Master-Essay:

Galacto-Oligosaccharides, Food Biotechnology & the EFSA

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Abstract

Functional foods are an emerging field in food biotechnology; amongst others, the food industries are highly interested in the field of probiotics and prebiotics. Such compounds preferably found in dairy products or fiber rich foods and many studies suggest and deal about their potential health beneficial aspects. The prebiotic galacto-oligosaccharides (GOS) gained more and more attention in the past years as they were found to resemble human milk oligosaccharide (HMO) and are already established to be beneficial in infant formula to mimic natural breast feeding. Current interest in GOS development is their authorization as health beneficial prebiotic beyond infant nutrition. Various studies have been conducted already that suggest the use of GOS when gastro-intestinal related problems occur. Out of many possible enzymes and processes to synthesize GOS, few companies worldwide established their production with fewer enzymes. Clasado Ltd. is one of these companies producing the GOS mixture Bimuno®. They are currently the only company, trying to receive the official authorization of a health beneficial prebiotic, that reduces bloating and intestinal pain collectively described as intestinal discomfort, by the European food safety authority (EFSA). This case shows the critical and crucial procedure of the EFSA in their approval of food related health claims. It provides further insight on expectations or complications for future applications on such food additives.

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1. Food Biotechnology & Functional Foods

Biotechnology is an innovative discipline that offers biological based concepts to and solutions in many business sectors. Different branches of biotechnology are commonly described by colors, each resembling the topic this branch is dealing with. The most prominent color is green, which represents agricultural biotechnology and is associated with genetic modified plants. The German website "biotechnologie.de" lists the three strongest branches separately in their annual surveys: health and medicine biotechnology (red), agricultural biotechnology (green) and industrial biotechnology (white). The fourth established color, marine biotechnology (blue), was not listed specifically. Also food biotechnology has not been listed as an own branch or color yet. However, green biotechnology can be counted as the predominant color for food biotechnology. Influenced by white biotechnology, the production and fermentation of food additives such as vitamins was made possible in a commercial way. Such advances broadened the opportunities for development of new food applications. (biotechnologie.de, 2013)

In the 1980´s "functional foods" were mentioned for the first time. Although this term is not universally accepted, it describes food that is part of the usual diet and comes with demonstrated physiological benefits besides providing basic nutrition (Sangwan, et al., 2011). Functional foods can contain various compounds intended to improve physical health: soluble fibers, omega 3-polyunsaturated fatty acids, anti-oxidants as well vitamins and minerals and more. Such food additives are mostly produced in biotechnological processes. Two prominent categories for functional foods are probiotics and prebiotics.

1.1. Probiotics

Probiotics are internationally described as live microorganism that my confer health benefits (Sanders, 2008; FAO/WHO; Araya, M.; Schlundt, J., 2001). This definition mostly contains suggestions of Roy Fuller who said that probiotics are a microbial live feed supplement that improves a host's intestine microbial balance. Older definitions only emphasized the role of probiotics as microorganisms that interact with the growth of another one by its secreted substances. Although dead microorganisms are found to lead to beneficial effects for the intestinal balance (Sanders, 2008), they are not considered as probiotics. Also isolated strains from the human commensal microbiota are not counted as probiotics.

Probiotic microorganisms are mainly consumed with fermented food, such as yoghurt or other dairy products. In that way, probiotics have to overcome the acid stomach in order to reach and function in the gut. However novel micro encapsulating techniques work as a vehicle for probiotics to ensure their survival through the stomach into the intestine (Islam, et al., 2010). Another problem for live probiotics and the probiotic containing products are environmental based. Especially dairy products come with a limited shelf live and require cold storing conditions to maintain the viability of its probiotics (FAO/WHO; Araya, M.; Schlundt, J., 2001).

The two most common probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* and are well researched and used in different therapeutic applications (Islam, et al., 2010). *Lactobacillus acidophilies* is a known microorganism living on humans. Strains that are used in yoghurt fermentation are considered as a probiotic (Ljungh, et al., 2006). Its probiotic benefit was observed as a significant reduction of *E.coli* in the intestine of cattle (Cull, et al., 2012). Another organism described for its supportive role for the commensal microbiota during antibiotic related diarrhea is *Saccharomyces boulardii* (Czerucka, et al., 2007).

1.2. Prebiotics

The definition for prebiotics resembles some characteristics of probiotics. The International Scientific Association of Probiotics and Prebiotics (ISAPP) states: "a dietary prebiotic as a selectively fermented ingredient that results in specific changes in the composition and/or activity of gastrointestinal microbiota, thus conferring benefit(s) upon host health" (6^{th} Meeting of the ISAPP, London, Ontario, Canada via (Roberfroid, et al., 2010))

Oligosaccharides and dietary fibers, mostly found vegetables or milk, are counted as prebiotics, whereas oligosaccharides are the best known ones and among dietary fibers not all are fibers are considered to be prebiotics (Slavin, 2013). Having a closer look at the requirements for prebiotics, the following characteristics for such food ingredients must be given: "(1) resists the gastric acidity, hydrolysis by mammalian enzymes and absorption in the upper gastrointestinal tract; (2) is fermented by the intestinal microflora; (3) selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being. (Slavin, 2013; Gibson, et al., 1995). While probiotics have a drawback in incorporation in products due to the requirement of being live microorganisms, prebiotic

ingredients do not have this limitation and are applicable in more various food products, however.

