The effect of water soluble fat replacers and fat reduction on the growth of *Lactobacillus sakei* and *Listeria monocytogenes* in broth and pork liver paté

Simbarashe Samapundo a, *, Ramize Xhaferi a, Slawomir Szczepaniak b, Olivier Goemare b, Liselot Steen b, Hubert Paelinck b, Frank Devlieghere a

*Ghent University, Faculty of Bioscience Engineering, Food2Know, Department of Food Safety and Food Quality, Laboratory of Food Microbiology and Food Preservation, Coupage Links 653, 9000 Ghent, Belgium
b Catholic University College Ghent, Research Group for Technology and Quality of Animal Products, Department Industrial Engineering, Gebroeders Desmetstraat 1, 9000 Ghent, Belgium

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**A B S T R A C T**

The major objective of this study was to evaluate the effect of water soluble fat replacers on the growth of bacteria of importance to processed meats. The first part of the study evaluated the effect of water soluble fat replacers (Beneo™ GR, Beneo™ HPX, STA-LITE WSIII™, Fibersol-2 and Nutriose) on the growth of *Lactobacillus sakei* and *Listeria monocytogenes*. Fibersol-2 appears to have the greatest potential for replacing fat without compromising microbiological stability as increase in its water phase concentration resulted in a significant increase in the lag phases of both *Lb. sakei* and *L. monocytogenes*. On the other hand, Beneo™ GR stimulated the growth of *L. monocytogenes*, implying that its use could compromise microbiological safety. In the second part of the study pork liver paté and low fat paté (30% less fat) were challenged with *L. monocytogenes*. The low fat paté was determined to be microbiologically less stable than the reference paté. This was a result of the low fat paté having significantly higher water activity and pH values than those of the reference paté. The results of this study are of importance to the meat processing industry to make more informed decisions when reducing and/or replacing fat.

1. Introduction

Fat reduction efforts in the food industry have largely been driven by the association of fat-rich diets with the increased risk of obesity, coronary heart disease and some types of cancer (AHA, 1996; Cengiz & Gokoglu, 2005). In The United States it has been recommended that the total dietary intake of fat should not exceed more than 30% of the total daily energy intake, with saturated fats contributing a maximum of 10% and polyunsaturated fats accounting for at least two-thirds of the total daily intake (USDA & USDAHHS, 1995). Although fat intake in industrialized countries has been reported to be declining due to the increased availability and popularity of low and reduced fat products, fat consumption is still above the recommended levels and the prevalence of the population classified as overweight is still increasing (Frazao, 1996).

Fat contributes to the flavour, texture, mouthfeel and overall sensation of lubricity of meat products (Mendoza, García, Casas, & Seglas, 2001). Therefore, reduction of the fat content can affect the acceptability of a product (Giese, 1996). Traditional techniques for reducing fat without significantly affecting the sensory and functional properties have been through the use of leaner materials and the addition of water (Drake & Swanson, 1995). Fat may also be replaced by reformulating the foods with lipid-, protein- or carbohydrate-based ingredients individually or in combination (Akoh, 1998; Drake & Swanson, 1995). These substances are collectively known as fat replacers and consist of two distinct groups – (i) fat substitutes and (ii) fat mimetics (Akoh, 1998). Fat substitutes are mostly lipid- or fat-based macromolecules that physically and chemically resemble conventional fats and oils and therefore can replace fat on a weight basis (ADA, 2005). Fat mimetics are substances that can imitate the organoleptic or physical properties of triglycerides. Unlike fat substitutes, fat mimetics...
cannot be used to replace fat on a weight basis (ADA, 2005). Fat mimetics are usually water soluble protein- or carbohydrate-based products with a reduced caloric content of 0–4 kcal/g. A large and growing number of fat substitutes and mimetics are now available commercially.

