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Monitoring the effect of high pressure and transglutaminase treatment of milk on the evolution of flavour compounds during lactic acid fermentation using PTR-ToF-MS

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ABSTRACT

In this study, the effects of thermal or high hydrostatic pressure (HHP) treatment of a milk base in the absence or presence of a transglutaminase (TGase) protein cross-linking step on the flavour development of yoghurt were investigated. The presence of several tentatively identified volatile flavour compounds (VOCs), both during the enzymatic treatment and the lactic acid fermentation of the milk base, were monitored using a proton transfer reaction time-of-flight mass spectrometer (PTR-ToF-MS). The formation of the major flavour compounds (acetaldehyde, diacetyl, acetoin, and 2-butanone) followed a sigmoidal trend described by the modified Gompertz model. The HHP treatment of milk increased significantly the volatile compound formation rate whereas it did not affect the duration of the lag phase of formation, with the exception of acetaldehyde and diacetyl formation. On the contrary, the TGase cross-linking of milk did not significantly modify the formation rate of the volatile compounds but shortened the duration of the lag phase of their formation.

1. Introduction

Transglutaminase (TGase, EC 2.3.2.13) is a transferase that catalyses the reaction between the γ-carboxyamide groups of peptide bound glutamyl residues (acyl donor) and several primary amines including ε-amino group of lysine, leading to protein cross-linking through the formation of both inter- and intramolecular isopeptide bonds (Motoki & Seguro, 1998; Özrenk, 2006). Non-globular milk proteins such as α-, β- and κ-caseins, and to a lesser extent whey proteins (α-lactalbumin, β-lactoglobulin) are favourable substrates for TGase, rendering possible the modification of several functional and structural characteristics of dairy products (Bönisch, Huss, Lauber, & Kulozik, 2007; Schorsch, Carrie, Clark, & Norton, 2000; Sharma, Zakora, & Qvist, 2002). The functionality of TGase in dairy systems, such as yoghurt gels, can lead to modification of protein solubility, hydration ability, water-holding capacity, emulsifying and rheological properties, enhancement of gelation in absence of thermal treatment, and changing of the elasticity, strength and microstructure of dairy gels. Also it can positively affect the nutritive value and quality characteristics of low fat or solid non-fat (SNF) unfortified products, and decrease the availability of amino acids, e.g., lysine and lipids or lipid-soluble materials which are responsible for deteriorative chemical reactions (Bönisch et al., 2007; Dickinson, 1997; Færgemand, Otte, & Qvist, 1998; Færgemand, Sørensen, Jørgensen, Budolfsen, & Qvist, 1999; Jacob, Nöbel, Jaros, & Rohm, 2011; Jaros, Jacob, Otto, & Rohm, 2010; Lauber, Henle, & Klostermeyer, 2000; Lorenzen, Neve, Mautner, & Schlimme, 2002; Myllärinen, Buchert, & Autio, 2007; Ozer, Kirmaci, Oztekin, Hayaloglu, & Atamer, 2007; Özrenk, 2006).

High hydrostatic pressure (HHP) processing is an alternative method of modifying milk proteins, towards improving the yoghurt-making properties of milk which are related to the formation of a tight and compact structure. The HHP treatment of milk also enables the inactivation of its pathogenic and spoilage microflora, while minimally affecting its endogenous enzymes and its quality characteristics and nutritional value (Cheftel, 1995; Moatsou et al., 2008; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002; Trujillo et al., 2000). With regard to the functionality of milk proteins and in contrast with the application of TGase in milk, it is shown that HHP treatment can cause changes in both caseins and whey

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proteins (Dumay, Lambert, Funtenberger, & Cheftel, 1996; Huppertz, Smiddy, Upadhyay, & Kelly, 2006; Iametti et al., 1997; Law et al., 1998; Lopez-Fandino, de la Fuente, Ramos, & Olano, 1998; Schmidt & Kooper, 1997). Previous studies on HHP implementation in yoghurt manufacture have shown that HHP-treated milk exhibits higher rate of acidification and coagulates at higher pH values; as a result, yoghurt prepared from HHP-treated milk exhibits lower amounts of whey separation and increased gel strength compared to those made from non-pressurised milk (Ferragut, Martinez, Trujillo, & Guamis, 2000; Harte, Amonte, Luedecke, Swanson, & Barbosa-Cánovas, 2002; Harte, Luedecke, Swanson, & Barbosa-Cánovas, 2003; Needs, Stenning, Gill, Ferragut, & Rich, 2000; Needs et al., 2000). HHP may also show a remarkable synergy with enzymatic cross-linking (TGase) of milk base proteins in order to achieve products with improved textural and sensorial attributes, without the need of addition of external protein sources or stabilisers (Anema, Lauber, Lee, Henle, & Klostermeyer, 2005; Tsevdou & Taoukis, 2012).