Consisting of single fructose molecules, the polysaccharide inulin from plants is well studied soluble dietary fiber and prebiotic compound (slavin 2009). Chicory represents the industrial relevant plant that is used for the extraction of fructan molecule chains: inulin (Roberfroid 2007 (Macfarlane, et al., 2006)). In a study on diabetes, Inulin showed a tendency on an improved overall controllability of patient s' diabetes situation. Although these data were obtained by blood samples only, an alternation and connection to intestinal microflora was discussed and a comparison between inulin and oligosaccharides pointed out. (Gargari, et al., 2013)

Besides polysaccharides also oligosaccharides can be prebiotic food ingredients. Fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are the most prominent members in this category and both are known to stimulate the gut microbiota. While FOS can be obtained e.g. from inulin degradation, GOS are found in milk of e.g. cows and humans and are of high interest for the dairy industry.

In this essay, the situation of prebiotics for food biotechnology is going to be analyzed. As an example we will focus on galacto-oligosaccharides. After a closer look at GOS itself and its current state of production, the focus will move towards the very well analyzed health benefits of these prebiotics and their viability and application in infant nutrition. Due to many and suggested health benefits parallel to heath claims promoted by the food industry, in a subsequent chapter the finding of the European Food Safety Authority on this topic are going to be discussed.

2. GOS: Galacto-oligosaccharides

In the field of prebiotics, oligosaccharides consisting of glucose and galactose, galacto-oligosaccharides, received more and more interest from research and industry over the last years. GOS prebiotics are recognized for their breadth of clinical evidence in supporting digestive and immune health (Sangwan, et al., 2011). Galacto-oligosaccharides were defined as "a mixture of those substances produced from lactose, comprising between 2 and 8 saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose and disaccharides comprising 2 units of galactose" (Tzortzis and Vulevic 2009).

The linkages between the single saccharides glucose and galactose are formed via beta glycosidic bonds (Torres, et al., 2010). Depending on the enzyme source, these linkages between two saccharides can vary in their position: in oligosaccharides the formation β1→2, β1→4 and β1→6 are the most common glycosidic bonds and are also found in commercial products. Furthermore the type of linkage seems to affect the GOS ability to be digested (Torres, et al., 2010; Hernández-Hernández, et al., 2012). All commercially available GOS are mixtures produced by different microorganism and enzymes. These GOS come with different glycosidic bonds and number of saccharide units, a summarized description of physicochemical properties was made:

Table 1: General physicochemical properties of GOS (Torres, et al., 2010)

Characteristic	Property
Solubility	Water-soluble, about 80% (w/w)
Appearance	Translucent/colorless
Viscosity	Similar to that of high-fructose syrup
Heat stability	Stable to 160°C for 10min at pH7; stable to 100°C for 10min at pH2; stable at 37°C at pH2 for several months
Freezing point	Reduces the freezing point of foods
Humectant properties	High moisture retaining capacity preventing excessive drying
Sweetness	Typically 0.3 to 0.6 times that of sucrose

Another valuable source of prebiotic oligosaccharides is milk. In Human milk oligosaccharides (HMO) make around 1% (w/v) of the content and compared to other domestic animals milk it is a factor of 100-1000 higher. HMO are galactose-based oligosaccharides that are of higher complexity than previously described GOS. They consist of whole lactose molecules with a repetitive core of galacotose and N-acetlyglucoseamine units. Furthermore α-glycosidic linkages of fucose or sialic acid are possible (Boehm, et al., 2007). Although HMO is of a higher complexity, its structural similarity to GOS stimulated

commercial interest in GOS as they are already in use in infant formula products. As for HMO, several publications describe health benefits for GOS, e.g. an increased number of bifidobacteria or a decreased number of pathogenic microorganisms (Sangwan, et al., 2011).

The current status of GOS as a food additive has been recognized as follows: in the USA their status is generally recognized as safe (GRAS) which allows their use on the market; Japan is one step further, with GOS accepted as food for specific health use (FOSHU); the EU categorized GOS as a non-Novel Food, which allows incorporation without special declaration and a customer safe acknowledged state, due to fact that GOS are already known since many years.

2.1. Production

Galacto-oligosaccharides production at industrial and commercial scale is done by only a few companies worldwide. The eight major producers of GOS are located in the UK (Clasado Ltd.; First Milk ingredients), Ireland (Dairygold Food Ingredients), The Netherlands (Friesland Foods Domo), USA (GTC Nutrition), and Japan (Yakult Honsha, Nissin Sugar Manufacturing Company, Snow Brand Milk Products). These companies offer GOS mixtures of individual composition based on the production process. Their products, carrying names like Cup-Oligo or Vivinal GOS, are enzymatically produced by β-galactosidase of *Cryptococcus laurentii, Aspergillus oryzae, Streptococcus thermophilus, Bacillus circulans or Bifidobacterium bifidum* origin. (Torres, et al., 2010; Sangwan, et al., 2011)

Although major GOS production is based on enzymes from a few organisms, the actual variety of β-galactosidases used in research is much broader. This enzyme´s actual catalytic reaction is the hydrolysis from lactose to glucose and galactose. However it is known since many years that this enzyme also performs a transglycosylation reaction and is able to synthesize GOS. But also other glycoside hydrolases came into focus for transglycosylating reactions. Therefore research on such enzymes and how to direct their transglycosylating abilities is not only of academic interest (Oliveira, et al., 2011).

In order to achieve sufficient GOS production yield with β-galactosidases, several factors have to be taken into account. As β-galactosidase is originally a hydrolase, the availability of water needs to be lowered, which favors the transglycosylation. One option to provide such conditions is a relatively high initial lactose concentration and reaction temperature, which also improves lactose solubility in water (Torres, et al., 2010). Another way is the use of reverse micelles that provide a micro environment where water concentration can be controlled. Compared to GOS yield with high initial lactose, similar

yields with lower initial lactose were achieved using reverse micelles (Chen, et al., 2003; Park, et al., 2010).

