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Control of listeria in meat emulsions by combinations of antimicrobials of different solubilities

Nino Terjung [a], Myriam Loeffler [a], Monika Gibis [a], Jörg Hinrichs [b], Jochen Weiss [a,*]

[a] Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany
[b] Department of Dairy Science and Technology, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 21, 70599 Stuttgart, Germany

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ABSTRACT

The antimicrobial efficacy of three antimicrobials with different aqueous solubilities (sodium lactate > lauric arginate (Nα-lauroyl-L-arginine ethyl ester, LAE > methylparaben)) was evaluated. The antimicrobials were added individually, and as binary or ternary mixtures to emulsion type sausages. The antimicrobial efficacy was evaluated against *Listeria innocua* on the surface of sliced sausages over 24 days of storage at 6 °C. Growth over time was determined, and fitted to a model with a shifted logistic function for evaluation and comparison of antimicrobial effectiveness. The maximum added concentration of LAE (2.0×10^3 μg/g) delayed growth for 1 day. In contrast, lactate and methylparaben (40 and 4.0×10^3 μg/g, respectively) delayed growth for 18 and 7 days, respectively. Five different mixing ratios were investigated for the three different binary combinations: LAE/lactate, LAE/methylparaben and lactate/methylparaben. Results showed that efficacy could be enhanced through mixing of antimicrobials and that the type of antimicrobials as well as their mixing ratio influenced the antimicrobial efficacy of combinations. For ternary combinations, concentrations of two antimicrobials were kept constant while the concentration of the third preservative was varied. To this purpose LAE/methylparaben, LAE/lactate and lactate/methylparaben combinations with concentrations of 0.3/3.3, 0.3/16.7 and $6.6/2.7 \times 10^3$ μg/g, respectively, were combined with increasing concentrations of lactate, methylparaben and LAE, respectively. Results indicated that a further increase in activity is possible, albeit there appears to be a critical concentration of the third component below which the ternary combinations were less effective than the binary combinations. Overall our study suggests that combining antimicrobials with different solubilities is a promising approach to enhance shelf life of structurally-complex products such as emulsion type sausages.

1. Introduction

Listeria innocua and *Listeria monocytogenes* are among the most frequently isolated microorganisms in "ready-to-eat" products (Karina, Julio, Leda, & Noemi, 2011). These pathogens have been shown to contaminate products mainly after thermal processing, and thus, can be found in ready-to-eat foods such as cooked ham or sausages where they pose a high risk to product safety and quality (Barmpalia et al., 2005). Cooked sausages – the latter of the two at-risk product classes – is one of the most frequently consumed meat product classes in Germany, and thus of particular importance to the German meat industry (Bundesverband der Deutschen Fleischwarenindustrie e.V., 2014).

Cooked sausages consist of three principal components, namely fresh pork trimmings (50 wt.%), fat (25 wt.%) and water (25 wt.%) and a number of minor components such as salt, spices, nitrite, phosphates and ascorbates. The ingredients are typically processed in a bowl chopper so that a continuous, viscous mass (the so-called meat batter) is formed. The batter is then stuffed into casings and heated. Structurally, such systems can be characterized as heterogeneous dispersions containing mixtures of particles (fat and meat) that are suspended in a continuous phase containing salt-solubilized meat proteins (Lautenschläger & Tröger, 2006). Upon heating, the solubilized proteins in the aqueous phase form a continuous network thereby causing a transition of the structure from a particle suspension to a particle-filled network.

Microbial growth in sausages, especially post-processing, may be controlled by addition of antimicrobials. There is an increasing awareness that the structural complexity of both the unheated meat batter as well as the heated sausage has a substantial impact on the activity of antimicrobials. For example, antimicrobial efficacy has been shown to depend on various product characteristics such as concentrations of fats, proteins, carbohydrates, and salt, as well as the pH of the product (Zhang, Kong, Xiong, & Sun, 2009). Ingredients such as fat have been suggested to "protect" microorganisms from antimicrobials apparently reducing their sensitivity to antimicrobials (Gutierrez, Barry-Ryan, & Bourke, 2009). In consequence, higher concentrations of antimicrobials are needed to achieve control of microorganisms. Recent research has shown that this is because antimicrobials may interact with proteins rendering them less available for interaction with microorganisms

* Corresponding author. Tel.: +49 711 459 24415; fax: +49 711 459 24446.
E-mail address: j.weiss@uni-hohenheim.de (J. Weiss).

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(Terjung, Loeffler, et al., 2014). To date, it is not yet fully understood which molecular properties truly govern these interactions that then render antimicrobials less effective. One of the key properties that has been suggested to play a dominant role is solubility, which causes compounds to be more or less heterogeneously distributed throughout a food matrix. Compounds may preferentially partition into certain phases (e.g. the fat particles) that then contain higher concentrations of antimicrobials than other phases. Thereby, the antimicrobial activity may be reduced due to the fact that microorganisms are predominately present in the aqueous phase of a food system. As such there exists a specific structure–function relationship that is not yet well understood.

Hydrophilic[1] antimicrobials, such as organic acids and their salts, have been successfully used in the food industry as antimicrobials. The application of hydrophilic antimicrobials in food is simple and efficient since they can be added to the commonly used minor constituents mentioned above (Mani-López, García, & López-Malo, 2012). Samelis et al. (2002) determined that a combination consisting of sodium lactate and sodium acetate (18 and 2.5×10^3 μg/g, respectively) inhibited the growth of *L. monocytogenes* in vacuum packaged frankfurters for 120 days (Samelis et al., 2002). The antimicrobial mechanism of lactic acid and many other antimicrobially active organic acids is based on them lowering the pH of the bacterial cytoplasm after permeation of the non-dissociated forms through the bacterial membranes (Crozier-Dodson, Carter, & Zheng, 2005). Since compounds are predominately present in the aqueous phase, this mass transport occurs rapidly making the compounds quite effective. In complex matrices such as sausages though, concentrations required to significantly prolong shelf life often lead to noticeable changes in sensory properties of products.

