Effect of low fat Dutch style dry fermented sausages elaborated with encapsulated linseed, algal and fish oil on the cytotoxicity and proliferation of Hepa-1C1C7 cells

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Abstract

The present consumption of meat and meat products contributes substantially to the daily fat intake; however, they contain predominantly saturated fatty acids. Therefore modification of the lipid profile of these products could help to improve the nutritional quality of the western diet, in particular by enhancing n-3 polyunsaturated fatty acids (PUFAs), due to their presumed protective effects against cardiovascular diseases, cancer and obesity. Dutch style dry fermented sausages were prepared containing 10 % and 30 % fat using beef meat and ostrich meat. The pork back fat was replaced by encapsulated fish, algal and linseed oils. Lipid fractions were extracted from the different dry fermented sausages at 0 and 60 days of storage. Hydrolysed (i.e. fatty acids) lipid samples were used to determine the effect on PUFAs on Hepa-1C1C7 cell lines. The MTT-assay, which detects the level of mitochondrial activity in living cells, was used to measure the effect of PUFAs on cytotoxicity and/or cell proliferation. In most of the samples cell cytotoxicity was observed when the concentration of fatty acids was increased above of 400 μg/ml, especially in sausages manufactured with ostrich meat.

Introduction

The field of functional foods is rapidly growing, aiming at the development of food that supports beneficial health effects including reduction of risks on diseases like cancer, cardiovascular diseases and obesity. As meat and meat products are some of the most important sources of dietary fat, modification of the lipid profile of such products, by enhancing n-3 polyunsaturated fatty acids (PUFAs), can help to improve the nutritional quality of the western diet (Ansorena & Astiasarán, 2004). The importance of PUFAs is due to their biological function in human health and presumed protective effect against some common cancers such as breast and colon cancer, rheumatoid arthritis and inflammatory bowels diseases (Alexander, 1998).

Dommels et al., (2003) and Van Beelen et al., (2007) have found that n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), which are present mainly in fish oil, inhibit mitochondrial and cell proliferation in Caco-2 cells. It was shown that this inhibitory effect was due to oxidative stress as α-tocopherol could reverse it. The same effect were seen in hydrolyzed samples of fish and algal oil and not in non-hydrolyzed samples (van Beelen, 2007).

The main goal of this study was to study the effect of n-3 PUFAs of encapsulated linseed, algal and fish oils content in low fat Dutch style fermented sausages elaborated with beef meat and ostrich meat on the mitochondrial activity of mouse liver hepatoma cells *in vitro* (Hepa-1C1C7) during storage period using the MTT-assay. This assay is also a good indication for effects on cytotoxicity and cell proliferation.

The oils from the sausages were extracted, hydrolyzed and tested according to Van Beelen et al., (2007). For comparison, the two fatty acids EPA and DHA were included.

Materials and methods

Sausage preparation: Dutch style fermented sausages (classic and low fat version), were manufactured at Wageningen University (The Netherlands). Lean beef, ostrich meat, pork back fat, encapsulated linseed, algal and fish oil were used as raw materials. 8 formulations of fermented sausages of about 1.5 kg each were prepared. Two controls (one manufactured with beef meat, and one manufactured with ostrich meat) were produced using 30% and 10% pork back fat respectively. Six formulation were produced with 10 % of total fat, in which pork back fat was replaced by encapsulated flaxseed, algal and fish oil, about 300 g (three samples were elaborated with ostrich meat and three samples were elaborated with beef meat.

Extraction of oil: Oils were extracted from 2 g of sample according to Bligh and Dyer Method (1959), with chloroform: methanol (2:1) and evaporated solvent till dryness.

Enzymatic hydrolysis to obtain fatty acids: Oils were hydrolyzed using amino lipase PS-C II (immobilized on ceramic) in a quantity of 0.75% w/w oil basis and later on glycerides and free fatty acids were extracted with hexane. The solvent was evaporated until dryness and the samples stored at -22 °C (Van Beelen et al., 2007).

Cells and treatments: The Hepa 1C1C7 cell line was obtained from the American Type Culture Collection. The cells were maintained in an atmosphere of 5% CO_2/95% air at 37 °C and a humidity of 100%. The cells were sub-cultured at a ratio of 1:5, after they had reached 70-90% confluence in 75 m^2 culture flasks. Hepa 1C1C7 cells were grown in α-MEM, supplemented with 10% FCS and 50 µg/ml gentamicin.

For the cytotoxicity test cells suspensions of $5x10^4$ cells were plated in a 96 wells plate (Greiner, The Netherlands) in 100 µl culture medium and incubated for 24 h to allow the cells to attach. After this incubation time culture medium was replaced by 100 µl exposure medium containing of fresh serum-free medium, supplemented with 1 mg ml^{-1} fatty acids free BSA and the indicated concentration of fatty acids, exposure time was 48 h. Fatty acids were dissolved in pure ethanol to give a final concentration of ethanol in the medium of either 0.5% or 1%. Control cells received serum-free medium supplemented with 1 mg ml^{-1} fatty acids free BSA plus either 0.5% or 1% ethanol (Van Beelen et al., 2007).

