Lactic acid bacteria fermentations in oat-based suspensions

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LACTIC ACID BACTERIA FERMENTATIONS IN OAT-BASED SUSPENSIONS

OLOF MÅRTENSSON

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Abstract

This thesis deals with the fermentation characteristics of lactic acid bacteria (LAB) in oat-based suspensions, with formulation work of fermented products based on oat and with nutritional studies of these products. Changes in structure in terms of viscosity and ropiness were studied when exopolysaccharide (EPS)-producing LAB strains, namely, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772, *Lactobacillus brevis* G-77 and *Pediococcus damnosus* 2.6 were grown in these oat-based suspensions. Differences in structure were seen after growth of EPS-producing bacteria, resulting in higher viscosity and ropiness in these products. The formulation work of fermented products was carried out by using commercial yoghurt cultures with and without the presence of an EPS-producing strain. The products developed were found to have sensory acceptance and good bacterial survival during storage. The bacterial survival of three probiotic strains, *Lactobacillus reuteri* ATCC 55730, *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20456, was also studied in different oat-based suspensions with and without the influence of a yoghurt culture during 30 days of storage. The highest survival was seen for the *Lactobacillus reuteri* strain. The co-fermentation in the presence of a yoghurt culture gave a lower pH in the final products and decreased the bacterial survival for the three strains.

Nutritional effects of fermented, oat-based products were investigated by using germfree and conventional rats and by performing a clinical study on 56 healthy volunteers. Physiological parameters such as changes in serum cholesterol, microbial conversion of cholesterol to coprostanol, and amount of faecal short chain fatty acids (SCFA) were studied. In addition, changes in the faecal flora were investigated. No changes in serum cholesterol levels or faecal excretion of cholesterol were seen in the rat models when the animals were fed on different, fermented oat-based products. The oat-based products gave a faecal SCFA pattern in conventional rats that differed from the group fed on rice. A decrease in plasma cholesterol was seen in the human subjects eating a fermented, ropy, oat-based product for five weeks, which gave a dietary intake of 3.5 g native β-glucans from oat per day. An increase in total bacterial count and *Bifidobacterium* ssp. was also seen in faecal samples from this group.

In conclusion, there is a potential for the development of fermented, non-dairy, oat- based products with a "ropy" structure containing soluble fibres of both native and microbial origin and with the lipid lowering effect that is generally associated with an intake of oat.

Key words: lactic acid bacteria, oats, exopolysaccharides, β-glucans, non-dairy, viscosity, ropy, ropiness, Adavena, probiotic bacteria, bacterial survival, cholesterol, SCFA, prebiotic

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"…between thought and expression
lies a lifetime…"
L R

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LIST OF PAPERS

This thesis is based on the following Papers, referred to the text by their respective Roman numerals (**I-VIII**). The papers are attached as appendixes at the end of the thesis.

Paper I: Lactic acid bacteria in an oat-based non-dairy milk substitute: Fermentation characteristics and exopolysaccharide formation.
Olof Mårtensson, Rickard Öste and Olle Holst.
Food Science and Technology/LWT (2000) 33, 525-530.

Paper II: Texture promoting capacity and EPS formation by lactic acid bacteria in three different oat-based non-dairy media.
Olof Mårtensson, Rickard Öste and Olle Holst.
European Food Research and Technology (2002) 214, 232-236.

Paper III: Comparison of growth characteristics and EPS formation of two lactic acid bacteria strains, *Pediococcus damnosus* 2.6 and *Lactobacillus brevis* G-77 in an oat-based, non-dairy, medium.
Olof Mårtensson, Maite Dueñas-Chasco, Ana Irastorza, Rickard Öste and Olle Holst.
(Submitted).

Paper IV: Formulation of a fermented product from oats and its comparison to yoghurt.
Olof Mårtensson, Carina Andersson, Kenneth Andersson, Rickard Öste and Olle Holst.
Journal of the Science of Food and Agriculture (2001) 81, 1413-1421.

Paper V: A fermented, ropy, non-dairy oat product based on the exopolysaccharide-producing strain *Pediococcus damnosus*.
Olof Mårtensson, Johan Staaf, Maite Dueñas-Chasco, Ana Irastorza, Rickard Öste and Olle Holst.
Advances in Food Sciences (2002) 24, 4-11.

Paper VI: The effect of yoghurt culture on the survival of probiotic bacteria in oat-based, non-dairy products.
Olof Mårtensson, Rickard Öste and Olle Holst.
Food Research International (2002) (in Press).

Paper VII: Effect of fermented, ropy, non-dairy, oat-based products on serum lipids and the faecal excretion of cholesterol and short chain fatty acids in germfree and conventional rats.
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(Submitted).

Paper VIII: Changes in plasma lipids and faecal *Bifidobacterium* spp. in humans after consumption of fermented, non-dairy, oat-based products for 5 weeks.
Gunilla Önning, **Olof Mårtensson**, Maria Biörklund, Adele Mbou Lambo, Maite Dueñas-Chasco, Ana Irastorza, Olle Holst, Elisabeth Norin, Gjalt Welling, Björn Åkesson and Rickard Öste.
(Manuscript).

CONTRIBUTION TO THE PAPERS

Paper I: The present author (O. Mårtensson) did the experimental work, took part in the evaluation of the results and wrote the manuscript.

Paper II: The present author (O. Mårtensson) did the experimental work, took part in the evaluation of the results and wrote the manuscript.

Paper III: The present author (O. Mårtensson) did part of the fermentations and the structural analyses, took part in the evaluation of the results and wrote the manuscript.

Paper IV: The present author (O. Mårtensson) devised part of the formulation strategy and the sensory analysis of the products, did the ropiness and colour measurements, took part in the evaluation of the results and wrote the manuscript.

Paper V: The present author (O. Mårtensson) did part of the fermentations and the structural analyses, devised part of the sensory analysis, took part in the evaluation of the results and wrote the manuscript.

Paper VI: The present author (O. Mårtensson) did part of the fermentations and the microbial enumeration of the different strains, took part in the evaluation of the results and wrote the manuscript.

Paper VII: The present author (O. Mårtensson) did the fermentations of the diets, took part in the termination of the animals, took part in the evaluation of the results and wrote the manuscript.

Paper VIII: The present author (O. Mårtensson) designed the formulations of the products, did part of the fermentations of the products, prepared the faecal samples for the FISH-analysis, took part in the evaluation of the results and wrote parts of the manuscript.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Mjölk anses som det enda flytande livsmedlet som på naturlig väg innehåller de tre fundamentala byggstenarna, fett, protein och kolhydrater, vilket definierar ett livsmedel näringsmässigt. Majoriteten av världens vuxna befolkning är dock tvungen att ha en begränsad konsumtion av mjölk då toleransen för dess kolhydrat, laktos, minskar med vuxen ålder. Detta beror främst på att aktiviteten av ett protein, enzymet β-galaktosidas, minskar och gör att en stor del av laktosmängden undergår en bakteriell nedbrytning, fermentering, i tjocktarmen. Detta kan leda till besvär i mag-tarm regionen i form av diarré, gasbildning och magknip. I Sverige är laktosintoleransen relativt låg, ca 3-4%, i Finland ca 15% medan den är ca 30% av den vuxna befolkningen i Storbritannien och ända upp till 100% i vissa delar av Asien. Således finns det alltså ett generellt behov av näringsmässigt goda alternativ till mjölk som bl.a. är fria från laktos. I Asien, och då främst Kina och Japan, har man länge använt soja som råvara för att på ett traditionellt sätt framställa vattenextraherad ”bönmjölk”. I början 1960-talet kom de första rapporterna om mer teknikbaserade processer att framställa en sojabaserad, mjölkliknande produkt. Soja kan av klimattekniska skäl inte odlas med god kvalitet på de skandinaviska breddgraderna. Istället är de stora grödorna på dessa breddgrader framförallt stråsäd i form av vete, korn och havre. Idén att göra ett skandinaviskt alternativ till mjölk, en ”cerealiebaserad mjölk”, föddes i slutet av 1980-talet. Detta genom att ett flertal rapporter som publicerades på 1970-80-talen i USA beskrev hur marknaden ökade för sojabaserade produkter. Havre valdes som den mest lämpliga cerealien för detta ändamål. Detta var till stor del baserat på den sensoriska aspekten, det näringsmässiga förhållandet mellan fett, protein och kolhydrater samt den relativt stora fraktionen av vattenlösliga fibrer, β-glukaner. Mycket forskning har fokuserats på just β-glukaner sedan början 1990-talet. Det är främst kring deras blodfettsänkande effekt som den nutritionella forskningssatsningen har gjorts. Höga blodfetter ökar risken för hjärtkärlsjukdomar som har blivit något av västvärldens folksjukdom. Ett godkännande från Food and Drug Administration (”FDA”)

i USA publicerades 1997 baserad på en vetenskaplig konsensus om att ett visst dagligt intag av just β-glukaner från havre sänker blodfettsnivån hos människa.

Under mitten av 1990-talet producerade den första havremjölken i Sverige. För att kunna erbjuda olika typer av mjölkfria mejeriliknande produkter baserade på havre är steget att använda mjölksyrabakterier i produktutvecklingsarbetet naturligt. Att studera hur mjölksyrabakterier växer, överlever och hur de ger både sensoriska samt strukturella effekter i havrebaser ger viktig kunskap i en långsiktig utveckling av fermenterade, mjölkfria, havrebaserade, flytande livsmedel.

Mjölksyrabakterier har spelat en allmän stor roll i utvecklandet av livsmedelsprodukter, då människan sedan urminnes tider har ”processat” livsmedel för att öka dess hållbarhet. Denna hantering har skapat produkter såsom surdeg, surkål och filmjölk. Ur en mejeriteknisk synvinkel har användandet av starterkulturer, d.v.s. en eller flera definierade bakterier som används för att skapa karaktäristika till en definierad kulturprodukt, s.k. fermenterad produkt, ökat markant. Idag finns det olika starterkulturer av mjölksyrabakterier för att skapa en karaktäristisk smak, arom och/eller struktur till olika typer av fermenterade produkter. Den karaktäristiska struktureffekt som vissa mjölksyrabakterier ger en del fermenterade produkter beror till stor del av deras förmåga att producera polysackarider, s.k. exopolysackarider (EPS). Forskningen kring detta område har fått ett ökat intresse, framförallt inom de livsmedels och näringsvetenskapliga disciplinerna. Främst p.g.a. dessa bildade polysackarider också kan tänkas bidra med en viss ökning av mängden lösliga fibrer i livsmedlet. Härtill hör även den ökande användningen av s.k. probiotiska bakterier som ofta är mjölksyrabakterier eller nära besläktade bakterier till dessa. De tillsätts eller används i olika fermenterad produkter för sina mer eller mindre dokumenterade fysiologiska effekt.

Avhandlingens innehåll

Forskningen som presenteras i denna avhandling är ett steg i ett långsiktigt mål att kunna erbjuda konsumenter fermenterade, mjölkfria och havrebaserade produkter, rika på fibrer av både nativt och mikrobiellt ursprung och med dokumenterade, fysiologiska effekter.
Arbetet som redovisas presenteras i 8 olika delarbeten (**artiklar I-VIII**). Dessa delarbeten visar resultat av studier som är gjorda i syfte att öka kunskapen kring utvecklandet av fermenterade, havrebaserade livsmedel och eventuella fysiologiska effekter av dessa. I **artikel I-III** är studier gjorda i avseende att undersöka hur olika mjölksyrabakterier, med

en potential att kunna användas i olika typer av havrebaserade livsmedel, växer i havrebaser som skiljer sig åt i avseende på deras kolhydratprofil och torrsubstanshalt. Fokusering gjordes på tre olika mjölksyrabakterier, *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772, *Lactobacillus brevis* G-77 och *Pediococcus damnosus* 2.6. Gemensamt för dessa tre bakterier är att de producerar exopolysackarider (EPS). Genom att mäta viskositeten i produkten före, under och efter tillväxt av respektive EPS-producerande bakterie ges en viss indikation på att EPS-bildning har skett. Ett annat sådant mått på strukturförändring i produkten är att mäta produktens ”trådighet”. Genom att använda båda dessa metoder kan information inhämtas om hur produktens struktur har förändrats efter det att en EPS-producerande bakterie har tillväxt och EPS har bildats. *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 tilläts växa i olika havrebaser med olika typer av kolhydratkällor. Strukturförändringar i havrebaserna studerades efter tillväxt i både renkultursystem och blandkultursystem där också icke EPS-producerande bakterier ingick (**artikel I** respektive **II**). I **artikel III** studerades denna strukturförändring under tillväxt av *Lactobacillus brevis* G-77 och *Pediococcus damnosus* 2.6. Det unika med dessa båda bakteriestammar är att de producerar en polysacckarid av β-glukan struktur. Genom att använda denna typ av mikroorganismer kan man skapa en framtida möjlighet att kunna erbjuda konsumenter fermenterade produkter som både innehåller nativa, d.v.s. β-glukaner från havre, och mikrobiellt bildade β-glukaner. I **artikel IV** gjordes formuleringsarbeten av en fermenterad produkt som till en viss del kan liknas vid yoghurt. I **artikel V** utvecklades en produkt baserad på en blandkultur, som förutom en traditionell yoghurtkultur även består av den EPS-producerande bakteriestammen *Pediococcus damnosus* 2.6. Detta gav en produkt med en karaktäristik struktur som närmast kan förknippas med den traditionella mejeriprodukten långfil. För att ytterligare undersöka de olika havrebasernas potential som substrat för mjölksyrabakterier gjordes en överlevnadsstudie med tre olika humanisolerad bakterier, *Lactobacillus reuteri*, *Lactobacillus acidophilus* och *Bifidobacterium bifidum* (**artikel VI**). Dessa humanisolerade bakterier kan förekomma som probiotiska bakterier i olika livsmedelssystem med krav på en viss mängd i den givna produkten, s.k. ”terapeutisk dos”. Därför är en god bakteriell överlevnad viktig under en viss given tidsram som kan liknas vid en tänkt, realistisk hållbarhetstid för en färdig produkt.

I **artikel VII** och **VIII** studerades den fysiologiska effekten av olika fermenterade, havrebaserade produkter. Utveckling av dessa baserades till stor del på den kunskap som

generats i **artiklarna I-VI**. Först gjordes en studie av olika fermenterad och ofermenterade produkter i två olika råttmodeller, en med ordinär tarmflora och en utan existerande tarmflora. Detta gav en första indikation på dessa produkters inverkan på fysiologiska parametrar såsom kortkedjiga fettsyror, som främst är produkter av bakteriell metabolism i tjocktarmen, och bakteriell nedbrytning/konvertering av kolesterol. En skillnad i fettsyramönstret kunde ses i de djur som åt havrebaserad diet i jämförelse mot de djur som åt risbaserad diet (**artikel VII**). I den andra fysiologiska studien studerades 56 personer, både män och kvinnor som under 5 veckor fick äta olika typer av fermenterade, havrebaserade produkter eller yoghurt (placebo produkt). Dessa havrebaserade produkter skiljdes åt i struktur, d.v.s. viskositet och ”trådighet” samt i halt av nativa, havrebaserade β-glukaner. En sänkning av blodkolesterolet kunde uppmätas för den grupp som åt en fermenterad, ”trådig”, havrebaserad produkt som gav ett intag av 3.5 g nativa β-glukaner per dag (**artikel VIII)**. I samma grupp sågs även en ökning av den fekala halten av bifidobakterier. Detta bakteriesläkte anses som en viktig byggsten i det som man allmänt kallar för ”den goda och hälsobefrämjande” tarmfloran.

INTRODUCTION

There is a general need for non-dairy products with an acceptable sensory quality and nutritive balance, as the majority of the world's population needs a restricted diet in dairy foods. The major raw material in the non-dairy field today is soy. In northern Europe, for instance Scandinavia, legumes, such as soy cannot be cultivated with a reasonable quality, whereas several cereals, such as wheat, barley and oats can. In addition, cereal-based foods are a major source of inexpensive, dietary energy nutrients worldwide (Salovaara, 1998). Oats, in particular have a great potential to be used as the raw material for different kinds of processed, non-dairy foods, including fermented products, as they are both a palatable cereal and generally have a good nutritional balance.

The use of lactic acid bacteria in fermented food and beverages has been practised for a long time, in fact the term lactic acid bacteria was used by early bacteriologists to describe the bacteria which grew spontaneously in milk. In the dairy field, starter cultures have been widely used, even before it was possible to grow all bacteria in pure cultures and before their taxonomy field was clarified (Hammes, 1991). The use of starter culture technology has expanded over the past decades and is not only concerned with the fact that the starter culture should contribute to the overall sensory characteristic of the product but that it should also improve the final consistency in a specific way, for instance by producing exopolysaccharides (EPS). Another example of this strategy is the use of high cell number inoculations of one or several specific bacterial strains of human origin that may improve gastrointestinal health, i.e., the use of probiotic bacteria.

Even if there have been reports concerning the development of fermented, cereal-based, non-dairy foods during the past decade (Molin *et al.*, 1993; Salovaara, 1996), the research input on lactic acid bacteria in this particular area, compared with the dairy field, must be regarded as limited.