Most of the studies on fat reduction and/or replacement in meat products have focused on the sensorial and functional aspects. In contrast, only a few studies have considered the consequences of fat reduction and/or replacement on the microbiological stability and safety of food products. These include Bloukas, Paneras, and Fournitzis (1997a, 1997b) who investigated the effect of replacing pork back-fat with liquid olive oil in fermented sausage formulations and Papadima and Bloukas (1999) who investigated the effect of reducing the fat level of traditionally processed Greek sausages on their quality during storage. With regards to commercial fat replacers, Nowak, von Mueffling, Grotheer, Klein, and Watkinson (2007) also evaluated the use of a long chain inulin fat mimetic, Fibruline XL® to replace fat in German-type mortadella sausages. They determined that the use of Fibruline XL® at levels up to 12% did not affect the microbiological stability of the sausages. In addition to this, some of these studies have also investigated the effect of fat replacers on the development and activities of beneficial microbial flora during the ripening of fermented meat products. Mendoza et al. (2001) evaluated the use of the fat mimetic Raftiline® (inulin) to replace fat in low-fat dry sausages with 25% less fat than the reference product. They reported that the use of Raftiline® did not affect the development and activities of the microbial flora during ripening.

dos Santos, Campagnol, Pacheco, and Pollonio (2012) evaluated the use of NutraFlora®, a fructooligosaccharide based fat mimetic, as a fat replacer in low fat cooked fermented sausages with 50% less pork back-fat. They reported that use of up to 9% NutraFlora® did not affect the microbial flora during production (fermentation). However, they determined that after 60 days of storage the lactic acid bacteria and total mesophilic counts on the cooked fermented sausages with 50% less pork back-fat and 6% NutraFlora® were significantly \( P < 0.05 \) less than those on the reference full fat sausages.

Given the increasing availability (and number) of commercial fat replacers, it has become necessary to assess the potential consequences of their use on the microbiological safety and stability of food products. This study had the major objectives of i) evaluating the effects of several types of selected water soluble fat replacers on the growth of Lactobacillus sakei and Listeria monocytogenes in broth and ii) determining the consequences of fat reduction on the stability of pork liver paté towards L. monocytogenes via challenge tests. L. monocytogenes has been reported to occur and grow to infective doses in paté (de Boer & Van Netten, 1990; Farber & Daly, 1994; Farber, McKellar, & Ross, 1995) whilst Lb. sakei is a commonly occurring psychrotrophic lactobacilli which becomes dominant when processed meat products are stored under anaerobic conditions (Devlieghere, Debevere, & Van Impe, 1998; Samelis, Kakouri, & Van Impe, 2000). This study is a follow-up to previous studies we have performed on the consequences of NaCl reduction and/or replacement on the microbiological stability and safety of cooked ham and white sauce (Samapundo et al., 2010, 2013).

2. Materials and methods

2.1. Isolates

L. monocytogenes LFMPF 235 and Lb. sakei LFMPF 221 (both isolated from cooked ham) were used in this study. These are maintained in the culture collection of the Laboratory of Food Microbiology and Food Preservation (Ghent University).

2.2. Evaluation of the effect of water soluble fat replacers on the growth of L. monocytogenes and Lb. sakei in broth

2.2.1. Growth medium
de Man Rogosa Sharpe (MRS, Oxoíd, Hampshire, United Kingdom) broth and nutrient broth (NB, Oxoíd, Hampshire, United Kingdom) adjusted to pH 6.2 were used as the basic growth mediums for Lb. sakei and L. monocytogenes, respectively. The growth medium was supplemented with 0, 3, 6 and 10% of the following commercially available fat replacers: Beneo® GR (92% inulin, 8% glucose, fructose and sucrose, Beneo-Orafti, Oreye, Belgium), Beneo® HPX (100% inulin, Beneo-Orafti, Oreye, Belgium), STA-LITE WSII® (polydextrose, Tate & Lyle, Erembodegem, Belgium), Fibersol-2 (min. 90% soluble dietary fibre (digestion resistant maltodextrin), Matsutani Chemical Industry Co. Ltd., Hyogo, Japan) and Nutriose (soluble dietary fibre, ROQUETTE, Lestrem, France). In all cases 99 ml of broth was placed in 250 ml Schott bottles and sterilized by autoclaving (121 °C for 30 min). Duplicates were prepared per condition studied. The \( w_0 \) values of the broths prepared were then measured by an AW SPRINT TH-500 Novasina Thermoconstanter (Novasina, Pfäffikon, Switzerland).

