingredients has been regarded as a particularly important aspect, since many nutraceutical compounds have natural bitter, astringent, or other off-

flavours (Drewnowski & Gomez-Carneros, 2000) that can lead to rejection of the product by consumers (LeClair, 2000).

Elaboration of edible films and coatings has been possible thanks to the filmogenic capacity of natural biopolymers. Hydrocolloids have good aptitude to form a continuous and cohesive matrix with adequate mechanical properties (Bourtoom 2009; Bourtoom 2008). Such ability is related to the chemical structure of these compounds, which allows the association through hydrogen bonding of their polymeric chains. The literature reports that the most common biopolymers used for edible film elaboration are polysaccharides (single or blend of several types), proteins (single or mixtures from different sources), and blends of carbohydrates and proteins. Although lipids such as waxes and fatty acids are mainly used to constitute edible coatings, they do not have a suitable stand-alone filmmaking nature. For this reason, lipids are often supported on a polysaccharide matrix to provide a film with mechanical strength (Bourtoom 2009). Lipids are incorporated to hydrocolloid-based films formulation to improve their water barrier characteristics or change their visual appearance (Karbowiak et al. 2007; Maftoonazad et al. 2007a). Regarding methodology to obtain edible films, a very high number of papers used casting technique, being less reported other methods like high pressure, extrusion, spread coating, or coacervation (Flores et al. 2010). In a first step, the material must be properly dispersed and/or dissolved into a solvent like water, alcohol, diluted acids solutions, or mixtures of solvents. In some cases, it is necessary to heat or adjust the pH of the slurry containing the hydrocolloids in order to dissolve the macromolecule (Vargas et al. 2008).

Once the hydrocolloids were dispersed, it is possible to add other substances, like functional elements, antimicrobials, antioxidants, flavorings, and colorants, to the film-forming solution in order to confer the desired functional property to the film or coating. The removal of the solvent in excess is the following step. The drying rate and environmental conditions will determine the final thickness and structural characteristics of the resultant films. In these sense, a very-well controlled drying process should be performed. As was previously mentioned, one of the most important characteristics of biopolymers is their ability to constitute a resistant network. As well, it is very

desirable that films have selective barrier properties to several gases. It has been reported the very low oxygen permeability of edible films, but it is also known that hydrocolloid-based films possess high water vapor permeability (WVP) (Buonocore et al. 2005). Many efforts have been made by the scientist to overcome this shortcoming. In general, lipid addition was the strategy selected for the majority of the researchers to reduce the water vapor transmission rate (Anker et al. 2001; Ayranci and Tunc 2003; García et al. 2000). Another possibility to reduce the interaction with water molecules is the modification of polymer structure by crosslinking reaction, photocrosslinking, gamma-irradiation, or reaction with polyvalent ions (Delville et al. 2003; Le Tien et al. 2000; Marques et al. 2006; Rhim 2004).

The resistance of films to water, determined by the solubility in water test, is critical for the potential application of films. Sometimes, high water solubility is desired. This is the case when the film or coating will be consumed simultaneously with the food. However, in other technological situations such as packaging application of films, a low solubility in water molecules is extremely necessary. As a consequence of the poor water vapor resistance and lower mechanical strength in comparison with synthetic polymers, edible films have still limited application in food packaging.

Polysaccharides are the most usual filmmaking materials. They form transparent and homogeneous edible films with moderate mechanical properties. However, the application of these films is limited by their water solubility and poor WVP. To solve this shortcoming, the blending with different biopolymers (Xu et al. 2005), the addition of hydrophobic materials such as oils or waxes (Anker et al. 2001; Ayranci and Tunc 2003; García et al. 2000), or chemical modification of polymer structure (Marques et al. 2006) have been proposed.

1.4.2 Microencapsulation

Microencapsulation with respect to a food application, involves the reversible capture of active bio-molecules in a stable core and the safely delivering of it to a given target. Functionality and bioavailability are the key factors driving the microencapsulation of food products. It is widely

used to preserve and control flavor, color, texture, functional properties and to maintain the potential health benefits. Probiotics, minerals, vitamins, phytosterols, fatty acids, lycopene and antioxidants are some of the compounds which have been delivered through microencapsulation in recent years. All the three forms of matter can be entrapped using microencapsulation techniques. Microcapsules are commonly spherical in shape but can take any random form with an outer layer. Sometimes they may be double walled depending on the carriers added (Gharsallaoui et al., 2007). Early on, microencapsulation was mainly used to mask off-flavors of food ingredients and for conversion of liquids to solids. Encapsulation helps in the physical separation of sensitive viable cells from the external adverse environment thus improving the viability of cells (Weinbreck et al., 2010). Protection through physical barrier is considered an easy and efficient way of protecting these sensitive micro organisms against adverse conditions (Krasaekoopt et al., 2003). These applications can be extended towards formulation of various food products and ingredients in the health food sector. Several methods of microencapsulating probiotics include: spray drying, freeze drying, extrusion, coacervation, chemical methods using Ca-Alginate, k-carrageenan, gums (xanthan, arabic, etc.), starch, etc. All methods have their pros and cons. The substance to be microencapsulated is called the core material or bioactive and surrounding it is the wall material. The sphere dimensions vary depending on the physico-chemical interactions between the core and wall and also the technique of microencapsulation. It could be a simple sphere or an irregular sphere or multiple core spheres, etc. The size of the microspheres usually ranges between 0.2 to 5000 micrometers. Challenges in bioavailability and viability of probiotic microorganisms inside the gut are of prime concern for the current health-food industry. The purpose of microencapsulation is not just a protection through physical barrier but also a controlled release of the functional probiotics passing through the stomach to effectively reach the intestines (Picot and Lacroix, 2004). Once the encapsulated bioactive cores reach the targeted organs, it is ideal for the microencapsulated matrix to release them in a controlled fashion. Undesirable interactions between bioactives and external medium can be prevented when they are microencapsulated. The release of

the product follows a predetermined kinetics when microencapsulated which can be modified depending on the system to be delivered. Microencapsulation technique is widely exploited to improve the shelf life and to retain probiotics health properties (Semyonov et al., 2010). Encapsulation of probiotics ensures active maintenance even under high water activity during storage (Weinbreck et al., 2010). The stability and activity of microcapsules in gastro-intestinal system is dependent on several factors like pH of the core and the gut, particle size, chemicals present in the microencapsulating material and enzymes present in the gut. Several synthetic polymer and microencapsulating agents were employed for an enhanced bioavailability of microorganisms but challenges still exist on survival against adverse and harsh conditions in gut. There were many successful attempts in microencapsulating probiotics in several media like Ca-alginate, k – carrageenan etc., but their microcapsule size may be a drawback for their incorporation into most powdered foods due to large size and undissolvability. The encapsulation efficiency and the microsphere stability are greatly dependent on the encapsulating material known as wall material. Ideally the wall material should be water soluble since most spray drying suspensions are water based and possess good mechanical strength, compatibility with the core materials, emulsification properties and film forming and low viscous properties (Reineccius, 2004). Biopolymers, natural gums (acacia, k-carrageenan, alginates, etc), low molecular weight carbohydrates and proteins (whey protein, gelatin, etc.) are generally considered as good wall materials (Reineccius, 2004). This of course varies from strain to strain, however the carriers (like arabic gum, inulin, FOS, maltodextrin, polydextrose, skim milk powder, soy milk protein etc) in the suspension may have a significant effect on the viability (Ananta et al., 2005; Corcoran et al., 2004; Santivarangkna et al., 2008a). Since these wall materials contain prebiotic sources, when mixed with probiotics the produced powders can be considered as synbiotics. Spray drying is one of the oldest methods of encapsulation since 1900s used initially for flavor capture (Gharsallaoui et al., 2007). It is a single step continuous processing operation. The process can produce purest and finest powders with high sterility reducing the post unit operation like grinding and conditioning

(Menshutina et al., 2010). Spray dried powder particles are relatively small and uniform in size and shape. Spray drying allows a uniform dispersion of powder particle by diluting the bioactive core when a low amount is required. Powders can be easily transported without any special requirements and they have a prolonged storage (Silva et al., 2005). Milk and coffee powders, dehydrated enzymes, fruit and vegetable powders, etc. are some of the spray dried food products currently available in the market. It is a controlled process where the, fluid to be spray dried is passed through a very fine nozzle which comes in contact with hot air in co-current or counter current direction. The process of spray drying, generally involves the following steps (Shahidi and Han, 1993):

1. Preparation of suspension/emulsion
2. Homogenization
3. Automated feed dispersion into the drying chamber through a fine nozzle
4. Dehydration of fine droplets (powder formation)
5. Collection of accumulated powder

The principal driving force behind the powder formation is the temperature difference between the surface of the particle and the surrounding air, which is usually considered as wet bulb temperature of inlet air. The size of droplet should be small and viscosity of the liquid should be low for prevention of air in droplets (Lu and Walker, 2001). High viscosity of liquid to be spray dried increases its inlet time which in turn increases the power consumption. Rapid evaporation occurs mainly during co-current air drying when heat sensitive products are safely dried (Peighambardoust et al., 2011). However counter air drying is more economical compared to co-current (Gharsallaoui et al., 2007). Feed temperature has a direct impact on drying efficiency because it modifies the viscosity of the liquid. Drying rate of microsphere, final moisture content, water activity and ease of agglomeration are dependent upon the feed temperature. If feed temperature is very high, volatile and sensitive compounds might be lost before they get microencapsulated. Cracks on the microspheres and premature release and destruction of the ingredients were observed when high

inlet temperatures were employed. Moisture content, color and other sensory properties are greatly affected by outlet air temperature of spray dryer (Koc et al., 2010). Spray drying of Lactobacillus cultures was first done in 1914 by Rogers but it was not adopted due to very low survival rate, difficulty in storage as wells as poor rehydration capacity. Spray drying yogurt to preserve Lactobacillus and dairy starter cultures have been long investigated (Gardiner et al., 2000). Dried and stable probiotic cultures are usually prepared by spray drying or freeze drying which are the most common processing techniques though spray dried samples had a better stability and storage life than freeze dried samples prepared under same composition of suspension and conditions of storage (Ying et al., 2010). Spray drying processing cost is approximately six times lower than that of freeze drying (measured as drying of water per liter). Yet there are difficulties like low survival rates of the probiotics during spray drying, poor rehydration properties of the resulting powders etc. Moreover, dried and concentrated probiotic powder allows the ease of incorporation into several foods, transport and handling.

1.5 Prebiotic and Synbiotic compounds: characteristics and health benefits

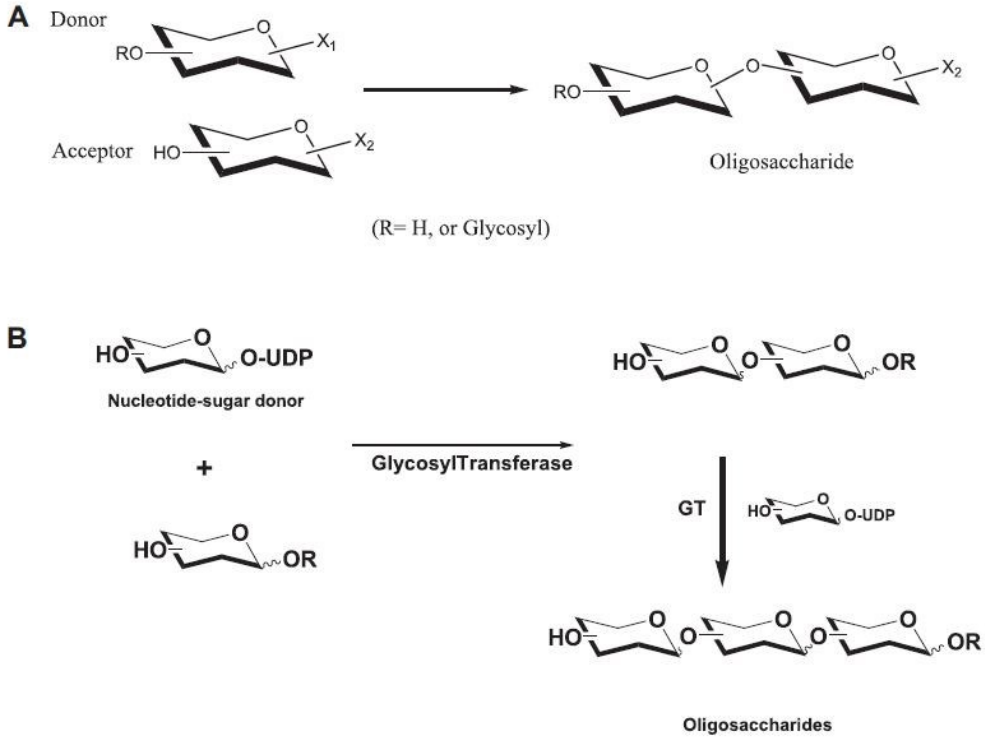
According to many authors (Rowland et al., 1992; Goldin et al., 1998), the low fibre diets of industrial societies are causative factors in the development of civilisation diseases like constipation, obesity, haemorrhoids, diverticulosis, cardiovascular disease, diabetes, colon cancer and others, whereas the better colonic activity achieved by high fibre diets protects against these diseases and can even cure some of them. Taking into consideration that many potentially health-promoting microorganisms, such as lactic acid and bifidobacteria, are already resident in the human colon, Gibson and Roberfoid (Gibson and Roberfroid, 1995) have introduced the prebiotic concept. According to them a prebiotic is »a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health«. Like probiotics, the prebiotics belong to a more general class of »colonic foods«, i.e. »foods entering the colon and serving as substrates for the endogenous

colonic bacteria, thus indirectly providing the host with energy, metabolihc substrate and essential micronutrients« (Gibson and Roberfroid, 1995). However, the relationship between the change in the number per gram of faeces of a particular bacterial species or strain and the dose of the prebiotic substrates is not yet clear. Indeed the initial number of the bacteria in faeces before any intake of the prebiotics seems to be a key parameter determining the multiplication factor (inverse relationship with the dose of the prebiotic), as well as the absolute increase in the number of bacteria (direct relationship) (Roberforid et al., 1998; Salminen et al., 1998). For a food ingredient to be classified as a prebiotic, it must:

1. neither be hydrolysed nor absorbed in the upper part of the gastrointestinal tract;
2. be a selective substrate for one or a limited number of potentially beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated;
3. consequently, be able to alter the colonic microflora towards a healthier composition, for example by increasing the number of saccharolytic species and reducing putrefactive microorganisms such as asaccharolytic clostridia and Enterobacteriaceae (Salminen et al., 1998; Gibson et al., 1997).