1.1 The scope of the thesis

This thesis deals mainly with the long-term goal to elucidate possibilities for the development of fermented, non-dairy, oat-based products with different textural attributes arising from the *in situ* production of exopolysaccharides, and to investigate the nutritional potential of these products. These goals were sought by using the current knowledge on lactic acid bacteria in dairy applications and in non-dairy raw materials such as soy, and use these in different kinds of oat-based suspensions, derived from a unique process (Lindahl *et al*., 1997) with tailor-made carbohydrate profiles.

The scope of the thesis was two-fold: In the first step, fermentation studies were carried out to study the characteristics of potential lactic acid bacteria for use in the different oat-based suspensions. This was combined with studies on the structural effects in these oat-based suspensions of lactic acid bacteria that produce exopolysaccharides with different types of structure (**Papers I-III**). In parallel, formulation work for different, fermented, oat-based products was done (**Papers IV** and **V**) with the aim of using these products to study their nutritional effects *in vivo* (the second step). In addition, the possibility to use fermented, oat-based products as carrier for lactic acid bacteria with a pronounced health-promoting potential i.e. probiotics, was also investigated (**Paper VI**).

In the second step, the nutritional effects of different, fermented, oat-based products were investigated by using rat models (**Paper VII**) and by performing a clinical study on 56 healthy volunteers (**Paper VIII**). The main physiological parameters studied were changes in serum or plasma lipids, microbial conversion of cholesterol to coprostanol and amounts faecal SCFA. In addition, changes in the faecal microflora were studied in **Paper VIII**.

VEGETABLE ALTERNATIVES TO MILK

Milk can be defined as the only true "liquid" food in that it contains in a natural way all the macronutrients, viz., fat, protein and carbohydrates. The carbohydrate component in milk, lactose, is a disaccharide composed of galactose and glucose. Tolerance to lactose is defined as the ability to express an enzyme, β-galactosidase (lactase), that degrades lactose to galactose and glucose, which are absorbed mainly in the upper intestine of the gastrointestinal tract (Scrimshaw and Murray, 1988). It is, however, only during the infancy that tolerance to lactose is high. During the weaning period and in the course of childhood lactase activity decreases, i.e., a down regulation of the β-galactosidase activity occurs that may lead to an insufficient digestion of lactose. This phenomenon is called lactose maldigestion, which is defined as a small increase in blood glucose concentration (<1.12 mmol l^{-1}) or in breath hydrogen (>20 ppm) after ingestion of 50 g lactose (Bayless, 1981). Lactose maldigestion is considered as a normal (physiologic) situation in mammals and humans (Sieber *et al.*, 1997). The lactase activity can reach such a low level that it will have a major impact on the general tolerance of milk and milk-based products. The symptoms associated with lactose intolerance include bloating, belching, flatulence, cramping and watery stools (Lasser *et al.*, 1975; McBean and Miller, 1998; Vesa *et al.*, 1998). There is, however, a great variation of this down-regulation of the β-galactosidase in humans, which is mainly due to ethnic background. With the exception of the population of Northern and Central Europe and its offspring in America and Australia, 70-100% of adults worldwide are lactose intolerant (Bayless, 1981; Johnson, 1981; Dahlqvist and Semenza, 1985). Thus, there is a general need to increase the alternatives and substitutes for dairy milk.

Non-dairy suspensions, i.e., re-diluted particles in water to make up a homogeneous solution, can also be a true liquid food, if it contains fat, proteins and carbohydrates but is free from milk components. Four raw materials are used as the major components in the commercial production of milk alternatives or milk substitutes and related products

today; these are soya, almond, rice and oats. On a commercial level these products are often fortified with vitamins (A, B_2, B_{12} and D_2), minerals (Ca) and fat (vegetable oils) to meet the requirements of a milk substitute. They are also often sweetened and/or flavoured to attain a sensory acceptance and are frequently launched as "lactose-free", "cholesterol-free" and "gluten-free" products. A comparison of non-dairy products based on these above-mentioned raw materials with dairy milk and their nutritional balance in terms of the amount of the macronutrients as part of the total energy intake (%) is shown in **Figure 2.1**. The recommended value of each macronutrient in terms of total energy intake on a daily basis for humans over 3 years of age is 10% for protein, 30% for fat and 57% for carbohydrates (Nordiska näringsrekommendationer, 1996).

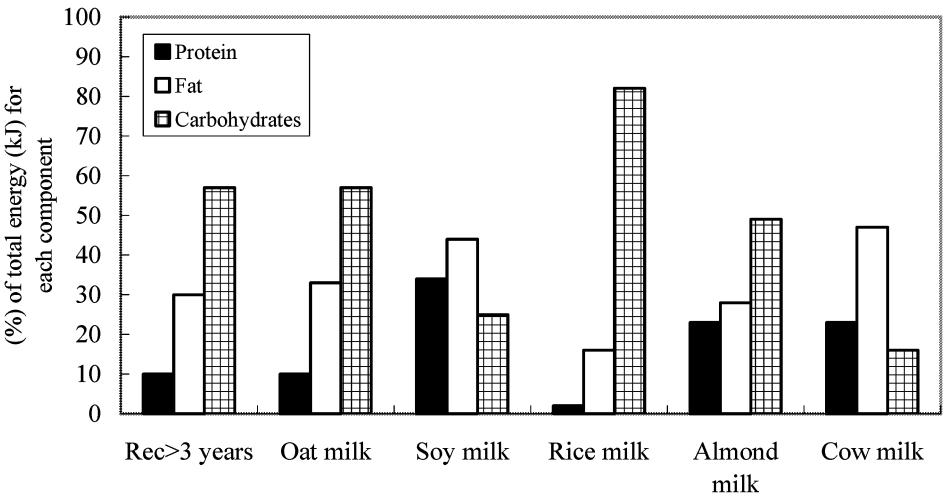


Figure 2.1 The nutritional balance in terms of protein (■), fat (□) and carbohydrates (⊞) in four different, commercial, non-dairy milk products based on oat, soy, rice and almond compared with cow milk (1.5% fat). All values are compared to the recommended intake (>3 years) (Nordiska näringsrekommendationer, 1996) of the above-mentioned macronutrients.

Hence, from the point of view of the balance of the macronutrients, oat milk seems to have a good potential to reach these recommended, nutritional values. Different varieties of oats, such as the high fatty oat type *Mathilda*, can be used in the production of oat milk to give a product with the same macronutrient balance but without any additional supplementation of oil (see **Chapter 4** for details).

OATS

The history of oats can be traced back to about 2000 B.C. The first evidence of oats was found in Egypt and among the lake dwellers of ancient Switzerland (Coffman, 1961). The main oat species include *Avena abyssinica*, *A. byzantina*, *A. fatua*, *A. nuda*, *A. sativa* and *A. strigosa*. The most commonly cultivated species in the world today are *A. sativa* and *A. nuda*, *A. sativa* making up more than 75% of the world's cultivars and is by far the most popular cultivated species (Coffman, 1961). Oats are grown as a multipurpose crop throughout the world from the 40th latitude in the southern hemisphere to the 60th latitude in the northern hemisphere, thus including countries such as New Zealand, Australia, China, North America, Argentina, Russia and related countries, and the countries of Scandinavia and Northern Europe (Schrickel, 1986). The world production of oats for the year 2000/2001 was in total about 25 million tonnes, grown over an area of 14 million hectares, most of which is used mainly for feed in animal production (Source of global oat information, 2002). Russia is the country that had by far the highest production of oats, followed by Canada, USA, Finland, and Sweden (**Figure 3.1**).

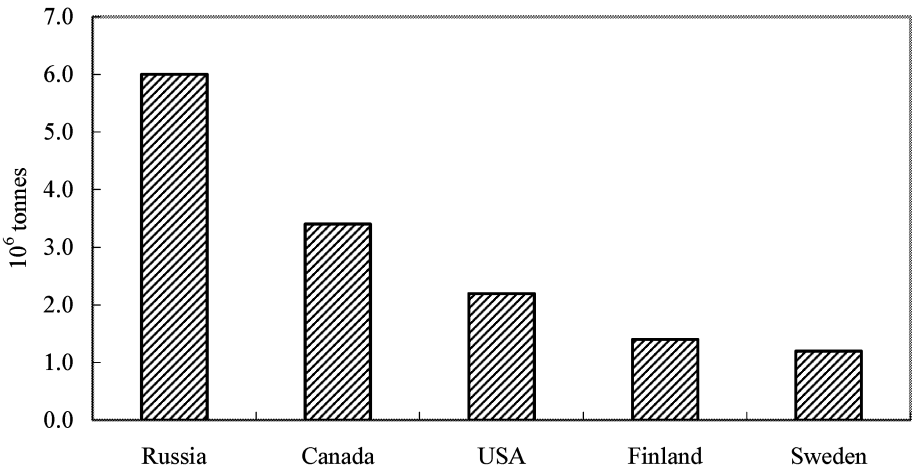


Figure 3.1 "Top five" of the world's greatest oat producing countries in million tonnes year 2000/2001 (Source of global oat information, 2002). (N.B. This figure reflects the production and not the consumption of oats in these countries)

The oat groat contains three major morphological and chemically distinct components, the bran, germ and the starchy endosperm. These are the traditional description of the commercial fractions and do not necessarily reflect the genetic, chemical or functional characteristics of the fraction (Fulcher, 1986). In the processing of oats for human consumption, the hulls are removed and the interior part of the groat is used as a whole grain. Oats contain the largest amount of protein in comparison with other cereal grains and have a carbohydrate fraction of high digestibility, except for the fibre fraction, which has other desirable, physiological properties (Lockhart and Hurt, 1986).

3.1 Nutritional value of oats

The nutritional composition of oats can be described as balanced. Oats contain a high percentage of protein with a good amino acid balance in contrast to other cereals. Oats also contain a high percentage of oat lipids that are highly unsaturated and contain substantial amounts of essential fatty acids. Oats are also a large source of a number of essential vitamins and minerals, viz., vitamin E and folic acid (Lockhart and Hurt, 1986). In contrast to other cereals, such as barley, wheat and rye, oats are now on the verge of being officially recognised as safe for individuals with gluten intolerance. This is due to the fact that oat proteins have a structure different from those in other cereals (Lockhart and Hurt, 1986).

3.1.1 β-glucan from oats and its physiological effects

The first attempt at isolating the non-starchy glucan from oats was made 1942 and showed it to be similar to lichenin from the lichen Iceland moss (Morris, 1942) that was later established by Acker et al. (1955a,b) and Peat et al. (1957). The non-starchy glucan was later known to be a (1 → 3) (1 → 4) β-D-glucan (Gagnair *et al*, 1975). This mixed-linked β-glucan from oat and barley belongs to the family of unbranched polysaccharides composed of glucopyranosyl units in varying proportions (**Figure 3.2**).

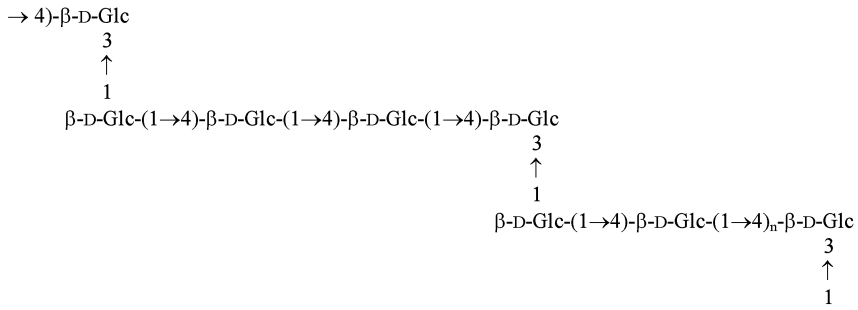


Figure 3.2 Main structural units of mixed-linked oat β-D-glucan, where n=1-20 units. However, although the tetrameric and pentameric units are repeated, their distribution along the polysaccharide chain is not known (Gagnair *et al.*, 1975).

The polysaccharide has been identified in a variety of tissues of the main commercial cereals (Preece and Hobkirk, 1953; Nevins *et al.*, 1978; Anderson and Stone, 1978) and rye grass (Smith and Stone, 1973), bamboo (Wilkie and Woo, 1976) and other grasses (Stinard and Nevins, 1980). It has also been suggested to be present in mung bean (Buchala and Franz, 1974) and in a number of lichens (Peat *et al.*, 1957; Takeda *et al.*, 1972; Nishikawa *et al.*, 1974). The main interest of the β-glucan from oats has been concentrated on the endospermic aleurone layer, oat β-glucan, which has the highest concentration of the β-glucan fractions known from oat, although non-endospermic β-glucan has also been studied (Wilkie and Woo, 1976; Wilkie, 1979).

The β-glucans from oat is known to actively lower the serum cholesterol levels in humans, thereby reducing the risk of coronary heart disease (Behall *et al.*, 1997), which is regarded as indisputable linked to high serum cholesterol levels (Boston and Cupples, 1996; Seman *et al.*, 1999). In January 1997, the USA Food and Drug Administration ("FDA"), allowed health claims to be made on products made from oats. It was the first time ever that such a food claim was permitted. The FDA stipulates that food with a certain nutritional balance, based on whole oats or oat bran, containing a minimum of 0.75 g of β-glucan fibre per serving (e.g. single serving of oatmeal) qualifies it for a direct heart disease relating label (FDA, 1996; 1997).

One of the first report concerning oats and its cholesterol lowering (hypocholesterolemic) effects in rats was published by DeGroot and co-workers (1963). These results have later been confirmed by other groups working with chicks (Fisher and Griminger, 1967) and rabbits (Hamilton and Carroll, 1976). Luyken et al.

(1965) was first to use human subjects to confirm the cholesterol-lowering effect of oats. Since then, many studies on humans have confirmed these preliminary data of the positive effects of oats on lipid metabolism (FDA, 1997). Even if much work have been focused on various biochemical aspects of lipid metabolism in general (Lairon, 1996) the biochemical and physiological effects of fibre-induced changes in cholesterol metabolism are still not fully understood. In the process to identify the mechanisms behind the lipid-lowering effects of β-glucans one can suggest a division of mechanisms into two major groups, the effect of β-glucan on the absorption of cholesterol and the effects of products derived from the microbial fermentation of β-glucans in the gastrointestinal tract. Viscous polysaccharides, such as β-glucans from oat have mechanistic effects that may be related to a lowering of both glucose and lipid absorption. The following have been suggested as possible mechanisms for the reduction of cholesterol by β-glucans from oat:

(i) *Cholesterol and bile acid excretion:* Studies have shown that the β-glucans can interact with and even bind to bile acids and/or other lipids and by this mechanism hinder the absorption and re-absorption of these products (Andersson and Bridges, 1986; Morgan *et al.*, 1993; Stedronsky, 1994). This mechanism will induce the synthesis of bile acids from cholesterol, whereby the blood cholesterol concentration is reduced. Subjects that have been given test meals containing oat bran have been shown to increase their excretion of bile acids, which supports this theory (Marlett *et al.*, 1994; Lia *et al.*, 1997).

(ii) *Rate of absorption:* Another mechanistic hypothesis is that β-glucans may increase the viscosity in the gastrointestinal tract and thereby reduce the lipid absorption (Trautwein *et al.*, 1998; Rieckhoff *et al.*, 1999; Gallaher *et al.*, 1999).

(iii) *Lipase activity:* There have also been suggestions that the β-glucans can interact with lipases and thereby depress their efficiency, leading to decreased lipid absorption. Increasing the dietary intake of fibre in rats resulted in an increased faecal excretion of fat (Isaksson *et al.*, 1983). Studies have also shown that dietary fibre can inhibit the intestinal lipolysis, albeit with a wide

variation, depending on the source of the fibre used (Hansen *et al*., 1987; Hendrick *et al*., 1992).

(iv) *Insulin secretion:* β-glucans from oats have also been shown to reduce insulin release after a meal. Insulin plays a role in lipid metabolism and may stimulate cholesterol synthesis and secretion of cholesterol ester complexes of cholesterol, such as very-low-density lipoproteins (VLDL) (Reaven and Bernstein, 1978). Soluble fibres, such as β-glucans reduce the rise of the glucose and insulin in blood (Jenkins *et al*., 1978, Jenkins, 1980; 1991) and the lower levels of insulin may lead to lower serum cholesterol levels. However to attain this effect, larger amounts are needed than for the lipid lowering effect.

The other major effect of β-glucans is mainly due to the production of short chain fatty acids (SCFA), which are produced solely from microbial fermentation in the large intestine (see **chapter 7**, **section 7.2.1** for details). The main SCFAs that are produced from microbial fermentation are acetic, butyric and propionic acid. These acids may modify cholesterol synthesis and thereby lower the serum cholesterol levels (Chen *et al*., 1984; Cummings, 1987; Bridges *et al*., 1992). It is suggested that the β-glucan can be fermented by the microflora in the large intestine and thereby increase the concentration of SCFA, which that may have an effect on the synthesis of cholesterol in the liver (Zhang *et al*., 1992; Berggren *et al*., 1996). The main SCFA contributor of this suggested effect is dedicated to propionic acid, which has been reported to inhibit cholesterol synthesis in rats (Wright *et al*., 1990) and to lower serum cholesterol levels in both rats and pigs (Topping, 1995). The main influence of propionic acid on lipid metabolism has been suggested to be by inhibiting the utilization of acetate for cholesterol synthesis (Wolever *et al*. 1991).