Another problem in GOS production is the self-inhibition of β-galactosidases by the by-product glucose. While GOS gets extended by the galactose molecule of lactose, the glucose is set free as a side product and causes inhibition of the enzyme activity. In a continuous process where side-products get filtered out of the reaction solution via cross-flow membrane ultracentrifugation, the enzyme inhibition problem could be addressed; this resulted in yield improvement as well (Czermak, et al., 2004). Generally a continuous process with constant composition of the substrate was found to improve GOS production. The inhibition problem can also be overcome via fermentative production. Here GOS are synthesized in cell suspensions where glucose and non-reacted substrates are metabolized by the microorganism. This co-metabolization, the fermentation of side products, was found to be an approach for a novel immobilization application where β-galactosidases got attached to cell surface of *S. cerevisiae*. (Li, et al., 2009). The fermentative purification of GOS mixtures with *S. cerevisiae* is already applied as a step in the production of GOS (Li, et al., 2008).

The previously described anchored β-galactosidases on *S. cerevisiae* represent one novel way of enzyme immobilization. However there are also other methods to prepare and immobilize these enzymes for re-useable GOS production. In the field of enzyme encapsulating, polyvinyl alcohol and sol-gel are known materials. However, polyvinyl alcohol found to be more efficient and reusable than sol-gel applications for β-galactosidases from *A. oryzae* (Park, et al., 2010).

Altogether these methods provide a valuable strategy to find optimal GOS production. Further process improvement can be achieved by selecting enzymes of microbial or fungi origin that favors cost efficient synthesis conditions and the desired GOS product. Many enzymes and applications were studied over the years. One system that showed solid characteristics was immobilized β-galactosidase from *Talaromyces thermophiles*. Working in an ultrafiltration micro-reactor at rather low 40 °C close to neutral pH, a yield of 50% GOS was the result. Besides the high yield, a productivity of 70 g per liter and hour by the use of a 20% (w/v) lactose solution was reported (Nakkharat, et al., 2006)). This study showed promising GOS production capabilities by the use of rather minimal resources compared with other systems (Park, et al., 2010; Torres, et al., 2010). However, enzyme engineering represents another powerful tool for further improvement of GOS synthesis.

As GOS are produced from lactose, milk represents the most available resource for their industrial synthesis. However, recent advances in GOS production set focus on the reusability of whey, the by-product of the cheese making process. Using these actual waste streams for

the GOS producing industry opens up the opportunity for its revaluation. (Jovanovic-Malinovska, et al., 2012). However, also soya beans are mentioned to be a resource for GOS production (Sangwan, et al., 2011), which seems to be interesting for e.g. Asian countries without a strong dairy industry compared to Europe.

2.2. Second Generation GOS

The first generation of prebiotics was considered to be more of fiber like properties, fibers obtained from plants such as inulin. Their functionality was rather low explored, like their bifidogenic stimulating effects and interaction with the bowel. The second generation of prebiotics however is known to have further interaction with the gut: a metabolic, immunology or disease beneficial orientation. (Ouwehand, et al., 2005). In a meeting of the ISAPP, this next generation of prebiotics was seen in the variety of oligosaccharides and other complex carbohydrates (International Scientific Association for Probiotics, 2002).

Such oligosaccharides or complex carbohydrates are existent in a broad variety in Japan. GOS are part of this diverse group, well known and showed to have beneficial effects in the gut. They belong to the group of second generation prebiotic, however a second generation GOS is not described yet. All advances on GOS synthesis in the last years did lead to constant improving of GOS itself in matter of purity, complexity and formulation. Still, a clear classification of different generations of GOS was not found, but it can be assumed that we have surpassed the first generation of GOS some time ago. Also the reusability of waste streams, such as whey, is considered to be advance prebiotics further. Other improvements aim on the enzymatic catalysis itself via enzyme engineering. This will allow higher complexity and broader substrate acceptance for the final GOS product and it might also become useful for future applications in the field of prebiotics.

In the next chapter, the current state of GOS and their use as prebiotic and health beneficial food application is highlighted. The focus will lie on Bimuno, a commercial GOS mixture that is promoted as a second generation prebiotic. But also the role of GOS in infant nutrition will be discussed there.

3. GOS in Health and Nutrition

Galacto-oligosaccharides experienced high interest as a valuable food additive over the last years. Many studies were conducted to research their effects as a deictic compound. GOS are prebiotics and therefore interact with the gastrointestinal microflora. One summarized description for a healthy gut and its microflora was recently published in Nature: "In the healthy state, [microbial communities] contribute nutrients and energy to the host via the fermentation of nondigestible dietary components in the large intestine, and a balance is maintained with the host's metabolism and immune system. Negative consequences, however, can include acting as sources of inflammation and infection, involvement in gastrointestinal diseases, and possible contributions to diabetes mellitus and obesity." (Flint, et al., 2012). Furthermore this publication pointed out the opportunity given by someone's diet or consumed food to influence actively the own gut microflora.

Among the more often found and described health beneficial effects due to GOS consumption are decreased pathogen adhesion in the gut, increased mineral uptake and decreased duration or severity of diarrhea. Other health beneficial effects that are found to stand in connection with GOS or other oligosaccharide prebiotics are on irritable bowel disease, inflammatory bowel disease or even cancer. However these connections are based on very few publications or have only been reported in animal studies yet. These findings are assumed to be caused by the selective fermentation of GOS by the intestinal microbial community into short chain fatty acids (SCFA): an interesting and diverse group of microbial metabolites with various functions. Further in depth research in this specific area is of high interest. (Macfarlane, et al., 2006; Roberfroid, et al., 2010)

Another more specific understanding of healthy or balanced microbiota considers the presence of bifidobacteria and lactobacilli as a significant factor; their saccharolytic capabilities allow SCFA synthesis and their species are not pathogen. Due to observation in high numbers of bacteria of these genera in feces, the consumption of GOS was found to have a simulative and selective function for these bacteria (Macfarlane, et al., 2006; Sangwan, et al., 2011).