MTT-assay: Mitochondrial activity was determined using the ability of mitochondrial enzymes to convert MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to an insoluble purple formazan salt. After 48 h of exposure 20 µl of MTT (5mg ml^{-1}) was added and incubated for 1 h at 37 °C. The medium was carefully removed and 100 µl DMSO (dimethyl sulfoxide) was added to each well to dissolve the formed formazan crystals. The absorbance of each sample was measured at 562 nm and 620 nm. Mitochondrial activity was expressed [Mean (562 nm-620 nm)$_{\text{exposed cells}}$/ Mean (562 nm-620 nm)$_{\text{blank cells}}$] x 100% (Van Beelen, et al., 2007).

Statistical analyses: Mean of 8 repetition and standard deviation were carry out for each sample.

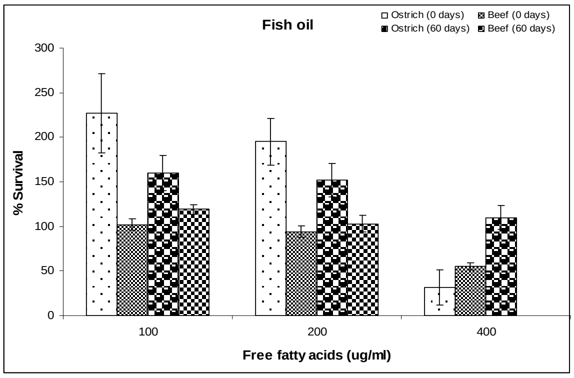
Results

Figure 1 shows the results of mitochondrial activity (% Survival) of Hepa 1C1C7 exposed to free fatty acids obtained from enzymatic hydrolysis of the fat extract from different dry fermented sausages elaborated with ostrich meat and beef meat, each with addition of linseed oil, algal oil, fish oil and pork back fat.

Differences in mitochondrial activity were found between sausages manufactured with ostrich meat and beef meat. Sausages with ostrich meat showed more mitochondrial activity at lower concentrations (100 µg/ml) of free fatty acids at the beginning of storage period. However, the addition of PUFAs apparently have not much influence on the cell proliferation as the type of meat used in the manufacture of the sausages.

No differences are observed between beef samples independent of PUFAs addition.

The cytotoxicity appears in most of the cases at highest concentrations of free fatty acids (400 µg/ml), especially in sausages manufactured with ostrich meat.



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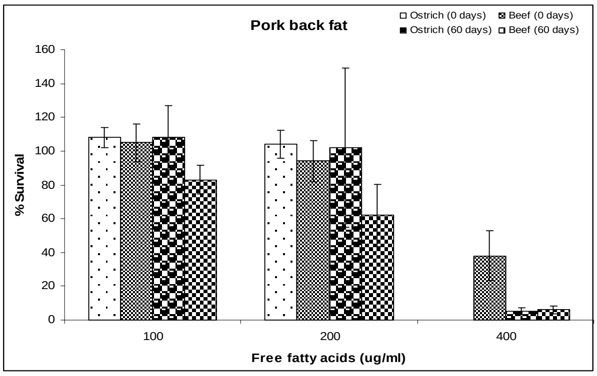

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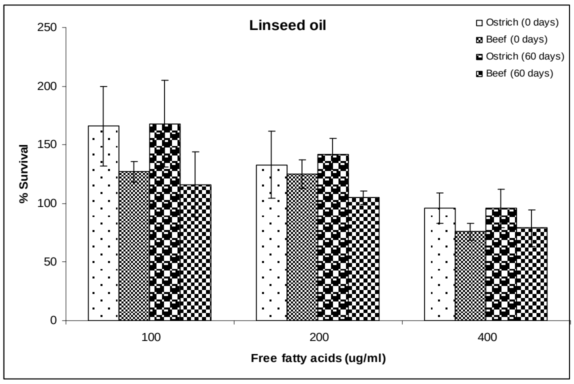

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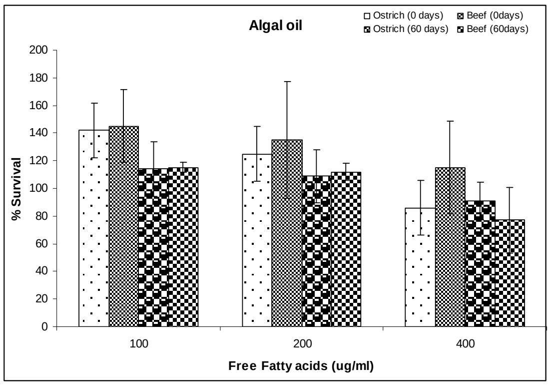

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Figure 1. Mitochondrial activity (% Survival) of Hepa 1C1C7 exposed to free fatty acids obtained by enzymatic hydrolysis of the fat extract of different Dutch style dry fermented sausages elaborated with ostrich meat and beef meat, both with addition of linseed oil, algal oil, fish oil and pork back fat.

Conclusions

1. Mitochondrial activity of Hepa 1C1C7 are higher at low concentrations of free fatty acids (100 µg/ml), especially in sausages elaborated with ostrich meat.
2. No effects were observed for the additions of PUFAs in sausages containing beef and ostrich..
3. Cytotoxicity appears at concentrations of free fatty acids of ca 400 µg/ml, especially in sausages elaborated with ostrich meat.

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