Influence of a limited enzymatic hydrolysis on the functional properties of β-lactoglobulin for microencapsulation

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INTRODUTION AND OBJECTIVE

Microencapsulation of sensitive lipophilic compounds has successfully been performed via spray drying using whey protein based matrices in the past (Hogan, 2001; Rodea-González, 2012). In this context, stability of the dispersed systems depends on the formation of viscoelastic films at the oil/water-interface respectively, with interfacial rheology being important during formation of the emulsions (Mahmoudi, 2010).

Limited enzymatic hydrolysis is a qualified means to modify the interfacial properties of proteins. However, the effect of this modification depends on various parameters, e.g. the type of enzyme used (Davis, 2005) and the degree of hydrolysis achieved, as high degrees can lead to a loss of functionality (Ipsen, 2001). Whey protein hydrolysates also contribute to the chemical stability of the sensitive lipophilic compounds by acting as radical scavengers (Elias, 2006; Peng, 2009).

Studies on the use of whey protein hydrolysates for encapsulation of these ingredients are still missing. Therefore, aim of the present study was to analyse the functional properties of β-lactoglobulin and hydrolysates thereof with a limited degree of hydrolysis related to their potential to encapsulate lipophilic food ingredients.

MATERIALS AND METHODS

A commercially available β-lactoglobulin (β-LG) (protein 97.9 % (dry matter)) was hydrolysed to a degree of hydrolysis of 6 % with bovine trypsin (DH6(T)) and Alcalase® (DH6(A)) using the pH-Stat method of Adler-Nissen, 1986. Subsequent characterisation of the interfacial properties at the oil/water (o/w)-interface were carried out on an automated drop tensiometer equipped with an oscillation unit as described elsewhere (Serfert, 2013). SDS-PAGE under reducing conditions was used to characterise the molecular weight distribution of the hydrolysates. Emulsions with protein solution, fish oil and dried glucose syrup (DE 38) were prepared using high shear and high-pressure homogenisation consecutively. Subsequent spray drying of these emulsions to encapsulate the fish oil was carried out on a pilot scale spray drier (Mobile Minor 2000, Niro A/S, Copenhagen, Denmark) at 180/70 °C inlet/outlet temperature and 4 bar with rotary atomization. Particle size distribution of emulsions before and after spray drying was determined via laser diffraction spectroscopy. Hydroperoxide content was analysed as described elsewhere (International Dairy Federation, 1991). Extractable oil content was determined gravimetrically after extraction with petrol ether (Westergaard, 2004).

RESULTS AND DISCUSSION

Limited enzymatic hydrolysis of β-LG with trypsin and Alcalase® resulted in the bulk of peptides being around 7 kDa in DH6(A), in contrast to DH6(T) consisting mainly of peptides between 3 and 5 kDa with no residual native proteins detectable respectively.

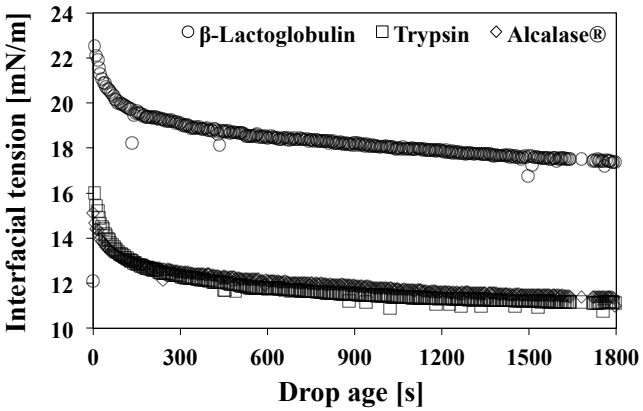

Figure 1: Change in interfacial tension of solutions of β-lactoglobulin and hydrolysates (ω = 0.1 wt%, pH 8.0) produced with trypsin and Alcalase® at the oil/water-interface over a timescale of 30 min

With respect to their ability to decrease the interfacial tension at the o/w-interface the hydrolysates differ only slightly among themselves, but a significant reduction can be detected in comparison to β-LG (Figure 1). This might be due to exposure of hydrophobic molecule parts and decrease in the size of the peptides caused by hydrolysis (Ipsen, 2001).