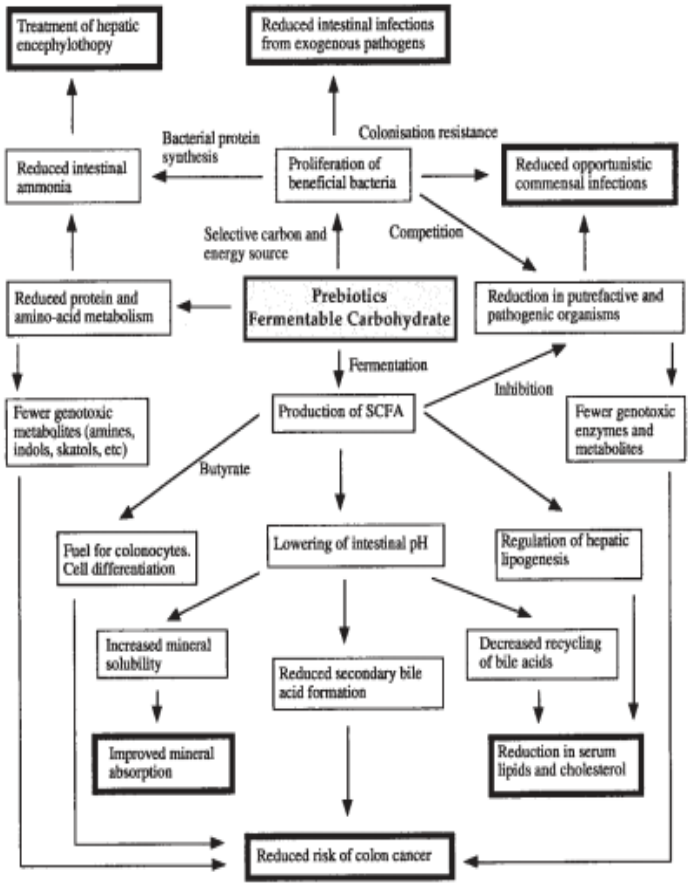
Most prebiotic oligosaccharides identified today are obtained either by extraction from plants (chicory inulin), possibly followed by an enzymatic hydrolysis (e.g., oligofructose from inulin) or by chemical synthesis by trans-glycosylation reactions from mono- or disaccharides. The glycosylation reaction (Figure 1.2) is obtained by inter-glycosidic condensation process between a protected glycosyl donor having an excellent leaving group such as halogenides at its anomeric position and a glycosyl acceptor possessing at least one free hydroxyl group.

Figure 1.2 Synthesis of oligosaccharides by glycosylation using A chemical process and B enzymatic process with glycosyltransferases.



The generation of specific glycosyl donor and acceptor needs many protections and deprotections steps to combinate high yields oligosaccharides production with high regio- and stereoselective processes (Barreteau, Delattre, & Michaud, 2006; Delattre, Michaud, Courtois, & Courtois, 2005). In enzymatic glycosylation strategies, a highly regio- and stereoselective glycosyltransferases and glycosylsynthetase enzymes are commonly used as biotechnological tools for oligosaccharides synthesis. Glycosyltransferases family (EC 2.4.x.y) catalyses transfer of sugar moieties from activated nucleotide glycosyl donors to specific glycosyl acceptors (Barreteau et al., 2006; Delattre et al., 2005; Takeo, Ohguchi, Hasegawa, & Kitamura, 1995; Weijers, Franssen, & Visser, 2008) allowing formation of glycosidic bonds as illustrated in Figure 1.3.

Figure 1.3 Proposed mechanisms of prebiotic action to improve human health (figure by courtesy of Dr. R.G. Crittenden, 2002).



This process has allowed the synthesis of bioactive oligosaccharides such as fructo-oligosaccharides from sucrose using fructosyltransferase or the formation of trans-galactosylated oligosaccharides or galactooligosaccharides from lactose (Crittenden & Playne, 2002) and cyclic non-reducing-end maltoligosaccharides named cyclodextrins (CDs) (Nigam & Singh, 1995) by using bacterial cyclodextrin glucosyltransferases (CGTase). Otherwise, lot of processes has been investigated to produce large amount of oligosaccharides by specific depolymerization of polysaccharide (Delattre et al., 2005). Several polysaccharides degradation mechanisms have been developed such as enzymatic, chemical treatments using acid and radical hydrolysis or physical treatments using g-

irradiations, thermal, microwaves, and ultrasonication degradation. Note that gamma, UV and others radiation process deliver high energy able to cleave glycosides linkages with short reactions times. Nevertheless, a long exposure time could cause degradation of osidic units. It was a non-random process and the depolymerization decrease with low-molecular weight fractions implicating the non-formation of oligosaccharides (Delattre et al., 2005). Therefore, enzymatic depolymerization of polysaccharides is the main approach currently used to prepare large amounts of oligomers. In these cases, procedures are investigated to depolymerize various polysaccharides (from plant, algae, microorganisms, etc.) by using regio-/stereospecific microbial enzymes such as polysaccharide lyases and polysaccharide hydrolases (Barreteau et al., 2006; Delattre et al., 2005; Michaud, Da Costa, Courtois, & Courtois, 2003). Using these selective, large scale of commercial prebiotic oligosaccharides with biological properties were prepared (Playne & Crittenden, 1996; Crittenden & Playne, 2002) such as malto-oligosaccharides from starch using a-amylases, fructo-oligosaccharides from enzymatic hydrolysis of inulin, isomalto-oligosaccharides with action of a,b-amylases and a-glucosidases and xylo-oligosaccharides from xylan cleavage with b-(1,4) xylanases. Namely, consumed probiotic bacteria must survive transit through the hostile conditions in the stomach and then adapt quickly to their new environment (survivability and colonisation may be a problem). On the contrary, prebiotics offer not only the potential to increase the number of beneficial bacteria, but also their metabolic activity through the supply of fermentable substrate. The increase in metabolic activity of autochthonous (probiotic) microorganisms is fundamental to many of the currently proposed mechanisms of health promotion by prebiotics. Moreover, the recent development of commercial prebiotic oligosaccharides and probiotic bacteria has led naturally to a new concept, that of symbiotic one, combining probiotics and prebiotics. The results of many researches (Nemcová et al., 1999; Gmeiner et al., 2000) point to a synergistic effect of probiotic and prebiotic combination on faecal microflora of experimental animals. This effect was demonstrated by increased total anaerobes, aerobes, lactobacilli, and bifidobacteria counts as well as by decreased clostridia, Enterobacteriaceae and E. coli counts.

Thus, the living microbial additions would be used in conjuction with a specific substrate for growth. The results of many researches (Nemcová et al., 1999; Gmeiner et al., 2000) point to a synergistic effect of probiotic and prebiotic combination on faecal microflora of experimental animals. This effect was demonstrated by increased total anaerobes, aerobes, lactobacilli, and bifidobacteria counts as well as by decreased clostridia, Enterobacteriaceae and E. coli counts.

The combination of probiotics and non-digestible carbohydrates may be a way of stabilisation and/or improvement of the probiotic effect. Such synbiotics indicate a realistic way of using biological preparations in the prevention of gastrointestinal diseases in humans and animals.

Such synbiotics indicate a realistic way of using biological preparations in the prevention of gastrointestinal diseases in humans and animals. Generally, there is a lack of studies on the development of symbiotic foods in dairy sector because probiotics and prebiotics effectiveness is investigated separately. The majority of the effects claimed by the prebiotics are associated with optimized colonic function and metabolism, such as an increase in the expression or change in the composition of shortchain fatty acids, increased fecal weight, a reduction in luminal colon pH, a decrease in nitrogenous end products and reductive enzymes, an increased expression of the binding proteins or on certain biomarkers in the field of lipid and mineral metabolism and immune system modulation (Bournet, Brouns, Tashiro, & Duvillier, 2002; Forchielli &Walker, 2005; Qiang, YongLie, & QianBing, 2009). All these effects on colonic flora and on the biochemistry and histology of the host bowel support the logic of the use of prebiotics for promoting health benefits (Table 1.3).

Table 1.3 types of prebiotic substrates and their chemical composition.

Type	Chemical composition
POLYOLS (sugar alcohols)	
Xylitol	$C_5H_{12}O_5$
Sorbitol	$C_6H_{14}O_6$
Mannitol	$C_6H_{14}O_6$ (stereoisomer of sorbitol)
DISACCHARIDES	Synthetic derivatives of lactose:
Lactulose	4-*O*-β-D-galactopyranosyl-D-fructose
Lactitol	4-*O*-β-D-galactopyranosyl-D-glucitol
OLIGOSACCHARIDES	
Raffinose	(α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru)
Soybean oligosaccharides	raffinose: (α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru) + stachyose:
(raffinose + stachyose)	(α-D-Gal-(1→6)-α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru)
Fructo-oligosaccharides (FOS)	(α-D-Glc-(1→2)-[(β-D-Fru-(1→2)-]^n n = 2-4
Oligofructose	(α-D-Glc-(1→2)-[(β-D-Fru-(1→2)-]^n n = 2-6
(mixture of oligosaccharides)	and β-D-Fru-(1→2)-[(β-D-Fru-(1→2)-]^n n = 1–6
Galacto-oligosaccharides (TOS – transgalactosylated oligosaccharides)	(α-D-Glc-(1→4)–[(β-D-Gal-(1→6)-]^n n = 2–5
OTHER NON-DIGESTIBLE OLIGOSACCHARIDES	
Palatinose	
Isomalto-oligosaccharides	
Lactosucrose	
Galactosyl lactose	
POLYSACCHARIDES	
Inulin	α-D-Glc-(1→2)–[(β-D-Fru-(1→2)-]^n
Resistant starch	$(C_6H_{10}O_5)_n$
(starch modifications that are resistant to the host endogenous glycolytic enzymes)	

Prebiotics such as FOS, TGOS (trans-galactooligosaccharides), Inulin as well as their symbiotic combination with probiotic bacteria (strains of L. plantarum, L. paracasei, or B. bifidum) increased bifidobacteria and lactobacilli or inhibited various human- and animal pathogenic bacteria strains (Clostridium spec., E. coli, Campylobacter jejuni, Enterobacterium spec., Salmonella enteritidis, or S. typhimurium) in vitro in mice (Asahara, Nomoto, Shimizu, Watanuki, & Tanaka, 2001), piglets (Bomba et al., 2002), or humans (Langlands, Hopkins, Coleman, & Cummings, 2004; Cummings & Macfarlane, 2002) . Moreover, a combination of prebiotics such as polydextrose and lactitol affects the microbial ecosystem of the gastrointestinal tract of rat and enhance the immune response by increasing the secretion of immunoglobulin A (IgA) (Peuranen et al., 2004). Furthermore, in treatment of colitis disorders, there are some experimental indications as to the beneficial effects of inulin in improving the distal colitis in the rat (Videla et al., 2001). In a randomized, controlled study, oligofructose and/or galactooligosaccharides were shown to have an effect on relapse of Clostridium difficile associated diarrhoea (Lewis, Brazier, et al., 2005; Lewis, Burmeister, et al.,

2005). Oligofructose administration to young childrens attending day care centre increased Bifidobacteria and decreased a potential pathogens, such as clostridia. These effects on gut microbiota were accompanied by less flatulence, diarrhoea, and vomiting (Waligora-Dupriet et al., 2007). The prebiotic foods are reported to enhance the immunity of the consumers. Indeed, the dietary components and their fermentation metabolites are in closely contact with the gut associated lymphoid tissue (GALT) which is the part of the vast intestinal immune system. The presence of food in the small intestine may be necessary for adequate function and development of GALT (Scheppach et al., 1992). Although, no information is available on how host organisms recognize ingested prebiotics in the process of expressing the immune modulating effects and subsequent events. Another mechanism on immunity system is assumed that innate defense responses can be activated through the interaction of sugar moieties with innate receptors on the plasma membrane of host cells, in particular in macrophages and dendritic cells (Arnold, Dwek, Rudd, & Sim, 2006; Nezlin & Ghetie, 2004). Indeed, Palma et al. (2006) have described that b-glucoseoligosaccharides stimulate innate immune reactions by binding to selective receptors (such as dectin-1) mainly expressed on M2 macrophages. Orally administrated inulin and oligofructose have recognised to modulate various parameters of the immune system, like the secretion of IL-10 and interferon (IFN-g) by Peyer's patch (Hosono et al., 2003), NK cell activity, the lymphocyte proliferation (Hoentjen et al., 2005), immunoregulation of intestinal IgA, increase of polymeric immunoglobulin receptor (pIgR) expression in both the small intestine and the colon in infant mice (Nakamura et al., 2004) and development of GALT (Pierre et al., 1997). Furthermore, inulin showed an anti-inflammatory effect on distal colitis induced in rats by dextran sodium sulphate and improves lesions of the intestinal mucosa (Videla et al., 2001). Schley and Field (2002) have reviewed some evidence of immune-enhancing effects of dietary fibres and prebiotic. Indeed, consumption of prebiotic fibres induces a variation of the intestinal microflora which may potentially mediate immune changes, via the short-chain fatty acids produced from the fibre fermentation, direct interaction of lactic acid bacteria or bacterial cell wall or cytoplasmic components with immune cells in the intestine and

finally by modulation of mucin production. Fermentation of prebiotics led to production of short-chain fatty acids (SCFA) which provide several effects on colonic mucosa.

Indeed, they directly or indirectly affect enterocyte proliferation, bowel inflammation, carcinogenesis, mineral availability, and colonisation by pathogens, enzyme activities and the production of nitrogenous metabolites. Butyric acid is used by the epithelial cells of the colon mucosa as energy source, being in addition a growth factor (Bugaut & Bentéjac, 1993). Recent preclinical studies have reported that butyrate might be chemopreventive in carcinogenesis (Scheppach & Weiler, 2004 or protector agent against colon cancer by promoting cell differentiation (Kim, Tsa, Morita, & Bella, 1982). In addition to butyrate, propionate can have anti-inflammatory effects on colon cancer cells. In addition, combination of probiotic L. rhamnosus and Bifidobacterium lactis with inulin enriched with oligofructose was shown to display an antitumorigenic action on azoxymethane-induced colon carcinogenesis in rats (Femia et al., 2002). Pool-Zobel (2005) has reported that inulin-type fructan may contribute to reduction of colorectal cancer risk. Prebiotic has also been proved to exert an effect on hepatic lipid metabolism. Inulin and oligofructan have shown a physiological effect on cholesterol and triglyceride levels in rats by decreasing postprandial cholesterolemia and triglyceridemia by 15% and 50% respectively (Delzenne, Daubioul, Neyrinck, Lasa, & Taper, 2002; Fiordaliso et al., 1995). In young adolescents, daily consumption of a combination of prebiotic short- and long-chain inulin-type fructans significantly increases calcium absorption and enhances bone mineralization during pubertal growth. Effects of dietary factors on calcium absorption may be modulated by genetic factors, including specific vitamin D receptor gene polymorphisms (Abrams et al., 2005).