With a growing number of possible applications of GOS in the health sector, industrial interest and commercialization increases as well. Although GOS mixtures are available on the market and GOS are already widely used in infant formulation they are not officially assessed as a health beneficial food compound in Europe. In the following two chapters, two current situations of GOS are going to be discussed – GOS resembles HMO as a deictic fiber in infant

nutrition and Bimuno as an example of a GOS mixture waiting for its health beneficial authorization from the European Food Safety Authority (EFSA).

3.1. GOS in Infant Nutrition

In the first months or years of infants, breast feeding with human breast milk is known to play an important role in growing up. Breast milk is found to be also a factor in the colonization process of gut microbiota and is reported to stimulate significantly the growth of bifidobacteria and leads to a more complex microflora compared to non-breast fed children (Buccigrossi, et al., 2013). The health beneficial effects are found to be mediated by HMO that are highly present in breast milk. As described previously, GOS resembles the structure of HMO in a close but less complex way (Barile, et al., 2013). These findings highlight the use of GOS in infant nutrition. It is one step closer in mimicking breast milk in formulations. The German company "Milupa" presents their development in infant nutrition from the beginnings towards recent products containing GOS in an online available brochure; this development was supported by scientific studies and public response in newspapers in the end (milupa GmbH).

Despite the HMO resembling characteristics of GOS, several other advantages of this type of prebiotics have been studied in infants. As allergies are always considered to be aware of in children, the effect of GOS supplemented nutrition has been found beneficial for this application. In a study where breast fed infants got in addition a prebiotic mixture containing GOS showed no change in relevant immune globulin A production in the subject´s feces, a positive difference was found however for non-breast fed infants. Such a promising prebiotic effect stimulates further research on GOS and their interaction with the immune system. Other evidence for the support of GOS in infant nutrition is connected with gastroenteritis and acute diarrhea. In a solid study, reduction of such incidences was found to be lower in subjects that were fed with a GOS mixture compared to standard formula. However these results are regarded critically due the open design of the study. In a not directly disease linked area, GOS was found to act as a mediator for mineral uptake, e.g. calcium. Calcium is an elemental and important mineral for the metabolism in humans e.g. bones. Although data and studies on prebiotic mediated calcium uptake in infants are still low, its opens up another interesting field of research on GOS, as bones require calcium for their growth and maintenance (Roberfroid, et al., 2010; Macfarlane, et al., 2006; Bruzzese, et al., 2006).

The current Directive of European Union on infant nutrition states that galacto-oligosaccharides are allowed to be supplemented in a combination with fructo-

oligosaccharides. The recommended ration is 9:1 (GOS : FOS) with a limit of 0.8g/100ml per preparation (Kommission der Europäischen Gemeinschaften, 2006), as there are many published scientific studies available. However health claims of GOS in infant nutrition are still not authorized (The Panel on Dietetic Producs, Nutrition and Allergies, 2009).

3.2. Bimuno® GOS effects on Health

Bimuno® is a commercial available mixture of GOS produced by Clasado Ltd. in the UK for the application as a second generation prebiotic food additive for adults. This mixture shows common described properties, like low sweetness low calorie stable under different conditions and not digested by the mouth microbiota. Its composition in dry matter is 50% galacto-oligosaccharides of a degree of polymerization (DP) form 2-5 monosaccharides, whereas the fraction size decreases with increasing DP (52%, 26%, 14% and 8%). The remaining 50% dry matter contains monosaccharides (15.3%) lactose (28%) as well as minerals, proteins and fats. The production of Bimuno® from lactose is performed by the *Bifidobacterium bifidum* strain, NCIMB 41171 isolated from the feces of a healthy human volunteer. Currently, Clasado Ltd. is trying to get the official authorization for their product as a health beneficial prebiotics from the EFSA. (Tzortzis, 2011; EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011; EFSA Panel on Dietetic Products, Nutrition and Allergies, 2013; Tzortzis, et al., 2005)

The health beneficial observations of Bimuno® are based on a range of different *in vitro* and *in vivo* studies in animals and humans. In first studies, the bifidobacterial stimulating effects of Bimuno have been found to be existent. These studies are based on fermentation experiments with conditions that resemble those in the colon, and a trial in pigs, an increased number of bifidobacteria was noted. Also studies in human demonstrated bifidobacterial benefits. A volunteer and elderly trial gave these hints when GOS were consumed. In the volunteer trial, a GOS mixture produced by *Bacillus circulans* ATCC 4516 based enzyme was compared and found to be less efficient than Bimuno® from *B. bifidum*. Both studies are based on a double blind cross-over and placebo controlled study design, which generally can be accepted as a suitable method to investigate the actual effect of the test compound. Further comments in these studies were that the enzyme origin reflected the higher efficiency of Bimuno® over the *B. circulans* product and that elderly people who consumed Bimuno® found to have intestinal microflora that resemble those of young people. (Depeint, et al., 2008)

Gastrointestinal disorders, such as inflammatory bowel disease (IBS) that follows gastroenteritis, are known to be induced by abnormal fermentations of the gut microbiota. In another study with Bimuno®, a single blinded, placebo-controlled, parallel crossover designed trial, aimed to investigate its effects on IBS. The findings stated positive effects, e.g. regaining a similar microflora as healthy human, for the selected subject with their suffering of IBS. Furthermore subjects that received Bimuno® in a lower dose even reported an improved overall comfort feeling. Positive observations of this study, namely concerning abdominal pain/discomfort, bloating/distension, and bowel movement difficulty, are part of Clasado´s application for the authority of Bimuno® related health claim by the EFSA. (Silk, et al., 2009)