2.2.2. Incubation, incubation and assessment of growth

to generate the inoculum, 10 ml of brain heart infusion (BHI, Oxoíd, Hampshire, United Kingdom) or MRS broth were inoculated from pure slant cultures of L. monocytogenes and Lb. sakei, respectively.

were incubated overnight at 30 °C. A second sub-culture of each isolate was made in 10 ml of the same type of broth and incubated for 16 h at 30 °C after which the tubes were transferred to 7 °C for 6–8 h to enable the isolates to adapt to the final storage temperature. Before inoculation, the temperature adapted cultures were first serially diluted to approximately \( 10^4 \) CFU/ml. Thereafter 1 ml was aseptically added to 99 ml of the sterilized broths with or without fat replacers to achieve a desired initial inoculation level of ca. \( 10^2 \) CFU/ml. 1 ml samples were immediately drawn-off aseptically from each bottle and serially diluted in physiological peptone saline (PPS) \( [8.5 \text{ g NaCl (Fluka, Germany)} + 1 \text{ g bacteriological peptone (Oxoíd, Hampshire, United Kingdom)}] \). The serial dilutions were then spread plated out on tryptone soy agar (TSA, Oxoíd, Hampshire, United Kingdom) for L. monocytogenes and poured plated (with an over-layer) on MRS agar for Lb. sakei to determine the exact initial inoculation levels. Resultant colonies were counted after incubation for up to 48 h at 30 °C.

The inoculated broths were stored at 7 °C. 1 ml samples were drawn of at regular intervals (dependent on the observed growth rate) and the counts of L. monocytogenes and Lb. sakei were determined as described above for the initial inoculation level. The pH of the media inoculated with the LAB was also determined at the end of the incubation period.

2.3. Challenge tests

Parallel to the experiments described above pork liver paté products with reduced fat levels were developed and optimized at the pilot plant of the Research Group for Technology and Quality of Animal Products (Catholic University College, Ghent, Belgium). Of several formulations investigated, only a paté with 30% less fat was selected for evaluation in the challenge tests as it most closely resembled the reference product on the basis of sensorial and functional quality (results not shown). The reference paté and paté with 30% less fat consisted of mixture of liver, pork back-fat and water at ratios of 30/40/30 and 30/13.3/56.7, respectively. Both paté’s also contained sodium nitrite (120 ppm), NaCl (18 g/kg), dextrose (5 g/kg), ascorbate (0.5 g/kg); sodium caseinate (10 g/kg),...
white pepper (2 g/kg), nutmeg (0.5 g/kg), ginger (0.5 g/kg), cardamom (0.2 g/kg) and onion powder (0.5 g/kg). The paté’s were produced as follows. Fresh pork liver was chopped for eight minutes after which the NaCl was added and chopping was done for a further two minutes. The resulting liver paste was then cooled to 7–10 °C. At the same time, the pork back-fat was broiled for 20 min and separated from the resulting cooking water. Sodium caseinate was added to the broiled pork back-fat before chopping was done for five minutes. The chopped liver paste and pork-back fat were then combined together with part of the cooking water in the ratios mentioned above, after which the rest of the ingredients were added and chopping was done at 40 °C for three minutes. The resulting liver paté emulsion was then filled into cans (250 g) which were sealed and cooked at 76 °C for three minutes. The resulting liver paté was then cooled to 4 °C.

The exact experimental details of the challenge tests were as follows. Reference paté and low fat paté were challenged with L. monocytogenes. On arrival two samples of each type of paté were collected to determine the q<sub>SW</sub> and the pH (SevenEasy pH meter, Mettler-Toledo, Urdorf, Switzerland). The initial microbiological quality of each type of paté was determined as follows. Two cans were randomly selected and samples (20 ± 1 g) were aseptically collected from each can and placed in a sterile stomacher bag. The samples were then serially diluted in PPS and spread plated out on PCA, pour plated (with an over-layer) on PCA, pour plated (with an over-layer) on MRS agar, and spread plated on YGC to determine the total aerobic, total anaerobic, lactic acid bacteria and yeast (and mould) counts, respectively. The plates were incubated at 30 °C for up to 48 h before the arising colonies were counted.