Allen (1913) was first to report on the beneficial effects of oats in the treatment of diabetes. Since then several studies have shown that soluble plant fibre, such as β-glucans from oats, effectively lowers the absorption of glucose and secretion of insulin after an oral glucose load, thereby giving a better physiological response to the food product. This response is estimated by the glycaemic index (GI). The glycaemic index (GI) concept was first initially proposed in 1981 (Jenkins *et al*., 1981). The GI value refers to the blood-glucose-raising potential of carbohydrate-foods (FAO/WHO,

1998). It has been shown that products with higher amounts of β-glucans had a higher viscosity in than control products, gave a more pronounced postprandial effect i.e., lower glycaemic index, on blood glucose and insulin levels. Thus the effect seems to be viscosity dependent (Jenkins *et al.*, 1997). A study with products containing a β-glucan concentration of 10% resulted in a 50% reduction in the postprandial glucose peak (Wursch and Pi-Sunyer, 1997). A summary of different studies on the relationship between the viscosity of oat β-glucans, their molecular mass and the effect on plasma glucose and insulin after an oral glucose load, showed that there was a positive, significant relation between the changes in blood glucose and the molecular weight of the β-glucans (Wood *et al.*, 2000).

3.2 The prebiotic concept

The microbial decomposition of dietary fibre in the colon leads to an increased stool volume, i.e., bulking, and an increase in the overall amount of bacteria. Due to the increased metabolic activity of these bacteria an increased formation of short chain fatty acids (SCFA) can be obtained (see **Chapter 7**, **section 7.2.1** for details). This elevated metabolic activity of the microflora can also lead to an increased formation of gases, which may cause flatulence. This may decrease with time as the subject becomes adapted to the fibre-rich diet (Schulze and Zunft, 1993). Based on the general criteria for dietary fibre, the prebiotic concept has been developed. This term prebiotic, was first introduced by Gibson and Roberfroid (1995), who defined prebiotics as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". This definition more or less overlaps or is very closely related to the latest suggested definition by Chung (2000). However the definition of dietary fibre encompasses a wider range of complex materials, which has been difficult to define and determine (Cummings and Roberfroid 1997). Nevertheless, soluble fibre, such as β-glucans from oats and several kinds of "limit"-dextrins can be included with these non-digestible carbohydrates.

In this concept a prebiotic, such as a dietary fibre, is fairly well defined dietary ingredient that reaches the large intestine (colon) intact and has a specific role in the metabolism there. This means that it is directed towards advantageous rather than adverse bacteria that can give rise to a selective fermentation in the larger intestine, so

called "prebiotic fermentation" (Gibson *et al*., 1999). This "prebiotic fermentation" should be directed towards potentially health-promoting bacteria, with indigenous lactobacilli and bifidobacteria currently being the preferred target organisms in the colon. This nutritional approach is similar to that suggested for dietary fibre, but with prebiotics this approach is more tailor made, which can lead to prebiotics that will also have the function of suppressing the numbers and activities of adverse organisms seen as pathogenic (Roberfroid, 1996). The main, prerequisite criteria for a food ingredient to be classified as a prebiotic have been suggested to include the following (Fooks *et al*., 1999):

(i) It must be neither hydrolysed, nor absorbed in the upper part of the gastro-intestinal tract.

(ii) It must be selectively fermented by potentially beneficial bacteria in the colon.

(iii) It must lead to an alteration in the composition of the colonic microbiota towards a healthier composition.

(iv) It must induce effects that are beneficial to the health of the host.

In general, not many dietary fibres have been thoroughly tested in humans on the basis of the existing criteria suggested for prebiotics.

THE ADAVENA OATBASE

4.1 Background and concept

To meet the increased demand from consumers for alternatives to milk, a foodbase (Adavena oatbase) derived entirely from oats and water via an enzymatic treatment was developed. The development was a joint collaboration at Lund University, Sweden, where research concerning the mechanisms behind lactose intolerance has been established since the 1960's. During the late 1980's, with support from Swedish grain producers a research team was formed. Its ultimate goal was to develop an oat-base, a non-dairy milk that apart from its function as a substitute or alternative to milk, would take advantage of the good nutritional and functional qualities of oats. Today this process of producing a foodbase entirely composed of oats and water by enzymatic treatments is patented (US patent 5,686,123) (Lindahl *et al.*, 1997). By using this oat-base there is a strong potential to develop and produce new foodstuffs with the same or better, nutritional composition and functional characteristics as dairy milk. One application of this unique process is a non-dairy milk with the main carbohydrate species being maltose and β-limit dextrins (**Table 4.1**). Clinical studies have also shown that products from this process have a lipid-lowering effect that is generally recognised in oats (Rytter *et al.*, 1996; Önning *et al.*, 1998, 1999).

The manufacturing process includes controlled conversion of the oat starch into a desired balance of different types of low molecular carbohydrates, mainly maltose or glucose, and dextrins. This makes it possible to tailor make properties such as sweetness and viscosity to suit different applications. The total fibre content and the balance between insoluble and soluble fibre can also be modified. Some of the fibre (50%) can be removed in the production process to obtain a more homogeneous and smooth, milk-like product. The fat content can be increased if required by adding vegetable oils, such as rapeseed oil or by using other oat variants, such as *Mathilda*. This oat type has a higher fat content than *Sang* and *Freja*, which are cultivated mainly for oat-based food products. Additions of vitamins and minerals are also possible during the manufacturing process.

The final product is either aseptically packed as Mill Milk™ or Oatly® (MM medium), which were used in **Paper I-II** and **VII**, spray dried as powder (M40 and G40 media) (**Paper II**, **III** and **VII**) or evaporated to a liquid base (M40 and G40 media) (**Paper IV-VI** and **VIII**).

Table 4.1 Chemical composition (%) based on dry matter, of two different oat-based suspensions, M40 medium (**Paper II**, **IV**, **VI** and **VIII**), and G40 medium (**Paper II**, **III**, **V-VIII**)

Component	M40 medium	G40 medium
Protein	12	12
Fat	8 (15)*	8
Glucose	2	44
Maltose	42	3
Carbohydrates	70	70
Total fibre	8	8
β-glucan	4	4

*Value in the commercial, aseptically packed product (MM-medium, that was used in **Paper I-II** and **VII**).

LACTIC ACID BACTERIA (LAB)

The term "lactic acid bacteria" (LAB) was coined already in 1919 as a concept to denote bacteria able to ferment and coagulate milk. The bacteria in this group are defined as those microorganisms, which produce lactic acid mainly from lactose (Orla-Jensen, 1919). As a result of these vague boundaries for this group, a more relevant description of LAB has been stated: Gram-positive, non-sporing, microaerophilic bacteria producing lactate as their main fermentation product from fermentable carbohydrates (Kandler, 1983). They have high acid tolerance and survive pH 5 and lower. This acid tolerance gives them a competitive advantage over other bacteria. Due to their lack of or limited ability to produce B-vitamins, nucleic acids and amino acids the nutrient requirements of LAB are somewhat complex. Most of them are generally regarded as safe ("GRAS"), except for some *Streptococcus* strains that are considered to be pathogenic. LAB commonly occurs in nutrient-rich environments, such as plant surfaces in plant decaying material, foodstuffs, such as milk, meat, and fish (Viniegra-González and Gómez, 1984). They are also inhabitants of the gastrointestinal tract of man and animals (Kandler and Weiss, 1986). LAB constitute a taxonomically changing group that today consists of: *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella* (Holzapfel and Wood, 1995; Stiles and Holzapfel, 1997). LAB are cocci, with the exceptions of *Lactobacillus* and *Carnobacterium* which are rods. Genera that resemble and which are phylogentically related to LAB are: *Areococcus*, *Bidfidobacterium*, *Brochotrix*, *Listeria*, *Staphylococcus* and *Sporalactobacteria* (Stiles and Holzapfel, 1997). The optimal temperature for growth of LAB varies from 20-30°C (*Leuconostoc*, *Pediococcus*), 30°C-37°C (*Streptococcus* and *Enterococcus*) to 25-45°C for *Lactobacillus* (Dicks *et al.*, 1995). Phylogenetically LAB can be divided into four super-clusters: (*Lactobacillus* (*Lb*) + *Pediococcus* (*Ped*));
(*Leuconostoc* (*Leu*) + *Weisella* (*Wei*) + *Oenococcus* (*Oen*));
(*Streptococcus* (*Str*) + *Lactococcus (Lc)* and

(*Enterococcus* (*Ent*) + *Tetracoccus* (*Tetr*) + *Carnobacterium* (*Carn*) + *Vagococcus* (*Vag*) (**Figure 5.1**).

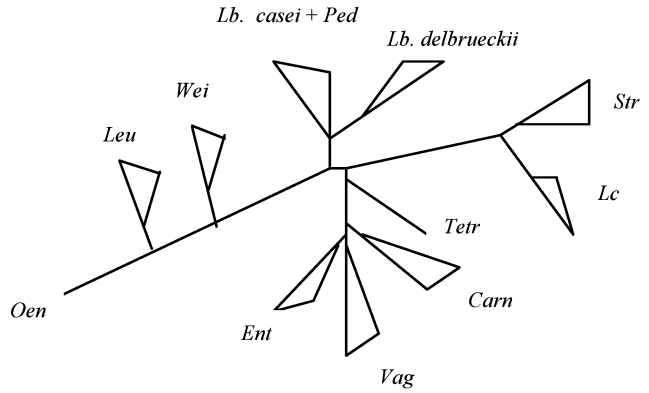


Figure 5.1 The phylogenetic tree of lactic acid bacteria (adapted from Stiles and Holzapfel, 1997).

The genus *Lactobacillus* is the largest with about 50 species (1993) (Hammes and Vogel, 1995). This group can either be subdivided into two groups, *Lb. delbrueckii* group and *Lb. casei* and *Ped.* group (Collins *et al.*, 1993) or into three groups based on fermentation type, as LAB in general ferment sugars via different pathways, resulting in homo-, hetero- or mixed acid fermentation (**Fig 5.2a-c**). Homofermentation (a) gives only lactic acid as the end product of glucose metabolism (Embden-Meyerhof-Parnas pathway (EMP)) (Thomas *et al*, 1979; Smith *et al.*, 1975). In heterofermentation (b) equimolar amounts of lactic acid, carbon dioxide and ethanol or acetic acid are formed from glucose via the phosphoketolase pathway (Axelsson, 1998). In the mixed acids fermentation (c) ethanol, acetic acid and formate can be formed in addition to lactic acid by homofermenting LAB under certain conditions, e.g., glucose limitation (Fordyce *et al.*, 1984).

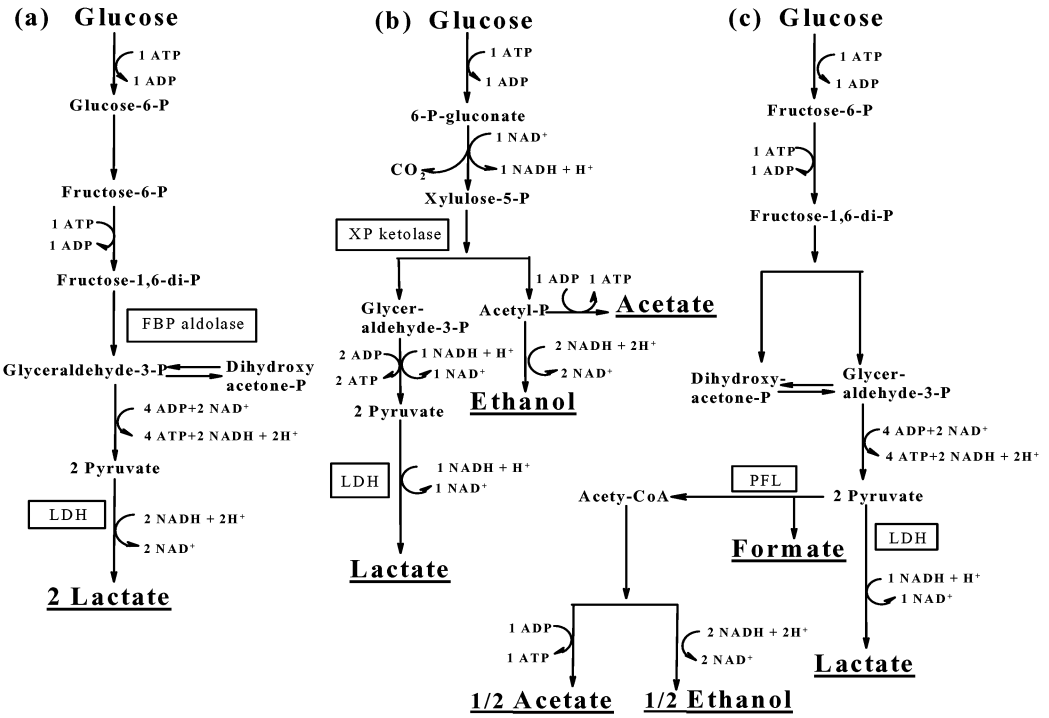


Figure 5.2 Major fermentation pathways of lactic acid bacteria. Homofermentation (Embden-Meyerhof) (a), heterofermentation (b) and mixed acid fermentation (c). *P*=phosphate, *BP*=biphosphate. Kew enzymes are marked as follows: *FBP*=fructose 1,6-biphosphate, *XP*= xylulose 5-phosphate, *PFL*=pyruvate fomate lyase, *LDH*=lactate dehydrogenase.

5.1 The use of lactic acid bacteria in fermented foods

Products fermented by lactic acid bacteria (LAB) have a long, culturally diverse history. The exact origin is difficult to establish, but they probably date back more than some 10.000 years to a time when populations evolved from food gatherers to food producers. Nevertheless, the natural fermentation process was crucial as a method to produce foods with an acceptable quality and taste.

Today, LAB are involved in the fermentation process of different kinds of food, such as dairy products, meat products, sour dough and other cereal products, wine, beer and various fermentable vegetables (Hammes, 1991). The fermentation process affects the food in a variety of ways, which are summarised in **Table 5.1**. The fermentation process can occur in two major ways, spontaneous or by using defined bacteria culture/cultures to obtain the desired property of the final food product.

Table 5.1 Effects of lactic acid bacteria (LAB) on the properties of fermented foods

Property	Metabolic reactions involved
Sensory (aroma and taste)	Formation and removal of volatile compounds. Fermentation of low molecular sugars with formation of weak acids (mainly lactic acid), turnover of acids and production of bitterness (peptides).
Texture	Softening (hydrolysis of polymers), formation of gas (CO_2, H_2). Thickening (production of polysaccharides).
Colour	Destruction of pigments (via formation of H_2O_2), formation of colour (formation of nitrosmyoglobin in meat products)
Shelf life	Reduction of pH by formation of acid, in combination with additional factors, e.g. reduced water activity and redox potential.
Safety	Removal of toxic compounds (e.g. from cassava, legumes and cabbage) prevention of the formation of nitrosamines, formation or removal of biogenic amines.
Nutritional value	Improved digestibility (destruction of trypsin inhibitors or phytine, hydrolysis of oligosaccharides (e.g. raffinose), formation of vitamins and the effects included in the "probiotic concept" (see **section 5.3.1**).

Adapted from Hammes (1991).

In the dairy industry LAB is the most frequent starter culture used today. The most common way to separate different starter cultures is by their optimal growth temperature where mesophilic microorganisms have an optimal growth temperature of <30ºC and thermophilic strains of >37ºC (Marshall, 1987). Since there has been a gradual evolution of locally based fermented milk products over the years, this has resulted in different products based on the local microflora and climate (Tamime and Robinson, 1988). In Scandinavia *Lactococcus* species were used for the production of

sour milk, whereas in the Middle East thermophilic strains were used, which gave rise to yoghurt products. Yoghurt, as a dairy product, is by far the most widespread fermented milk product globally. It also a highly defined product in most countries i.e. it is covered by a *Codex Alimentarius Standard* which stipulates dry matter, incubation temperature, starter culture, addition of ingredients etc. In traditional productions of yoghurt two thermophilic bacteria are used, namely, *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* subsp. *salivarius* (Mäyrä-Mäkinen and Bigret and, 1998). These two bacteria act symbiotically in such a way that the *Lb. delbrueckii* subsp. *bulgaricus* degrades the casein in milk to peptides, which promotes the growth of the *S. salivarius* subsp. *thermophilus*. This grows quickly at first and in return, produces lactic acid and lesser amounts of formic acid, reducing the pH to an optimal level for the growth of *Lb. delbrueckii* subsp. *bulgaricus* (Hamann and Marth, 1983). During growth important aromatic compounds are produced. Both of the strains produce diacetyl, whereas acetaldehyde is produced mainly by *S. salivarius* subsp. *thermophilus* (Keenan and Bills, 1968; Bottazzi and Vescovo, 1969). The aromatic compounds mentioned, the formation of acid, together with the strain production will give the unique characteristics of yoghurt in terms of flavour, texture and consistency (Marshall, 1987).

In addition to the above-mentioned, other bacteria, such as *Lb. acidophilus* and *Bifidobacterium* ssp., are commonly used in the production of yoghurt nowadays (Tamime and Robinson, 1999). Fermented, non-dairy products, which contain one or several LAB that also can be recognised in yoghurt products have been developed recently (Molin *et al*., 1993; Salovaara, 1996; Salovaara and Kurka, 1997; **Paper IV** and **V**). In this thesis a yoghurt culture (V2), which is often used in the production of commercial yoghurt products in Sweden, was used in the fermentation of different oat-based products (**Papers IV-VIII**).

