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A feasibility study on the application of a laccase-mediator system in stirred yoghurt at the pilot scale

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ABSTRACT

The crosslinking of milk proteins by laccases, often in the presence of a small phenolic mediator, has been demonstrated in model systems. In this study, the pilot scale application of a laccase-mediator system in stirred milk yoghurt in a post-processing step was assessed. Stirred skim milk yoghurt with 4.6% protein was treated with two laccase dosages (1 U/g or 3 U/g yoghurt) and vanillin concentrations (1 mM and 5 mM) at 20 °C for 24 h in a screening step. Mainly protein fragmentation was observed via SDS-PAGE due to laccase only while higher molecular weight oligomers were formed with laccase-vanillin. The storage modulus, viscosity and particle size were lower after laccase treatment, revealing a degradation in the gel structure. In a second step, laccase-vanillin combinations which resulted in the greatest structural modifications, namely 3 U laccase/g yoghurt or 5 mM vanillin, were applied to stirred skim and full milk yoghurt for sensory and structural analysis. Lower in-mouth viscosity and characteristic taste were evaluated for the laccase containing yoghurts, an effect exacerbated in the presence of vanillin. Additionally, lower structural parameters were observed, independent of vanillin. The storage modulus and apparent viscosity of the full milk yoghurt decreased by 18–29%. In line with this observation, a three- to four-fold higher concentration of smaller peptides was detected by fluorescence spectroscopy. Thus, the laccase-mediator system caused simultaneous protein crosslinking and fragmentation with an overall negative impact on the fermented milk gel structure and sensory perception.

1. Introduction

The enzymatic crosslinking of milk proteins, e.g. with microbial transglutaminase (Menéndez, Schwarzenbolz, Rohm, & Henle, 2004) and oxidoreductases such as peroxidase and fungal laccase (Hiller & Lorenzen, 2009), is of high interest to the dairy industry. The higher molecular weight oligomers formed can be applied to modify the structure of acidified milk gels and improve structural gel properties, namely gel strength, storage modulus and apparent viscosity (Ercili-Cura et al., 2009; Menéndez et al., 2004). Thus, structural losses arising from post-processing of the shear sensitive milk coagulum, e.g. due to pumping, cooling and packaging, can be compensated for enzymatically rather than via stabilisers or higher dry matter contents (Sodini, Remeuf, Haddad, & Corrieu, 2004).

Laccases from fungi (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) catalyse the formation of covalent bonds between phenolic amino acid residues in milk proteins, primarily tyrosine, via a radical mechanism with the concomitant reduction of oxygen to water. Owing to the acidic pH optimum of laccases (Mokoonlall, Pfannstiel, Struch, Berger, & Hinrichs, 2016; Stoilova, Krastanov, & Stanchev, 2010), an alternative to pre-treatment of the milk as studied by Hiller and Lorenzen (2011) is to add the enzyme in a post-processing step after fermentation. In a recent study, Mokoonlall et al. (2016) observed mainly a degradation in the structure of fermented milk gels on application of a laccase preparation only, attributed to protein fragmentation.

Since phenolic amino acid residues in larger proteins often have poor steric accessibility, a small phenolic mediator with typically

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one to four carbon rings can be applied to enhance the crosslinking reaction. It forms a strongly oxidising intermediate which in turn reacts with larger proteins (Fabbrini, Galli, & Gentili, 2002). The use of phenolic mediators in combination with laccase, so-called laccase-mediator systems, has been widely investigated at laboratory scale. This has been typically performed in model systems comprising individual milk proteins suspended in buffer systems at near-neutral or neutral pH. For example, ferulic acid has been often added to α-casein, caseinate and β-lactoglobulin to promote the formation of laccase induced higher molecular weight polymers (Ercili-Cura et al., 2009; Selinheimo, Lampila, Mattinen, & Buchert, 2008; Steffensen, Andersen, Degn, & Nielsen, 2008). Catechin, quercetin and chlorogenic acid had a similar effect on α-casein and bovine serum albumin (Kim & Cavaco-Paulo, 2012). Other laccase-mediator combinations were also found to lead to more effective protein crosslinking, e.g. β-casein with caffeic acid (Stanic et al., 2010), whey protein isolate to which vanillin was chemically attached (Ma, Forssell, Partanen, Buchert, & Boer, 2011) and α-lactalbumin with chlorogenic acid (Færgemand, Otte, & Qvist, 1998).

Since mediators enhance laccase induced protein crosslinking reactions so that more oligomers are formed, laccase-mediator systems are expected to improve the structure of fermented milk gels. A sodium caseinate suspension with laccase and ferulic acid resulted in an acidified set gel with a higher gel strength (Ercili-Cura et al., 2009). Conversely, Hiller and Lorenzen (2011) found a decrease in the gel strength and viscosity of set yoghurt made from laccase-mediator treated skim milk. This occurred despite a lower percentage of protein monomers and a higher percentage of oligomers after incubation of the yoghurt milk with laccase and chlorogenic acid or green tea phenolics. At the laboratory scale, vanillin, vanillic acid and caffeic acid as mediators in combination with laccase in a post-processing step promoted protein crosslinking. As a result, the viscoelastic properties of a commercial skim milk yoghurt increased (Struch, Linke, Mokoonlall, Hinrichs, & Berger, 2015).