Since Bimuno® is found to exert positive effects on the intestinal microflora, further interest of immunomodulatory properties is existent. In cell culture experiments, a decreased inflammatory response, based on several measured cellular responses such as NFκB, was observed when Bimuno® was present in the treatment. Also in the previous mentioned study of Bimuno® in elderly people. There an anti-inflammatory cytokine pattern was measured: an increase in interleukin 10 and a decrease in pro-inflammatory factors such as TNFα. A correlation between natural killer cell activity, phagocytosis and number of bifidobacteria in the subject strengthened the positive connection between Bimuno® and the subject's immune response. Other positive health benefits go towards the protection from pathogens. In trials with mice and parallel in cell culture, the added pathogen *Salmonella enterica serovar* Typhimurium was less able to attach when additional Bimuno® was added to the culture or fed in mice. The significant results led to the suggestion of a human trial where Bimuno® was used on subjects travelling to developing countries where bacterial infections resulting in travelers' diarrhea are potentially occurring. The results of this study where positive, a significant lower number of incidence where observed in subjects travelling with the prebiotic. (Tzortzis, 2011; Vulevic, et al., 2008)

All in all, Bimuno® has shown to exhibit health beneficial potential. It suggests working positively in the gastro intestinal area with effects on several fields. Bimuno® is a well-defined and commercial available mixture of GOS; Clasado´s interest in further research, proof and authorization of the found health benefits is still going on. Since a few years, the company applies for the EFSA´s official approval of so far reported health claims associated with Bimuno®. Very recently the EFSA rejected second try of Bimuno® acceptance as health beneficial food product for adults. In the following chapter, this case between the EFSA and Clasado is going to be discussed. The focus will lie on the claimed health benefits of the prebiotic and the reason for their rejection.

4. The EFSA: Health Claims in Food

The European Food and Safety Authority (EFSA) is an institution of the European Union dealing with all kind of questions, issues and problems related with food and food products. In order to protect European customer's health and advice the European Commission in this field, the EFSA conducts own non-experimental based research or asks for expertise to validate innocuousness of e.g. food additives for their application on the market. In the growing field of prebiotics as a health beneficial and economically interesting food additive, the EFSA conducts its own surveys. Although many studies suggest certain benefits of prebiotic supplemented diets, "further research is required" or similar conclusive remarks are very often found in such published studies. The food industry aims to offer their products along with different health beneficial effects, but often the actual proof is low, insufficient or does not exist at all. In such cases, the EFSA´s interest of customer protection lies in the approval or rejection of such health claims by conducting research using its own expertise.

4.1. Functioning of the EFSA

The responsibility in the field of functional foods lies by the EFSA´s panel on Dietetic Products, Nutrition and Allergies (NDA). In this field, the guideline EC No. 1924/2006 on nutrition and health claims made on foods represents the most relevant document established and used by the NDA panel on the assessments for pro- and prebiotics as well as for any other claims on food products (European Food Safety Authority, 2006). This guideline provides the applicant with all relevant information needed when a request on a product is going to be submitted. For example, a health claim is defined as: "any message or representation, which is not mandatory under Community or national legislation, including pictorial, graphic or symbolic representation, in any form, which states, suggests or implies that a food has particular characteristics that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health" (Article 2(2.1+5)). More precise regulations are made in other Articles: the intended amount of food that needs to be consumed for achieving profit by the health claim (Article 5(1d)) and other statements do not allow a health claim that comes from disadvantages by not consuming the promoted food product (Article 12). But also considerable obvious statements about health claims are made in this guideline; beverages of 1.2% (v/v) alcohol cannot hold health claims (Article 4(3)).

In article 6 of the panel demands scientific evidence on each health or nutrition that is made by the applicant or other evidence or date for its justification. However, this article remains vague in details; there is no statement the amount and kind of data and scientific proof that is required for the validation of a health claim. In this consensus it remains the applicant's task to provide the EFSA with sufficient scientific material for their products approval. (European Food Safety Authority, 2006)

4.2. Evaluation a GOS Health Claim: Bimuno®

Although the topic of GOS as a health beneficial food additive is still emerging, the EFSA already proved its relevance as an important and a to be convinced institution for the food industry. In the case of 3000 health claims in all fields that were reported and commented by the EFSA, only 20% were approved as providing sufficient scientific proof for their claims, but none of these were dealing with probiotics. The responses to this high number of rejections were mostly negative (Katan, 2012). Further support for claim-rejection by Katan argues the price margins between food with and without claimed health benefits. The high prices for health beneficial food are in contrast to the actual invest for development, compared to regular food. Another valid point: pharmacy companies have to overcome high hurdles to get permission for their health beneficial products for the market. However, similar requirements are not existent for health claims in food products, but should be taken into consideration. This discussion supports the EFSA´s demand for sufficiently well carried out studies and research to proof a health claim in food (Katan, 2012).

In the application process of Bimuno® Article 13, namely "Health claims other than those referring to the reduction of disease risk and to children's development and health", is the most referred one in the official published opinions ending evaluation processes. Essentially, this article deals with the scientifically correctness and customer understandable declaration of food related health claims. Health claims in this article deal with growth, development and other body functions, as there are stated psychological and behavioral functions. Also weight controlling, hunger sensing and other effects that affect a diet are considered in this article. However, the health claim of Clasado´s application is described as "reduced bloating and intestinal pain that can be described collectively as intestinal discomfort" is not listed. Therefore, such a claim needs to be approved and authorized according to Article 13 in this case. (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011) (European Food Safety Authoritiy, 2012) (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2013)

The case for Clasado´s product authorization consists of two applications and one comment towards the decision of the first application at present. On the other side, the EFSA answered the process by publishing two scientific opinions and a technical report in form of a response towards the comments on the first scientific opinion. The whole application can be followed via EFSA´s official publications on the case. The main focus will be on the two mentioned published opinions, whereas the second opinion mostly repeats itself due to no addition or providing new trials or research on the product.