1.6 Dairy and meat sector: state of art of probiotics and/or prebiotics enriched food products

Fermented dairy products enriched with probiotic bacteria are one of the most studied and optimized functional foods (Saxelin et al., 2005). Among these, cheese has been suggested as a better carrier product to deliver probiotic bacteria than fermented milk. The higher pH and fat content, and the solid matrix of cheese, may protect bacteria more efficiently than a fluid environment during the storage of the food and its transit through the human body (Ross, Fitzgerald, Collins, & Stanton, 2002). Besides, cheese is a lactose-free food, which represents an advantage over other dairy products, as many consumers are lactose-intolerant. Probiotic bacteria, such as lactobacilli, bifidobacteria and enterococci, have been incorporated into different cheese varieties: Gouda (Gomes, Malcata, Klaver, & Grande, 1995), Argentinean fresco cheese (Vinderola, Prosello, Ghiberto, & Reinheimer, 2000), white cheese (Kasimog˘ lu, Go¨ ncu¨ og˘ lu, & Akgu¨ n, 2004), Cheddar (Gardiner, Ross, Collins, Fitzgerald, & Stanton, 1998), Cottage (Blanchette, Roy, Be´langer, & Gauthier, 1996), and Crescenza (Gobbetti, Corsetti, Smacchi, Zocchetti, & De Angelis, 1998). The viability of probiotics is considered important for their ability to mediate the health benefits to the consumer and it is highlighted in the probiotic definition (FAO/WHO, 2002). In order to provide health benefits, probiotics bacteria must be present at a minimum level of 10^6 CFU/g of food product (Doleyres and Lacroix, 2005) or 10^7 CFU/g at the time of consumption (Lee and Salminem,1995) or they must be eaten in a sufficient amount to yield a daily intake of 10^8 -10^9 CFU/100g (Lopez-Rubio et al., 2006). Several factors have been claimed to affect the viability of probiotic cultures in food products including, temperature (Ostlie Hilde, Treimo, Narvhus, & Judith, 2005; Vinderola, Bailo, & Reinheimer, 2000), pH, (Corcoran, Stanton, Fitzgerald, & Ross, 2005), concentration of lactic and acetic acids (Samona, Robinson,& Marakis, 1996), the type of food matrix, processing conditions (Jaana, Alakomi, Vaari, Virkajärvi, & Saarela, 2006) and the presence of prebiotics to enhance their viability.

Until now there have been no more studies about the inclusion of probiotics and prebiotics by means of an alginate coating in a typical southern Italian dairy product: Fiordilatte mozzarella

cheese. Fiordilatte is the name used for Italian high moisture traditional mozzarella. It is a pasta filata cheese obtained by stretching acidified curd complemented or not, by the addition of lactic acid bacteria (LAB) (Faccia et al., 2013). The curd is obtained after coagulation of milk by rennet and/or coagulant enzymes (Kindstedt 2004). Fiordilatte cheese shows up to 60% of humidity, pH between 5.1 and 5.3 when obtained by acidification with LAB and between 5.6 and 5.8 when produced by chemical acidification (Faccia et al. 2009), high fat content (> 45% on dry matter; Salvadori del Prato 2001), and maximum 2% of salt (Anon 1997b). Then turns out to be a perfect substrate for probiotics and prebiotics addition. The advantage of food products such as dairy products is that they may additionally provide essential nutrients (e.g. calcium, proteins) and the addition of probiotics to these products is a natural way to enhance their functionality (Weichselbaum, 2009). Orally ingested probiotics have to survive adverse conditions during their passage through the GI tract to be able to influence the human gut microflora. Nevertheless, there are still some problems with respect to the low survival of probiotic bacteria in dairy foods as well as gastrointestinal conditions (Homayouni, Ehsani, Azizi, Razavi, & Yarmand, 2008b). This subject encouraged researchers to investigate different techniques for survival improvement of probiotics (Pourjafar, Mirzaei, & Manafi, 2007).

Meat is frequently associated with a ‘‘negative’’ health image due to its ‘‘high’’ fat content and in the case of red meat is seen as a cancer-promoting food. Therefore a low meat intake, especially red meat is recommended to avoid the risk of cancer, obesity and metabolic syndrome. However, this discussion overlooks the fact, that meat is an important source for some micronutrients such as iron, selenium, vitamins A, B12 and Folic acid. These micronutrients are either not present in plant derived food or have a poor bioavailability. In addition, meat as a protein rich and carbohydrate ‘‘low’’ product contributes to a low glycemic index which is assumed to be ‘‘beneficial’’ with respect to obesity, diabetes development and cancer (insulin resistance hypothesis). Taken together meat is an important nutrient for human health and development. As an essential part of a mixed diet, meat ensures adequate delivery of essential micronutrients and amino acids and is involved in

regulatory processes of energy metabolism. Meat is an important source for methyl donors such as folate and vitamin B12 and transfer factors methionine and choline. Folate and methionine as methyl donors influencing methyl group availability may also been associated with colon cancer incidence. It is frequently argued that the increased risk of different types of cancer resulting from low intake of fruits and vegetables is a result of a folate deficient diet, because fruits and vegetables are important sources for folate. While this is true, however, it has to be considered that the bioavailability of folate from meat and liver is much better than from fruits and vegetables Selenium is found largely in grains, fish and meats and enters the food chain through plants at geographically variable rates dependent on selenium concentration of the soil. The best known biochemical role for selenium is as part of the active site of the enzyme glutathione peroxidase (GPx). The metabolic function of this enzyme is vital for cells, as it is part of a mechanism responsible for the metabolism and detoxification of oxygen. It is assumed that GPx can protect DNA from oxidative damage and consequently from mutation leading to neoplastic transformation of cells (Combs & Clark, 1985). At relatively high levels, selenium protects against the action of certain carcinogens in various animal models (Halliwell & Gutteridge, 1989). In the US Selenium is mainly supplied by cereals, breads, meats and meat products. Beef alone is estimated to contribute approximately 17% of the total selenium in the American diet. Two recent studies in humans showed that meat was as good a source of selenium as wheat . Bioavailability of selenium from beef is higher than, or at least equal to, that of selenite and slightly lower than that of L-selenomethionine. Taken together meat is an important source for bioavailable selenium. Zinc is a component of some metalloenzymes and is important for cell growth and replication, osteogenesis and immunity. It may further contribute to the overall antioxidative defence. The primary dietary sources of zinc are red meat, sea food, poultry, grains, dairy, legumes and vegetables (Groff & Grooper, 2000). Read meat is a rich source of readily available zinc, whereas cereals contain different levels of phytic acid, a potent inhibitor of zinc absorption. Indeed, the recommendation to decrease or even avoid meat intake may result in a low zinc status as recently documented in

women from New Zealand (Gibson, Heath, & Limbaga, 2001). A mixed and balanced diet, including meat and meat products, is the best way to ensure sufficient intake of all essential and potentially cancer preventive components.

Meat consumption, especially red meat, is not carcinogenic per se, even if it contains components which, based on epidemiological and animal experiments, are assumed to contribute to cancer formation. The reason why meat is an essential source for some micronutrients is due the fact that meat is either the only source or has a much higher bioavailability for some micronutrients. Two important micronutrients occur only in meat: vitamins A and B12. Both cannot be compensated for by plant-derived provitamins. Provitamin B12 does not exist and the provitamin A, b-carotene, has to be taken in high amounts due to a poor conversion rate (1:12). Iron has a higher bioavailability when derived from meat as heme iron than plant-derived iron. Similarly folic acid has a nearly 10-fold higher bioavailability from meat (especially liver) and eggs than from vegetables.

Consequently, a low intake of meat (including liver) is associated with a risk for deficiencies in selected micronutrients.

It has been assumed, based on per capita protein intake and colon cancer risk, that total protein, is related to colon cancer risk. However, the majority of epidemiological cohort and case control studies could not confirm these assumptions assumptions. Only for red meat derived protein is there some evidence that risk increases if red meat is consumed twice a day and more (MacIntosh & Le Leu, 2001) or processed (boiled or fried). Whether the non-fat matrix of meat, e.g., the amino acid composition or the amount of heme iron plays a role in carcinogenesis is not understood. McIntosh and coworkers observed a non-significant increase from 33% to 59% in the incidence of DMH-induced (differential methylation hybridization) intestinal tumours in mature rats when barbecued beef was substituted for whey protein concentrate against a high fat (20%) diet background (MacIntosh, Royle, & Le Leu, 1998). Whether the concentration of protein in a diet determines the risk for cancer is controversial (MacIntosh, & Le Leu, 2001). In contrast the type of protein seems more important with respect to carcinogenesis. A high methionine diet, determined by type of

protein as well as quantity ingested, has been reported to lead to increased circulating insulin which has been assumed to contribute to colon carcinogenesis (McKeoween-Eyssen, 1994). Diets with a high glycemic index are thought to be associated with or to favour insulin resistance (Frost, Leeds, & Trew, 1998). Red meat, however, has a low glycemic index and may not contribute to the metabolic syndrome as long as its fat/energy content does not contribute primarily to the daily energy intake. Koohestani, Tran, and Lee (1997) showed that a high fat diet promotes aberrant crypt formation (ACF) in IR rats as an important step in colon carcinogenesis. Based on their results they conclude that: diets high in energy, saturated fat, and glycemic carbohydrate and low in ω3-fatty acids could deleteriously affect cell signaling in colonic cells in ways that lead to IR and colon cancer. Dietary intervention that reduces IR may also reduce colon cancer risk. So far low fat meat seems not to contribute to colon cancer. The other meat nutritional component put under examination for its negative role is fat. It is more or less generally suggested that animal fat rich in saturated fat is more closely related to cancerogenesis and plantderived mostly unsaturated fat (PUFA) is more protective. In animal models, the tumour promoting effect of fat intake has been observed primarily for PUFA (Hopkins & Carroll, 1979; Hopkins, Kennedy, & Carroll, 1981). A couple of studies show that polyunsaturated ω6 fatty acids (linoleic acid) enhance cancer development in rodents (Carroll, 1991; Fay, Freedman, & Clifford, 1997). The suggestion that consumption of red meat as a source of dietary fat increases risk of colon cancer is based on the rather simple fat-colon cancer hypothesis, which is based on the premise that dietary fat promotes excretion of bile acids which can be converted to carcinogens (Reddy, 1981). The controversial results from different studies and the fact that meta analyses show that fat might have a rather minor role, if even any, in cancerogenesis of the colon or in other cancer sites might be explained in different ways. The fat content of red meat varies in a wide range and shows different patterns. The fat content of red meat varies in a wide range and shows different patterns. Palmitic acid but not stearic acid present in different amounts in red meat has been shown to be a strong mitogen of adenoma cells in culture (Friedman, Isaksson, & Rafter, 1989). Fat, derived from red meat, might

be less absorbed, due to either its composition (stearic acid) or due to matrix (muscle) interactions. Polymorphisms of genes involved in the expression of cleavage and re-esterification of triglycerides may also play important roles regarding individual susceptibility. Finally, components not belonging to lipids might contribute to carcinogenesis such as HCAs (heterocyclic amines) or at least the iron content of meat. Finally carcinogens and promoters, e.g., HCAs are formed when meat is fried or cooked and may contribute more or less to the individual cancer risk, especially in colorectal, breast and prostate cancer.

Due to increasing concerns for health, efforts have been made by food industries in many countries to develop new foods with tertiary functions. Such foods having tertiary functions are regarded as functional foods. Although there have been limited studies of tertiary functions of meat until recently, it should be possible to develop new meat products with potential health benefits by increasing or introducing bioactive properties (Arihara, 2004). Such meat products would open up a new market in the meat industry. There are diverse possible strategies for developing healthier meat and meat products, including functional foods. Items listed below are strategies suggested by Jime´-nez-Colmenero et al. (2001).

1. Modification of carcass composition.

2. Manipulation of meat raw materials.

3. Reformulation of meat products.

– Reduction of fat content.

– Modification of the fatty acid profile.

– Reduction of cholesterol.

– Reduction of calories.

– Reduction of sodium content.

– Reduction of nitrites.

– Incorporation of functional ingredients.

Although numerous low-fat meat products have been developed in many countries, the meat industry in most countries has been hesitant to adopt the functional trend and to introduce additional physiologically functional properties into meat products. Utilization of functional ingredients is one approach to the development of functional meat products. Such ingredients include vegetable proteins, fibers, antioxidants, probiotics and prebiotics. In fact, dietary fiber from oats, sugar beet, soy beans, apples, peas, and probiotic lactic acid bacteria have been used in the formulation of meat products (Ferna´ndez- Gine´s et al., 2005; Jime´nez-Colmenero, Reig, & Toldra´, 2006). The following nine FOSHU meat products, four sausage products, one sliced ham product, two hamburger steak products and two meat ball products, have been approved in Japan (Arihara, 2004). Dietary fibers or soy proteins have been utilized as functional ingredients in these FOSHU products. several meat products with additional fibers, proteins and calcium have been marketed in Japan. Vegetable proteins have been used in meat products for their nutritional value and functional value. Soy proteins are typical of such proteins with health-enhancing activity. They are thought to be effective for preventing cardiovascular diseases, cancer and osteoporosis. A reduced-fat sausage formulated with a modified potato starch has been marketed in the United States (Pszczola, 2002). Such dietary fiber contributes to the improvement of intestinal microflora and the reduction of fat intake. As described above, the functional properties of meat products can be improved by adding ingredients considered beneficial for health or by eliminating components that are considered harmful. The items of functional modification in meat and meat products listed below have recently been reviewed by Ferna´ndez-Gine´s et al. (2005):

– Modification of fatty acid and cholesterol levels in meat.

– Addition of vegetal oils to meat products.

– Addition of soy.

– Addition of natural extracts with antioxidant properties.

– Sodium chloride control.

– Addition of fish oils.

– Addition of vegetal products.

– Addition of fiber.

Dietary fibre (DF) is, currently, considered to correspond to the edible parts of plants or analogous carbohydrates that are resistant to digestion by human enzymes such and to absorption in the human small intestine with complete or partial fermentation in the large intestine. Well-documented studies acknowledge that diets enriched with DF play a significant role in the prevention of several disorders such as colon cancer, constipation, obesity and cardiovascular diseases (Roberfroid & Slavin, 2000; Rodrı´guez et al., 2006).

Among the components of dietary fibre we can find the fructooligosaccharides (FOS), recognised as a natural food ingredient and classified as DF in almost all European countries (Flamm et al., 2001). They are considered as a prototype prebiotic which stimulates the growth of colonic microbiota (bifidobacteria and lactobacilli) which ferment it and produce short-chain carboxylic acids that enhance mineral absorption (Bounik et al., 1996; Coussement & Franck, 2001; Flamm et al., 2001; Harland & Narula, 2001). As only part of the FOS is digested, its available energy content is around 40–50% that of a digestive carbohydrate, giving energy value of 1.5 kcal g)1. Consequently, FOS unites all the characteristics to be considered a very interesting ingredient of functional foods, mainly as a component of a low-calorie diet (Roberfroid & Slavin, 2000; Cho & Dreher, 2001; Coussement & Franck, 2001; Flamm et al., 2001).

Short-chain FOS (sc-FOS) are synthetically derived from FOS on a commercial scale from sucrose using a fungal b-fructosyl transferase; sc-FOS have been considered as Generally Recognized as a Safe (GRAS) due to their potent prebiotic properties, good gastrointestinal tolerance and the absence of genotoxic potential (ILSI, 1999). The prebiotic effect of sc-FOS on lactic acid bacteria is well documented (Louis, 2007). Moreover, FOS and mainly the sc-FOS have been used for processed foods (soft drinks, cereals and candies, ice creams and dietetic products) because of their low caloric value and the formation of viscous solutions that simulate fat (Roberfroid & Slavin, 2000; Rodrı´guez et al., 2006). They are also suitable for use in meat products as a good fat replacer

and have been successfully used in meat emulsion products with favourable sensory results due to they favour water retention, reduce cooking losses and have a neutral flavour (Desmond & Troy, 2002; Ca´ ceres et al., 2004; Selgas et al., 2005; Garcı´a et al., 2006).