This paper investigates modifications induced by a laccase-mediator system in stirred yoghurt at pilot scale under conditions replicating industrial production. The mediator used is vanillin as it is an accepted flavour compound in yoghurt with a low detection threshold value of 0.9 ng/L in air and 0.02 mg/L in water (Belitz, Grosch, & Schieberle, 2004). Both skim and full milk yoghurts were tested since not only proteins but also fatty acids can undergo oxidation reactions. Changes in the protein pattern were analysed. The structural gel properties, namely viscoelastic properties, apparent shear viscosity, particle size and microstructure were evaluated. The effect of the laccase-mediator system on sensory attributes related to texture and taste was also assessed.

2. Materials and methods

2.1. Laccase

Laccase C, isolated from the *Trametes* species as a light brown powder, was bought from ASA Spezialenzyme GmBH (Germany). The laccase activity A_{lac} in U/mg was quantified prior to each experiment as described by Mokoonlall et al. (2016) to ensure a standardised dosage. Briefly, the measurement was performed in sodium tartrate buffer at pH 4.5 and 25 °C at 420 nm with ABTS as substrate ($\varepsilon_{420} = 36\,000\ M^{-1}\ cm^{-1}$).

2.2. Manufacture of stirred yoghurt

Bovine raw milk from the Dairy Research Station Meiereihof (University of Hohenheim, Germany) was separated into skim milk and cream in-house in the Dairy of Research and Training, University of Hohenheim, Germany. The skim milk was pasteurised at 74 °C for 30 s and the protein content determined with a Lacto-Scope FTIR Advanced (Delta Instruments B.V., The Netherlands).

For skim milk yoghurt, the protein content was adjusted to 4.60 ± 0.02% protein with low heat skim milk powder (type Instant C, 34% total protein, Schwarzwaldmilch GmbH, Germany). For whole milk yoghurt, the protein and fat content were adjusted to 4.00 ± 0.02% and 4.00 ± 0.10% with low heat skim milk powder and cream respectively. Both yoghurt milks were heated 95 °C for 256 s in the pilot scale tubular heat exchanger (150 L/h, Asepto GmbH, Germany). The whole milk was additionally homogenised at 150/30 bar (Asepto GmBH, Germany). After cooling to 35 °C, the milk was inoculated with 0.02% (*w*/w) of the starter culture FD-DVS YoFlex® 812, kindly provided by Chr. Hansen GmbH (Germany). It was fermented in a stainless steel can at 35 °C to a pH of 4.5. Fermentation was stopped by cooling to 20 °C.

For the initial screening step with stirred skim milk yoghurt, 2 kg of the standardised skim milk was fermented and the resulting milk gel was sheared as given in Mokoonlall et al. (2016).

The skim and full milk yoghurt was produced at the pilot scale, namely 15 kg, for the sensory evaluation in triplicate. A needle valve with a gap of 6 mm for the skim milk yoghurt and 2.5 mm for the whole milk yoghurt was used to shear the set milk gel.

2.3. Laccase treatment

First, a screening step was performed with stirred skim milk yoghurt. Two dosages of the commercial laccase preparation were added at 1 U/g or 3 U/g yoghurt. Additionally, 1 mM or 5 mM of vanillin (Sigma–Aldrich, Germany) was used as mediator. The parameter combinations assessed are given in Table 1. All the milk gels, standard and laccase containing, were treated at 20 °C for 24 h, then cooled to 10 °C.

Based on the results from the screening step, the following four variations of skim and full milk yoghurt were produced at the pilot scale: standard (S), standard with 5 mM vanillin (V5), laccase treated with 3 U/g yoghurt (L3) and laccase-mediator treated (3 U laccase/g yoghurt +5 mM vanillin; L3_V5). All the milk gels were stirred in the same way so that they had a similar shear history. After filling in 125 mL pots, the yoghurts were kept at 20 °C for 24 h for the post-processing enzymatic treatment step, after which they were cooled and stored at 6 °C.

2.4. SDS-PAGE

The protein pattern was visualised for the skim milk yoghurt with all parameter combinations in the screening step. The skim milk yoghurts were diluted in bidistilled water prior to sample preparation for SDS-PAGE according to Mokoonlall et al. (2015). Briefly, a 10–20% Tris–HCl polyacrylamide gel (Bio-Rad

Table 1
Laccase dosage and vanillin concentration combinations for post-processing treatment of stirred yoghurt.

Laccase dosage (U/g yoghurt)	Vanillin concentration (mM)	Nomenclature
0	0	S
0	1	V1
0	5	V5
1	0	L1
3	0	L3
1	1	L1_V1
1	5	L1_V5
3	1	L3_V1
3	5	L3_V5