5.2 Production of exopolysaccharides (EPS)

Like many other microorganisms LAB can also may produce polysaccharides which may be present that can either as a capsule, bound to the cell wall around the bacterial cell e.g. capsular polysaccharides (CPS) or secreted from the cell, or loosely attached to the cell as slime, such as exopolysaccharides (EPS) (Cerning, 1990; Cerning, 1995). Cell wall polysaccharides (WPS) are another type of bacterial polysaccharides, which are associated with the cell envelope, which is not secreted into the medium

(Delcour *et al.*, 1999). The functions suggested of microbial polysaccharides are (Cerning, 1995):

(i) To be an energy reserve

(ii) To provide protection against phagocytosis or phage attack.

(iii) To give adhesion to solid surfaces in liquid environments.

(iv) To provide protection against drying by binding water.

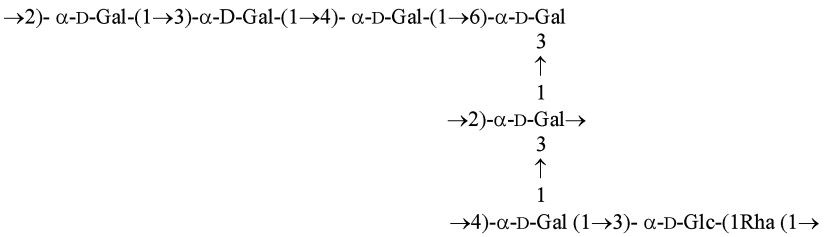
(v) To be an agent in the interactions with plants and other organisms.

(vi) To increase adsorption of nutrients and metal ions (Sutherland, 1972).

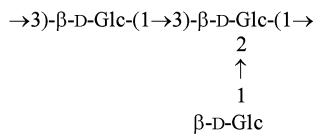
The capacity of LAB to produce EPS is an important function in the production of fermented foods. Most of the EPS-producing LAB that have been studied in detail have been isolated from different dairy products, such as Scandinavian fermented, ropy milk (Forsén, 1966; Macura and Townsley, 1984; Nakajima *et al.*, 1990) and various yoghurt products (Manca De Nadra *et al.*, 1985; Cerning *et al.*, 1986; Cerning *et al.*, 1988; Ariga *et al.*, 1992; Degeest *et al.*, 1997). The production of EPS during fermentation is of importance for the characteristics of the final product, as the EPS produced contribute to the texture, mouth-feel, taste perception and stability of the final product (Crescenzi, 1995; Bouzar *et al.*, 1997; Cerning and Marshall, 1999; Christiansen *et al.*, 1999).

Three strains of LAB known to produce EPS were used in this thesis (**Papers I-III, V, VII-VIII**). The structures of the different EPS, which these bacteria are known to produce, are shown in **Figure 5.2a-c**.

(a)



(b)



(c)

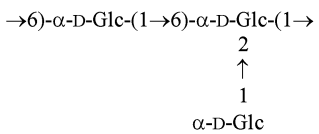


Figure 5.2 Structure of exopolysaccharides (EPS) produced by *Lactobacillus delbrueckii* subsp *bulgaricus* NCFB 2772 (a) (Grobben *et al.*, 1997, used in **Paper I**, **II**, **VII**), *Pediococcus damnosus* 2.6 (b) (Dueñas-Chasco *et al.*, 1997, used in **Paper III**, **V**, **VII**, **VIII**) and *Lactobacillus brevis* G-77 (b+c) (Dueñas-Chasco *et al.*, 1998, used in **Paper III** and **VII**).

5.2.1 Homopolysaccharides

A large variety of EPS can be produced by LAB used to ferment food products. These polysaccharides can be divided into two major groups: homopolysaccharides and heteropolysaccharides. Homopolysaccharides are composed of one monosaccharide moiety only and can be of the fructan (fructose homopolysaccharides) or the glucan (glucose homopolysaccharides) type. The fructan-types of polysaccharides can be divided into *levans* and *inuline*-types. The best-known LAB producing *levans* are strains of the oral flora, such as *Streptococcus mutans* and *S. salivarius*, which are known to be a key actor in the development of caries (Shiroza and Kuramitsu, 1988; Giffard *et al.*, 1993). Strains of *S. mutans* and *Lactobacillus reuteri* have been shown to produce *inuline*-types of fructooligosaccharides, containing a β-2,1 glucosidic bond (Ebisu *et al.*, 1975; Van Geel-Schutten, 2000). The glucan-types of polysaccharides can be divided into four major groups, *dextran*, *mutan*, *alternan* and β-1,3 *glucan*. Pasteur discovered dextran as early as 1861 in a gelification of cane sugar (Pasteur, 1861). The microorganism responsible for this production was later isolated and named *Leuconostoc mesenteroides* (Van Tieghem, 1878). The most widely used dextran-producing strain today is *Leu. mesenteroides* NRRL B-512F, producing a polysaccharide containing 95% of α-1,6 linkages for which the main field of

application is in the production of chromatography supports for gel permeation separation (Sephadex®), for instance, and in the production of blood plasma substitutes and blood coagulation prevention (Groenwall and Ingelman, 1948; Soetaert *et al.*, 1995). *Leu. mesenteroides* strains are also associated with the production of *mutans* together with several *Streptococcus strains* (Sidebotham, 1974; Mooser, 1992). *Mutans* are water insoluble polysaccharides containing more than 50% of α-1.3 glucosidic linkages. *Alternan*, contains an alternating 1,6 and 1,3 glucosidic linkages and is known to be produced by only a few *Leu. mesenteroides* strains (Jeanes *et al.*, 1954; Seymour and Knapp, 1980; Cote and Robyt, 1982). *β*-1.3 glucan has been reported to be produced by *Lactobacillus brevis* and *Pediococcus damnosus* (Dueñas-Chasco *et al.*, 1997, 1998). These strains were isolated from spoiled, i.e. "*ropy*" ciders. Other *Ped. damnosus strains*, producing similar kinds of polysaccharides, have been isolated from spoiled wines (Llaubère *et al.*, 1990).

5.2.2 Heteropolysaccharides

Heteropolysaccharides from LAB are composed of different sugar moieties, such as glucose, galactose, rhamnose, mannose, *N*-acetylglucosamine and glucuronic acid (Cerning *et al.*, 1990; Grobben *et al.*, 1997; Faber *et al.*, 1998). EPS with a heteropolysaccharide structure can be divided into two major subgroups, namely, the mesophilic group, such as *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Lactobacillus casei*, *Lb. sake*, *Lb. rhamnosus,* and a thermophilic group consisting of *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus* and *S. thermophilus* (De Vuyst and Degeest, 1999). From the point of view of applications of heteropolysaccharides in food and especially dairy products, most focus has been on the latter group, as they have played a major role in the production of fermented milk products.

5.2.3 Biosynthesis of EPS in LAB

The biosynthesis of EPS by LAB has been studied to some extent. Generally EPS are synthesized at different phases of growth and under different conditions, which are more or less strain specific (Ricciardi and Clementi, 2000). The genetic organization of the genes involved in the synthesis has been studied thoroughly in Gram-negative miroorganisms (Sutherland, 1990) and strong similarities between the Gram-positive

microorganisms such as LAB, and the Gram-negative biosynthesis systems have been found (Ricciardi and Clementi, 2000).

Extracellular enzymes, such as glycansucrases produce most of the well-known homopolysaccharides (*dextrans*, *levans* and *mutans*) (Monsan *et al.*, 2001). The biosytnthesis of the β-glucan and the α-dextran produced by LAB found in cider (Dueñas *et al.*, 1997, 1998) and wine (Llaubère *et al.*, 1990) is, however, not yet known. Heteropolysaccharides are synthesized by a more complex, intracellular system, which involves among others, enzymes in the cytoplasmic membrane and several intracellular precursors (De Vuyst *et al.*, 2001).

5.2.4 Physiological activity of EPS from LAB

In addition to the technological benefits of using EPS-producing LAB as texture generators and rheology promoters, it has also been claimed that these EPS have beneficial physiological effects (German *et al.*, 1999). The suggestion has been made that the increased viscosity of a product fermented with EPS-producing bacteria may increase the residence time in the gastrointestinal tract and that these products could therefore be good vehicles for probiotic bacteria into the gastrointestinal tract (German *et al.*, 1999). Other suggestions have been put forward concerning the generation of short chain fatty acids (SCFA) upon the degradation of the EPS in the gut by the colonic microflora i.e. a similar function as has been reported for dietary fibre (Harris and Ferguson, 1993; Cummings and Englyst, 1995) (see **sections 3.2** and **7.2.1**). EPS produced by LAB have been shown not to be degraded by intestinal bacteria *in vitro* (Ruijssenars *et al.*, 2000) and *in vivo* (Looijesteijn *et al.*, 2001). Anti-tumor effects have also been postulated by EPS produced by certain *Lactobacillus* strains (Oda *et al.*, 1983), together with cholesterol-lowering effect of fermented, ropy milk produced for EPS-producing LAB (Nakajima *et al.*, 1992) and immuno-modulatory effects (Hosono *et al.*, 1997). Until recently, most interest in EPS from LAB has been focused on their physical-chemical properties, but these polysaccharides have now caught a new general interest due to their potential in nutritional and health-promoting applications.

5.3 The role of lactic acid bacteria in the functional food concept

During last decades, there have been an accelerated pace of new products in order to satisfy the nutritional, technological, and quality requirements of consumers. More information is continuously provided on which nutrients and other food constituents that are needed to achieve a certain nutritive goal. In this context food is not only seen as a way to prevent nutritional deficiencies but also to promote good health in various stages of a human's development. This insight has led to the development of the concept of functional foods (FF). Based on a consensus document on the scientific concepts of FF in Europe, the following features of functional foods are outlined (Diplock *et al*., 1999.):

(i) They should be conventional or everyday food consumed as part of the normal diet.

(ii) They should be composed of naturally occurring components, sometimes in increased concentration or present in foods that would not normally supply them.

(iii) Their positive effects on target functions beyond basic nutrition should have been scientifically demonstrated.

(iv) They should provide enhancement of the state of well-being and health to improve the quality of life and /or reduce the risk of disease.

(v) Scientifically, authorised claims should have been proved.

5.3.1 Probiotics

The most common way to date to develop LAB-fermented products with a FF-approach has been to use probiotic LAB cultures. The term *probiotic*, "for life", was first used by Lilly and Stillwell (1965) to describe a "substance secreted by one microorganism which stimulates the growth of another", thus as a contrast to the term *antibiotic*. The term was later more widely used and defined by Parker (1974), and further improved by Fuller (1989) with the following definition: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". This definition have later been slightly revised (Schaafsma, 1996) to its latest wording suggested by Schrezenmeir and de Vrese (2001) "Foods

containing live and defined bacteria, which when given in sufficient numbers, exert beneficial effects by altering the microflora in the host".

Ever since the work of Metchnikoff (1907), lactic acid bacteria have been assumed to possess probiotic effects. In fact, the first commercial yoghurt was sold in pharmacies because they were believed to have a therapeutic effect. Today the probiotic concept is widely spread in the scientific and industrial fields (Shortt, 1999). The lactic acid bacteria primarily associated with probiotic recognition are lactobacilli and bifidobacteria. For the selection of probiotic microorganisms three theoretical bases, including safety, and functional and technological aspects have been suggested (Salminen *et al.*, 1998; Adams, 1999):

(i) Strains for human use should be preferably of human origin.

(ii) Strains should have a history of being non-pathogenic and have no association with diseases.

(iii) Strains should not deconjugate bile salts (Marteau *et al.*, 1995).

(iv) Strains should not carry transmissible antibiotic resistance genes.

In addition to the safety requirements for probiotics also functionality aspects are required, which must first have been established by *in vitro* methods (Saarela *et al.*, 2001):

(i) Acid tolerance and tolerance to human gastric juice.

(ii) Bile tolerance.

(iii) Adherence to epithelial surfaces and persistence in the human GI-tract.

(iv) Immunostimulation but no proinflammatory effect.

(v) Antagonistic activity against pathogenes such as *Heliobacter pylori*, *Salmonella* spp., *Listeria monocytogenes* and *Clostridium difficile.*

(vi) Antimutagenic and anticarcinogenic properties.

The technological aspects concerning probiotic strains concerns the manufacturing process, which includes the interaction of the probiotic cultures with other existing cultures in the product, such as yoghurt culture (German *et al*., 1999). To obtain the "probiotic" effect in consumers there is a therapeutic minimum in terms of viable cells in which have to be consumed. In order to attain the required effect it is suggested that about 100 g of product, containing more than 10^6 cfu per gram product, should be consumed (Kailasapathy and Rybka 1997). The number of bacteria used to inoculate the product and its stability i.e. the bacterial survival of the probiotic culture in the product are crucial parameters. Most commercial probiotic cultures today are like other commercial starter cultures, supplied in a highly concentrated form, and most of them are constructed for DVS (direct vat set) application, either in the freeze-dried form or as deep-frozen cultures (Honer, 1995).

Food containing both probiotic and prebiotics (non-digestible carbohydrates) are called synbiotics (Roberfroid, 1998). Due to their general chemical properties prebiotics might in various ways influence the growth and survival of the probiotic bacteria. However, the concept of synbiotic is quite new and not many specific studies have been carried out.

LACTIC ACID BACTERIA IN OAT-BASED SUSPENSIONS

To monitor and determine the growth of lactic acid bacteria (LAB) during the production of fermented products the parameters such as decrease in pH, the amount of residual fermentable carbohydrate e.g. glucose, maltose, lactose, the amount of produced lactic acid, and colony-forming units (cfu) can be studied. For practical reasons, the only parameter measured during real production situations of yoghurt is for instance, decrease in pH with fermentation time. To investigate the potential for LAB to be used in the development of fermented, oat-based products, different LAB from food as well as from human isolates, were used to study the fermentation characteristics in different oat-based suspensions by using the above-mentioned parameters (**Table 6.1**). Measures of structural changes in the oat-based suspensions when EPS-producing LAB were used, and the bacterial survival in different laboratory products were also studied. The oat-based suspensions used throughout this thesis were two maltose-rich media, MM-medium (10% dry matter) (**Paper I-II, VII**) or M40 medium (16-20% dry matter) (**Paper II, IV, VI, VIII**) and a glucose-rich medium, G40-medium (16-20% dry matter) (**Paper II, III, V-VIII**) (see **chapter 4** for details). In addition, a mixture (1:1 ratio) of G40 and M40 medium (MG20 medium) was also used in **Paper VI**.

6.1 Growth of LAB in oat-based suspensions

In the first approach to investigate LAB and their ability to ferment these non-dairy, oat-based suspensions, the growth of nine strains was studied in the MM medium (**Paper I**). With the exception of three strains, *Lb. delbrueckii* subsp. *bulgaricus*, *S. salivarius* subsp. *thermophilus* and *Propionibacterium propioniacidici*, all strains were able to reduce the pH to 4.5 or lower from the initial level of 7.1. A final pH of 4.0-4.5 after fermentation has been reported as suitable in the production of yoghurt and yoghurt-like products (Tamime and Robinson, 1999). The same pH range was

obtained when probiotic cultures were grown in different oat-based suspensions, with the exception of the *Bifidobacterium bifidum* strain, which yielded a considerably higher pH (**Paper VI**).

Table 6.1 Lactic acid bacteria and commercial cultures used in this thesis

Organism	Source	EPS-producer	ref.
Lb. delbrueckii subsp. *bulgaricus* NCFB 2772	Yoghurt	+	**Paper I, II, VII**
Lb. kefiri DSM 20485	Kefir grains	-	**Paper I**
Lb. delbrueckii subsp. *bulgaricus* DSM 20081	Yoghurt	-	**Paper I, II**
Lb. acidophilus DSM 20079	Human small intestine	-	**Paper VI**
Lb. acidophilus La5	Human small intestine	-	**Paper VIII**
Lb. reuteri ATCC 55730	Human mother's milk	+	**Paper VI**
Lb. brevis DSM 1269	Lager beer yeast	-	**Paper I, II**
Lb. brevis G-77	Cider	+	**Paper III, VII**
S. thermophilus DSM 20259	Yoghurt	-	**Paper I, II**
Ped. damnosus DSM 20331	Lager beer yeast	-	**Paper I**
Ped. damnosus 2.6	Cider	+	**Paper III, V, VII, VIII**
P. propioniacidici DSM 20272	Emmental cheese	-	**Paper I**
Leu. mesenteroides DSM 20240	NI[a]	+	**Paper I**
Leu. dextranicum NCFB 2706	NI[a]	+	**Paper I**
B. bifidum DSM 20456	Faeces from infant	-	**Paper VI**

Mixed cultures	Source	Industrial application	ref.
ABT *Lb. acidophilus* *S. salivarius* subsp. *thermophilus* *Bifidobacterium* spp.	Christian Hansen A/S, Hørsholm, Denmark	Non-dairy products	**Paper IV**
V2 *Lb. delbrueckii* subsp. *bulgaricus* *S. salivarius* subsp. *thermophilus*	Visby Tønder A/S Tønder, Denmark	Yoghurt products	**Paper IV, V, VI, VIII**

[a]NI=No information.