However, until now but there are no works on the use of prebiotics in fresh meat products such as burgers, and on the influence of these components on the sensory and nutritional properties of meat.

Natural sources of prebiotics also include cereals. Oat, among other cereals, has received considerable attention for its high soluble and insoluble fibres content that may promote several beneficial physiological effects (Mälkki & Virtanen, 2001). In addition, these oat constituents fulfill the prebiotic concept and can be used as fermentation substrates resulting in considerably high probiotic cell concentrations (Angelov, Gotcheva, Hristozova, & Gargova, 2005). Oat fiber source of β-glucan has been generally added to different meat products to counteract the problems caused by fat reduction, because fiber is not only desirable for its nutritional properties but also for its viscosity and then for its ability to improve rheological properties and stability (Anonymus, 2001). Hence, the development of new functional oat-based food products, that combine the beneficial effects of oat fiber with its technological properties, is a challenging issue.

Whey proteins are a by-product of cheese and casein manufacture, which contains approximately 20% of the original milk protein. Such proteins include a-lactalbumin, b-lactoglobulin, lactoferrin, lactoperoxidase, immunoglobulins, glycomacropeptide, and a variety of growth factors. These proteins have been implicated in a number of biological effects observed in human and animal studies, ranging from anti-cancer activity to influence on digestive function. Moreover, these same proteins, once partially digested, serve as a source of bioactive peptides with further physiological activity (Regester et al., 1996). Furthermore, whey protein have the ability to form a thermally induced gels when heated above 70 °C (Langley and Green, 1989) which can positively affect meat products stability and texture. Whey proteins contain a few proline amino acids, and numerous disulphide bonds, for this reason they are surface-active globular proteins and can be absorbed at water/fat interface stabilizing the water binding and the final texture (Shie, 2004). There is no

consensus among researches concerning the minimum FOS consumption that is needed to ensure prebiotic activity but according to some studies, a daily intake close to 5 g /day (Roberfroid and Salvin, 2000) is sufficient to stimulate the growth of bifidobacteria. The most important consideration that must be taken into account during the development of a functional meat product is not only the achievement of a perfect final prebiotic retention but also the maintenance of good textural and sensory properties in the final product. FOS and inulin have a neutral taste and are stable over a wide range of pH and temperature; therefore, they have great potential to be used for food applications. However, to the best of our knowledge a few studies from the literature deal with ingredients to ground beef to improve texture and palatability (Trout et al, 1992). Hence, on the basis of the above considerations, the current study is focus on the technological optimization of functional beef burger loaded with FOS, inulin and oat bran loaded protein foam. To this aim, the influence of functional addition on cooking quality, nutritional and sensory properties of meat burgers has been studied.

PhD Aim

Therefore, in this PhD thesis both cheese and meat were enriched with health promoting compounsds; in particular, prebiotic and probiotic substances were simultaneously added to Fiordilatte and FOS were added to meat burgers. The goal was to formulate a symbiotic cheese by means of an edible sodium alginate coating as carrier of probiotic (*Lactobacillus rhamnosus*) and prebiotic substances (FOS). Microbiological, sensory and functional quality indices were monitored to prove the viability of the probiotic bacterium and its effects on product quality during storage. As regards meat, the study was aimed to develop beef burgers enriched with prebiotic compounds to enhance the nutritional value of meat. In the specif, the prebiotics substances used were: fructo-oligosaccharides (FOS) and inulin alone and respectively combined with an oat bran protein foam. In order to optimize burger formulation and provide an acceptable product various technological strategies were adopted. The judgment of trained panelists was used to assess the effects of the different technological options used during processing. Once an acceptable meat product was realized, a complete characterization was carried out in terms of cooking characteristics, physic and chemical properties and meat composition.

The main goals of the project were:

- The study of the functional elements interaction with the food matrices from a technological, microbiological, nutritional and sensorial point of view.
- The development of technological solutions to ensure good cell survival and prebiotic retention in the food matrices.
- The study of the storage impact on cell viability and product shelf life.

2. Materials and Methods

2.1 Fiordilatte samples preparation

Fiordilatte samples were purchased from a local cheese factory "Capurso Azienda Casearia SPA" (Gioia del Colle, Bari, Italy) and transported to the laboratory in polystyrene boxes containing ice. Then, samples were dipped into sodium alginate solution (Farmalabor, Canosa di Puglia, Italy) prepared by dissolving sodium alginic acid (2% wt/vol) both in distilled water and in a solution made of 2% (wt/vol) of pure freeze dried *Lactobacillus rhamnosus* GG (LGG) (Granarolo, Italy), 14% (wt/vol) of fructo-oligosaccharides (FOS) (Granarolo, Italy) and distilled water. The coated samples were immersed into a 5% (wt/vol) calcium chloride ($CaCl_2$) (Sigma–Aldrich Milan, Italy) for 1 min, to physically crosslink the polymeric matrix. All samples were dried at room temperature for 2 min and packaged in commercially available polypropylene bags with brine. The control samples consist in Fiordilatte cheese without coating packaged in trays with the traditional brine (2% of NaCl solution). The experimental analyses were conducted in three different trials, using three different production batches of samples stored at three different temperatures: 4°C, 9°C and 14°C. Samples will be named as follows: CNT (control sample consisting in Fiordilatte cheese without coating), COAT (Fiordilatte cheese with a 2% sodium alginate coating without probiotics and prebiotics), and FUNC (Fiordilatte cheese with a 2% sodium alginate coating containing probiotic and prebiotics).

2.2 Fiordilatte analyses

2.2.1 Microbiological analyses

Twenty grams of Fiordilatte were aseptically removed from each package, diluted with 180 mL of NaCl solution 0.9% in a stomacher bag and homogenized with a Stomacher LAB Blender 400 (Pbi International, Milan, Italy). Subsequently, decimal dilutions of homogenates were made using the same diluent and the dilutions were plated on appropriate media in Petri dishes. Enterobacteriaceae were determined on Violet Red Bile Glucose Agar (VRBGA) (Oxoid) after incubation at 37°C for

18-24 h. Lactic acid bacilli (LAB) were plated on MRS Agar (Oxoid), supplemented with cycloheximide (0.1 g L-1106, Sigma) and incubated anaerobically at 30°C for 2-4 days. Pseudomonas Agar Base (Oxoid), added with SR103 E selective supplement (Oxoid) and incubated at 25°C for 48 h for *Pseudomonas* spp. All analyses were performed in duplicate on two different samples. To prove the functional characteristics of cheese, the count of viable lactic acid bacteria for the sample named FUNC was carried out using the whole coated Fiordilatte cheese, hereinafter named $FUNC_{WHOLE}$ and also in the sole functional coating indicated as $FUNC_{COAT}$.

In order to quantitatively determine the microbial acceptability limit (MAL), a modified version of the Gompertz equation was fitted to the experimental data, as reported in previous works (Conte et al., 2009; Del Nobile et al., 2009).

In order to quantitatively determine the microbial acceptability limit, a modified version of the Gompertz equation was fitted to the experimental data, as reported in previous works (Conte et al., 2009; Del Nobile et al., 2009).

(1)

where N(t) is the viable cell concentration at time t, A is related to the difference between the decimal logarithm of maximum bacteria growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration, μmax is the maximal specific growth rate, λ is the lag time, Nmax is the microbial threshold value, **MAL** is the microbiological acceptability limit [i.e., the time when N(t) is equal to Nmax], and t is the storage time. The value of Nmax for Pseudomonas spp. was set to 10^6 cfu/g of cheese and 10^5 CFU/g for Enterobacteriaceae ($MAL^{Enterobacteriaceae}$). The latter is imposed by the DPR 54/97 (European Union, 1997).

At each sampling time the pH was also measured by a pH meter (Crison, Barcelona,Spain). Each value was the average of two measurements.

2.2.2 Sensory analysis

Sensory analysis was conducted according to a method reported in the literature (Chiavari et al., 2006). A quantitative descriptive analysis was used for the comparison of samples (UNI 10957:2003 Sensory analysis – Method to define the sensory profile of foods and beverages). The analysis was divided in four phases:

1. Dictionary definition of sensory descriptors
2. Development of reference standards for each descriptor, corresponding to the maximum intensity on the rating scale used
3. Assessment of the intensity of each descriptor in the product concerned
4. Statistical analysis and interpretation of the results

In accordance with the standard UNI 10957:2003, eight testers of the Food Packaging laboratory were selected on the basis of international standards ISO 8586-1:1993 and ISO 8586-2:1994. The selection was made considering various aspects: interest and motivation, eating habits (consumption of Fiordilatte), ability to communicate sensations, time available for analysis sessions, ability to concentrate and performance training. Eight sessions of one hour each were required to define the sensory profile, with the frequency of three meetings a week. The sessions were used to familiarize the testers with the characteristics of Fiordilatte samples in terms of odor, color, texture and overall quality. After training, Fiordilatte samples were presented to each panelist without brine. The panelist was asked to evaluate odor, color, texture and overall quality every day for the entire period of observation, by using a scale from 0 to 7, where 4 was the minimum threshold for cheese acceptability. To judge the overall quality of cheese the following product characteristics were also taken into account: white porcelain, smooth surface, tight shut-off, elastic release of buttermilk after cutting, lack of holes and typical milk smell. The texture consistence has been determined using hands. Each taster evaluated a set of four samples each labeled with a random three-digit code. Samples have been stored in the sensory analysis laboratory at room temperature before tasting and the order of presentation was different for each accepted subject, to avoid mutual interference.

Before evaluating, each coated Fiordilatte cheese was deprived of the coating and immersed in water at room temperature for a few minutes, in order to tie these samples to wet uncoated cheese. The tasters used individual tasting booths in the hall of sensory analysis of the Food Packaging laboratory. The quantitative analysis was performed with a number equal to three replicates sessions on different days at the same hour of each day. In order to determine the sensory acceptability limit (SAL), that represents the storage time to reach the sensory attribute threshold, a modified version of the Gompertz equation was fitted to the experimental data (Conte et al., 2009; Del Nobile et al., 2009).

(2)

where SA(t) is the sensory attribute at time t, A^{SA} is related to the difference between the sensory attribute attained at the stationary phase and the initial value of sensory attribute, μ^{SA} max is the maximal rate at which SA(t) decreases, λ^{SA} is the lag time, SA_{min} is the sensory attribute threshold value, **SAL** is the sensory acceptability limit (i.e., the time at which SA(t) is equal to SA_{min}), and t is the storage time. As reported above, the value of SA_{min} is equal to 4.

2.3 Production of functional meat burgers

Adult beef medium fat for slaughter was used to produce the hamburgers. Meat was purchased from the farm Cucugliato S.r.L (Vernole, Lecce, Italy) and transported to the laboratory vacuum packaged in a frozen state (-20°C). The meat has been thawed for 24 hours at 2 °C and minced with a mincer (Everest, Sbarlati and C., s.n.c Rimini, Italy) equipped with a 4 mm grinding plate.

2.3.1 Production of meat burgers with FOS and Inulin

FOS (Beneo Orafti, Belgium) and inulin (Beneo Orafti, Belgium) were added to minced meat containing salt (1g) and oregano (0,3 g), in the form of powder (6% and 9%) (w/w) (FOS 6%-POWD; FOS9%-POWD; INUL6%-POWD; INUL9%-POWD), and in the form of water solution. The water solutions were obtained dissolving 6g of FOS and Inulin powder in 15 mL of distilled

water (FOS6%-WS and INUL6%-WS) and 9g of FOS and Inulin powder in 22,5 mL of distilled water (FOS9%-WS; INUL9%-WS). Hamburgers with sole minced meat with salt and oregano were also realized as control samples (CNTR). The experimental burgers were cooked according to the American Meat Science Association methodology (AMSA, 1995) in the oven (Moulinex Activys, France) to an internal end point temperature of 71°C, recorded at the geometrical centre of each patty by using hypodermic probe-type thermocouple (Model HVP-2-21-V2-TO-48-OCT-M Omega, Stanford, CT). Each formulation was prepared in duplicate.

2.3.2 Production of meat burgers with oat bran

The formulations with oat bran were prepared by adding to the minced meat, containing salt (1g) and oregano (0,3 g), oat bran soaked in water or in oil. In particular, for the first sample, 5g of oat bran powder (Di Minno Dario and c.s.r.l., Milano, Italy) were soaked with 15mL of distilled water (Oat-Hydr) and for the second meat sample, 5g of oat bran powder were soaked with 8mL of olive oil (Oat-Oil). Hamburgers with sole minced meat with salt and oregano were also realized as control samples (CNTR). The experimental burgers were cooked as previously described. Each formulation was prepared in duplicate.

2.3.3 Production of meat burgers with the whey protein foams

The formulations realized with the addition of foams were prepared by adding to the minced meat, containing salt (1g) and oregano (0,3 g), different kinds of protein foams: a simple whey protein foam (WPF) (Farmalabor, Canosa di Puglia, Italy) and 3 whey protein foams with different percentages of oat bran (20%, 30% and 40% w/w) (Oat20%WPF; Oat30%WPF; Oat40%WPF). The foams were prepared according to a previous work of Del Nobile et al. (2009), briefly described as follows. The foams with and without the desired amount of oat bran were prepared by mixing 60 g whey protein, 4g NaCl, 10g Na_2CO_3 and 110 mL distilled water. This mixture was cooked at 160 °C for 40 min in the oven (Moulinex Activys, France). After cooking the foam was minced by a Sterilmixer (PBI International, USA), reduced to crumb, soaked with oil (7,5 mL) and added to the minced meat. Each meat formulation was homogenized for a few minutes with hands

in order to obtain a final beef burger of 100g. Control samples based on sole minced meat with salt and oregano were also realized (CNTR). The experimental burgers were cooked as previously described. Each formulation was prepared in duplicate.

2.4 Meat burger characteristics evaluation

2.4.1 Cooking characteristics evaluation:

Cooking yield

Cooking yield of beef patties was determined by measuring the weight of six patties for each treatment/batch and calculating weight differences for patties before and after cooking, as follows:

Cooking yield (%) = (cooked weight/raw weight)x100

Diameter reduction

Change in beef patties' diameter was determined using the following equation:

Diameter reduction (%) = (raw beef patties diameter – cooked beef patties diameter / raw beef patties diameter)x 100

Cooking loss

Cooking loss of beef patties was determined by measuring the weight of six patties for each treatment/batch and calculating weight differences for patties after and before cooking as follows:

Cooking loss = (raw weight - cooked weight / raw weight)x100

2.4.2 Physico-chemical analysis

Water activity was determined with a Decagon CX1 dew point hygrometer (Decagon Devices, Pullman, WA, USA) at 20 °C. pH was measured in homogenate prepared with 1 g of burger and 9 mL of distilled water, using a Crison 2001 pH-meter.