The inoculum of L. monocytogenes used in the challenge tests was prepared and temperature adopted as described above for the tests performed in broth. Inoculation was done in a laminar flow, where 100 µl of a serial dilution of the L. monocytogenes subculture at 7 °C with approximately 10<sup>6</sup> CFU/ml was added to 100 g of paté to achieve an initial inoculation level of 10<sup>5</sup> CFU/g. The paté was then thoroughly mixed (manually) before being distributed as 50 g portions in high oxygen barrier bags (dimensions = 25 cm × 30 cm × thickness of 110 µm; O<sub>2</sub> Transmission Rate = 2 ml O<sub>2</sub> (m<sup>2</sup> × 24 atm.) at 23 °C and 90% relative humidity). 32 such portions were prepared per type of paté to enable at most 16 duplicate analyses to be done in during storage. Two samples from each type of paté were collected at this point to determine the exact initial inoculation level and counts of general microbiological parameters i.e. total aerobic, total anaerobic, lactic acid bacteria and yeast counts. The methods used are described further below.

The 50 g portions were then packaged under a modified atmosphere with 30% CO<sub>2</sub> (+70% N<sub>2</sub>) using a MULTIVAC A300/42 machine (Sepp. Haggenmüller KG, Wolfertschwenden, Germany). A gas to product ratio of 2/1 was applied. 32 packages of each type of paté which were not inoculated were also prepared and packaged in the same atmosphere. After packaging the paté was placed at 7 °C. During the storage two samples (2 × packages) of each type of paté were randomly selected. The paté which was not inoculated were sampled as frequently as the inoculated paté during the first two weeks of storage, after which they were sampled once every week. 10–12 g samples were collected aseptically from each of the packages and serially diluted by PPS in sterile stomacher bag. The total aerobic, total anaerobic, lactic acid bacteria and yeast (and mould) counts were then determined as described above for the experiments performed in broth, whilst the total Listeria counts were determined by spread plating of the decimal dilutions on Agar Listeria acc. Ottaviani & Agosti (ALOA, BioMérieux Industry, Marcy L’etoile, France).

2.4. Data analysis

The flexible growth function of Baranyi and Roberts (1994) was fitted to the growth data – log<sub>10</sub> CFU/ml or log<sub>10</sub> CFU/g as a function of time (d), enabling the determination of the maximum growth rate (μ<sub>max</sub>, log<sub>10</sub> CFU/ml/d or log<sub>10</sub> CFU/g/d) and the lag phase (λ, d) for each experimental condition. The fitting was done using SPSS® version 15.0 (SPSS Inc., Chicago, Ill., USA). Significant differences were determined by comparing the 95% confidence intervals (CI) for overlap or lack thereof.

3. Results and discussion

3.1. Effect of water soluble fat replacers on the growth of Lb. sakei in broth

The estimated growth parameters of Lb. sakei, μ<sub>max</sub> (log<sub>10</sub> CFU/ml/d) and λ (d), and their 95% CI’s are shown in Table 1. As can be seen in Table 1, addition of the water soluble fat replacers at a level of 10% resulted in an insignificant (P > 0.05) increase in the μ<sub>max</sub> of Lb. sakei. It was also determined that no significant differences (P > 0.05) occurred between the effects of the different fat replacers investigated on the μ<sub>max</sub> at the same WPCs.