The evaluation process by the EFSA is based on a literature search that was provided by the applicant in order to support the acceptance of their product. The results were a few numbers of trials in human and animals. More precisely: eight human intervention studies, two human observational studies and three non-human studies. In both opinions, an unpublished study, performed by the applicant and another study published by Silk et al. (Silk, et al., 2009) are considered to be of higher relevance in the case. Other provided scientific proof for the health claim is based on research about other GOS mixtures or animal and in vitro studies. These publications got rejected in the opinion with only few sentences by stating, that a bifidogenic effect does not provide sufficient evidence to conclude a health beneficial effect of Bimuno®. This decision is very reasonable, as the company applies for a health claim in humans and the *in vitro* and animal studies only provide a positive effect on bifidobacteria. Any prediction on the effectiveness is hard to make. The same counts for studies on GOS that are not Bimuno®. Although these studies' results lead to a better understanding in the interaction of GOS in human, still the tested substance was not the product that the company wants an approval for. There is no guarantee that the observed effects will be the same when Bimuno® is applied. However, in the same category there is also a study with Bimuno® tested on human, but still does not support the application towards the desired outcome. There, Bimuno® was tested as a mixture preventing traveler diarrhea (Drakoularakou, et al., 2010). Indeed this study shows positive effect of the product in this frame of research, but it reflects the functionality of Bimuno® in this context only – as a product that seems to help travelers during their vacation. Also it is based on one single study with only a low number of participants. They were travelling to different countries around the globe, where there are different local conditions to face. This can be argued as a reasonable point that lowers the studies´ overall level of confidence after all, e.g. in statistical matters. In the later applications, these publications are listed only and the EFSA´s panel refers to the previous opinion. This can be seen as a re-statement of the low significance of these studies for the authorization of Bimuno®.

A more detailed discussion is done on two publications during the first application process. The studies, conducted by Clasado itself and Silk et al., performed as a human

intervention trial on subjects suffering from either gastrointestinal discomfort with a probability towards functional bowel disease or subjects with irritable bowel syndrome (IBS). Although the EFSA was provided with preliminary information and a statistical analysis for the first application in 2011, the study performed by Clasado on gastrointestinal discomfort still remains unpublished in July 2013. Here the panel criticizes the short duration of 2 weeks over the recommended 4 weeks, as it was further discussed in the response to the EFSA´s scientific opinion for the first application. Also the statistical analysis was a negative point on this study and even considered to be inappropriate for the study design – a randomized, double blind cross-over and placebo controlled study. Providing new data and a re-evaluation by the applicant were not able to change the outcome. All together and the fact that this study is still not published might give a supportive indication for eventual rejection on the case by the EFSA in all their publications.

The other important study in this case was performed by Silk et al. and was designed in the same way like the previous one by Clasado itself, but it was an interventional one where subjects were diagnosed with IBS. Also here the panel criticized several points in the study that lead to not convincing conclusion for the case. The dropout rate lowered the trial population to a low number, only 44 people in a range between 18 and 80 were assessed with the prebiotic/placebo from original 60. Although cross over was performed in the study, the panel pointed out the difference according to regular cross-over studies. The study was designed that each group starts with the placebo in the first period of treatment and only in the second period either Bimuno® or placebo were applied. Original cross over includes also treatment in reverse order or more stages of treatment. However, this way of treatment was reasonable argued by the intention to avoid any carry on effects, when the first stage of treatment would have had contained the prebiotic already. Further observations due to proper cross over would provide more substance to the found results on the other hand. The statistical data analysis was found to be insufficient in this study as well; the panel was not able to consider this publication for the health claim. Even additional data that were requested were unable to support this study for a successful application in the second try. The author states, that this study was the first of its kind for use of prebiotic in the treatment of IBS, he also points out that further research is required to confirm the first successful, yet by the EFSA unflavored, results in this field.

The case between the EFSA and Clasado´s prebiotic Bimuno® reveals the current situation on health claim evaluation in the European Union. A low number of official scientific studies on the beneficial functionality of well characterized GOS mixture - Bimuno® - stand in opposite of the strict regularities of the EFSA. The outcome, considering this matter is not surprising after all. On the one hand, Clasado enters the application process

with a few actual studies on their product, mostly first trials on a possible application of their GOS product in a health beneficial way. On the other hand there is the EFSA working according to their regulation established their NDA panel. However, just these regulations remain vague in the demanding kind of scientific proof that it needs for a successful evaluation of a prebiotic health claim. The EFSA´s intention is to evaluate health claims on individual scientific evidence, although both opinions and the response on the case picturing a tendency towards EFSA´s expectation of sufficient scientific evidence:

- Human trials, with or without interventional character
- Aim of the study should include/address the health claim
- Randomized, cross over double blinded and placebo controlled study design
- High statistical analysis, e.g. intention to treat analysis
- Consideration of the dropped out patients in further detail

Furthermore both evaluations provide further expertise for both sides and third parties. The EFSA build up knowledge on GOS prebiotics for upcoming cases also submitted by other producers. Clasado Ltd. and any other companies are able to consider these cases for their study design on similar trial and eliminate methodological errors. This should allow a successful submitting and authorization of GOS-related health beneficial claim on food products – which will come.

5. Conclusive Opinion

"It is still early days for prebiotics, but evidence increasingly suggests that they offer the potential to modify the gut microbial balance in such a way as to bring diect health benefits cheaply and safely" (Macfarlane, et al., 2006).