Colour

The colour was measured with a Chroma Meter CR-200 colorimeter (Minolta Co., Osaka, Japan) using the colour space CIE L*a*b* system and calibrated with a rose tile (L* 44.88, a* 25.99, b* 6.67) and a D-65 light source. Hue angle (tonality) and saturation index (vivacity) were also

estimated. Measurements were performed at room temperature on the surface of each hamburger (ten measurements for each burger).

Textural analysis

Burger sample firmness was determined instrumentally by means of compression test using a Zwick/Roell model Z010 texture analyzer (Zwick Roell Italia S.r.l., Genova, Italy). Samples of each batch were of 1 cm high and 2.5 cm diameter and were placed between the parallel plates: an insert plate fixed in the universal work platform (100×90× 9 mm) and a compression die (75 mm diameter). The force required to compress slices of burger to a predetermined level of penetration against a rigid back plate using a cylindrical plunger was recorded for each sample tested. Trial specifications were as follows: pre-load of 0.3 N; load cell of 1 kN; percentage deformation of 50%; and crosshead speed constant of 100 mm/min. Ten measurements were carried out for each sample.

2.4.3 Proximate composition

Fat content was determined according to Soxhlet method; protein (Kjeldhal nitrogen), moisture (oven air-drying method) and ash (muffle furnace) (AOC, 1990) procedures. Fructooligosaccharides (FOS), Inulin and dietary fiber in the oat bran crumb were determinate by an enzymatic kit (Megazyme, Ireland 2011).

2.4.4 Sensory evaluation

Sensory analysis was performed by a ten member in house taste panel that evaluated the beef burgers as described by Jeffery and Lewis, 1983. The panel was chosen from a pool of 16 selected assessors based on their experience in sensory analysis of meat products and on their availability. Training consisted of a presentation of the treatments in three preliminary sessions to let the panelists familiarize with the characteristics to be evaluated in accordance with the American Meat Science Association (AMSA, 1983) guidelines. Tenderness, oilness/juiciness and meat flavor were evaluated by means of a nine-point structured scale where 1, extremely dislike; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely (Patsias et al., 2006). According to the scale, a

score of 5 was taken as the lowest limit of acceptability. The panel test was conducted using individual booths (located away from the sample preparation area) under red-filtered incandescent light to avoid bias, due to potential color differences among samples. Each patty was cut into four wedges, assigned with three-digit random numbers and served to each panelist. A glass of water and unsalted crackers were provided to cleanse the palate among samples. Experimental samples were evaluated in a total of four sessions held over 2 days with a randomized serving order.

2.5 Statistical analysis

Experimental data were compared by one-way Anova analysis. A Duncan's multiple range test, with the option of homogeneous groups ($P<0.05$), was used to determine significance among differences. To this aim, Statistica 7.1 for Windows 152 (StatSoft Inc.,Tulsa, OK, USA) was used

3. Results and Discussion

3.1 Fiordilatte cheese

As reported above, a new method to produce a symbiotic mozzarella cheese was proposed in this study. In particular, the innovation consists in the application of an alginate coating on the surface of the product as carrier of probiotic and prebiotic substances. The addition of probiotics and prebiotics to obtain a functional edible coating for dairy products has not yet been reported. To assess the functional characteristics of the product, the lactic acid bacterial count was monitored for the entire experimental period to prove that probiotic level remained acceptable to produce beneficial effects on the gut (10^7 CFU/g). In addition, microbial and sensory attributes were also monitored. In the following results obtained were reported and discussed separately.

3.1.1 Fiordilatte microbiological quality

Figure 5.1 shows the viable cell concentration of LAB as a function of storage time for all the investigated samples, during the three experimental trials at 4°C (a), 9°C (b) and 14°C (c). As it can be seen, the cell load for both coating ($FUNC_{COAT}$) and coated Fiordilatte ($FUNC_{WHOLE}$) samples was above the functional acceptability limit (10^7 CFU/g) during the entire observation period in all the experimental trials. In fact, the initial cell load at 4°C for $FUNC_{COAT}$ was about 1.23 x 10^9 CFU/g and for $FUNC_{WHOLE}$ was about 4.52 x 10^7 CFU/g while CNT and COAT samples showed a concentration of about 2.03 and 2.15 x 10^3 CFU/g. The lactic acid bacteria in the lyophilized probiotic added to the coating were about 10^{11} CFU/g. The same values were recorded in the experimental step carried out at 9°C and 14 °C. The 2-log-cycle decrease recorded in the $FUNC_{WHOLE}$ compared to the concentration of the original lyophilized probiotic was due to a dilution effect. The survival and the maintenance of LAB in the coated product ($FUNC_{WHOLE}$) may be considered satisfactory, as their values remained above the functional acceptability limit whereas the CNT and the COAT were below this threshold. Data listed in table 5.1 highlight that the FAL (functional acceptability limit) for the $FUNC_{WHOLE}$ was > 8 days at 4°C, > 6 days at 9°C and > 5 days at 14 °C, while for the CNT and the COAT was 0 because their cell load was always below the

FAL. This experimental evidence assessed that the innovation process consisting in the sodium alginate coating enriched with probiotic and prebiotic was effective in the functionalization of Fiordilatte cheese. When a functional probiotic coating is applied on a food product, the effectiveness is evaluated through the enumeration of the indigenous and inoculated microbial population during the storage period (Mitrakas et al., 2008; Moreira et al., 2009; Seol et al., 2009; Martins et al., 2010). The probiotic addition to each trial was established as a function of the expected real human consumption of Fiordilatte cheese, at least 100g day^{-1}. Our symbiotic cheese respects the minimal counts suggested by several authors to produce beneficial effects on the gut, representing $10^8 – 10^9$ CFU $100g^{-1}$ of daily product consumption (Hoier et al., 1999; Vinderola et al., 2000a; Vinderola et al., 2000b; Vinderola and Reinheimer, 2000). Besides the satisfactory probiotic viable counts, a protective behavior by the prebiotic added to the Fiordilatte cheese was expected because FOS are known to increase the survival of the probiotic organisms, being a nutritional substrate (Shin et al., 2000; Bruno et al., 2002; Özer et al., 2005; Capela et al., 2006).

Figure 5.2 shows the evolution of *Pseudomonas* spp. count in Fiordilatte cheese during the three experimental trials 4°C (a), 9° (b) and 14 °C (c). Bishop and White (1986) stated that a *Pseudomonas* spp microbial load equal to 10^6 cfu/g of cheese represents the contamination level at which the alterations of the product start to appear. As can be seen in the figure a, there was a gradually increase in the microbial count for all the three samples from day 2 till the end of storage, but the CNT and COAT samples reached the microbiological acceptability limit ($MAL^{pseudomonas}$) at day 5 and 5.5 respectively and so, faster than the $FUNC_{WHOLE}$ which match the threshold value at day 6.5 (table 5.1). In figure b, the result obtained in the trial at 9°C was confirmed, in fact also in this trial CNT and COAT reached the microbial limit ($MAL^{pseudomonas}$) at day 3.5 and 2.5, respectively, while the $FUNC_{WHOLE}$ at day 4.5. Figure c remarks the previous considerations, also in this trial there was a faster achievement of the microbial limit by CNT and COAT, reached by both of them at day 2, while the $FUNC_{WHOLE}$ at day 3.3. However, there is an additional consideration, in this case the temperature played an important role in the fast development of target microorganism.

In the product, microbial growth started yet at the first day for all the three samples. So, the increase of the storage temperature influenced the durability of the product. The delay in reaching the MAL in the $FUNC_{WHOLE}$ sample may be explained by the presence of the probiotic coating over the product. The addition of probiotic and prebiotic exerted a sort of antimicrobial action, probably due to the acidification of the substrate and the production of some metabolites such as organic acids and bacteriocins (Pithava et al., 2011). A reduction of pH value was recorded for all trials in the brine liquid (data not shown) and this is a commonly observation in dairy products enriched with probiotics because of the continuous production of organic acids by fermentation that lower the pH of the environment and consequently inhibit bacterial pathogens growth (Pithava et al., 2011).

Figure 5.3 shows the evolution of *Enterobacteriaceae* population in Fiordilatte cheese during the three experimental trials 4°C (a), 9°C (b) and 14°C (c). Also for this microbial group the addition of probiotic and prebiotic in the coating, combined with the low storage temperature, proved to be effective in slowing down bacterial count in the final product. In figure (a) there was an increase of the microbial group for CNT and COAT samples starting from day 4, that reached the acceptability limit ($MAL^{Enterobacteriaceae}$) at day 7, while for the $FUNC_{WHOLE}$ sample the threshold was reached at day 9.5 and the bacterial growth started on day 5. In the second trial (b) the increase of temperature at 9°C caused an immediate proliferation for all the three samples, in fact the COAT sample reached the microbial threshold at day 4 while the CNT sample overlapped the microbial limit at day 5. The $FUNC_{WHOLE}$ cheese also in this case presented a delay in overlapping the microbial limit (at day 6.5). The trial at 14°C (c) demonstrated that this temperature was a condition of thermal abuse because all three samples reached the microbial limit faster respect to those previously described, the CNT and the COAT at day 2.2 and 3, respectively and the $FUNC_{WHOLE}$ at day 3.5. The probable antimicrobial effect of the probiotic culture added to the product was clearly demonstrated for *Enterobacteriaceae* microorganisms. It can be suggested, on the basis of the microbial considerations, that the addition of probiotic and prebiotics in the coating surface of the

Fiordilatte cheese is an optimal and innovative way to functionalize the product and at the same time, with a combination of an optimal storage temperature, to prolong its microbial quality.

3.1.2 Fiordilatte sensory quality

Edible films and coatings are usually consumed with the coated product. Therefore, the incorporation of compounds such as probiotics and prebiotics should not affect consumer acceptance. The addition of probiotics to obtain functional edible films and the effect of this addition to the food product has been barely studied (Rojas-Grau et al., 2009). The taste of these nutraceutical ingredients has been regarded as a particularly important factor since several authors found that probiotics cause the acidification of the substrate and the production of some metabolites such as organic acids and bacteriocins (Pithava et al., 2011). As reported above, a reduction of pH value was also recorded for all trials in the brine liquid. Furthermore, the increase of fermentation processes can also lead to a change in the product-structure. Fiordilatte cheese is considered a traditional Italian product with a characteristic sensory property that leads this product to the concept of "natural-traditional-product". Thus, it is necessary to preserve these attributes also when probiotic and prebiotic were added. It is important that treatments applied to functionalize also allow maintaining the appearance (i.e. color and integrity) and the flavor characteristics, being the first factor that the consumer perceives as product quality (Faccia et al., 2013). Figure 5.4 shows the evolution during storage of the overall quality of Fiordilatte cheese during the three experimental trials at 4°C, 9°C and 14 °C (a, b, c). The curves were obtained by fitting Eq. (2) to the experimental data, whereas the horizontal dashed line is the sensory threshold value. In all the three figures there was a steadily decrease of overall quality more pronounced in both the control samples. At 4°C (a), samples CNT and COAT reached the sensory acceptability limit ($SAL^{o.q.}$) at day 7.4 and 7.3, respectively while the $FUNC_{WHOLE}$ remained over the threshold since day 8 (table 5.1). The trend was confirmed in the second trial at 9°C (b). Also in this case, the $FUNC_{WHOLE}$ was the last to reach the sensory limit (at day 6.5), while the CNT and the COAT samples match the threshold line at day 5 and 4, respectively (table 5.1). In the final trial, under thermal abuse (c), the

trend of the $FUNC_{WHOLE}$ overall quality decreased faster compared to the previous trials, in fact the sample reached the sensory limit at day 5.5 day but later respect to the CNT and the COAT samples that reached the threshold at day 3.5 and 4, respectively. The trend of the overall quality coincides with that of the odor, thus proving that this attribute represented the factor limiting cheese storability. Texture and color attributes did not affect the overall quality of the product; indeed, the panelists expressed their judgments with a score above the acceptability limit during the entire storage time (data not shown). The sensory evaluation confirmed the considerations of the microbial quality: the increment of the storage temperature speeds up the microbial deterioration of the product, also causing the faster decrease in the overall quality attribute. The addition of probiotic and prebiotics in the coating creates a sort of protection against microbial and sensory deterioration of Fiordilatte cheese. The panelist highlighted a typical milk odor of sample coated with probiotic and prebiotics substances, which conceded with the natural characteristics of this Italian traditional product. This consideration probably determined the higher $SAL^{O.Q.}$ for the $FUNC_{WHOLE}$. A study conducted by Mirzaei et al. (2012) confirmed that the addition of free or encapsulated probiotics had no significant effect on sensory properties of a probiotic Iranian cheese, in fact the total evaluation in terms of color, texture and taste of samples were good and did not have any marked off-flavor during the storage and samples enriched with probiotics recorded an overall quality better than that with no probiotic cells.

3.1.3 Fiordilatte shelf life

The Fiordilatte shelf life is listed in table 5.1 for each sample tested in this study. For samples without functional coating (CNT and COAT) it was calculated as the lowest value among $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$, and $SAL^{O.Q.}$ (Conte et al., 2008), while for the sample with the functional coating ($FUNC_{WHOLE}$) it was the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$, $SAL^{O.Q.}$ and FAL. As it can be seen from the table, the FAL for samples without functional coating (CNT and COAT) coincides with 0, because these samples were below the functional acceptability limit, since they did not possess any probiotic or prebiotics. It can be

emphasized from data that microbial quality limited the shelf life of the three samples with a major contribute of *Pseudomonas* growth. In the first trial (4°C) the shelf life of CNT and COAT was set at 5 and 5.5 days, respectively while the $FUNC_{WHOLE}$ revealed a longer storability, about 6.5 day, probably for the improvement of the microbial and sensory characteristics of the functional addiction. With the increase of temperature there was a decrease in shelf life for all the samples but always faster in samples without the functional coating. This effect may be explained by the increment of microbial growth and as a consequence of the sensory deterioration. At 9°C of storage temperature the shelf life for CNT and COAT was set at 3.5 and 2.5 days, respectively, while $FUNC_{WHOLE}$ recorded a longer shelf life, about 4.5 day. Under conditions of thermal abuse (14°C) the shelf life further decreased, reaching a value of 2 days for CNT and COAT samples and 3.3 days for the $FUNC_{WHOLE}$. The functional characteristic (the viable count of LAB) remained constant for the entire observation period and it did not match the threshold line during the experimental period, thus assessing a FAL value longer than the storage period in all the experimental trials. To sum up, the application of an edible sodium alginate coating enriched with probiotic and prebiotics to Fiordilatte cheese improved its microbial, sensory and functional characteristics, contributing also to extend its shelf life.

3.2 Meat burgers

This work was aimed to develop beef burgers enriched with prebiotic compounds to enhance the nutritional value of meat. In order to optimize burger formulation and provide an acceptable product from a sensorial point of view, various technological strategies were adopted. The judgment of trained panelists was used to assess the effects of the different technological options used during processing. Once sensory acceptable meat product was realized, a complete characterization was carried out in terms of cooking characteristics, physic and chemical properties and meat composition. To better present the work and the different experimental plans carried out, results are presented in three different steps.