It is well known that LAB produces less lactic acid during growth in vegetable-based substrates than in milk (Gehrke and Weiser, 1948ab). This is due mainly to a combination of the limitations in fermentable carbohydrates and the lower buffering capacity in these substrates. This was shown by chemically acidifying the MM-medium with glucono-δ-lactone at the same concentration used in dairy milk (Van Marle and Zoon, 1995). The pH in the dairy milk reached 4.3 after the addition of glucono-δ-lactone, whereas in the MM-medium it decreased to as low as 2.9 when the same concentration was used (**Paper I**). This was also confirmed by the final concentration of lactic acid, which varied from 0.14-0.44% in the MM-medium, and which is of the same order that has been reported concerning LAB fermentation of soymilk (Angeles and Marth, 1970), but lower than in sour milk and yoghurt products (0.8-1.4%) (Tamime and Robinson, 1999). Lactic acid concentration has been reported to be in the range of 0.4-0.9% in thin, oat-based pastes when dairy products, such as yoghurts and kefir were used as inoculum (Salovaara *et al.*, 1991). Similar values have also recently been reported in fermented oat mash mixed with fat-free milk (Bekers *et al.*, 2001).

The MM-medium used in **Papers I** and **II** and the M40 medium used in **Paper IV** and **VI** contain mainly maltose as the low molecular carbohydrate constituent. None of the strains used in these studies are known to be able to ferment maltose. However, small amounts of glucose (~0.4-0.6%) may be present in the medium after the enzymatic hydrolysis of the starch (see **Chapter 4** for details). Thus, small amounts of glucose, vitamins and various micronutrients in combination with a low buffering capacity, are sufficient to support growth and decrease the pH in the MM and M40 media to appropriate levels. This was also confirmed when using commercial yoghurt cultures in the M40 medium, as both pH and cfu showed that an appropriate growth had occurred (**Paper IV** and **VI**). However, support for growth also comes from the "carry-over" effect from the growth medium during the inoculation procedure. These media contain high levels of nutrients for growth. But even when this "carry-over" effect is minimized, appropriate growth occurs (**Paper VI**). The G40 medium contains an excess of glucose when it contains dry matter of 16-20%. As a consequence, good growth in terms low pH and a high number of colony-forming units after growth was obtained for all strains tested in this medium (**Paper III**, **V-VIII**).

The influence of the temperature is known to be crucial to the growth of LAB (Hammes and Vogel, 1995). This was confirmed when a commercial yoghurt culture (V2) was grown at a lower temperature (28°C) than the optimal one (37°C) and as a consequence, a lower cfu value was obtained (**Paper V**).

6.2 Formation of exopolysaccharides (EPS) by LAB in oat-based suspensions

The formation of exopolysaccharides (EPS) by LAB in milk has been thoroughly studied (Cerning, 1990; Cerning *et al*., 1995). Only a few reports have focused on the formation of EPS-producing LAB in non-dairy food products, namely in Mettwurst salami, Prosciutto ham and black olives (Ludbrook *et al*., 1997). It is well known that when the formation of EPS occurs in milk it contributes to an increased viscosity (Macura and Townsley, 1984; Manca de Nadra *et al*., 1985; Marshall and Rawson, 1999) and also ropiness (Hess *et al*., 1997).

The term ropiness can be defined as one or several threads remaining attached to a spoon when it is lifted from the surface of the product. Viscosity can be described as the capacity of a material to resist deformation. In the case of fermented liquid foods yoghurt, this can also to some extent describe the slimy behaviour of the product (Duboc and Mollet, 1999). The influence of EPS-producing strains on viscosity and ropiness of different, fermented, oat-based suspensions are listed in **Table 6.2**.

The use of structural measurements, such as viscosity and ropiness, is suggested to be too im-precise to measure the formation of EPS in dairy milk. This is mainly due to precipitation of the proteins in the milk, as the microstructure of yoghurt consists mainly of aggregated casein particles, which interfere with the viscosity measurements. When dairy milk was acidified chemically using glucono-δ-lactone, an increase in viscosity could also be seen (Bouzar *et al*., 1997). This was, however, not the case when the pH was decreased chemically in the MM medium (**Paper I**). Thus, the measurements of viscosity and also ropiness can be sufficient parameters to measure and detect the formation of EPS in oat-based suspensions.

Table 6.2 EPS-producing strains and their effect on viscosity and ropiness after growth in different oat-based media

Organism	Oat-base medium	Ropiness (cm)	Viscosity (mPas)	ref.
Lb. bulgaricus NCFB 2772	MM	ND*	22[a]	**Paper I**
		ND*	28[b]	**Paper I**
		ND*	58[c]	**Paper I**
		ND*	120[d]	**Paper II**
	M40	ND*	176[e]	**Paper II**
	G40	5.0	523[e]	**Paper II**
Lb. brevis G-77	G40	7.6	1 211[f]	**Paper III**
Ped. damnosus 2.6	G40	13.2	1 423[f]	**Paper III**
	G40	28.2	>2 400[g]	**Paper V**

ND=Not Determined.

[a] Measured with a shear rate of 1 207 s^{-1} after 24 h of fermentation.

[b] Measured with a shear rate of 1 207 s^{-1} after 24 h of fermentation with the addition of 5% (w/v) of glucose in the medium.

[c] Measured with a shear rate of 1 207 s^{-1} after 72 h of fermentation with the addition of 5% (w/v) of glucose in the medium

[d] Measured with a shear rate of 129 s^{-1} after 24 h of fermentation with the addition of 5% (w/v) of glucose in the medium.

[e] Measured with a shear rate of 129 s^{-1} after 24 h of fermentation using G40 medium (dry matter 20%) based on powder.

[f] Measured with a shear rate of 0.06 s^{-1} after 24 h of fermentation using G40 medium (dry matter 20%) based on powder.

[g] Measured with a shear rate of 10.5 s^{-1} after 24 h of fermentation using G40 medium (dry matter 20%) based on evaporated oat-base.

6.2.1 Formation of EPS by pure cultures

The EPS-producing strain *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772, known to produce a heteropolysaccharide (Grobben *et al.*, 1997), was studied for its effectiveness to change the viscosity in the MM medium under different growth conditions, by using different temperatures and carbon sources (**Paper I**). The ability of this strain to change the viscosity has earlier been studied in milk (Garcia-Garibay and Marshall, 1991). It was shown that when the MM medium was not supplemented with any source of carbon only a poor increase in the viscosity occurred. When glucose was added to a concentration of 5% in combination with a low incubation temperature (25°C), an increase in viscosity was obtained that was higher than in the yoghurt that was used as control product. That sub-optimal growth conditions, such as low incubation temperatures, can be favourable for the production of EPS has been

reported earlier (Cerning *et al.*, 1992; Gancel and Novel 1994; Moreira *et al.*, 2000), although reports concerning *Lb. delbrueckii* subsp. *bulgaricus* strains have shown that good EPS-production can also be obtained at optimal temperatures (37-43°C) (Grobben *et al.*, 1995; Mozzi *et al*, 1994). The incubation time was also shown to be important to obtain an increased viscosity in the MM medium. A relatively long incubation time (72 h) gave a higher final viscosity than after 24 h (**Table 6.2**, **Paper I**). Longer fermentation time than 72 h gave no further improvement of the viscosity of the MM medium.

Elasticity i.e. the property of the food material to recover after a deformation was also measured. Deformation occurs when viscosity is measured. In general, elasticity is an attribute of firm body and gives a gum-like appearance. The elasticity was not as high as in the yoghurt control but comparable with other data from experiments with milk products (Rawson and Marshall, 1997). When *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772 was grown in either the M40 or the G40 medium with a dry matter of 20% (consisting of about 9% maltose or 9% glucose, respectively) (**Paper II**) a higher viscosity was observed in the G40 medium. These data confirm the results from **Paper I**, where the MM medium supplemented with glucose gave a higher final viscosity after fermentation (**Table 6.2**). A fermentation time of 24 h was sufficient to obtain a desired viscosity. Longer fermentation time in the G40 medium did not yield any increased viscosity compared to the case of the MM medium (**Paper I**). This shows that also the dry matter of the medium plays a role in the effectiveness of the EPS produced to affect the viscosity in these complex media.

Fermentation in the G40 medium was also carried out by *Pediococcus damnosus* 2.6 and *Lactobacillus brevis* G-77, known to produce two types of homopolysaccharides (Dueñas *et al.*, 1997, 1998, **Paper III**) (see **chapter 5**, **section 5.2** for details). In this thesis work an Instron instrument was used to detect '*ropy*' behaviour of the fermented products after fermentation of these strains (**Figures 6.1a,b**). Similar instruments for measuring ropiness have been used earlier in dairy products (Hess *et al.*, 1997; Marshall and Rawson, 1999).

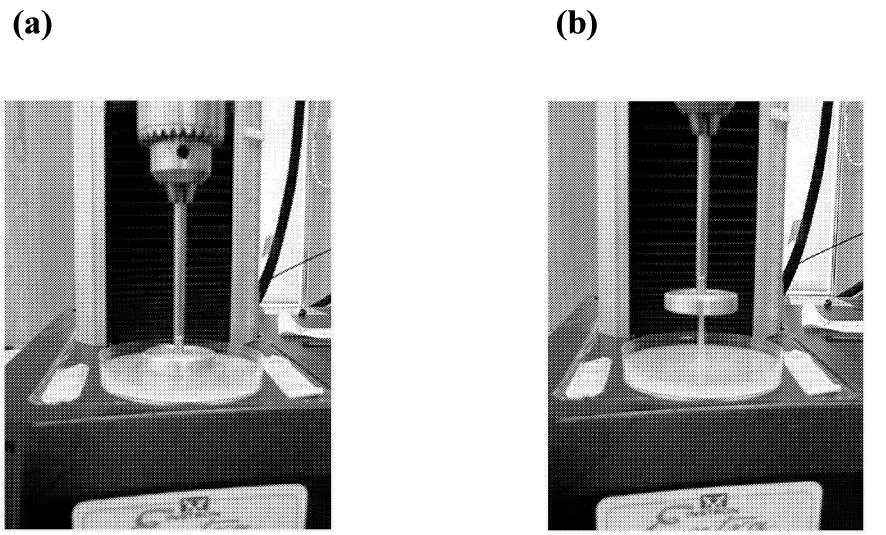


Figure 6.1 Measurement of ropiness. The probe is placed on the surface of the product (a) and then lifted up at a speed of 100 cm min^{-1} (b). The length of the thread that is attached to the probe until it breaks is measured.

The ropiness in the G40 medium after growth of *Ped. damnosus* 2.6, *Lb. brevis* G-77 or *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772 is shown in **Figure 6.2**. These three '*ropy*' products were used as diets in **Paper VII**.

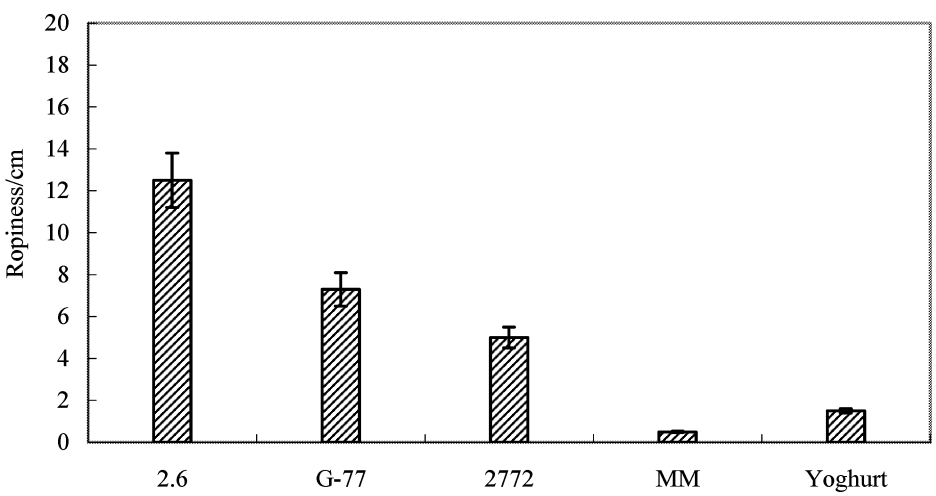


Figure 6.2 Ropiness (cm) in the G40 medium after growth of *Ped. damnosus* 2.6, *Lb. brevis* G-77 and *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772, compared with an unfermented control (MM medium) and a commercial yoghurt product (**Paper III**, **VII**).

The effect of *Ped. damnosus* 2.6 and *Lb. brevis* G-77 on the structure of the G40 medium was studied in detail in **Paper III**. The change in structure was obvious after about 6 h of fermentation and reached its peak after about 16-18 h of fermentation and decreased thereafter (**Figure 6.3a,b**).

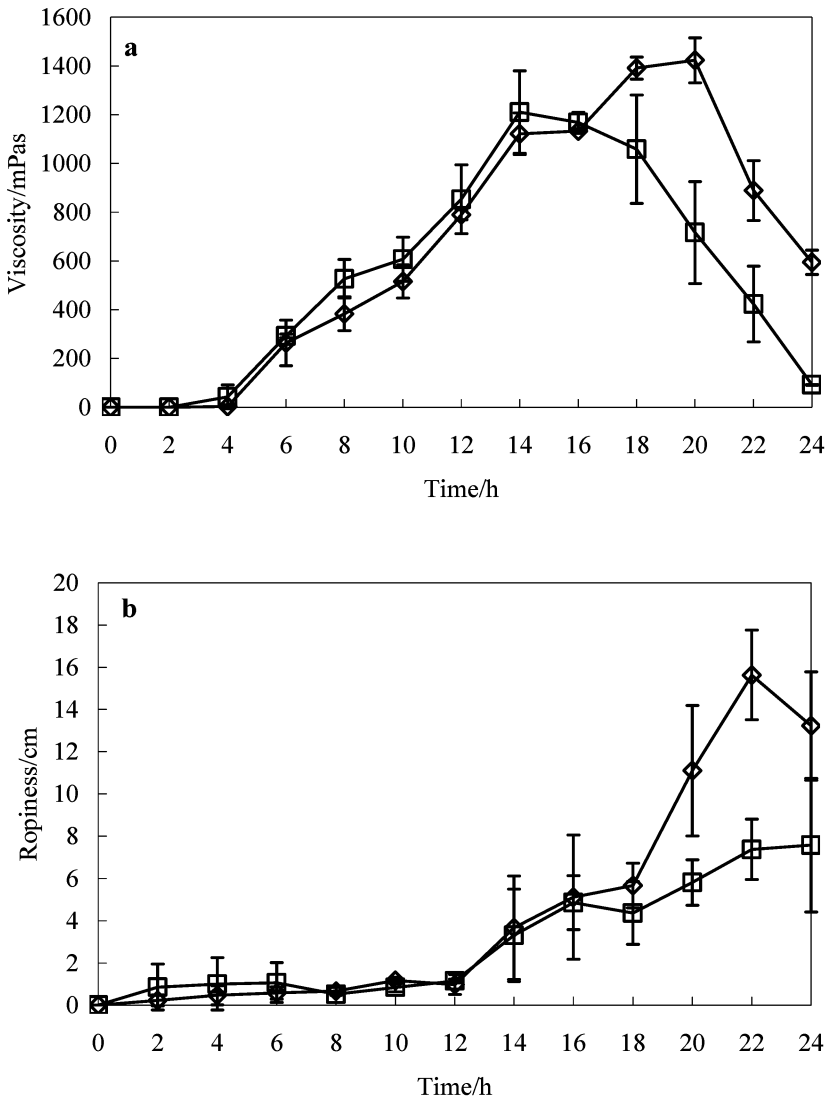


Figure 6.3 Viscosity (a) and ropiness (b) during growth of *Pediococcus damnosus* 2.6 (◇) and *Lactobacillus brevis* G-77 (□) in G40 medium at 28°C for 24 h (**Paper III**).

The decrease in viscosity during the last 6-8 h of the fermentation may be an effect of inhomogenity in the medium at this stage of the fermentation period. This inhomogenity may effect the structural measurements to an extent that the results do not fully reflect the real structure of the medium. This decrease was, however, not seen to the same extent in the ropiness measurements. Thus these two parameters, viscosity and ropiness, measure two different structural behaviours of the medium. That the fermentations were proceeded without stirring may be one reason for this inhomogenity. By running the fermentations with a continuous stirrer speed is found to decrease the impact of the EPS-formation on both viscosity and ropiness. The same problem has been reported when using EPS-producing bacterial strains in milk e.g. during the production of set yoghurts, which are often fermented in the container (Tamime and Robinson, 1999).

6.2.2 Formation of EPS in mixed cultures

To achieve a proper fermented product in terms of acidity, flavour and texture, mixed starter cultures are often used. It is therefore of importance from the point of view of product development, to investigate the formation of EPS when these EPS-producing strains are combined as a mixed culture consisting of non-EPS-producing bacteria (**Paper II and V**). It has been reported earlier that when an EPS-producing strain was grown in milk together with a non-EPS-producing strain, a faster acidification and a higher final viscosity was obtained (Bouzar *et al.*, 1997). In **Paper II**, *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772 was grown in the presence of other LAB. These were selected on the basis that they did not affect the structure of the MM medium during growth. These mixed cultures were grown in media consisting of maltose (MM and M40 media) and glucose (G40 medium). The viscosity was higher after fermentation with the mixed cultures in the medium containing maltose (M40 and MM) than when the bacteria were grown as pure cultures. However, no significant difference in the final viscosity was seen between pure and mixed cultures when they were grown in the G40 medium (**Figure 6.4**). None of these strains have been reported to ferment maltose. This suggests that when there are no or very low fermentable carbohydrates as, in the M40 and MM cases, the co-operative growth between bacterial strains is more important in terms of EPS production than when there is an excess of fermentable carbohydrates, as in the G40 case.