Some differences were observed with regards to the effect of the water soluble fat replacers on the estimated lag phase durations of Lb. sakei. In increase in concentration of Beneo HPX from 0 to 10% resulted in gradual but an statistically insignificant (P > 0.05) decrease in the lag phase of Lb. sakei from 0.3d to 0.24d. Increase in the WPCs of Beneo GR and Nutriose from 0 to 3% resulted in an statistically insignificant (P > 0.05) increase in the lag phase durations from 0.3d to 1.9d and 0.8d, respectively. Further increase in the concentrations of both Beneo GR and Nutriose from 3% to 10% resulted in a gradual but statistically insignificant (P > 0.05) decrease in the lag phase durations to 1.6 and 0.2 days, respectively. The estimated lag phases of Lb. sakei at Beneo GR and Nutriose at water phase concentrations of 6% and 10% were not significantly different (P > 0.05) to the lag phase estimated at 0%. Increase in the WPCs of STA-LITE® and Fibersol-2 from 0 to 10% resulted in an increase in the lag phases of Lb. sakei, from 0.3d to 0.7d and 2.97d, respectively. However, whilst the lag phase extension observed with STA-LITE® was not statistically significant (P > 0.05), a statistically significant increase (P < 0.05) was observed when the WPC of Fibersol-2 was increased from 0% (0.3d) to either 3% Fibersol-2 (1.5d) and 6% Fibersol-2 (2.4d). Statistically significant differences (P < 0.05) were also observed between the estimated lag phases of Lb. sakei at 3 and 10% of Fibersol-2. No significant differences (P > 0.05) were observed between the estimated lag phases of Lb. sakei at Fibersol-2 concentrations of 3 and 6% and 6 and 10%.

Some differences were also observed between the effects of the water soluble fat replacers on the lag phase duration of Lb. sakei at water phase concentrations of 6 and 10%. At a WPC of 6%, the estimated lag phase durations of Lb. sakei were significantly longer (P < 0.05) in broth supplemented with Beneo GR (1.8d) and Fibersol-2 (2.4d) than they were in broth supplement with Beneo HPX (0.2d), STA-LITE® (0.7d) and Nutriose (0.8d). At a WPC of 10%, the estimated lag phase durations of Lb. sakei were also significantly longer (P < 0.05) in broth supplemented with Beneo GR and Fibersol-2 than they were in broth supplement with Beneo HPX, STA-LITE® and Nutriose. The q<sub>SW</sub> values of all the broths prepared did not differ significantly (P > 0.05) from those of the controls (results not shown). Additionally, none of the components have any known antimicrobial activities, indicating that the differences observed were largely a result of the ability of Lb. sakei to utilize them as a carbon or nutrient source.
Only a few studies have been performed in which the effect of a commercial fat replacer on the growth of lactic acid bacteria has been explicitly reported. Ramchandran and Shah (2008a) reported that Versagel® (a modified whey protein-based fat replacer) improved the growth of Streptococcus thermophiles and inhibited the growth of Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus casei and Lactobacillus acidophilus, which are used as starter cultures in yoghurt production. As a result of differences in the ratios of lactic and acetic acid produced by the L. delbrueckii ssp. bulgaricus and L. acidophilus in the presence of Versagel®, Ramchandran and Shah (2008a) suggested that Versagel® shifted the metabolic pathways of these microorganisms. Ramchandran and Shah (2008b) reported that the use of Versagel® to produce low fat yoghurt reduced the fermentation time by 20 min compared to the control; indicating its improvement the growth of the starter culture cocktail consisting of S. thermophilus and delbrueckii ssp. bulgaricus. Mendoza et al. (2001) evaluated the use of Rafitline® (inulin) as a fat replacer in low fat dry sausages with 25% less fat than the reference product. They reported that the use of Rafitline® did not affect the development and activities of the microbial flora during ripening. Nowak et al. (2007) evaluated the use of a long chain inulin, Frubrine XL®, to replace fat in German-type mortadella sausages. They reported that the use of Frubrine XL® at levels up to 12% did not affect the microbiological stability of the sausages. dos Santos et al. (2012) evaluated the use of NutraFlora®, a fructooligosaccharides, as a fat replacer in low fat cooked fermented sausages with 50% less pork back-fat. They determined that use of up to 9% NutraFlora® did not affect the microbial flora during production (fermentation). However, it was determined that after 60 days of storage the lactic acid bacteria and total mesophilic counts on the cooked fermented sausages with 50% less pork back-fat and 6% and 9% NutraFlora® were significantly (P < 0.05) less than those on the reference full fat sausages.