Flavour is among the most important parameters affecting the perceived quality of fermented milks, being influenced by compositional and processing factors (Tamime & Robinson, 2007). Although more than 60 volatile compounds have been reported to contribute to the formation of the flavour profile of yoghurt, acetaldehyde, diacetyl (2,3-butanedione), 2-propanone, acetoin (3-hydroxy-2-butanone), 2-butanone, ethanol, and 2,3-pentanedione are considered as the key components for the development of the typical yoghurt aroma (Kneifel, Ulberth, Erhard, & Jaros, 1992; Ott, Fay, & Chaintreau, 1997; Ott, Germond, Baumgartner, & Chaintreau, 1999). Milk base fortification (e.g., addition of external protein source, stabilisers etc.) can induce important changes in the release of endogenous volatile organic compounds (VOCs), due to kinetic and thermodynamic factors such as air/product partition coefficients and diffusion, lipophilic–hydrophobic character of flavour compounds, matrix–VOCs interactions, etc. (Déléris, Lauverjat, Tréléa, & Souchon, 2007; Guichard, 2002; Soukoulis et al., 2010; Tamime & Robinson, 2007). Moreover, it is well established that individual processing steps, such as homogenisation, heat treatment, incubation and cooling may also change flavour development and release (Tamime & Robinson, 2007). For example, it has been previously reported that severe milk heat treatments may lead to important changes in evolution profiles of the major endogenous flavour compounds during fermentation and the development of off-flavours as well (Labropoulos, Palmer, & Tao, 1982; Vazquez-Landaverde, Torres, Velazquez, & Qian, 2005). Recently, Serra, Trujillo, Guamis, and Ferraga (2009) reported that the application of high pressure homogenisation of milk instead of the conventional thermal treatment may affect the flavour quality of the final products proportionally to the pressure conditions. However, there is still a lack of knowledge on the potential differences and advantages, concerning the evolution and release of flavour, which could be achieved by the application of high pressure and enzymatic treatment of fermented milk products.

Proton transfer time-of-flight mass spectrometry (PTR-ToF-MS) is a novel direct injection mass spectrometric technique that allows the rapid monitoring of VOCs, based on the hydronium ion (H_3O^+) transfer reaction (Jordan et al., 2009). PTR-ToF-MS allows the non-invasive real time detection of VOCs with higher proton affinities than that of water (e.g., carbonyl compounds, carboxylic acids, alcohols, esters, sulphur and nitrogen compounds, ammonia) at the low pptv level. Therefore, PTR-ToF-MS can be considered as an efficient technique for the measurement of flavour release studies in complex food systems or in matrices undergoing time-dependent transformations, as in the case of dairy gels formation (Fabris et al., 2010; Lauverjat, de Loubens, Déléris, Tréléa, & Souchon, 2009; Soukoulis et al., 2010) or of food mastication (Aprea, Biasioli, Gasperi, Märk, & van Ruth, 2006).

The effects of HHP and TGase milk base pre-treatment on the physicochemical, textural, rheological and structural properties of yoghurt have been extensively studied over the last years. However, to the best of our knowledge there are no studies dealing with the impact of TGase and HHP on flavour development during the lactic acid fermentation of milks. For the purposes of the present study, PTR-ToF-MS was implemented for the non-destructive monitoring of the endogenous flavour compounds changes of thermally or HHP-treated milk in the presence or absence of a TGase milk base pretreatment step.

2. Materials and methods

2.1. Milk treatment

Homogenised milk (protein content of 3.0% and standardised fat content of 4.0%) was provided directly from the plant of a dairy company. Milk was either subjected to thermal (85 °C for 30 min) or HHP (600 MPa at 55 °C for 10 min) treatment. For thermal treatment, milk was put into beakers of 2000 mL capacity, preheated in a microwave and, placed in a water-bath maintained at the desirable temperature for the appropriate time. Samples were then stored at 4 °C overnight until use.

HHP treatment was performed using a laboratory-scale HHP system with a maximum operating pressure of 1000 MPa (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Netherlands), consisting of an HHP unit with a pressure intensifier, an HHP vessel of 1.5 L volume and a multi-vessel system consisting of six vessels of 42 mL capacity each. All HHP vessels were surrounded by a water-circulating jacket connected to a temperature control system. The pressure-transmitting fluid used was polyglycol ISO viscosity class VC 15 (Resato International BV). Milk samples (750 mL) were put into multilayer (PP, foil, PE) packaging and placed in the 1.5-L chamber for processing. The desired value of pressure was set and, after pressure build-up (approximately 20 MPa s^{-1}), the pressure vessel was isolated; this point defined the zero time of the process. The pressure of the vessel was released after a preset time interval (10 min pressurisation time) by opening the pressure valve (release time <3 s). The initial temperature increase during pressure build-up (about 3 °C per 100 MPa) was taken into consideration, in order to achieve the desired operating temperature. Pressure and temperature were constantly monitored (intervals of 1 s) and recorded during the process.

2.2. Enzymatic cross-linking with transglutaminase

For the on-line monitoring of ammonia evolution during transglutaminase treatment, thermally and HHP-treated milks were heated to 42 °C, inoculated with TGase at a concentration of 2.2 U/g protein (ACTIVA YG, Ajinomoto, 100 U/g transglutaminase activity), shared into 120-mL glass vials (30 mL of milk sample) and, incubated in a water-bath maintained at the aforementioned temperature (42.8–43.1 °C) for 180 min. Duplicate samples were taken every 30 min and zero time was set 30 min after the inoculation of the enzyme, in order to achieve a desirable headspace for the samples.

For the monitoring of volatile aroma compounds during lactic acid fermentation, thermally and HHP-treated milks were heated to 42 °C, inoculated with TGase at a concentration of 2.2 U/g protein (ACTIVA YG, Ajinomoto, 100 U/g transglutaminase activity) and, incubated in a water-bath maintained at the aforementioned temperature for 180 min. Incubation of milk with the enzyme was carried out into 2 L sterile beakers, which were placed into a water-bath of controlled temperature (43 ± 0.2 °C). Afterwards, milk was