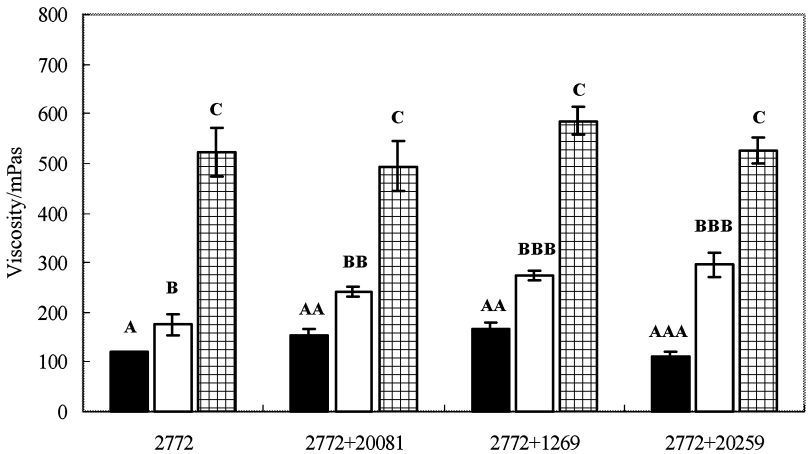


Figure 6.4 Viscosity in the three different oat-based suspensions, MM medium supplemented with 5% glucose (■), M40 medium (□) and G40 medium (⊞) after fermentation with *Lb. delbrueckii* subsp. *bulgaricus* 2772 as pure culture or in a mixed culture with non-EPS-producing strains, *Lb. delbrueckii* susp. *bulgaricus* DSM 20081, *Lb. brevis* DSM 1269 or *S. thermophilus* DSM 20259. Means marked with the same letter did not differ at $p<0.05$ (**Paper II**).

When *Ped. damnosus* 2.6 was grown in the G40 medium together with a commercial yoghurt culture, V2, it was shown that the incubation temperature had a greater influence on the final viscosity than the presence of the yoghurt culture (**Paper V**). Obviously the co-operative growth that occurs in mixed cultures has a greater effect when there is a limitation of fermentable carbohydrates, as in the MM and M40 cases. None of the tested strains have any reported ability to ferment this disaccharide. This may also be one explanation why the difference in viscosity after growth of an EPS-producing strain used as pure culture or combined with a mixed culture, was lower in dairy milk (Bouzar *et al.*, 1997) than in MM and M40 (**Paper II**). The study by Rawson and Marshall (1997) showed that when two ropy strains grew together a lower firmness and viscosity was obtained than when a ropy strain was grown together with a non-ropy one, as in our case. This suggests that the combination of several ropy strains does not necessarily improve the final texture and that it is not only the amount of the polysaccharide produced that is important for the rheological properties but also the type of EPS produced (Hess *et al.*, 1997). This shows the complexity of using mixed cultures in complex media and that more research in this area is required.

6.2.3 Isolation of the soluble polymer fraction from fermented oat-based suspensions

Several studies have attempted to isolate EPS from dairy milk and reported yields have varied from 60-150 mg l^{-1} (Garcia-Garibay and Marshall, 1991; Toba *et al.*, 1992) up to over 2 000 mg l^{-1} (Cerning *et al.*, 1986; Mozzi *et al.*, 1996) for some *Lb. delbrueckii* subsp. *bulgaricus* strains. Generally, two methods have being reported for use in isolation of the EPS fraction from complex dairy milk media. These are precipitation of the proteins by trichloracetic acid (TCA) (Garcia-Garibay and Marshall, 1991) followed by ethanol precipitation and centrifugation or by using deproteinization technique, i.e. enzymatic degradation of the milk proteins with enzymes, such as Pronase® (Cerning *et al.*, 1986, 1988, 1990, 1992). In the complex oat-base suspensions not only proteins can interfere with the analysis procedure but also both the insoluble and soluble fibre from oats. No method is the obvious choice to separate these two types of soluble polysaccharide, the β-glucans from oats and the EPS produced by the bacteria. An attempt was made to isolate a polymer fraction from the fermented oat-base suspensions that is suggested to include both of these polymers. This was made by a combination of Alcalase® and Esperase® followed by ethanol precipitation and centrifugation (**Paper II**, **V**).

6.3 Development of fermented, oat-based, non-dairy products

Many countries, especially those in Africa and Asia, have a long tradition of spontaneously fermented vegetable-based foods where LAB plays a major role in the final quality of the product. The use of defined starter cultures of LAB in the production of non-dairy foods is, however, not so common. Several attempts have been made to develop yoghurt-like products from legumes, such as soy (Pinthong *et al.*, 1980ab; Chopra *et al.*, 1982; Lee *et al.*, 1990 Buono *et al.*, 1990) and cowpea blends (Rao *et al.*, 1990; Sanni *et al.*, 1999). The main encountered problem with products developed from legumes is that there is no natural presence of fermentable sugars and that a flavour, often defined as “beany”, is obtained in the final product (Chopra and Prasad, 1990; Lee *et al.*, 1990). Among cereals both rice (Shin, 1989) and oat (Salovaara, 1996; Salovaara and Kurka, 1997; **Paper IV** and **V**) have been used as the main raw material in the development of fermented, non-dairy products.

To characterise the ability of a food-base to function in a formulation process it is of importance that it interacts in a suitable way with ingredients or additives, such as stabilisators, fat, flavours and starter cultures. In the development of semi-viscous, liquid foods, such as yoghurt-like products the dry matter is crucial, not only to obtain desired structure but also to provide a good energy balance and a substrates for the starter culture. The influence of dry matter solids in yoghurt production has been thoroughly investigated and its concentration normally ranges between 11-16% (Tamime and Robinson, 1999, Lankes *et al*., 1998). Sensory evaluation is of importance in the course of determining product characteristics of the final product. The nine-point hedonic scale method (Jones *et al*., 1955; Peryam and Pilgrim, 1957) was used for the measurement of acceptance-preference compared to reference products (**Paper IV** and **V**) together with the descriptive test (Stone *et al*., 1974) using intensity scales and references (**Paper IV**). These methods have been reported as accepted sensory assessment of both plain and flavoured commercial yoghurt products (Barnes *et al*., 1991; Harper *et al*., 1991). All oat-bases used for the formulation work were freshly made and evaporated to increase the dry matter to a sufficient level as these oat-bases have a more general freshness compared with the re-diluted oat-bases from powder.

6.3.1 Oat-based products fermented with yoghurt cultures

Apart from traditional yoghurt cultures consisting of *Lb. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*, other bacteria, such as *Lb. acidophilus*, *Bifidobacterium* subsp., are also frequently used in different yoghurt applications. These types of bacteria have also been reported to be starter cultures for yoghurt-like products consisting mainly of vegetable raw materials, such as soy (Lee *et al*., 1990; Nsofor and Chukwu, 1992), rice (Shin, 1989), oats (Salovaara, 1996; Salovaara and Kurka, 1997; Bekers *et al*, 2001, **Papers IV-VIII**) and different mixed vegetable blends (Shirai *et al*., 1992ab). To obtain sufficient flavour in a fermented product a decrease in pH is crucial. A difference in pH level, which may appear to be small (3.8-4.2), can have a major impact on the acceptance of the product. Two different commercial yoghurt cultures, V2 and ABT, were used in the course of fermenting the maltose-rich, oat-base suspension (M40 medium) (**Paper IV**). V2, which is a traditional yoghurt culture, consists of *Lb. delbrueckii* subsp. *bulgaricus* and *S.*

salivarius subsp. *thermophilus*, whereas the ABT culture, developed for use in non-dairy applications, consists of three different strains, *Lb. acidophilus*, *S. salivarius* subsp. *thermophilus* and *Bifidobacterium* subsp. (**Table 6.1**). By using these two different cultures two products were obtained, one with low acidity (fermented by the V2 culture) with a pH around 4.4-4.5 and a more acid product (fermented with the ABT culture) with a pH ranging between 3.8-4.0. The ABT-culture has also been reported to grow well in an enzymatically hydrolysed oat mash with fat-free milk included in the formula (Bekers *et al.*, 2001). A summary of the different products described in **Paper IV** and **V** and their general characteristics are shown in **Table 6.3**. A dry matter content of 16% gave the most yoghurt-like consistency (**Paper IV**). It was obvious that by using a microbial polysaccharide, xanthan gum, an improved stability and texture of the product was achieved. Also, the addition of fat gave a smoother and improved surface to the final product. The impact of jam was evident in the test ranking for preference (**Paper IV**).

Table 6.3 Characteristics of the fermented, oat-based products developed in this thesis

Culture	Oat-base	Dry matter (%)	Viscosity (mPas)	Ropiness (cm)	pH	ref.
ABT[a]	M40	18	83[b]	1.5	4.0	**Paper IV**
		16	78[b]	1.7	3.9	**Paper IV**
V2[c]	M40	18	67[b]	1.8	4.5	**Paper IV**
		16	43[b]	1.7	4.4	**Paper IV**
V2[c]	G40	16	450[d]	1.8	3.9	**Paper V**
V2 +2.6[e]	G40	16	958[d]	3.1	3.6	**Paper V**
V2 +2.6[f]	G40	16	>2 400[d]	22.6	4.1	**Paper V**

[a]Fermented with a commercial culture consisting of *S. salivarius* subsp. *thermophilus*, *Lb. acidophilus* and *Bifidobacterium* ssp.
[b]Measured with a shear rate of 1 207 s^{-1} after 24 h of fermentation.
[c]Fermented with a commercial yoghurt culture consisting of *Lb. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*.
[d]Measured with a shear rate of 10.5 s^{-1} after 24 h of fermentation.
[e]Mixed culture consisting of a yoghurt culture (V2) and the EPS-producing strain, *Ped. damnosus* 2.6 grown at 37°C.
[f]Mixed culture consisting of a yoghurt culture (V2) and the EPS-producing strain, *Ped. damnosus* 2.6 grown at 28°C.

In addition, supplementation of glucose to the M40 medium was also done to achieve a fairly equal energy load compared to the other products, which were all used in the clinical study (**Table 6.4**, **Paper VIII**) (**see chapter 7** for details).

Table 6.4 Composition of a fermented, ropy, oat-based product (Product A) and a fermented, non-ropy oat-based product (Product B) and a fermented, dairy-based product (Product C). All products were used in the clinical study presented in **Paper VIII**

Components (g/100g)	Product A[a)]	Product B[b)]	Product C[c)]
Energy (kJ)	378	373	315
Fat	1.2	1.2	1.3
Protein	1.9	1.7	3.5
Carbohydrates	18.0	18.3	12.4
β-glucan	0.6	0.5	<0.05
Lactic acid	3.8	3.7	7.7
pH	4.1	4.1	4.1

[a)] Fermented, ropy, non-dairy oat-based product produced by yoghurt culture (V2) and *Pediococcus damnosus* 2.6 (**Paper V** and **VIII**).
[b)] Fermented, non-ropy oat-based product produced by yoghurt culture (V2) and *Lb. acidophilus* La5 (**Paper VIII**).
[c)] Fermented dairy-based product (yoghurt) (**Paper VIII**).

It was also obvious that the typical oat flavour was still pronounced in the fermented products (**Paper IV**). The use of the V2 culture together with the *Lb. acidophilus* La5 strain, gave also a sufficient decrease in pH (to ~4.0) when glucose was added in appropriate amounts (~1%) to the M40 medium (**Paper VIII**).

6.3.2 An oat-based product fermented with the an EPS-producing strain

With the knowledge obtained from **Paper IV** a fermented product based on the EPS-producing strain, *P. damnosus* 2.6 was developed (**Paper V**). The use of EPS-producing LAB strains has been reported in the development of "ropy" laboratory yoghurts (Marshall and Rawson, 1999). The glucose-rich oat-base (G40 medium) was used according to earlier studies (**Paper II** and **III**). The use of the *Ped. damnosus* 2.6 strain gave a low pH value (~4.1) even at sub-optimal incubation temperature (28°C) for a thermophilic yoghurt culture (V2) (**Table 6.3**). The EPS-producing *Ped. damnosus* 2.6 strain gave a product with a characteristic texture, i.e., high viscosity and ropiness. The chosen reference product in the sensory preference test was a commercial, fermented, ropy, traditional, dairy milk ("långfil"). This product has a

of flavour from yoghurt, as it is not fermented with yoghurt cultures but with a complex mixed culture that contains different lactococci and *Leuconostoc* strains. However, by using sufficient amounts of jam (16%) these flavours originating from the fermentation process could be masked.

6.4 Bacterial survival in oat-based products during storage

The survival or viability of the starter culture in the food product can be a measure of how suitable the food product is as a carrier for the bacteria. This measure has been an important parameter mainly for yoghurt products. In fact, an international standard has been established for a recommended minimum value of yoghurt bacteria per ml of product as a requirement for the product to be defined as yoghurt (Hamann and Marth, 1984). This has been even more pronounced as there has been an increased development of products with high numbers of certain bacteria strains with more or less documented health beneficial effects, i.e., probiotic bacteria (see **section 5.3.1** for details). In this case the food product also has the function as a "vehicle" for the bacteria to provide high concentration of bacterial cells into the gastrointestinal tract.

6.4.1 Survival of yoghurt cultures during storage

Milk is the food product on which most of investigation has been done on the survival of yoghurt cultures during storage. Apparently these cultures that consists mainly of *S. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* survive in high numbers in milk, with a bacterial survival that ranges between 10^5-10^8 cfu ml^{-1} after 20 days depending on fermentation temperature and temperature during storage (Hamann and Marth, 1984). The yoghurt culture (V2) was found to have a good survival in the oat-based products (**Table 6.5**, **Paper IV-VI**).

The incubation temperature during fermentation had a major influence on the survival during storage. In **Paper IV** the survival rate of both of *S. salivairus* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* was about 10^7 cfu ml^{-1} after a fermentation at 43ºC (**Paper IV**), whereas the survival was 10^5 cfu ml^{-1} for these strains after the same storage period but when the fermentation temperature was as low as 28ºC (**Paper V**). When the fermentation temperature was 37ºC the survival after 20 days was about 10^7 cfu ml^{-1} for both strains when grown in the M40 medium or in the mixed medium consisting of both maltose and glucose (MG20 medium)

(**Paper VI**). The survival decreased faster, especially for the *S. salivairus* subsp. *thermophilus* strain, when grown in the G40 (**Table 6.5**, **Paper VI**).

Table 6.5 Bacterial survival during storage (20 and 30 days) in different fermented oat-based suspensions with different pH values

Bacterial strain	Oat-base	pH	Bacterial survival Log (cfu/ml)/- Initial value (0)	Days of storage (20)	(30)	ref.
Lb. bulgaricus (V2)	M40	4.4	7.8	6.7	6.7	**Paper IV** **Paper VI**
	MG20	3.9	8.0	7.5	7.4	**Paper VI**
	G40	3.9	8.0	7.1	6.1	**Paper V** **Paper VI**
S. thermophilus (V2)	M40	4.4	6.6	7.2	6.5	**Paper IV** **Paper VI**
	MG20	3.9	7.2	7.1	5.7	**Paper VI**
	G40	3.9	9.0	6.1	4.9	**Paper V** **Paper VI**
Ped. damnosus 2.6	G40	4.1	8.9	8.9	8.7	**Paper V**
Lb. acidophilus DSM 20079	M40	4.3	7.6	5.0	4.8	**Paper VI**
	MG20	4.3	7.9	3.7	3.1	**Paper VI**
	G40	4.4	7.3	3.2	3.2	**Paper VI**
Lb. reuteri ATCC 55730	M40	5.2	8.0	8.7	7.7	**Paper VI**
	MG20	4.5	8.1	8.3	7.6	**Paper VI**
	G40	4.6	8.2	8.1	8.0	**Paper VI**
B. bifidum DSM 20456	M40	6.6	9.1	5.8	5.4	**Paper VI**
	MG20	5.0	8.2	6.4	5.3	**Paper VI**
	G40	4.9	8.1	5.4	4.9	**Paper VI**

6.3.2 Survival of Pediococcus damnosus *2.6 in an oat-based product*

The viability of the EPS-producing strain, *Ped. damnosus* 2.6, was also studied in a fermented, oat-based product when grown in combination with a yoghurt culture (V2) (**Paper V**). The initial cell numbers after fermentation at 28ºC was 10^8 cfu ml^{-1} and after 30 days of storage at 6ºC there was no apparent change in the bacterial cell level

of the *P. damnosus* strain (**Table 6.5**, **Paper V**). Thus, the G40 medium is not only a good support for growth of *P. damnosus* 2.6, it is also a suitable carrier for this strain during a proposed shelf life period of 30 days.