### 3.2. Effect of water soluble fat replacers on the growth of L. monocytogenes in broth

The estimated growth parameters of L. monocytogenes, μmax (log10 CFU/ml/d) and λ (d), and their 95% CI’s are shown in Table 2. As can be seen the responses of L. monocytogenes to the water soluble fat replacers were in some instances different to those of Lb. sakei. With regards to the effect of the concentration of the fat replacers on the μmax, it can be seen that only the addition of Beneo GR and Beneo HPX increased the μmax of L. monocytogenes relative to the control (0%). Moreover, when Beneo HPX was added at concentrations ≥3%, the estimated μmax of L. monocytogenes were significantly (P < 0.05) faster than those at 0%. Although the μmax increased gradually from 0.560 log10 CFU/ml/d at 3% Beneo HPX to 0.665 log10 CFU/ml/d at 10%, this increase was not statistically significant (P > 0.05). Increase in concentration of Beneo GR also had a similar effect on the μmax of L. monocytogenes, however, the increase observed in this case was not significant. STA-LITE, Nutriose and Fibersol-2 had a significant effect (P < 0.05) on the μmax of L. monocytogenes. However, unlike Lb. sakei, an increase in

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<th>Fat replacer</th>
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<th>Concentration</th>
<th>μmax (log10 CFU/ml/d)</th>
<th>λ (d)</th>
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<tr>
<td>Beneo HPX</td>
<td></td>
<td>0%</td>
<td>0.525⁹&lt;sup&gt;a&lt;/sup&gt; (0.456–0.593)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.57⁹&lt;sup&gt;a&lt;/sup&gt; (3.15–5.98)</td>
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<td>Beneo GR</td>
<td></td>
<td>0%</td>
<td>0.525&lt;sup&gt;**&lt;/sup&gt; (0.456–0.593)</td>
<td>4.57&lt;sup&gt;a&lt;/sup&gt; (3.15–5.98)</td>
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<td>STA-LITE</td>
<td></td>
<td>0%</td>
<td>0.525&lt;sup&gt;**&lt;/sup&gt; (0.456–0.593)</td>
<td>4.57&lt;sup&gt;a&lt;/sup&gt; (3.15–5.98)</td>
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<td>Fibersol-2</td>
<td></td>
<td>0%</td>
<td>0.525&lt;sup&gt;**&lt;/sup&gt; (0.456–0.593)</td>
<td>4.57&lt;sup&gt;a&lt;/sup&gt; (3.15–5.98)</td>
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<td>Nutriose</td>
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<td>0%</td>
<td>0.525&lt;sup&gt;**&lt;/sup&gt; (0.456–0.593)</td>
<td>4.57&lt;sup&gt;a&lt;/sup&gt; (3.15–5.98)</td>
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<sup>*estimated growth parameter, **95% confidence interval, ***different superscript letters indicate where significant differences (P < 0.05) occurred between the various concentrations evaluated of a particular fat replacer, ****different superscript numbers indicate where significant differences (P < 0.05) occurred between the effects of the fat replacers at a particular concentration.