3.2.1 Optimization of FOS and Inulin addition

This first experimental step was related to the choice of the best FOS and Inulin amounts to be added to the burgers and how to add them into the final products, to ensure not only the highest final prebiotic concentration but also a good product taste. To this aim, different beef burger formulations were prepared: beef burgers without any prebiotic addition (CNTR), and beef burgers with FOS and inulin, at 6% and 9% (w/w) respectively, in the form of powder (FOS6%-POWD) (FOS9%-POWD) (INUL6%-POWD) (INUL9%-POWD) and in the form of water solution (FOS6%-WS) (FOS9%-WS) (INUL6%-WS) (INUL9%-WS). The used amounts of prebiotics were selected because it has been reported that the prebiotic effect is achieved at a minimum daily intake close to 3g for 100g of food product (European Parliament, 2006). A panel test was carried out to choose the best sensory prebiotic amount between 6% and 9% and also the best way to introduce it in the final formulation, choosing between powder and water solution. The panelists evaluated raw and cooked hamburgers in terms of flavor, oiliness, juiciness, tenderness and overall quality. To summarize the findings, the sole overall quality of cooked samples was reported (figure 5.5), since it well represents the trend of the other evaluated sensory attributes and better underlines the differences between samples. As regards the raw samples (data not shown), the addition of FOS and inulin in the form of powder improved the oiliness and the juiciness, determining a more compact structure but at the same time it compromised the overall quality because the visual aspect was negatively affected by the presence of little white spots, corresponding to the added powder. For this reason, panelists rejected the use of FOS and inulin in the form of powder. On the contrary, the addition of these components in the form of water solution improved the sensory quality; in fact, in this way, the structure was juicier and tender also respect to the CNTR sample and the overall quality was more positively perceived respect to the previous formulations. Most probably, this is because water solubilization of FOS and inulin avoids the white spots on the burgers. The most important differences between tested samples were recorded after cooking. The addition of FOS and inulin in the form of water solution improved the overall quality because samples appeared more

similar to the CNTR sample, camouflaging the prebiotic addition and the flavor was recorded less sweet respect to the same compound added in the form of powder. As a result of this panel test it was decided to introduce FOS (6%) and inulin (9%) in the form of water solution (FOS6%-WS) (INUL9%-WS) because they turned out to be less sweet and with absence of white spots.

3.2.2 Optimization of oat bran addition

Taking into account that oat fiber is a good source of fibers, this experimental step was aimed to further improve the prebiotic content of meat burgers, optimizing the amount of oat bran to be added, considering not only the final prebiotic retention but also the sensory acceptability. To this aim, the beef burgers were prepared by adding to ground beef the oat bran by different technological methods. First, oat bran powder 5% (w/w) soaked with water (Oat-Hydr) or with oil (Oat-Oil) was added into the meat matrix. The second strategy involved the use of different kinds of foams based on simple whey protein (WPF) or on whey protein with different amounts of oat bran (20%, 30% and 40% w/w) (Oat20%WPF, Oat30%WPF, Oat40%WPF). As reported for the previous step, also in this case the panel test assessed the sensory quality of all raw and cooked meat burgers. Figure 5.6 reports data of the overall quality of all cooked meat products. As can be observed, cooked meat formulations containing the foams, showed better sensory scores in terms of general acceptability, this was mainly because of their improved structure. A higher score for the flavor attribute was observed for samples loaded with oat bran loaded foam respect to the addition of oat bran as powder. Moreover, the figure highlights that the overall quality score increased as the concentration of oat bran increased (see Oat40%WPF), thus suggesting the relevant importance of foams properly enriched with oat bran (40%) to enhance product quality. The only appreciable consideration on raw samples (data not shown) is that the use of oat bran in the form of powder determined an excessive compact structure with low tenderness and juiciness, while the use of all the protein foams improved the tenderness and the juiciness without compromising the flavor. For these reasons the sample Oat40%WPF was used for the subsequent work, to be combined with FOS and Inulin.

3.2.3 Combination of all the prebiotic compounds

In this last step the best prebiotic amounts chosen in the first experimental plan (FOS6%-WS) (INUL9%-WS), were combined with the ingredient individuated in the second step (Oat40%WPF) to further increase the meat burger prebiotic concentration. In fact, two types of prebiotic meat burgers (FOS6%-WS-Oat40%WPF; INUL9%-WS-Oat40%WPF) were realized. Results of panel test carried out on these new formulations, where the oat bran loaded foam was combined with FOS and inulin, properly solubilized in the water solution (table 5.5), suggested that both samples were well accepted because the scores were above the sensory acceptability limit. The combination of all the ingredients further improved the overall quality of both raw (data not shown) and cooked samples (figure 5.7) and allowed recording better score than the control meat. As regards raw samples, a better structure, a good tenderness and a pleasant juiciness were recorded (data not shown). Cooked samples appeared with a pleasant flavor, more appreciated than in the previous steps. As regard oiliness, it is important to underline that all the samples appeared similarly appreciated, except the sample INUL9%-WS-Oat40%WPF that recorded a slightly low score. No off-flavor were perceived and a slight better sensory score was recorded for the sample FOS6%-WS-Oat40%WPF due to the fact that combination of FOS, inulin and oat foam had a more neutral taste than meat without foam (Caceres et al., 2004). To sum up, the two samples FOS6%-WS-Oat40%WPF and INUL9%-WS-Oat40%WPF revealed high sensory scores probably due to their high water holding capacity, the ability of fiber to create a soft gel, the soft final texture and the liked meat flavor. These results confirmed the findings of earlier studies where fiber has been used to improve the sensory characteristics of meat products (Thranathan and Mahadevamma, 2003; Ylmaz, 2004).

Once defined the best burgers formulations, the influence of the selected ingredients on the chemical, physical, nutritional and technological properties of burgers were assessed. To better highlight differences between samples with and without foams, the following four formulations

were taken into account: FOS6%-WS, INUL9%-WS, FOS6%-WS-Oat40%WPF, INUL9%-WS-Oat40%WPF.

3.2.4 Cooking characteristics

Meat performance during cooking is reported in table 5.2. From data reported in the table it can be inferred that all meat burgers had a reduction in diameter that is directly related to the amount of water lost during cooking. Samples without oat bran loaded foam, recorded the highest values of cooking loss and, as a consequence, the greatest diameter reduction. These samples requested an additional quantity of water used to solubilize FOS and inulin that might have led to these results. On the contrary, the two samples with foam (FOS6%-WS-Oat40%WPF, INUL9%WS-Oat40%WPF) recorded the lowest shrinkage and cooking loss value, most probably due to the water binding properties of oat fiber combined with FOS and inulin and the presence of the foam, which helps to retain water during cooking. These samples revealed the highest cooking yield, suggesting that fiber has the ability to create hydrogen bounds with water and in this way, it could retain moisture and keep meat from drying out during cooking. This effect could be enhanced by the ability of whey protein present in the protein foam to interact both with fiber and with water. These results are in line with other works on beef patties containing fibers (Trout et al, 1992). The ability of fiber to retain water and the less cooking yield of these samples makes them more succulent. Samples that have lost more water during cooking (CNTR and INUL9%-WS) were assessed as less juicy and less tender. These findings are also recorded in sausages (Desmond at al., 1998; Caceres et al, 2004), thus suggesting that the addition of prebiotics fiber improved the cooking characteristics of the investigated burgers.

3.2.5 Physical and chemical characteristics

As it can be seen in table 5.3, the incorporation of prebiotic fiber did not affect the pH and the a_w of raw and cooked samples. In fact, the values of both parameters were similar in all samples, with no statistically significant differences among them. Color properties of samples enriched with FOS, inulin and oat foam are quite different compared to the control sample. In fact, these burgers

recorded lower values of L^* and a^* and higher b^* value, both in raw and in cooked samples. These findings can be explained by the FOS ability to interact with the protein meat component creating a sort of gel that coexists with the other meat components yielding a linked and consistent unit that caused the loss of lightness (Matuszek, 2001). The reduction of redness and the increase of yellowness in FOS6%WS-Oat40%WPF, INUL9%WS-Oat40%WPF was confirmed in a similar work of Ylmaz (2002) and it should be a consequence of the synergic effect of FOS, inulin and oat bran foam. As can be deduced from the sensory data, the color change did not cause loss of sensory quality in the final product.

Texture profile parameters of both samples with and without prebiotics are also shown in table 5.3 As regards the compression of raw samples, no significant differences were found between samples. The most evident difference is that after cooking, the shear force increased for all meat burgers. This is, most probably, due to the contemporary occurrence of several factors such as water loss, resulting protein rearrangement and meat hardening. As far as the cooked samples is concerned, a different trend was found for both FOS or inulin respectively combined with the oat bran foam. In this case the shear force was significantly lower if compared to the control and to the other samples without foams. FOS6%WS-Oat40%WPF and INUL9%WS-Oat40%WPF were affected by the double water binding effect of FOS, inulin and oat bran that, creating a stable and soft gel (Dos Santos et al., 2012), resulted in a reduction of the force required to compress the sample. These results are in line with other works (Mendoza et al., 2001; Caceres et al., 2004) where FOS and inulin have been used as fat replacer, just for their ability to modify and soften meat and to reproduce fat creaminess. The changes detected in the textural assay and in the cooking analysis are clearly correlated with the sensory evaluation, mainly to tenderness and juiciness. In the case of tenderness, the scores increased in samples with FOS and inulin combined with oat foam (FOS6%WS-Oat40%WPF and INUL9%WS-Oat40%WPF). The softening revealed by textural analysis was confirmed by the tenderness of these samples, also due to oat/FOS and oat/inulin lower cooking loss (table 5.5).

3.2.6 Burger composition

Data listed in table 5.4 describe the composition of all raw and cooked beef burgers. Moisture ranged from 63.32% in the raw CNT sample to 71.48% in FOS6%WS-Oat40%WPF sample. Most probably this is due to the extra amount of water added to this sample. For the meat sample obtained by combining FOS in the form of water solution and the oat bran foam, an higher water content was found compared to the formulations without foams due to the addition of water required to achieve the fiber solution and that required to obtain the foam. Also cooked burgers with the prebiotic combined with the oat bran foam (FOS6%WS-Oat40%WPF and INUL9%WS-Oat40%WPF) recorded the highest values of moisture, probably because these samples showed the lowest value of cooking loss. In fact, oat bran present in the foam has the ability to retain moisture during cooking and keeps meat from drying out (Pszola et al., 1991). As regards fat, protein and ash composition in raw burgers, no differences were noticed among the investigated samples. On the other hand, in the cooked burgers an increase of fat, protein and ash was found for all samples, probably due to a concentration effect related to the water loss during cooking (Egbert et al., 1991). It can be seen that FOS6%WS-Oat40%WPF and INUL9%WS-Oat40%WPF recorded the lowest content of fat, protein and ash, probably because to the low dehydration caused by the cooking process. Looking at the raw and cooked samples, it is evident that there is a little loss of fibers with the addition of FOS and inulin in the form of water solution, while combining them with the oat foam there is a better retention of the same components over cooking. Therefore, according to the Regulation No. 1924/2006 of the European Parliament (2006), all the four developed meat products could be considered as 'fiber sources' because all of them contain more than 3 g of dietary fiber per 100 g of food. In particular, samples with FOS and inulin combined with oat foam contain 5.60 g and 7.55g (w/w) of prebiotics, respectively.

6. Conclusions

The first goal of my PhD thesis was the functionalization of Fiordilatte cheese by means of a sodium alginate edible coating as a carrier of probiotic and prebiotics. The addition of probiotics and prebiotics to obtain functional Fiordilatte cheese has been scarcely studied. As discussed above, to define a product as functional it must ensure a concentration of LAB not less than 10^7 CFU/g that has to be preserved till the end of the final consumption of the product. Indeed, this concentration has to be considered in a normal daily quantity of the product. Our approach has proved that viability of LAB remained over the imposed limit for the entire observation period and in all experimental trials: about 4.52 x 10^7 CFU/g at 4°C, 3.42 x 10^7 at 9 °C and 4.62 x 10^7 at 14 °C. This means that with a daily consumption of 100g of coated Fiordilatte cheese a person would assume a quantity of LAB equal to 10^9 CFU/100g. Furthermore, the addition of probiotic and prebiotics substances in the coating showed a little antimicrobial activity against *Pseudomonas* spp. and *Enterobacteriaceae*, improving the final taste of the product and prolonging the shelf life. The other goal of my PhD thesis was the functionalization of beef burgers by means of fructooligosaccharides and inulin, alone and respectively combined with oat bran loaded foam. The final concentration of fibers in the products developed in the current study was above the minimal level imposed (3 g per 100 g of food product). In the specific, samples with FOS and inulin properly hydrated presented a prebiotic content after cooking of 5.07% and 6.66% (w/w), respectively. This study has shown that combination of FOS and inulin with oat bran loaded foam is an interesting way to minimize the loss of these compounds during cooking, in fact FOS6%WS-Oat40%WPF and INUL9%WS-Oat40%WPF recorded the highest prebiotic values: 5.60% and 7.55% (w/w), respectively. The prebiotic addition in presence of foam properly enriched with oat bran also improved the technological and sensory characteristics of meat, giving products that appear to be very prized.

7. Tables and Figures

Table 5.1
Shelf life (days) of Fiordilatte samples as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and $SAL^{O.Q.}$

Temperature	Samples	Microbial quality (day)		Sensory quality (day)	Functional quality (days)	
		$MAL^{Pseudomonas}$	$MAL^{Enterobacteriaceae}$	$SAL^{O.Q.}$	FAL	Shelf life
T=4°C	CNT	5	7	7.4	0	5
	COAT	5.5	7	7.3	0	5.5
	$FUNC_{WHOLE}$	6.5	9.5	8	>8	6.5
T=9°C	CNT	3.5	5	6.5	0	3.5
	COAT	2.5	4	5.5	0	2.5
	$FUNC_{WHOLE}$	4.5	6.5	7	>6	4.5
T=14°C	CNT	2	2.2	3.5	0	2
	COAT	2	3	4	0	2
	$FUNC_{WHOLE}$	3.3	3.5	5.5	>5	3.3

CNT= control sample (Fiordilatte cheese).
COAT= samples dipped into sodium alginate solution without probiotics.
$FUNC_{WHOLE}$= samples dipped into sodium alginate solution with probiotics and prebiotics.
$MAL^{Pseudomonas}$ = microbiological acceptability limit for *Pseudomonas* spp
$MAL^{Enterobacteriaceae}$ = microbiological acceptability limit for *Enterobacteriaceae*
$SAL^{O.Q.}$ = sensory acceptability limit related to the overall quality attribute.
FAL= functional acceptability limit

Table 5.2 *Cooking characteristics of burgers.*

Cooking characteristics	CNT	Fos6%-WS	Inul9%-WS	Fos6%WS-Oat40%WPF	Inul9%WS-Oat40%WPF
Diameter Reduction (%)	22.37 ± 2.28 a,b	26.30 ± 4.68 b,c	30.23 ± 2.68 c	15.57 ± 2.70 a	16.83 ± 5.25 a
Cooking Yield (%)	67.50 ± 0.65 b	68.57 ± 1.44 b	60.9 ± 0.17 a	72.47 ± 2.08 c	75.02 ± 2.77 c
Cooking Loss (%)	33.50 ± 0.65 a	36 ± 0.87 b	43.43 ± 1.29 c	32.38 ± 1.57 a	32.67 ± 0,31 a

[a-b-c]Data in the same column with different superscript upper cases are significantly different (P<0.05).
Abbreviations:
CNT, meat burgers with only minced meat with salt and oregano;
Fos6%-WS, meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL;
Inul9%-WS, meat burgers with Inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL;
Fos6%WS-Oat40%WPF meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL combined with oat bran proteic foam 40% (w/w);
Inul9%WS-Oat40%WPF, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL combined with oat bran proteic foam 40% (w/w).