6.4.3 Survival of probiotic cultures in oat-based products

The use of probiotic cultures, i.e., health promoting microorganisms, in various types of food, especially in fermented, dairy products has increased (Lourens-Hattingh and Viljoen, 2001). It is generally accepted that fermented, semi-liquid foods, such as yoghurt, are excellent carriers for probiotic bacteria, as these products are rich in nutrients, have a high water activity (0.98-0.99%) and fermentation is a natural part of their development (Shah and Ravula, 2000). The main requirement for a food carrier of probiotic cultures is that it can "carry" the bacteria in large numbers into the intestine, i.e., the microorganisms shall have a good survival in the food product throughout its entire shelf life so that they are maintained active and viable until the food reaches the intestine of the consumer.

In general, the most common probiotic bacteria used in different fermented products are *Lb. acidophilus* and *Bifidobacterium bifidum*. A "therapeutic minimum" requirement is for a food carrier to have a bacterial load of about 10^6 cfu ml^{-1}, and that the potential volume consumed is at least 100 g per day (Kailasapathy and Rybka, 1997). This is based on a suggested therapeutic dose of probiotic bacteria at a level of 10^8-10^9 cells per day (Kurman and Rasic, 1991). Even if most of the products on the market today are of dairy origin, cereal-based products or products containing cereal-based components have been developed during recent years with a focus on being products for "probiotic delivery", (Molin *et al.*, 1993; Langhendries *et al.*, 1995; Salovaara, 1996). Two *Lactobacillus* strains, *Lb. reuteri* ATCC 55730 and *Lb. acidophilus* DSM 20079, and *Bifidobacterium bifidum* DSM 20456, all microorganisms recognised as having a high potential to be probiotic cultures, were used to study the ability of three oat-based products to act as carriers for probiotic cultures (**Paper VI**).

In most cases these probiotic bacteria of human origin, are not suitable to alone develop a good sensory characteristic in terms of flavour and aroma. They are in most cases used as dietary adjuncts in products and must undergo a co-fermentation with at least one culture, e.g., yoghurt culture (Kailasapathy and Rybka, 1997). Therefore, the

survival of these probiotic cultures was investigated both when used as pure cultures but also when they were co-fermented with a traditional yoghurt culture (V2) (**Paper VI**). The National Yoghurt Association (NYA) in USA has recommended that fermented products should have at least 10^8 cfu ml^{-1} at the time of manufacture, in order to be permitted to place "live and active culture" on the label (Roberts and Maust, 1995). Except for the *Lb. acidophilus* strain in the M40 and the G40 media, the probiotic bacterial strains that were studied fulfilled this criterion when used as pure cultures (**Table 6.5**, **Paper VI**). As most of these fermented products are used as semi-fresh products it is suggested that a good survival (~10^6 cfu ml^{-1}) after 30 days of shelf life is appropriate. The *Lb. reuteri* strain the bacterial strain had the highest survival over the storage period. *Lb. reuteri* can ferment maltose, the main fermentable carbohydrate in the M40 medium and has also been reported to grow well in an oat-based soup product (Johansson *et al.*, 1993). *B. bifidum* has been reported to grow poorly in milk and needs in general cooperative growth of *Lb. acidophilus* to reach reasonable numbers (Klaver *et al.*, 1990).

In the different oat-based products there was good growth of the *B. bifidum* strain when grown as a pure culture (**Paper VI**). It is well known that *Bifidobacterium* species metabolise a wide range of indigestible polysaccharides and oligosaccharides, including isomaltooligosaccharides, to mainly acetic and lactic acid (Mitsuoka, 1992). As the oat-based suspension (16% dry matter) contains about 3% maltodextrins and 0.6% β-glucans, these components seem to be good supports for the growth of these bacteria, which has also been confirmed by Jaskari et al. (1993, 1998). The fact that the β-glucan concentration decreased after fermentation of the *B. bifidum* strain also supports these reports. As unaffected levels of β-glucans are desirable in these oat products it is of importance that bacterial strains are screened for their ability to affect the β-glucan before they are introduced in new, fermented, oat-based products.

One of the major hurdles in terms of maintaining high survival of probiotic bacteria in fermented dairy products during storage is the low pH level (<4.5) and the risk of "over acidification" mainly due to a possible growth of *Lb. delbrueckii* subsp. *bulgaricus* if present. This is mainly caused by an increased accumulation of D(-)-lactic acid (Marshall, 1991). The temperature during storage is therefore crucial to avoid this undesirable production of lactic acid during storage (Kneifel *et al.*, 1993). A storage temperature of <4°C is ideal in terms of high bacterial survival rates, but a

temperature of about 6ºC, as used as storage temperature in **Paper V** and **VI** may be a more realistic condition. When the yoghurt culture (V2) was included as a starter culture the final pH decreased to about 3.9-4.1 for all products. The survival for all of probiotic strains decreased in this case (**Figure 6.5**, **Paper VI**). This was most evident for the *B. bifidum* strain that was found in low numbers (~10^3 cfu ml^{-1}) after co-fermentation with the yoghurt culture. This result confirms reports saying that growth of *B. bifidum* is retarded below pH 5 (Costello, 1993). It is generally accepted that the growth of *Lb. acidophilus* and *B. bifidum* is inhibited in the presence of high numbers (>10^6 cfu ml^{-1}) of *Lb. delbrueckii* subsp. *bulgaricus*. This is not only due to the production of lactic acid but also to the amount of hydrogen peroxide that is produced (Gilliand and Speck, 1977).

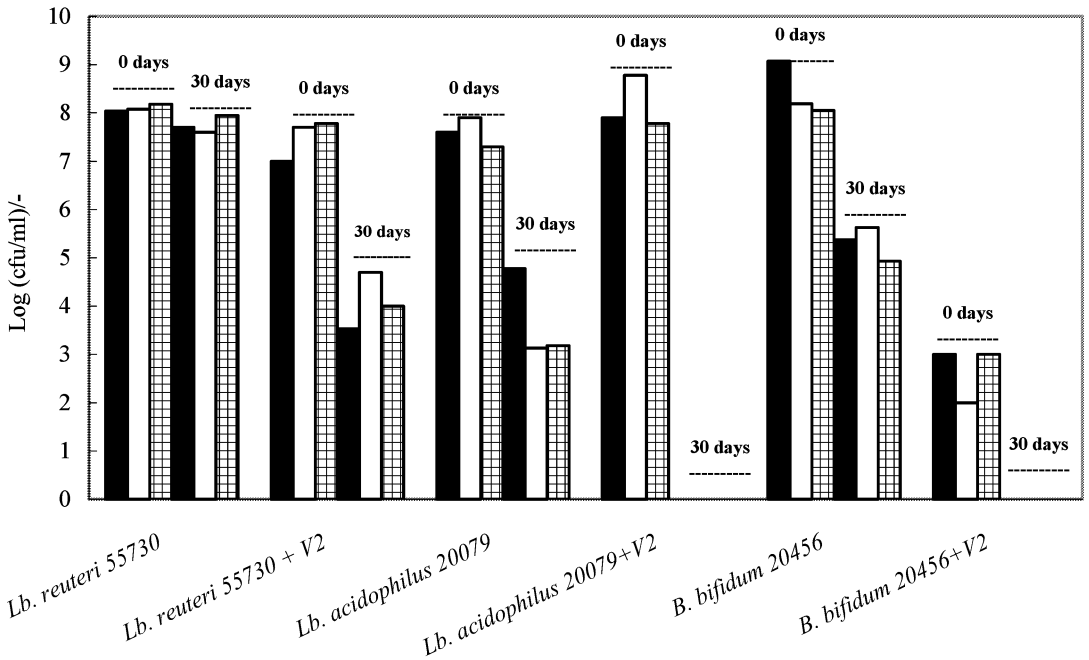


Figure 6.5 The effect of an yoghurt culture (V2) on the survival of probiotic cultures in M40 (■), MG20 (□) and G40 (⊞) after 16 h of fermentation (0 days of storage), with these cultures and after 30 days of storage at 6°C (**Paper VI**).

6.4.3.1 Process technology to increase the survival of probiotic bacteria in foods

Several technological approaches have been suggested to increase the survival rates of probiotic bacteria in fermented food products. By terminating the fermentation process at pH levels higher than 5, in combination with very low temperature (1-2ºC) during the storage period has been shown to increase the survival of *Lb. acidophilus* and *B. bifidum* (Sakai *et al.*, 1987). Using a two-step fermentation process, in which the probiotic culture is inoculated first, followed by the yoghurt culture, has been shown to give similar results (Lankaputhra and Shah, 1997). Other approaches that have been suggested to increase the survival of probiotic bacteria are to improve the buffering capacity of the food (Suriadi *et al.*, 1994) the use of hydrostatic pressure to prevent after-acidification (Tanaka and Hatanaka, 1992) or a "heat shock" procedure (58ºC for five minutes) (Marshall, 1992). Using incubation temperatures at the lower range (35-37ºC) has been shown to increase the viability of bifidobacteria (Costello, 1993). The use of microencapsulation of bacterial cultures has attracted increased interest in recent years (Siuta-Cruce and Goulet, 2001), with some promising results on survival in different food systems for both LAB and *Bifidobacterium* strains (Ravula and Shah, 1999; O´Riordan *et al.*, 2001).

6.4.4 Media for the enumeration of different LAB in oat-based products

The methodology for the enumeration of yoghurt bacteria has been established earlier (IDF, 1983). These suggested media were used when enumerating the yoghurt bacteria used in **Paper IV-VI**, i.e. MRS (De Man *et al.*, 1960) for *Lb. delbrueckii* subsp. *bulgaricus* and M17 (Terzaghi and Sandine, 1975) for *S. salivairus* subsp. *thermophilus*. The *P. damnosus* 2.6 has been reported to grow well at a relatively high concentration of ethanol (6% (v/v)) (Dueñas *et al.*, 1997) and as this ethanol concentration inhibits the growth of yoghurt bacteria, it was a suitable medium to use for the enumeration of this strain (**Paper V**). Many different media have been proposed for the enumeration of probiotic bacteria (Shah, 2000). A standard methodology for the enumeration of *Lb. acidophilus* in fermented milk products has been suggested (IDF, 1995) which includes MRS with the supplementation of bile salts (Collins, 1978) This modified medium was also used for the enumeration of *Lb.*

reuteri (**Paper VI**). The *B. bifidum* strain used in our study was sensitive to bile salts even at small concentrations. Bifidobacteria and their sensitivity to bile salts have been reported earlier (Kociubinski *et al*., 1999), who showed that more than 80% of the investigated strains showed sensitivity to bile. In general, media for the enumeration for *B. bifidum* have been disputed and many different methodologies have been suggested (Vinderola and Reinheimer, 1999). One recent study showed that the medium suggested by Lapierre et al. (1992) gave effective enumeration of *B. bifidum* strains in the presence of yoghurt cultures (Vinderola *et al*., 2000). This medium was also used in **Paper VI**.

Finally, enumeration of bacteria, especially when selected from a mixed culture, should not be generalised. The bacterial growth on a selective medium can be strain dependent. A new technique for the viability assessment of probiotic bacteria in food products is currently being reported using *in situ* assessment with a laser microscopy technique (Auty *et al*., 2000). However, these techniques are not yet realistic for use in a microbiological laboratory in the food industry.

NUTRITIONAL EFFECTS OF FERMENTED, OAT-BASED PRODUCTS

To study the nutritional effects of different fermented, oat-based products two approaches were taken. Firstly, the influence of the diets on the microflora was studied by measuring changes in the conversion of cholesterol to coprostanol, the amounts of faecal, short chain fatty acids (SCFA) and the concentrations of serum cholesterol in germfree (axenic) rats (GF) and conventional (holoxenic) rats (CONV) (**Paper VII**). Secondly, a clinical intervention-study on 56 healthy volunteers was carried out for 8 weeks. Changes in plasma cholesterol concentrations, blood glucose, and conversion of cholesterol to coprostanol were measured together with changes in the faecal microflora both in terms of total bacterial count and the count of bifidobacteria (**Paper VIII**).

7.1 Effect of oat-based products on cholesterol metabolism

Fat transport in all mammals proceeds by the action of lipoproteins, which consist mainly of proteins, cholesterol, phospholipids, triglycerides, esters of cholesterol and fat-soluble vitamins (Friedman and Nylund, 1980). Generally, there are four major classes of lipoproteins: Chylomicrons (CM), very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Of the mentioned lipoproteins, LDL contains the highest concentration of cholesterol (about 45%). The main role of the LDL particle is to provide the cells with cholesterol (Friedman and Nylund, 1980). It is well-known that a high concentration of LDL in blood plasma is one of the main factors causing *atherosclerosis*, which may be a first stage in the developing of *ischaemic heart disease* (IHD) (Boston and Cupper, 1996; Seman *et al.*, 1999).

In fact, based on clinical data, it has been suggested that each 0.026 mmol/l increment in LDL cholesterol causes an increased risk of coronary disease by 1% (Mensink and Katan, 1992; Pearson, 1998). Efforts to reduce low-density lipoprotein (LDL) cholesterol in plasma by diet therefore play an important role in the prevention of IHD (FAO/WHO, 1982).

Oat-based diets were fermented with *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772, *Lb. brevis* G-77 or *Ped. damnosus* 2.6, to study their effect on cholesterol metabolism in two different rat models, germfree (GF) rats and conventional (CONV) rats (**Table 7.1**, **Paper VII**). The potential of oat-based products to decrease the concentration of cholesterol has been well documented over recent years in rats (Shinnick, 1990; Mälkki *et al.*, 1992) but also in other kinds of rodents, such as hamsters (Wang *et al.*, 1997; Rieckhoff *et al.*, 1999). Fermented milk and its effect on cholesterol has been investigated thoroughly by animal studies (Rao *et al.*, 1981; Gilliand *et al.*, 1985; Sharpe *et al.*, 1994; Beena and Prasad, 1997) and involved intake of including fermented, ropy, dairy milk (Nakajima, *et al.*, 1992). All rats used in **Paper VII** had been on ordinary rodent diet before starting with the fermented, ropy, oat-based diets (**Table 7.1**).

Table 7.1 Composition (g/100 g) of four experimental diets (with a dry matter of 95-97%) and one ordinary diet for rodents (**Paper VII)**

	Diets					
Components	2.6[1]	G-77[2]	2772[3]	MM[4]	RD[5]	R36[6]
Protein	12.2	12.1	11.6	11.7	9.3	18.5
Dietary fibre	7.1	7.0	6.7	7.0	1.2	3.5
β-glucans	3.8	4.2	4.1	3.8	ND[7]	ND[7]
Carbohydrates	76.5	75.8	76.7	71.9	70.3	70.7
Fat	6.6	5.7	6.4	11.3	7.3	4.0

[1]G40 product fermented with *Pediococcus damnosus* 2.6.
[2]G40 product fermented with *Lactobacillus brevis* G-77.
[3]G40 product fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772.
[4]Mill Milk™ product (oat-based control product).
[5]Rice Dream® product (rice-based control product).
[6]R36, ordinary diet for rodents (Lactamin, Vadstena, Sweden).
[6]Not determined.

No reduction in serum cholesterol was seen in the different groups (**Paper VII**). It is well known that mice and rats have a cholesterol metabolism that gives higher levels of HDL compared to LDL and are relatively resistant to hypercholesterolemia (Paigen *et al.*, 1990, 1994), whereas it is the other way around in humans and particularly men (Kirby *et al.*, 1981).

Human studies have been carried out to study the effect of fermented dairy products on cholesterolemia since the 1970s (Hepner *et al.*, 1979). Despite all clinical studies conducted since then, the effects of fermented dairy milk on serum cholesterol concentrations are somewhat ambiguous (St-Onge *et al.*, 2000).

The clinical study presented in **Paper VIII** showed that an intake of a fermented, ropy, oat-based product that gave 3.5 g β-glucans per day for 5 weeks decreased the levels of plasma cholesterol by approximately 5% compared with the group eating the placebo product (yoghurt) (see **Chapter 6**, **section 6.3** for details concerning the fermented products). This reduction is at the same level as was seen in previous studies on different oat-based, non-dairy milk products (Önning *et al.*, 1998, 1999).

7.1.1 Effect of oat-based products on the microbial conversion of cholesterol to coprostanol

Intestinal cholesterol can either be absorbed or eliminated or undergo a microbial conversion, with the major end metabolite being coprostanol (Midtvedt, 1999). The intestinal microflora is very active in metabolising sterols and steroid hormones, resulting in a microbial transformation of cholesterol to coprostanol (Danielsson and Gustafsson, 1959). This microbial transformation of cholesterol to coprostanol has been related so far to only a few species, namely, *Eubacterium lentum* and *E. coprostanoligenes* (Sadzikowski *et al.*, 1977; Eyssen *et al.*, 1973). In humans these coprostanol-forming microorganisms are not active until several months after birth (Gustafsson, 1982). GF rats lack this elimination of cholesterol by microbes, described as the microbial excretion-knife (Midtvedt, 1987). Higher levels of serum cholesterol have been determined in GF than in CONV rats (Danielsson and Gustafsson, 1959). Since, as already mentioned, the microflora is active in metabolising sterols and steroid hormones, this microbial conversion of cholesterol to coprostanol may be of importance for the serum cholesterol level (Danielsson and Gustafsson, 1959). The intestinal microbial conversion of cholesterol decreased in

male rats fed on the G-77 and the MM diet, whereas in female rats this decrease was not evident (**Paper VII**). This difference between male and female rats in the microbial conversion of cholesterol to coprostanol was, however, not verified by the serum lipid analysis. However, an indication was observed that the amount of coprostanol was higher in the male rats than the female rats fed on the diet fermented with the *Ped. damnosus* 2.6 strain (**Paper VII**). This difference between the sexes was also seen in the clinical study, in which the amount of coprostanol was higher in the male subjects than in the female subjects after eating the product fermented with the *Ped. damnosus* 2.6 strain (data not shown). A trend towards higher faecal coprostanol concentration was, however, seen for the whole group eating the fermented, ropy oat-based product (**Paper VIII**). This may be an indication that the rate of microbial conversion of cholesterol to coprostanol may be influenced by diet and that there can be a difference between male and female in the microbial conversion of cholesterol.