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<td>4.03&lt;sup&gt;b&lt;/sup&gt; (2.41–5.65)</td>
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<td>4.00&lt;sup&gt;b&lt;/sup&gt; (2.41–5.65)</td>
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<td>3.73&lt;sup&gt;b&lt;/sup&gt; (2.41–5.65)</td>
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<td>3.67&lt;sup&gt;b&lt;/sup&gt; (2.86–4.36)</td>
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<td>0.546&lt;sup&gt;b&lt;/sup&gt; (0.419–0.673)</td>
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<td>1.94&lt;sup&gt;b&lt;/sup&gt; (1.20–2.63)</td>
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<tr>
<td>STA-LITE</td>
<td></td>
<td>10%</td>
<td>0.512&lt;sup&gt;b&lt;/sup&gt; (0.465–0.558)</td>
<td>2.54&lt;sup&gt;b&lt;/sup&gt; (1.99–3.48)</td>
</tr>
<tr>
<td>Fibersol-2</td>
<td></td>
<td>10%</td>
<td>0.413&lt;sup&gt;b&lt;/sup&gt; (0.350–0.476)</td>
<td>1.94&lt;sup&gt;b&lt;/sup&gt; (1.20–2.63)</td>
</tr>
<tr>
<td>Nutriose</td>
<td></td>
<td>10%</td>
<td>0.444&lt;sup&gt;b&lt;/sup&gt; (0.388–0.500)</td>
<td>1.94&lt;sup&gt;b&lt;/sup&gt; (1.20–2.63)</td>
</tr>
</tbody>
</table>
the concentration of the dietary fibre based products, Nutriose and Fibersol-2, resulted in a decrease gradual decrease in the μmax of L. monocytogenes from 0.525 log10 CFU/ml/d in the control to 0.413 and 0.444 log10 CFU/ml/d, respectively, at a concentration of 10%. This could reflect differences in the capacity of the two isolates to utilize the fibre based fat replacers as an energy or carbon source.

With regards to the effect of the fat replacers on the lag phase, it can be seen that only the addition of Beneo GR and Beneo HPX gradually decreased the lag phase of L. monocytogenes from 4.57d (in the control) to 2.73d and 3.17d, respectively, at a WPCs of 10%. However, the reduction observed was not significant (P > 0.05) in both cases. The addition of Nutriose and Fibersol-2 had the opposite effect, increasing the lag phase from 4.57d in the control to 6.47d and 8.55d, respectively. The lag phases estimated for the growth of L. monocytogenes in the presence of up to 10% Nutriose were determined to be statistically non-significant (P > 0.05) from the control whilst those in media supplemented with Fibersol-2 were determined to be significantly longer (P < 0.05) than the controls as from a WPC of 3%. The presence of STA-LITE® did not have any significant effect on the lag phase of L monocytogenes and no particular trends could be noted.

It appears from these results that Beneo HPX and Beneo GR enhance the growth of L. monocytogenes, whilst Nutriose and (especially) Fibersol-2 partially retard growth. Beneo GR contains 8% sugars (glucose, fructose and sucrose) which can be readily utilized by L. monocytogenes, resulting in enhanced growth compared to the control, whilst the dietary fibres (Nutriose and Fibersol-2) were most likely not utilizable by L. monocytogenes. The aw values of all the broths prepared did not differ significantly (P > 0.05) from those of the controls, implying that the differences observed, especially those in the presence of Fibersol-2, relative to the controls were due to their potential to be used as a carbon source as none of the components have any known antimicrobial activities.

No studies were found in literature in which the effect of commercial fat replacers on the growth of L. monocytogenes has been evaluated.

3.3. Challenge tests

Fig. 1 show plots of the total aerobic bacteria and inoculated L. monocytogenes (counts on ALOA) as a function of time on the different types of paté evaluated. Table 3, shows the estimated growth parameters (μmax, log10 CFU/ml/d and λ, d) and their 95% confidence intervals. The reference paté and the reduced fat (−30%) had aw values of 0.976 ± 0.001 and 0.986 ± 0.001 and pH values of 6.48 ± 0.01 and 6.54 ± 0.02, respectively. According to a paired t-test performed in SPSS, the aw and pH values of the reference paté were significantly lower (P < 0.05) than those of the reduced fat paté.

The canned paté was determined from the initial samples to have total aerobic, lactic acid, yeast and Listeria counts below the detection limit i.e. <1 log CFU/g. The total aerobic, lactic acid, yeast and Listeria counts on uninoculated samples remained below the detection limit throughout the incubation period (results not shown). This indicated that the growth we observed on the inoculated pork liver paté was that of the inoculated microorganism. This assumption is also supported by the close agreement between the total aerobic and Listeria (ALOA) counts during the storage period. It can be seen in Fig. 1 and Table 3 that a significantly (P < 0.05) longer lag phase occurred on the reference paté (35d) than on the low fat paté (13d). However, the estimated μmax in the low fat paté (0.205 log10 CFU/ml/d) was insignificantly (P > 0.05) faster than that estimated for growth on the reference paté (0.152 log10 CFU/ml/d). The differences observed in the growth of L. monocytogenes may be due to the differences in the aw and pH values which were both significantly higher (P < 0.05) in the low fat paté. It can be inferred from the results of the challenge tests that reduction of the fat level by 30% results in paté which is far less stable with regards to L monocytogenes. This could potentially be the case for most pathogens.

