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The effect of cysteine on production of volatile sulphur compounds by cheese-ripening bacteria

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Abstract

The effect of cysteine on the ability of smear cheese-ripening bacteria (*Brevibacterium linens* and *Arthrobacter spp*) to produce volatile sulphur compounds (VSC) from methionine was studied. These bacteria were cultivated in a synthetic medium supplemented with various cysteine concentrations with or without methionine. Cultures with only cysteine showed slightly lower levels of VSC produced and an unpleasant odour like rotten eggs, resulting from hydrogen sulphide production. The levels and profiles of VSC produced with supplemented methionine–cysteine mixtures had strain-dependant behaviours. However, the highest levels of dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide were observed when increasing the cysteine concentration from 0.2 to 1.0 gl^{-1} at the same methionine concentration (1.0 gl^{-1}). In contrast, production levels of thioesters, especially *S*-methylthio acetate, were reduced by 50 and 80% under such conditions. An initial sensory approach showed that such an effect could have a strong impact on the global odour of ripened cheeses.

Keyword: L-cysteine; L-methionine; Volatile sulphur compounds; *Brevibacterium*; *Arthrobacter*; Cheese-ripening microorganisms; Polysulfides; Thioester

1. Introduction

Volatile sulphur compounds (VSC) are of great importance in the flavour of ripened cheese. Methanethiol, dimethyl disulphide (DMDS), dimethyl trisulphide (DMTS), dimethyl tetrasulphide and several thioesters, mainly *S*-methylthio acetate (SMTA), are widely recognized as responsible for cheese flavour (Cuer, 1982). It is accepted that methanethiol is at the origin of other identified VSC in cheese. SMTA and thioesters are produced by esterification of methanethiol with an acyl-CoA (Helinck et al., 2000). By oxidation, methanethiol produces several polysulfides, mainly DMDS. Chin and Lindsay (1994) proposed that hydrogen sulphide (H_2S), the main product of cysteine catabolism, is involved in DMTS and DMQS formation. Methanethiol production from methionine varies among microorganisms, but at least two pathways are recognized. An inducible methionine γ-lyase (MGL), also named demethiolase, which produces methanethiol and α-keto-butyrate from methionine, has been identified and purified in *Brevibacterium linens* (Dias and Weimer, 1998). This enzyme is induced by methionine in *B. linens* strains (Ferchichi et al., 1985; Cholet et al., 2007) but not in *Lactoccocus lactis* strains (Dias and Weimer, 1998). This enzyme is also suspected to be present in several other cheese surface bacteria, such as *Micrococcus luteus*, *Arthrobacter* sp., and *Staphylococcus equorum* (Bonnarme et al., 2000). In other surface bacteria, in lactic bacteria and in several cheese-ripening yeasts (*Geotrichum*, *Kluyveromyces*, *Yarrowia*), methionine degradation is initiated by a transamination or desamination step with the α-keto-γ-methyl thiobutyrate (KMBA) as intermediate (Yvon et al., 1997; Bonnarme et al., 2001a; Arfi et al., 2007). These two pathways are nevertheless not mutually excluded and can therefore be used by a same microorganism.

Since cysteine is present in the cheese curd in the α_{SN2} and κ-casein which are only slightly hydrolyzed during ripening (Sousa et al., 2001), it appears likely that part of the methionine

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is transformed into cysteine by the transsulfuration pathway (Soda, 1987). The main enzymes on this metabolic pathway are cystathionine β-lyase (CBL) and cystathionine γ-lyase (CGL) which catalyze the formation of homocysteine or cysteine from cystathionine. These two enzymes also take part in the biosynthesis of methionine from cysteine (Hwang et al., 2002). In addition to their anabolic activity, it has been demonstrated that CBL and CGL are able to produce methanethiol from methionine and, in the case of CGL, H_2S from cysteine. Their catabolic activity seems however to be controlled by the methionine and cysteine their self. Indeed, Dias and Weimer (1998) observed that the total cystathionase activity (cystathionine β−γ lyase, CBGL) of *L. lactis* subs *cremoris* S2 was reduced when the methionine concentration of culture media was increased. These authors observed the same effect at slightly increased levels (0.002%) of cysteine concentration in culture media of *L. lactis* subs. *cremoris* S2 and *B. linens* BL2.

Fortuitously we observed, when developing a screening test for the quantification of H_2S, that VSC production by *B. linens* strains falls when cysteine is added to culture media (López del Castillo-Lozano et al., 2007). To our knowledge, the effect of cysteine on the VSC production by surface cheese bacteria has not been studied, perhaps due to the greater importance of methionine in cheese. Nevertheless, given the reactivity of H_2S and cysteine, we thought that it was important to consider the effect of cysteine on methionine degradation and VSC production.