7.2 Effect of oat-based products on faecal short chain fatty acids (SCFA)

7.2.1 Background and physiological importance of SCFA

Short-chain fatty acids (SCFA) are a common name of a group of monocarboxylic acids. Quantitatively, the main SCFA in the intestinal tract are acetic, propionic and butyric acids. These acids constitute to about 90% of the total SCFA concentration in man (Cummings, 1981). SCFA are products of anaerobic microbial (anabolic and catabolic) metabolism in the intestine of carbohydrates and proteins, both of dietary and endogenous origin (Cummings, 1981; Fleming and Arce, 1986). More than 95% of the SCFA are rapidly absorbed and metabolised by the organism, which utilises SCFA as an energy source that would otherwise be lost and excreted in the faeces (Ruppin *et al.*, 1980; Holtug *et al.*, 1995). The main absorption occurs in the colon and is concentration-dependent. Under normal circumstances about 95-98% of the amount of SCFA generated in the colon is absorbed (Ruppin *et al.*, 1980; Fleming and Arce, 1986 Sellin *et al.*, 1994). For butyrate the epithelial tissue of the digestive tract takes nearly all up and only trace amount enters the blood circulation (Bergman, 1990). The importance of SCFA production as a process of digestion is apparent in ruminants i.e. foregut fermentors, in which about 70-80% of the energy requirements

in the animal are met in this way (Bugaut, 1987; Bergman, 1990). In hindgut fermentors e.g. rats, rabbits and horses, energy from SCFA, which covers about 30% of their maintenance energy requirements (Glinsky *et al.*, 1976; Imoto and Namioka, 1978). In humans it is much less amounts, about 5-10% of the energy requirements that are covered from SCFA in healthy individuals on a Western diet (McNeil, 1984).

The microbial production of SCFA from fermentation of indigestible carbohydrates is an important mechanism for carbohydrate and energy conservation and may play a role in various diseases of the colon (Fleming and Arce, 1986; Høverstad, 1989; Cummings and Macfarlane, 1991; Mortensen and Clausen, 1996). Among others SCFA reduces the osmotic load and there is increasing evidence that the individual SCFA have specific roles, including beneficial health implications (Henningsson, *et al.*, 2001).

It has been suggested that the significance of SCFA on the host has five major aspects:

(i) *Regulation of the microflora.* SCFAs are know to have an antimicrobial effect and may therefore be an important factor in the establishment of a balanced ecosystem in the gut, preventing colonization by pathogenic microbes such as *Salmonella* and *Shigella* (Hentges, 1983; Prohaszka *et al.*, 1990).

(ii) *Maintenance of fluid and electrolyte balance in the gut:* During the absorption of SCFAs there is a stimulation of the uptake of sodium chloride and water, which is associated with a rise in pH and bicarbonate concentration in the luminal contents (Argenzo *et al.*, 1975; Cummings and Macfarlane, 1991; Sellin, 1994).

(iii) *Provision of energy to the host:* The composition of the diet has however, a strong influence. In a diet rich in fibre the contribution of the colonic fermentation to the overall energy balance in man can be as high as 20-25%, whereas in a typically Western diet the figure is suggested to be nearer 5-7% (McNeil, 1984; Cummings and Macfarlane, 1991).

(iv) *Nutrition to the epithelial cells:* SCFAs and especially butyric acid, are the main source of fuel, about 70% of the requirements of the colocytes (Roediger, 1990; Cummings and Macfarlane, 1991; Mortensen and Clausen, 1995). The lack of

SCFAs or diminished cellular utilisation of n-butyric acid has been implicated in the pathogenesis of colonic disorders such as ulcerative colitis and irritable bowel symptoms (Mortensen *et al.*, 1987; Rabassa and Rodgers, 1992; Cummings 1997).

(v) Colonic cancer: n-Butyric acid has been shown to affect cellular enzymes associated with the cell differentiation. Its effect, namely, prolongation of the doubling-time and reduction in growth rates of several human colorectal adenocarcinoma cell lines has been shown *in vitro* (Mortensen and Clausen, 1995; Scheppach *et al.*, 1995) as has the stimulation of apoptosis (programmed cell-death) (Hague *et al.*, 1995).

7.2.2 Concentration of faecal SCFA in conventional and germfree rats fed on oat-based products

Even if SCFA in faeces comprise only a minor fraction of the total amount generated by the intestinal ecosystem, the faecal concentration may carry valuable information about the latter. In GF animals, faeces do not contain SCFA (Høverstad *et al.*, 1985) and the establishment of a microflora, for instance, in infants, is accompanied by a major increase of SCFA in the faeces (Midtvedt and Midtvedt, 1992). The addition of fibre to a low fibre diet increases concentrations of faecal SCFA while normal dietary variations in fibre intake do not lead to significant changes in SCFA concentrations (Cummings and Macfarlane, 1991). This can explain why no change in the total amount of SCFA was seen in rats after being fed on the oat-based diets as the ordinary rodent diet also contained a considerable amount of fibre (**Table 7.1**, **Paper VII**). The amounts of SCFA contained in the various experimental diets ranged from 15-30 mmol kg^{-1} when distributed to the rats. The same relative amount of SCFA was seen in the GF rats after 21 days on the different diets. The SCFA measured in the faeces of the GF rats can therefore be considered as being solely of dietary origin, taking into account the amounts that have been absorbed during the gastrointestinal transfer. The amounts of SCFA in faecal samples from the GF rats were significantly lower than in the CONV rats, which is in line with the fact that SCFA are products of microbial fermentation (Høverstad and Midtvedt, 1986). Depending on the diet different amount of SCFA originating from the diet will reached the colon (**Figure 7.1**). A higher amount of acetic acid was found in the faeces of the rats fed on the rice-based diet

compared to the groups on the oat-based diet. A tendency towards a higher amount of butyric acid in the faeces from the groups on the oat-based diets was also found compared to the group on the rice-based diet (**Paper VII**). This indication follows the results from Bach-Knudsen et al. (1993), which showed an increase of butyric acid in the large intestine of pigs on a diet that consisted mainly of oat bran.

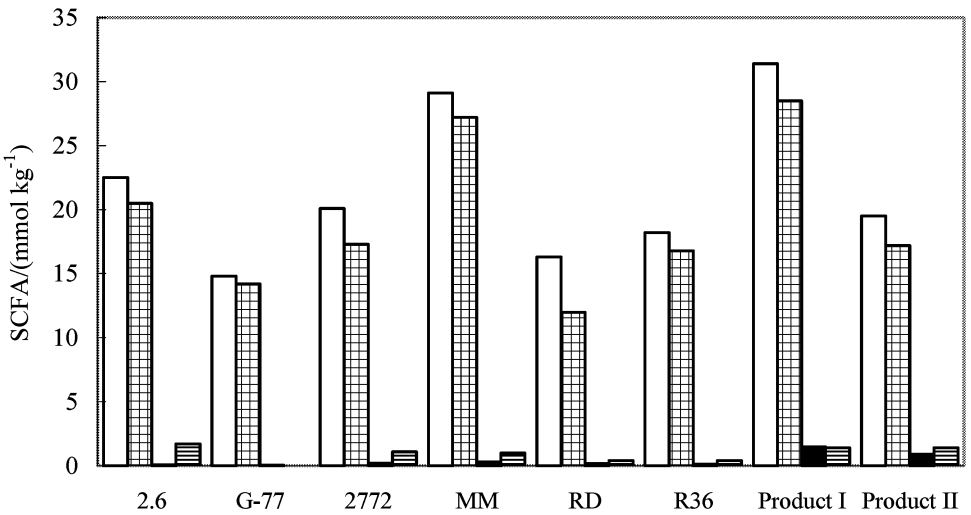


Figure 7.1 Total concentrations (mmol kg^{-1}) of SCFAs (□) and of acetic (⊞), butyric (■) and propionic (▤) acids in the three different fermented, ropy, oat-based diets (2.6, G-77, 2772), two commercial, non-dairy products based on oats (MM) and rice (RD), and a commercial diet for rodents (R36, Lactamin, Vadstena Sweden) all used in **Paper VII**, compared with two other commercial rodent products (product I and product II, Ewos AB, Södertälje, Sweden).

7.3 Effect of oat-based products on the faecal microflora

7.3.1 Background and methodology

The intestinal tract of humans contains a complex bacterial ecosystem that is usually referred to as the normal existing microflora. This flora consists mainly of obligatory anaerobic bacteria (Finegold *et al.*, 1983; Hill, 1995). It is generally considered that the normal intestinal microflora flora plays an important role in human health and disease through its involvement in the nutrition, pathogenesis and immunology of the host (Gibson and Roberfroid, 1995). The normal flora provides resistance to

colonization and might stimulate immune responses to potentially pathogenic bacteria (Goldin and Gorbach, 1992; Kimura *et al.*, 1997). The diet has, undoubtedly, a major impact on the intestinal microflora and effects the composition of the ecosystem in the gastrointestinal tract in various ways depending on its nutritional composition of the diet (Gibson and Roberfroid, 1995). To investigate the effect of the different, fermented, oat-based products on the ecosystem in the GI-tract, samples of faeces were used in an attempt to investigate changes in microbial composition in faeces after the subjects had included these products in their diet (**Paper VIII**).

By using molecular tools it is possible to study the composition of intestinal flora in a culture-independent way based on r-RNA (Amann *et al.*, 1995). The detection of 16S r-RNAs, or corresponding genes, in the GI-tracts of humans and different mammals by using PCR amplification or dot blot hybridisation with specific probes also by studying the gut ecology has been successful when tracking pathogenic or probiotic strains (Yamamoto *et al.*, 1992; Kaufmann *et al.*, 1997; Lin *et al.*, 1997). Molecular tools have been used to demonstrate the biodiversity of the intestinal flora and the diversity and dynamics of *Bifidobacterium* and *Lactobacillus* strains isolated from faecal samples (Wilson and Blitchington, 1996; McCartney *et al.*, 1996; Kimura *et al.*, 1997). In the human intestinal microflora, *Bifidobacterium*, is the third most common genus after the genera *Bacteroides* and *Eubacterium* (**Figure 7.2**) (Macfarlane *et al.*, 1995).

Due to the potential use of bifidobacteria in the treatment and prevention of gastrointestinal disorders (Orrhage *et al.*, 1994) developments in molecular techniques have been made to enumerate bifidobacteria to a high accuracy in faecal samples. Here, a more direct method for the identification of single cells within complex ecosystems is fluorescent *in situ* hybridization (FISH) with specific 16S r-RNA-based oligonucleotide probes (Amann *et al.*, 1995; Harmsen *et al.,* 1997; **Paper VIII**). This technique has been used to quantify bifidobacteria in the human gut (Langendijk *et al.*, 1995) and for the detection of *Salmonella* in the gut of mouse (Licht *et al.*, 1996). This use of the FISH technique to detect bifidobacteria has shown that these are an important group of bacteria and can be enumerated at least to the same level of accuracy as conventional and more laborious cultivation techniques (Langendijk *et al.*, 1995).

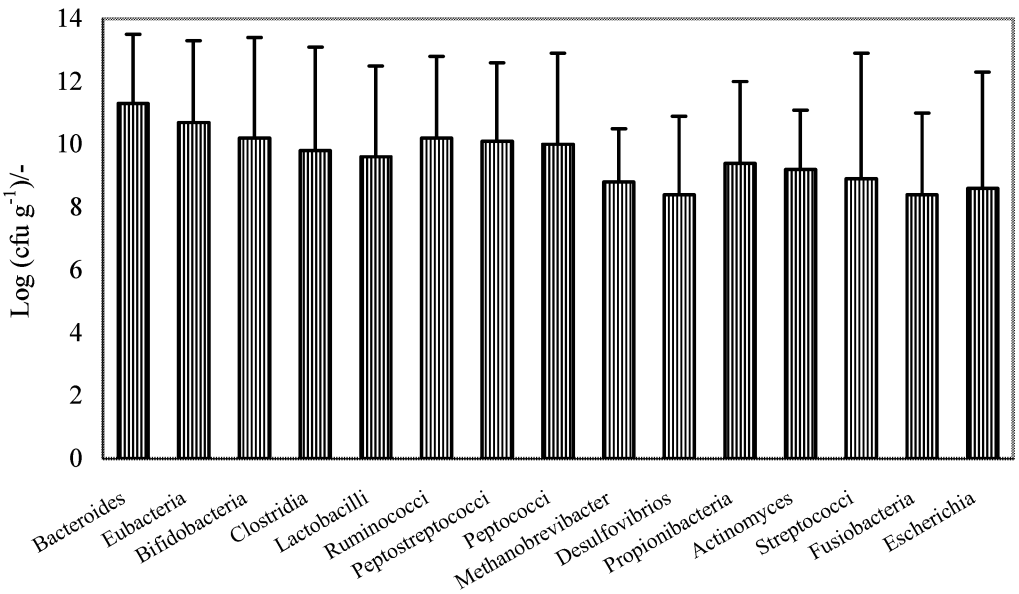


Figure 7.2 Main types of bacteria (log cfu g^{-1} dry weight of faeces) found in the human large intestine and their different range. Bacteroides, Desulfovibrios, Fusiobacteria and Escherichia are considered as gram negative (G-), all other bacteria are gram positive (G+) (Adapted from Macfarlane *et al.*, 1995).

7.3.2 Total count of bacteria in human faecal samples

In rat experiments oat bran with no added bacterial cultures, has been shown to increase the content of *Lactobacillus* and also bifidobacteria in the faecal flora (Ryhänen *et al.*, 1993, 1996). There was a significant increase of total bacterial count in the faecal samples from both of the groups, eating the fermented, ropy oat-based product or the dairy-based product (yoghurt) (**Paper VIII**). This indicates that a continuous administration of fermented products during 8 weeks increases the total bacterial count in the gastrointestinal tract.

7.3.3 Total count of Bifidobacterium *spp. in human faecal samples*

In vitro studies have shown that isolated β-glucan stimulates the growth of *Bifidobacterium* strains (Jaskari *et al.*, 1993, 1998). In **Paper VI** it was shown that *Bifidobacterium bifidum* DSM 204 56 decreased the β-glucan content in an oat-based product during fermentation. In the clinical study there was a significant increase of

bifidobacteria in the faecal samples from the group eating the fermented, ropy oat-based product. No change of faecal bifidobacteria was seen in faecal samples from the group eating the dairy-based product (yoghurt) (**Paper VIII**). The initial values of bifidobacteria that were obtained in the faecal samples before the administration of the test products were high compared to what were obtained in a earlier study (Johansson *et al.,* 1998). The variation of *Bifidobacterium* in human faeces can, however, be considered as great (**Figure 7.2**). Nevertheless, this indicates that a fermented, oat-based product containing both native and microbial β-glucans can increase the faecal count of bifidobacteria and may therefore be considered as a product with a prebiotic potential towards these microorganisms in the gastrointestinal tract.

CONCLUDING REMARKS

The main conclusions of this work are the following:

A non-dairy, oat-based product with maltose as the main fermentable carbohydrate source was able to support the growth of different kinds of lactic acid bacteria (LAB) (**Paper I**).

It was possible to change the structure in oat-based suspensions using EPS-producing strains both as pure cultures (**Paper I-III**) and in combination with other LAB that do not producing EPS (**Paper II**).

A higher viscosity was observed in the oat-based suspension containing mainly maltose as the fermentable carbohydrate source when an EPS-producing strain was combined with non-EPS-producing LAB (**Paper II**).

It was possible to formulate a fermented, non-dairy product with a texture close to that of yoghurt (**Paper IV)** and also one with a ropy characteristic using an EPS-producing strain (**Paper V**).

Oat-based, non-dairy suspensions with different carbohydrate profiles were shown to be suitable carriers for different LAB. This was shown for yoghurt cultures (**Paper IV-VI**), an EPS-producing strain (**Paper V**) and human isolated strains recognised as probiotics (**Paper VI**).

Oat-based diets gave a faecal SCFA pattern in conventional rats that differed from the group fed on rice (**Paper VII**). No effect on the serum cholesterol in two different rat models, conventional and germfree rats, was however observed.

A fermented, ropy, non-dairy product decreased total cholesterol and LDL in healthy volunteers compared to the control group (**Paper VIII**). This fermented product provided a total intake of 3.5 g native β-glucans from oats per day. An increase in

faecal *Bifidobacterium* spp. was also seen this group. This shows that a fermented ropy, oat-based product can give a lipid-lowering effect that is normally recognised in oats and in addition, function as potential prebiotic product, i.e., be substrate for bacteria that are considered as a part of the beneficial microflora in the gastrointestinal tract.

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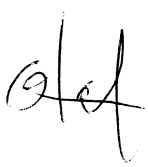

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