As mentioned earlier only a handful of studies have reported the potential microbiological consequences of fat reduction in meat products. These include Bloukas et al. (1997a) who reported that low fat frankfurters (9% fat) produced with olive oil had a significantly higher moisture content than control (high fat) frankfurters (27% all animal fat), resulting in the low fat frankfurters having a shorter microbiologically determined shelf-life of three weeks at 4 °C compared to four weeks for the control. Bloukas et al. (1997b) investigated the effect of replacing 10 and 20% of pork back-fat with liquid olive oil in fermented sausage formulations. They determined that the moisture content after production did not differ significantly between the controls and sausages in which 10 or 20% of the pork back-fat had been replaced. They also reported that no significant differences occurred between the lactic acid bacteria and Micrococcaceae counts as a function of the level of pork back-fat replacement during a 26 day processing period. However, no further microbiological examinations were done after the processing period to determine if differences in the microbial shelf-stability occurred. López-López, Cofrades, and Jimenez-Colmenero (2009) determine that the use of olive oil to replace 50% of the pork back-fat in low fat frankfurters resulted in products which were microbiologically less stable. Higher total viable counts were found on the frankfurters produced with olive oil from the 14th day of storage at 4 °C. Papadima and Bloukas (1999) also reported an increase of the moisture content with a decrease in the fat level of traditionally processed Greek sausages. Sausages with 10, 20 and 30% fat had moisture contents of 69.8, 63.8 and 59.1%, respectively.

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th>Reference paté</th>
<th>Low fat paté</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmax (log10 CFU/g/d) estimate</td>
<td>0.152</td>
<td>0.205</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.133–0.171*</td>
<td>0.157–0.252*</td>
</tr>
<tr>
<td>λ (d) estimate</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>95% CI</td>
<td>33–37*</td>
<td>10–16*</td>
</tr>
</tbody>
</table>

*different superscript letters indicate where significant differences (P < 0.05) occurred between the conditions evaluated.

Fig. 1. Evolution of the counts of total aerobic bacteria and L. monocytogenes on the reference and reduced fat paté's stored at 7 °C.
Whilst the differences in moisture content did not affect the lactic acid bacteria counts during storage, it was determined that lower counts of Gram negative bacteria were obtained on sausages with 20 and 30% fat compared to those with 10% fat. As no $a_w$ measurements are stated in any of these studies it is difficult to compare any of these studies with our findings on liver paté.

4. Conclusions

At the concentrations studied, water soluble fat replacers appear to have a largely species dependent effect on bacterial growth. Fibersol-2 appears to have the greatest potential for replacing fat without compromising the microbiological safety and stability. Beneo GR could potentially reduce the safety as it stimulated the growth of $L$. monocytogenes. Reduction of fat by 30% in liver paté reduced the safety of this product with regards to $L$. monocytogenes. This was mainly a result of a significant increase in both the $a_w$ and pH in the reduced fat paté. However, it has to be mentioned that this study did not evaluate the effect of a water soluble fat replacer on the microbial stability and safety in a real food product. The actual effect of fat replacers may be different in a real food product where other factors influencing growth are present and interacting i.e. atmosphere, preservatives, competing flora etc. Therefore, future experiments evaluating fat replacers should preferentially be in the form of challenge tests.

The results from this study are of importance to the meat processing industry as they can be used (together with the more widely available data on the effects of fat replacers on the technological and sensorial quality of meat products) to make more informed decisions when reducing and/or replacing fat in meat products.

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References