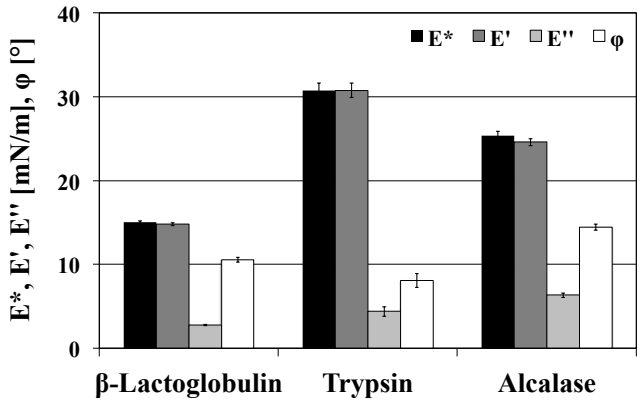

Figure 2: Dilatational rheology of solutions of β-lactoglobulin and hydrolysates produced with trypsin and Alcalase® (ω = 0.1 wt%, pH 8.0, f = 0.07 Hz) at the oil/water-interface.

Enzymatic hydrolysis also resulted in alteration of the interfacial rheological for all samples (Figure 2).

Due to phase angles (φ) of maximum 15 degrees all samples showed primarily elastic behaviour, which is a generally recognised attribute for whey proteins. An increase in the interfacial dilatational modulus E* for both hydrolysates could be detected, being more pronounced for DH6(T).

Table 1: Percentile of oil droplet sizes in liquid and reconstituted emulsions (means; β-LG - β-lactoglobulin; DH6(A)/(T) - hydrolysates of β-lactoglobulin with degree of hydrolysis of 6 % produced with Alcalase® (A) or bovine trypsin (T)

Sample	Percentile of oil droplet size [µm]		Span
	50th	90th	
Liquid emulsions before spray drying			
β-LG	1.04 ± 0.03	1.64 ± 0.03	1.03
DH6(A)	0.81 ± 0.03	1.58 ± 0.07	1.55
DH6(T)	0.83 ± 0.01	1.40 ± 0.01	1.27
Reconstituted emulsions after spray drying			
β-LG	1.08 ± 0.01	1.71 ± 0.00	1.02
DH6(A)	0.80 ± 0.04	1.81 ± 0.06	1.87
DH6(T)	0.87 ± 0.03	1.48 ± 0.01	1.25

The microencapsulation efficiency for the hydrolysates (88.7 - 90.7 %) differed only slightly compared to β-LG (91.6 %). The oil droplet sizes of the emulsions before and after spray drying (Table 1) remained almost unchanged, thus all proteins stabilised the oil droplets well during atomisation and drying.

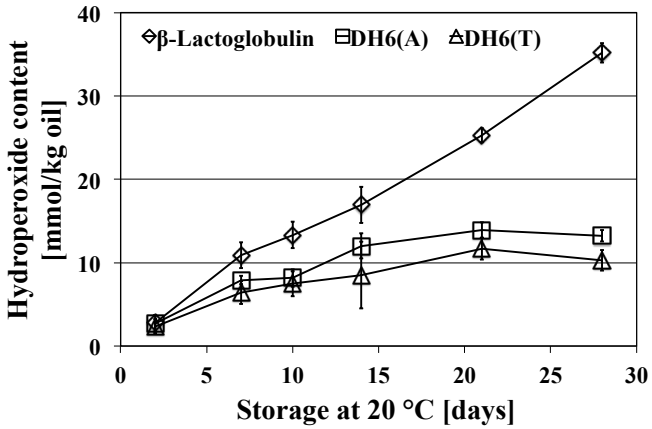

Figure 3: Development of hydroperoxide content in spray dried emulsions of β-lactoglobulin and hydrolysates produced with trypsin (DH6(T)) and Alcalase® (DH6(A)) during storage at 20 °C (and 33 % relative humidity).

Analysis of the hydroperoxide content of the spray dried powders showed similar values for the hydrolysate-stabilised samples and a significantly increased rate of hydroperoxide formation for β-LG (Figure 3). This cannot be explained by interfacial properties and their effect on oil droplet size and microencapsulation efficiency, but rather by the antioxidative potential of whey protein hydrolysates (Elias, 2006; Peng, 2009).

CONCLUSIONS

Enzymatic hydrolysis altered molecular weight profile and interfacial properties of β-lactoglobulin. However, the limited degree of hydrolysis ensured that parameters important for microencapsulation like oil droplet size distribution and microencapsulation efficiency were not negatively affected. Free sulhydryl groups resulting from the hydrolysis contributed to an improved stability of the encapsulated lipid over a period of four weeks.

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