Table 5.3 Physical and Chemical Properties of raw and cooked burgers

	Physical and Chemical Properties	CNT	Fos6%-WS	Inul9%-WS	Fos6%WS-Oat40%WPF	Inul9%WS-Oat40%WPF
Raw	pH	5.68 ± 0.11 a	5.57 ± 0.08 a	5.60 ± 0.07 a	5.55 ± 0.06 a	5.56 ± 0.05 a
	Aw	0.99 ± 0.00 c	0.97 ± 0.01 a,b	0.97 ± 0.00 a,b	0.98 ± 0.01 b,c	0.96 ± 0.01 a
	Colour					
	lightness L	49.23 ± 1.05 b	51.04 ± 1.26 b	55.08 ± 1.51 c	42.45 ± 0.75 a	41.34 ± 0.77 a
	Redness a	13.58 ± 1.59 c	12.49 ± 2.17 a,b	11.53 ± 1.13 a,b	10.65 ± 0.47 a	10.36 ± 0.83 a
	Yellowness b	6.04 ± 1.69 a	6.21 ± 1.89 a	6.35 ± 1.50 a	9.18 ± 0.6 b	9.29 ± 0.78 b
	Shear Force value (N)	6.79 ± 1.18 b	4.50 ± 0.51 a	3.97 ± 0.40 a	4.73 ± 0.17 a	4.06 ± 0.46 a
Cooked	pH	5.86 ± 0.05 a	5.80 ± 0.23 a	5.76 ± 0.03 a	5.75 ± 0.03 a	5.78 ± 0.05 a
	Aw	0,97 ± 0.02 a	0.96 ± 0.01 a	0.96 ± 0.00 a	0.97 ± 0.02 a	0.95 ± 0.01 a
	Colour					
	lightness L	48.64 ± 1.97 b	48.67 ± 1.95 b	48.07 ± 0.98 b	45.32 ± 1.52 a	44.43 ± 1.23 a
	Redness a	8.43 ± 0.26 b	8.52 ± 0.43 b	8.56 ± 0.34 b	5.61 ± 0.24 a	6.04 ± 0.39 a
	Yellowness b	4.68 ± 0.75 a	6.95 ± 0.57 b	7.31 ± 0.33 b	12.39 ± 0.56 c	11.11 ± 0.57 d
	Shear Force value (N)	20.25 ± 1.60 b	19.68 ± 2.04 b	20.35 ± 1.57 b	15.50 ± 1.25 a	16.05 ± 1.19 a

[a-b-c-d]Data in the same column with different superscript upper cases are significantly different ($P<0.05$).

Abbreviations:

CNT, meat burgers with only minced meat with salt and oregano;

Fos6%-WS, meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL;

Inul9%-WS, meat burgers with Inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL;

Fos6%WS-Oat40%WPF meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL combined with oat bran proteic foam 40% (w/w);

Inul9%WS-Oat40%WPF, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL combined with oat bran proteic foam 40% (w/w).

Table 5.4 Proximate composition of raw and cooked burgers.

	Proximate composition	CNT	Fos6%-WS	Inul9%-WS	Fos6%WS-Oat40%WPF	Inul9%WS-Oat40%WPF
Raw	Moisture (%)	63.32 ± 1.58 a	65.58 ± 2.65 a,b	67.98 ± 1.21 b	71.48 ± 1.16 c	71.42 ± 1.58 c
	*Fat (%)	15.51 ± 0.88 a	15.48 ± 0.75 a	15.41 ± 0.32 a	15.36 ± 0.72 a	15.32 ± 0.48 a
	Protein (%)	19.32 ± 0.36 a	19.23 ± 0.56 a	19.20 ± 0.57 a	19.36 ± 0.21 a	19.34 ± 0.38 a
	Ash (%)	2.35 ± 0.11 a	2.38 ± 0.36 a	2.37 ± 0.22 a	2.43 ± 0.26 a	2.46 ± 0.16 a
	Fos and Inulin (g/100g)	-	5.75 ± 0.10 a	8.10 ± 0.16 b	5.90 ± 0.11 a	8.74 ± 0.10 c
	Fiber (g/100g)	-	-	-	0.21 ± 0.04 a	0.20 ± 0.05 a
Cooked	Moisture (%)	59.83 ± 0.71 a	62.86 ± 1.20 b	61.16 ± 1.49 a,b	68.59 ± 1.63 c	68.34 ± 1.22 c
	*Fat (%)	22.10 ± 0.16 b	22.05 ± 0.17 b	22.12 ± 0.36 b	20.86 ± 0.42 a	21.02 ± 0.39 a
	Protein (%)	27.22 ± 0.46 b,c	27.44 ± 0.34 c,d	28.14 ± 0.31 d	26.10 ± 0.39 a,b	26.16 ± 0.48 a
	Ash (%)	3.32 ± 0.68 a	2.76 ± 0.54 a	2.67 ± 0.13 a	2.80 ± 0.71 a	2.70 ± 0.41 a
	Fos and Inulin (g/100g)	-	5.07 ± 0.36 a	6.66 ± 0.17 c	5.60 ± 0.06 b	7.55 ± 0.06 d
	Fiber(g/100g)	-	-	-	0.27 ± 0.03 a	0.25 ± 0.04 a

* Fats were calculated as a percentage of the dry weight of the sample

[a-b-c-d]Data in the same column with different superscript upper cases are significantly different (P<0.05).

Abbreviations:

CNT, meat burgers with only minced meat with salt and oregano;

Fos6%-WS, meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL;

Inul9%-WS, meat burgers with Inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL;

Fos6%WS-Oat40%WPF meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL combined with oat bran proteic foam 40% (w/w);

Inul9%WS-Oat40%WPF, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL combined with oat bran protein foam 40% (w/w).

Table 5.5 Sensory characteristics of cooked burger.

Sensory characteristics	CNT	Fos6%-WS	Inul9%-WS	Fos6%WS- Oat40%WPF	Inul9%WS- Oat40%WPF
Juiciness	5.42 ± 0.58 a,b	6.00 ± 0.45 a,b,c	5.10 ± 0.89 a	6.62 ± 0.22 c	6.42 ± 0.38 b,c
Tenderness	5.25 ± 0.69 a	5.50 ± 0.55 a	6.17 ± 0.26 a,b	6.80 ± 0.27 b	6.60 ± 0.55 b
Oiliness	5.70 ± 0.84 a,b	5.83 ± 0.26 a,b	5.58 ± 1.07 a,b	7.00 ± 0.00 b	6.10 ± 0.74 a
Flavour	5.10 ± 0.74 a	5.67 ± 0.61 a	5.60 ± 0.55 a	7.10 ± 0.22 b	6.00 ± 0.61 a
Overall Quality	6.00 ± 0.00 a	6.67 ± 0.41 b	6.93 ± 0.49 b,c	7.60 ± 0.42 c	7.58 ± 0.20 c

[a-b-c]Data in the same column with different superscript upper cases are significantly different ($P<0.05$).
Abbreviations:
CNT, meat burgers with only minced meat with salt and oregano;
Fos6%-WS, meat burgers with FOS at 6% (w/w) in the form of watersolution with an amount of water of 15 mL;
Inul9%-WS, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL;
Fos6%WS-Oat40%WPF meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL combined with oat bran proteic foam 40% (w/w);
Inul9%WS-Oat40%WPF, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22.5 mL combined with oat bran protein foam 40% (w/w).

CAPTURE TO FIGURES

Figure 5.1 Evolution of Lactic acid bacterial count in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c).

Figure 5.2 Evolution of *Pseudomonas* spp count in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c).

Figure 5.3 Evolution of *Enterobacteriaceae* in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c).

Figure 5.4 Evolution of the overall quality in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c).

Figure 5.5 CNT, meat burgers with only minced meat with salt and oregano; Fos6%-POWD, meat burgers with FOS at 6% (w/w) in the form of powder; Fos9%-POWD, meat burgers with FOS at 9% (w/w) in the form of powder; Fos6%-WS, meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL; Fos9%-WS, meat burgers with FOS at 9% (w/w) in the form of water solution with an amount of water of 22,5 mL; Inul6%-POWD, meat burgers with inulin at 6% (w/w) in the form of powder; Inul9%-POWD, meat burgers with inulin at 9% (w/w) in the form of powder; Inul6%-WS, meat burgers with inulin at 6% (w/w) in the form of water solution with an amount of water of 15 mL; Inul9%-WS, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22,5 mL. Error bars indicate means ± SD of 6 replicates.

Figure 5.6 CNT, meat burgers with only minced meat with salt and oregano; Oat-Hydr, meat burgers with oat bran powder 5% (w/w) soaked with water (15mL); Oat-Oil, meat burgers with oat bran powder 5% (w/w) soaked with oil (8 mL); WP-Foam, meat burgers with the whey protein-based foam; Oat20%-Foam, meat burgers with oat bran loaded foam 20% (w/w); Oat30%-Foam, meat burgers with oat bran loaded foam 30% (w/w); Oat40%-Foam, meat burgers with oat bran loaded foam 40% (w/w). Error bars indicate means ± SD of 6 replicates.

Figure 5.7 CNT, meat burgers with only minced meat with salt and oregano; Fos6%WS-Oat40%WPF, meat burgers with FOS at 6% (w/w) in the form of water solution with an amount of water of 15 mL combined with oat bran foam 40% (w/w); Inul9%WS-Oat40%WPF, meat burgers with inulin at 9% (w/w) in the form of water solution with an amount of water of 22,5 mL combined with oat bran foam 40% (w/w). Error bars indicate means ± SD of 6 replicates.

Figure 5.1 *Evolution of Lactic acid bacterial count in Fiordilatte cheese during the three experimental trials 4°C (**a**), 9°(**b**) and 14°C (**c**) $FUNC_{COAT}$ (●), LYOPHILIZED PROBIOTIC (1g) (◆), CNT (■), COAT (■), $FUNC_{WHOLE}$ (▲).*

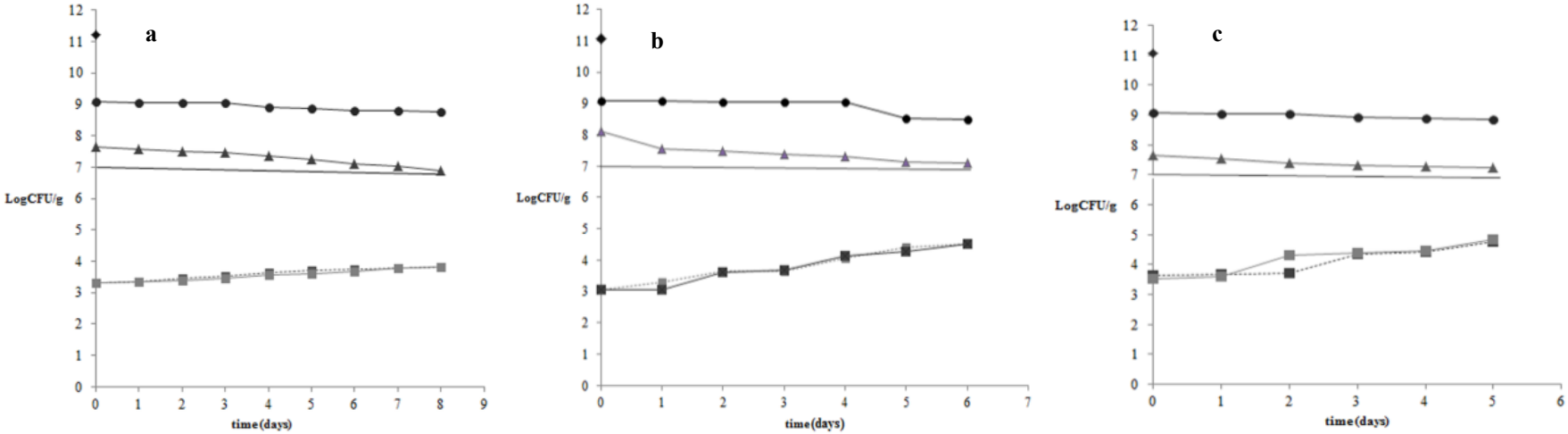


Figure 5.2 Evolution of *Pseudomonas* spp count in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c). *CNT (◆), COAT (■), $FUNC_{WHOLE}$ (▲).*

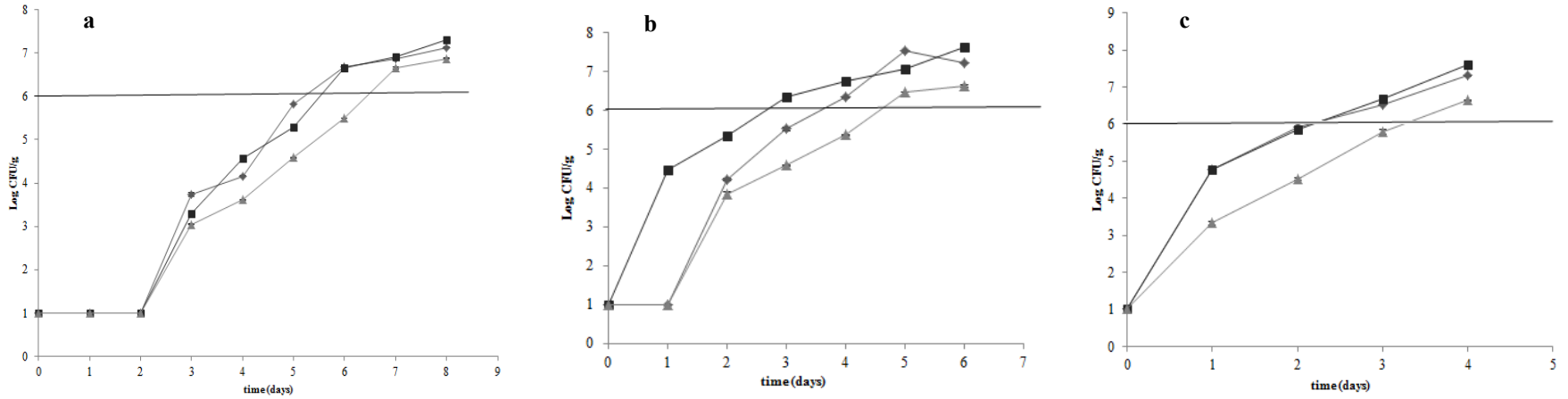


Figure 5.3 Evolution of *Enterobacteriaceae* count in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c). *CNT (◆), COAT (■), FUNC$_{WHOLE}$ (▲).*