We therefore studied the effect of different cysteine concentrations on VSC production by cheese-ripening bacteria.

Furthermore, we decided to compare the aromatic properties of a strain of *Arthrobacter sp.* and a strain of *B. linens*. As a matter of fact, even if the *B. linens* specie is recognized as the major producer of VSC among the various cheese-ripening bacteria, it is now widely reported that *B. linens* is not a dominant specie of the ripening flora although it is still used as the major component of ripening cultures (Brennan et al., 2002; Feurer et al., 2004). In contrast, recent results have shown that *Arthrobacter* strains are often dominant on the cheese surface after ripening (Feurer et al., 2004) and play an important role in ripening processes, such as colour development (Leclercq-Perlat et al., 2004).

2. Materials and methods

2.1. Microorganisms and culture conditions

Arthrobacter spp. strain 7(2) and *B. linens* CNRZ918 were stored in 10% glycerol-skim milk (BD-Difco, Le Pont de Claix, France) at −80 °C. These strains, isolated from French cheeses, were selected from among 17 bacterial strains for their ability to produce large quantities of H_2S from cysteine (López del Castillo-Lozano et al., 2007). A preculture of each microorganism was grown in a 100 ml flask containing 20 ml of brain heart infusion broth (BHI, Biokar Diagnostic, Beauvais, France) inoculated with 200 µl of the strain stock suspension for 48 h at 25 °C under agitation (150 rpm). Precultures were subsequently used to inoculate (1%) BHI culture medium for the production of volatile sulphur compounds (VSC) and trap flasks when H_2S was quantified.

VSC were produced by growing the bacterial strains in 500 ml flasks in 100 ml of BHI culture medium supplemented with L-cysteine (1.0 gl^{-1}), L-methionine (1.0 gl^{-1}) or L-methionine-L-cysteine (1.0–1.0 and 1.0–0.2 gl^{-1}). Cultures were agitated (150 rpm) at 25 °C for 48 h. After this time, 5 ml of each microbial culture were transferred into solid phase microextraction (SPME) vials at 4 °C and stored at −80 °C until analysis. Each bacterial culture was prepared in triplicate.

2.2. H_2S production and quantification

Trap flasks were prepared as described previously (López del Castillo-Lozano et al., 2007). BHI medium (5 ml), supplemented with L-methionine, L-cysteine or L-methionine-L-cysteine mixtures as described above, was introduced into trap flasks and inoculated (1%) with each strain. This culturing was carried out for 48 h at 25 °C and 90 rpm. The H_2S produced was quantified by the modified methylene blue reaction (Fogo and Popowsky, 1949) as previous described (López del Castillo-Lozano et al., 2007).

2.3. Preparation of cell-free extracts

Cell-free extracts (CFEs) were prepared as described previously (Bonnarme et al., 2001b). CFEs were maintained at −20 °C and employed in a short period of time (less than 1 week). Protein content was determined with the method of Bradford (1976) using bovine serum albumin as a standard.

2.4. Enzymatic activities

Methionine γ-lyase (MGL) activity was determined as described previously (Ferchichi et al., 1985; Bonnarme et al., 2000) using L-methionine as the substrate. The produced methanethiol reacted with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to produce the thionitrobenzoic acid that can be detected at 412 nm by spectrophotometry. A standard curve was obtained with different concentrations of sodium methanethiolate. Specific activity was expressed as (nmol of methanethiol) $(g\ protein)^{-1}\ s^{-1}$.

Total cystathionine β−γ lyase (CBGL) activity of CFE was determined in the same conditions except that L-methionine was substituted by cystathionine as the substrate. In this case, the hydrolysis of cystathionine released a thiol (cysteine or homocysteine) which then reacted with the DTNB. Specific activity was expressed as (nmol of free thiol) $(g\ protein)^{-1}\ s^{-1}$.

For both enzymatic activities, controls without CFE and with heat denaturated CFE (95 °C, 30 min) were included. No enzymatic activity was measured in any of the performed controls.

2.5. Cysteine and methionine quantification

Initial and residual L-cysteine was quantified by the ninhydrine acid method as reported by Gaitonde (1967). The L-cysteine being spontaneously oxidized to cystine in solution, we quantified the total residual cysteine (cysteine+cystine) using dithiotritol (DTT) to promote the reduction of cystine to cysteine before quantification: 500 µl of the sample were mixed