Influence of additional stabilizers on protection against rumen biohydrogenation of PPO-stabilized emulsions in order to increase oil content

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Introduction

Several studies have shown the beneficial effect of omega-3 unsaturated fatty acids on the reproductive performances of ruminants.[1–3] However, due to the presence of anaerobic microorganisms, polyunsaturated fatty acids (PUFA) are readily biohydrogenated in the rumen.[4] As a consequence, several techniques have been developed to protect PUFA from biohydrogenation. Thereof treatment of a casein stabilized oil-in-water emulsion with formaldehyde has shown to be the most efficient.[5] Yet, due to the high toxicity of formaldehyde and strict regulations this protection method is rarely used in practice. Recently, research in our laboratory, focusing on the use of polyphenol oxidase (PPO) to protect PUFA, has shown promising results as an alternative for the former method. Initially, red clover PPO has been suggested to protect PUFA against degradation, both in silage as in the rumen.[6] Further research has led to the formation of PPO-stabilized oil-in-water emulsions, using a protein extract, containing PPO.[7] Addition of a PPO substrate, such as 4-methylcatechol, in the presence of oxygen leads to the formation of electrophilic quinones which are suggested to readily react with the nucleophilic groups of the protein adsorbed to the oil droplets, forming a network of crosslinked protein surrounding the emulsion droplets and protecting the oil against the microbial activity in the rumen. Increasing the oil level leads to larger droplet sizes and a lower degree of protection, which suggests the amount of protein in the red clover extract is too limiting to stabilize higher oil levels. Although casein addition resulted in lower droplet sizes, the protection efficiency was detrimentally affected, most probably due to competition between casein and PPO for adsorption at the oil-water interface. Hence, noncompeting stabilizers are necessary. The aim of this research is to verify if bovine serum albumin (BSA) and/or pectin might act as stabilizer of PPO-containing emulsions (10% oil) without an adverse impact on the protection against biohydrogenation.

Material and methods

The formation of (un)protected PPO-stabilized emulsions occurred in three steps. First, a PPO-containing protein extract was prepared, in this case using potato tuber peels as a source of PPO. After preparation, the PPO activity was measured spectrophotometrically using 4-methylcatechol (4MC) as a substrate.[8] Subsequently, the protein extract was homogenized with linseed oil to obtain an oil-in-water emulsion (10/90 (v/v)). The emulsion droplet size distribution was determined by laser diffraction. Lastly, after emulsification PPO substrate or buffer was added to obtain protected or unprotected emulsions, respectively, after 24 h of reaction in the presence of oxygen. Three different treatments were applied: I) Pure protein extract, II) addition of BSA to the protein extract (0.30 mg/mL), III) addition of pectin to the protein extract (0.15 mg/mL).

Finally after 24 h reaction, the degree of protection against ruminal biohydrogenation was evaluated by simulation of the rumen metabolism through 24 h *in vitro* batch incubation. During incubation, three batches of hay were used as a substrate for microbial growth (1 g/ 100 mL). Emulsions were added (0.4 mL/ 100 mL) and unprotected emulsions served as a control. After incubation, extraction and methylation of the fatty acids was performed before GC-analysis. Biohydrogenation was calculated by determining the disappearance of α-linolenic acid (C18:3 n-3).[7]

The following statistical model was used to analyze the results: $Y_{ijk} = \mu + T_i + P_j + T_i x P_j + S_k + \varepsilon_{ijk}$, with Y_{ijk}, the degree of biohydrogenation of C18:3 n-3, T_i the fixed effect of treatment (no additive, BSA or pectin), P_j the fixed effect of protection (addition or no addition of 4MC) and S_k the random effect of hay batch (hay 1, 2 or 3).

Results and discussion

Unexpectedly, the results showed no significant differences in protection against biohydrogenation between the different treatments ($p = 0.93$). Only a significant effect of protection ($p < 0.0001$) was observed (Figure 1A). Furthermore, for all three treatments an average protection degree of 70% or more was found, while less than 50% protection was expected with this level of oil, based on extrapolation of previous results.[7] This difference in protection might be due to the use of a different PPO source: In previous experiments red clover was used, while in this experiment an extract was prepared using potato tuber peels. In his review, Mayer discussed the great variation of amino acid sequences of PPO from different organisms,[9] which results in different conformations and sizes. The latter inevitably leads to differences in adsorption kinetics at the oil-water interface of the emulsions,[10] which plays an important role in the protection efficiency. Additionally, the amount, size and conformation of the enzyme will not only differ between plant species but will also depend on biotic and abiotic stress the plant undergoes during lifetime, postharvest processing and storage.[9] Furthermore, other proteins are also present in the extract, that might compete with PPO for adsorption to the oil-water interface of the emulsion droplets. Again, the presence of these proteins will depend on different factors and might therefore differ each time an extract is made. However, previous results have shown that similar protection efficiencies can be found for different red clover extracts despite differences in harvest time and duration of storage.[7] Figure 1B shows extract characteristics from red clover and potato tuber peel. In contrast to the high difference in protection efficiency (<50% versus 70%, respectively), only a small difference in protein content and PPO activity was found, most probably due to the presence of less competing protein in the potato tuber peel extract.

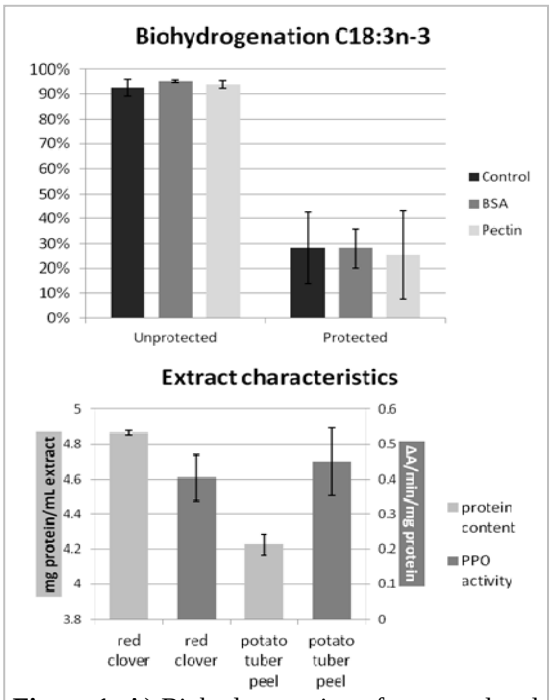

Figure 1: A) Biohydrogenation of protected and unprotected emulsions of different treatments, B) Comparison of extract characteristics of red clover and potato tuber peel

In conclusion, the addition of BSA or pectin, at present concentrations, had no enhancing effect on the PPO protection mechanism when potato tuber peel extract was used. On the other hand, also no adverse effect is found, which suggests that both BSA and pectin don't compete with PPO to adsorb to the oil-water interface. Further research will be executed to evaluate the potential of potato tuber peels as a valuable source of PPO for an application of rumen by-pass PUFA, taking in account its great availability, as a by-product of the food industry.

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