In this essay I discussed the current situation of galacto-oligosaccharides development as prebiotic and its potential use as a health beneficial food additive. On the one side there are advancing production methods, providing improved ways for synthesizing GOS mixtures with different enzymes and conditions and on the other side there are increasing numbers of possible health beneficial applications of them. Although many publications and research are available, the detailed understanding and proof is still needed to be researched. From my point of view while dealing with this topic, many trials on the health beneficial effects are rather well performed proofs of concept that support the positive picture of GOS as a functional food only. The variety of available mixtures due to source of the enzyme, production process or substrate and studies on different types of GOS all together strengthen this picture. However long term or multiple studies on large number of subjects with the same health benefits is still waiting to be conducted or published, especially considering the case of Clasado´s Bimuno®. Their health claim´s authorization application was basically based on only few studies, which were also different in their set up. In my opinion, the EFSA´s rejection was very reasonable due to that and my knowledge.

In pharmaceutical research where real drugs are under development for actual health improvement, large studies have to be performed in order to get an official approval for the market. Positive Animal and *in vitro* studies for example allows a pharmacy company to conduct actual clinical trials in human. In the last phase of clinical trials, a number of at least 1000 subjects have been tested with the drug. Every time one and the same test substance before it can be released, hopefully. But GOS are no drugs, yet they want to claim the same benefits without going the long way through numerous studies and trials. GOS occur naturally mostly in milk, modern biotechnology allows their industrial synthesis and availability. No doubt, their background, e.g. part of breast milk, and current research put GOS into the right direction and indicate possible fields of application beyond their nutritional value. The commercial success of such health beneficial GOS enhanced food products might be foreseeable already. In my opinion it is simply more convenient to incorporate a GOS-yoghurt into your daily diet than taking pills to improve your general well-being. And here begins the responsibility of the EFSA by examining such processes, health claims or issues on the food

market. They have to make sure that such claims are based on sufficient evidence in order to protect the customer who might not have the knowledge and/or time to develop its own opinion on this topic.

Also, generally speaking, nutrition is a highly diverse topic. To my experience as an international student, the way we eat differs tremendously around the globe. Also education and social status influences our diet and eventually health. I think this is an affecting factor in pro- and prebiotic research besides age. However, it was not clear to me to what extent this is considered in clinical trials or is of actual significance in the end.

Another very prominent example how health beneficial food claims interact on the market shows the case of Actimel that was discussed extensively in the press. This probiotic drink containing a proclaimed beneficial *Lactobacillus* that strengthens the immune system. Numerous commercials on TV, which addressed children as well as adults, aimed to establish the health beneficial perception of Actimel. However, the health claims eventually had to be retracted due to insufficient scientific evidence. From a commercial point of view, Actimel was a success. An annual report of Danone states sales of around 1 billion euros for Actimel in 2006 (while being on the market for more than 10 years at that time, (media.corporate-ir.net, 2006)) – the year when the EFSA´s NDA panel set up their regulations on health claims on food. Anyways, customers paid increased prices for something that turned out to be not true.

I think it needs this kind of general awareness to ensure the prevention of any potential misleading proclamation based on insufficient data at the expense of the customers – especially for things that regards and affects all of us on daily base: food and nutrition.

Literature

Barile, Daniela and Rastall, Robert A. 2013. Human milk and related oligosaccharides as prebiotics. *Current Opinion in Biotechnology.* 2013.

biotechnologie.de. 2013. Die deutsche Biotechnologie-Branche 2013. *biotechnologie.de*. [Online] 2013. [Cited: July 21, 2013.] http://www.biotechnologie.de/BIO/Redaktion/PDF/de/umfrage/2013-umfrage,property=pdf,bereich=bio,sprache=de,rwb=true.pdf.

Boehm, Günther and Stahl, Bernd. 2007. Oligosaccharides from Milk. *The Journal of Nutrition.* 2007.

Bruzzese, E., et al. 2006. Impact of prebiotics on human health. *Digestive and Liver Disease*. 2006.

Buccigrossi, V, Nicastro, E und Guarino, A. 2013. Functions of intestinal microflora in children. *Current Opinion in Gastroenterology.* 2013.

Chen, C. Will, Ou-Yang, Chao-Chih and Yeh, Chih-Wei. 2003. Synthesis of galactooligosaccharides and transgalactosylation modeling in reverse micelles. *Enzyme and Microbial Technology*. 2003.

Cull, C. A., et al. 2012. Efficacy of a vaccine and a direct-fed microbial against fecal shedding of Escherichia coli O157:H7 in a randomized pen-level field trial of commercial feedlot cattle. *Vaccine.* 2012.

Czermak, P, et al. 2004. Membrane-assisted enzymatic production of galactosyl-oligosaccharides from lactose in a continuous process. *Journal of Membrane Science*. 2004.

Czerucka, D., Piche, T. and Rampal, P. 2007. Review article: yeast as probiotics - Saccharomyces boulardii. *Alimentary Pharmacology & Therapeutics.* 2007.

Drakoularakou, A, et al. 2010. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers´diarrhoea. *European Journal of Clinical Nutrition.* 2010.

EFSA Panel on Dietetic Products, Nutrition and Allergies. 2013. Scientific Opinion on the substantiation of a health claim related to Bimuno GOS and reducing gastro-intestinal discomfort. *EFSA Journal.* 2013.

EFSA Panel on Dietetic Products, Nutrition and Allergies. 2011. Scientific Opinion on the substantiation of a health claim related to Bimuno GOS and reducing gastro-intestinal discomfort. *EFSA Journal.* 2011.

European Food Safety Authoritiy. 2012. Response to comments on the Scientific Opinion on the substantiation of a health claim related to Bimuno GOS and the reducing gastro-intestinal discomfort. *Supporting Publications.* 2012.

European Food Safety Authority. 2006. Regulation (EC) No 1924/2006 on nutrition and health claims made on foods. *Regulation of the European Parliament and of the Council.* 20. December 2006.