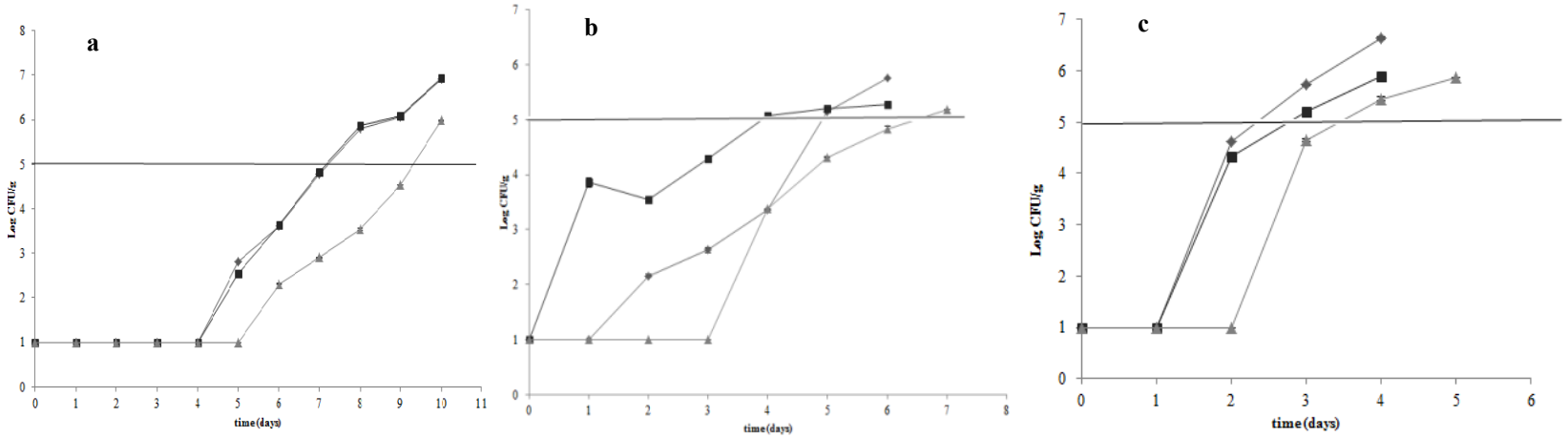


Figure 5.4 Evolution of the overall quality score in Fiordilatte cheese during the three experimental trials 4°C (a), 9°(b) and 14°C (c). *CNT (◆), COAT (■), FUNC$_{WHOLE}$ (▲).*

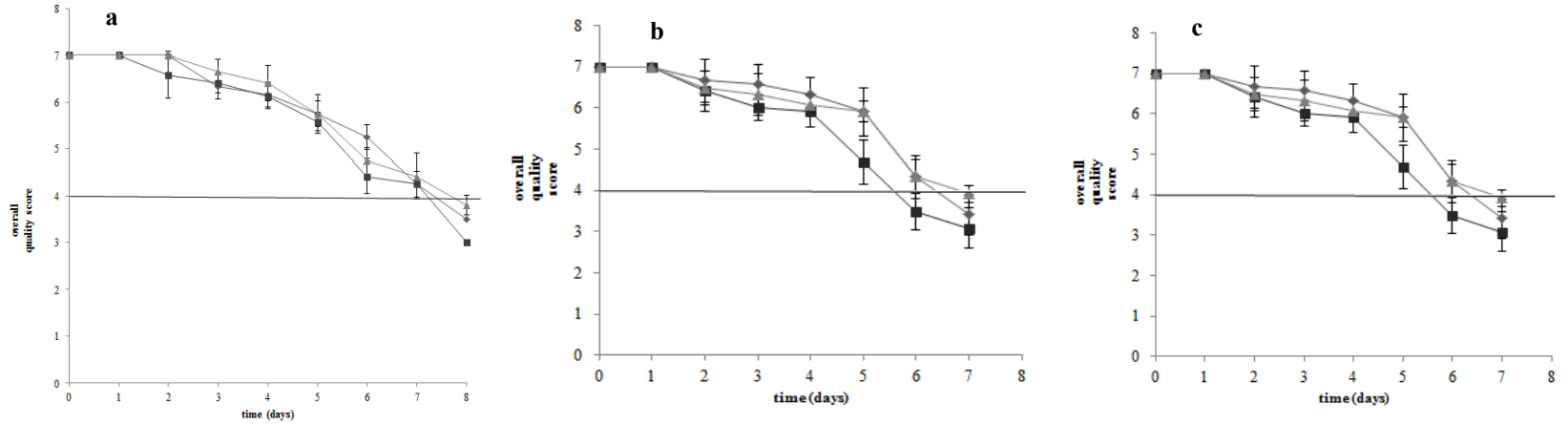


Figure 5.5 Overall quality of cooked burgers.

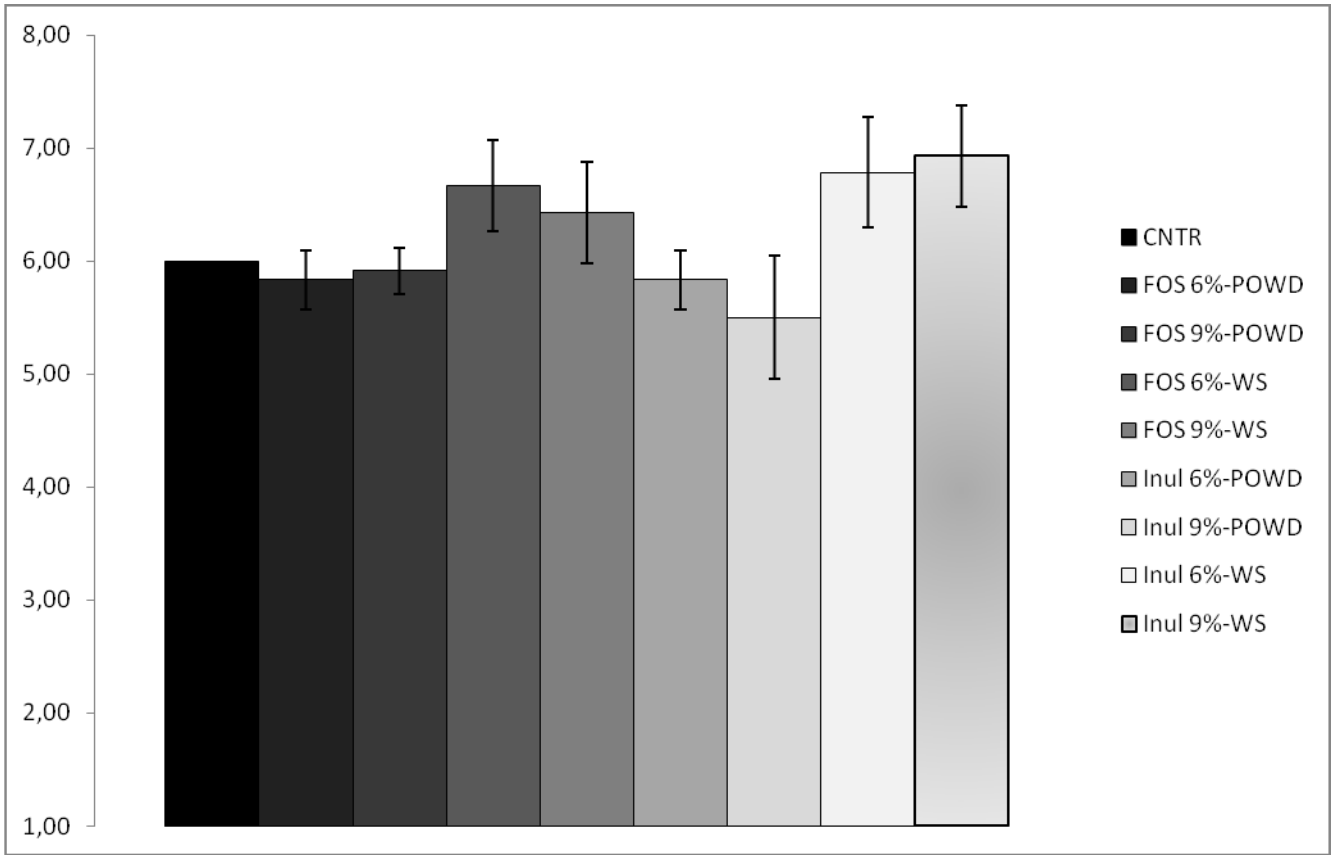


Figure 5.6 Overall quality of cooked burgers.

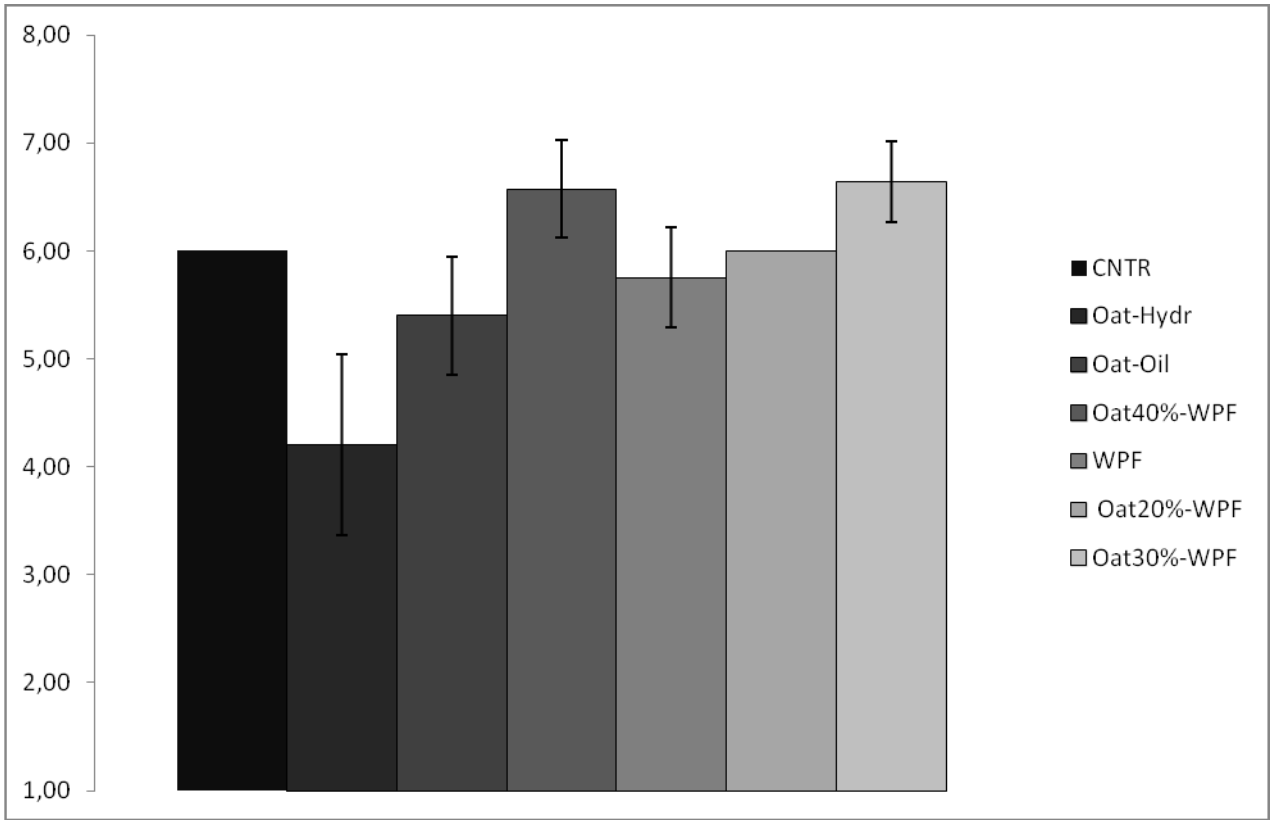
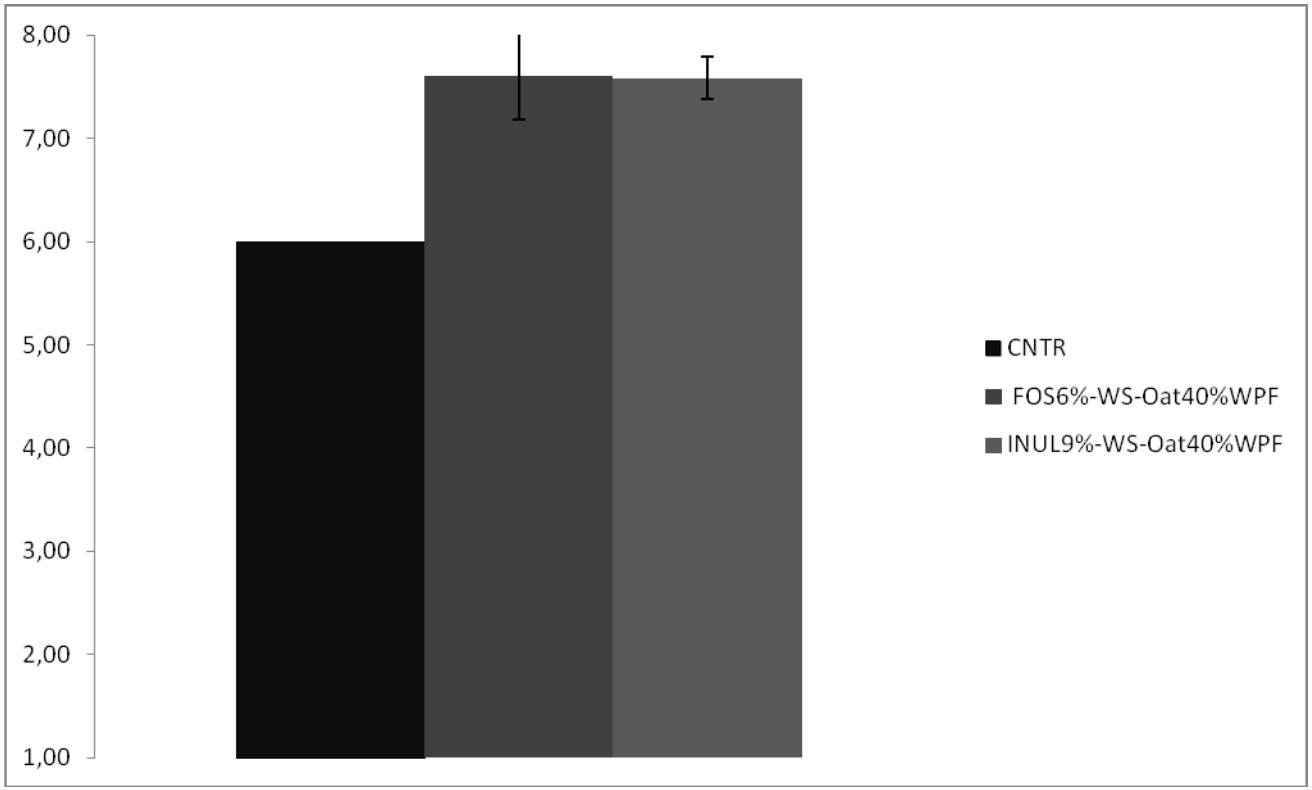


Figure 5.7 Overall quality of cooked burgers.



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