FAO/WHO; Araya, M.; Schlundt, J. 2001. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. *Joint FAO/WHO Expert Consultation.* 2001.

Flint, H J, et al. 2012. The role of the gut microbiota in nutrition and health. *Nature Reviews Gatroenterology & Hepatology*. 2012.

Gargari, Bahram Pourghassem, Dehghan, Parvin and Jafar-abadi, Mohammad Asghari. 2013. Effects of High Performance Inulin Supplementation on Glycemic Control and Antioxidant Status in Women with Type 2 Diabetes. *Diabetes & Metabolism Journal.* 2013.

Gibson, G. R. and Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition.* 1995.

Hernández-Hernández, O., et al. 2012. Monomer and linkage type of galacto-oligosaccharides affect their resistance to ileal digestion and prebiotic properties in rats. *The Journal of Nutrition.* 2012.

International Scientific Association for Probiotics. 2002. Report from the Second Generation Prebiotic Working Group. *www.iaspp.net.* [Online] 2002. [Cited: July 17, 2013.] http://www.isapp.net/docs/2nd_gen_prebiotics_mscrpt.pdf.

Islam, M. A., et al. 2010. Microencapsulation of live probiotic bacteria. *Journal of Microbiology and Biotechnology.* 2010.

Jovanovic-Malinovska, Ruzica, et al. 2012. Galacto-oligosaccharides Synthesis from Lactose and Whey by ß-Galactosidase Immobilized in PVA. *Applied Biochemistry and Biotechnology.* 2012.

Katan, M. B. 2012. Why the European Food Safety Authority was right to reject health claims for probiotics. *Beneficial Microbes.* 2012.

Kommission der Europäischen Gemeinschaften. 2006. Richtlinie 2006/141/EG der Komission über Säuglingsanfangsnahrung und Folgenahrung und der Änderung der Richtlinie 1999/21/EG. *Europäische Behörde für Lebensmittelsicherheit.* s.l. : Amtsblatt der Europäischen Union, December 22, 2006.

Li, Y, et al. 2009. Cell surface engineering of a beta-galactosidase for galactooligosaccharide synthesis. *Applied Environmental Microbiology.* 2009.

Li, Z Y, et al. 2008. Production of non-monosaccharide and high-puritiy galactooligosaccharides by immobilized enzyme catalysis and fermentation with immobilized yeast cells. *Process Biochemistry.* 2008.

Ljungh, A. and Wadström, T. 2006. Lactic acid bacteria as probiotics. *Current Issues Intestinal Microbiology.* 2006.

Lozupone, Catherine A., et al. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature.* 2012.

Macfarlane, S., Macfarlane, G. T. and Cummings, J. H. 2006. Review article: prebiotics in the gastrointestinal tract. *Alimentary Pharmacology & Therapeutics.* 2006.

media.corporate-ir.net. 2006. Danone Annual Report 2006. *media.corporate-ir.net.* [Online] 2006. [Zitat vom: 25. July 2013.] http://media.corporate-ir.net/media_files/irol/95/95168/annualreports/AnnualReport_2006.pdf.

milupa GmbH. milupa Mediathek: Broschüren. *www.milupa-gmbh.de.* [Online] [Cited: July 27, 2013.] http://www.milupa-gmbh.de/mg/media/pdf/milupa_forschungsbroschuere.pdf.

Nakkharat, P. and Haltrich, D. 2006. Lactose hydrolysis and formation of galacotooligosachharides by a novel immobilized beta-galactosidase from the thermophilic fungus Talaromyces thermophilus. *Applied Biochemistry and Biotechnology.* 2006.

Oliveira, Carla, Guimaraes, Pedro M. R. and Domingues, Lucilia. 2011. Recombinant microbial systems for improved beta-galactosidase production and biotechnological applications. *Biotechnology Advances.* 2011.

Ouwehand, Arthur C., et al. 2005. Prebiotics and other microbial substrates for gut functionality. *Current Opinion in Biotechnology.* 2005.

Park, Ah-Reum and Oh, Doek-Kun. 2010. Galacto-oligosaccharides production using microbial ß-galactosidase: current state and perspectives. *Applied Microbilogy and Biotechnology.* 2010.

Roberfroid, M., et al. 2010. Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition.* 2010.

Sanders, Mary Ellen. 2008. Probiotics: Definition, Sources, Selection, and Uses. *Clinical Infectious Diseases.* 2008.

Sangwan, Vikas, et al. 2011. Galactooligosaccharides: Novel Compounds of Designer Food. *Journal of Food Science.* 2011.

Silk, D B.A., et al. 2009. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics.* 2009.

Slavin, Joanne. 2013. Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients.* 2013.

The Panel on Dietetic Producs, Nutrition and Allergies. 2009. Scientific substantiation of a healthclaim related to "Follow-on formulae with fixed combination of short-chain galacto-oligosaccharides (GOS),acidified milk, nucleotides and beta-palmitate" and intestinal ailments. *The EFSA Journal.* 2009.

Torres, Duarte P.M., et al. 2010. Galacto-Oligosaccharides: Production, Properties, Application and Significance as Prebiotics. *Comprehensive Reviews in Food Science and Food Safety.* 2010.

Tzortzis, George. 2011. Development and Evaluation Bimuno, a Novel Second-Generation Prebiotic Galactooligosaccharide Mixture. [Buchverf.] Teri M Paeschke und William R Aimutis. *Nondigestible Carbohydrates and Digestive Health.* s.l. : Blackwell Publishing Ltd. and Institute of Food Technologies, 2011.

Tzortzis, George, Goulas, Athanasios K. and Gibson, Glenn R. 2005. Synthesis of prebiotic galactooligosaccharide using whole cells of novel strain Bifidobacterium NCIMB 41171. *Applied Microbial Biotechnology.* 2005.