Lipophilic[2] antimicrobials, such as parabens, have been used as antimicrobials in cosmetics, pharmaceutics and food for more than 50 years (Soni, Carabin, & Burdock, 2005). The application as methylparaben has been permitted by the European Food Safety Authority (EFSA). Parabens have a broad spectrum of antimicrobial activity over a pH range of 3 to 8. At pH >8, the molecules ionize which reduces their efficacy (Soni et al., 2005). Parabens have been suggested to inhibit the synthesis of DNA and RNA (Nes & Eklund, 1983; cited in: Nguyen, Clare, Guo, & Martinac, 2005). They have also been suggested to interfere with the activity of key enzymes such as ATPases and phosphotransferases (Soni, Burdock, Taylor, & Greenberg, 2001). Furthermore, parabens can cause damage to the microbial membrane without causing lysis (Freese, Sheu, & Galliers, 1973). Lauric arginate (LAE) is an amphiphilic[3] antimicrobial that has shown to possess very high antimicrobial activities in model microbiological systems i.e. the minimum inhibitory concentration for *L. monocytogenes* has been reported to be as low as 8 μg/g (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) 20649) (Becerril, Manso, Nerin, & Gómez-Lus, 2013). In food systems LAE has been found to effectively reduce initial levels of microorganism but this is then often followed by an adaptation of cells leading to a full recovery (Stopforth, Visser, Zumbrink, van Dijk, & Bontenbal, 2010). For example, Luchansky et al. (2005) added 0.4 ml of a 5% LAE solution to shrink-wrap bags. Cooked hams (1.5 kg) inoculated with 7 log CFU/ham of *L. monocytogenes* were then stored in these bags over 60 days at 4 °C. The authors reported that levels of *L. monocytogenes* on ham surfaces initially decreased by 6.5 log CFU/ham, but this was followed by growth within the storage period (Luchansky et al., 2005).

In systems containing anionic compounds, LAE may form complexes that have reduced antimicrobial activities (Asker, Weiss, & McClements, 2009). Terjung et al. also reported a reduction of the loss of antimicrobial efficacy of LAE due to it interacting with lipid surfaces (Terjung, Monville, et al., 2014).

In this study we hypothesized that the lack of effectiveness of the above described antimicrobials in a complex matrix is because they do not match the properties of all the phases that may be present in a complex food matrix (aqueous phase, lipid phase and interface). Instead, combinations of antimicrobials that possess hydrophobic, hydrophilic and amphiphilic properties may be needed to control contaminations. In order to test our hypothesis we used the above mentioned antimicrobials methylparaben, lactate and LAE alone or in binary or ternary combinations as agents to inhibit growth of *L. innocua* on the surface of emulsions type sausages. Growth over time data was modeled with a shifted logistic function to obtain parameters that could be used to assess and compare antimicrobial effectiveness.

2. Materials and methods

2.1. Materials

Mono- and diglycerides of edible fatty acids (E 471; melting point 54 to 64 °C), Mirenat NSM (85.5 g/100 g maltodextrin and 14.5 g/100 g LAE) and methyl 4-hydroxybenzoate were purchased from Meat Cracks Technology GmbH (Mühlen, Germany). Sodium lactate solution (50% in H_2O) was purchased from Sigma Aldrich (Steinheim, Germany). Fresh lean pork meat and pork back fat were purchased locally (Mega, Stuttgart, Germany). Nitrite curing salt containing 0.4–0.5 g sodium nitrite and 2.5 mg potassium iodine in 200 g sodium chloride was obtained from Zentrag (Frankfurt am Main, Germany), disodium dihydrogen phosphate from Fibrisol-Muscalla GmbH (Viernheim, Germany), and ascorbic acid and the spices "Meisterklasse-S" from Frutarom Savory Solutions GmbH (Viernheim, Germany). All chemicals and ingredients were used without further purification. The microbial test strain (*L. innocua*) was obtained from the culture collection of the Department of Food Microbiology, University of Hohenheim (Stuttgart, Germany). Standard I nutrient broth and Standard I nutrient agar were purchased from Merck (Darmstadt, Germany). Double-distilled water was used in the preparation of all solutions.

2.2. Methods

2.2.1. Antimicrobial application systems

Methylparaben was added directly as a powder to the meat batter (see below). Lactate was added as an aqueous lactate solution due to its hygroscopic properties. Solutions were prepared by dilution of 50% sodium lactate solution in double-distilled water to the concentrations desired. LAE was applied as solid lipid particles (SLP) that had previously been shown to be the most effective LAE application system for meat-based systems (Terjung, Loeffler, et al., 2014). There, a coarse pre-emulsion was prepared by homogenizing 10 wt.% melted (80 °C) mono- and diglycerides (E 471) with hot (80 °C) 90 wt.% emulsifier solution (2 wt.% E 471, 27.56 wt.% Mirenat NSM (4 wt.% LAE, pH 3.7)) in a high-shear blender (Standard Unit, IKA Werk GmbH, Staufen, Germany) at 24,000 rpm for 2 min. The hot pre-emulsion was passed through a high-pressure homogenizer equipped with an H-interaction chamber (diameter: 100 μm) (M110-EH-30, Microfluidics International Cooperation, Newton, USA) three times at 150 MPa. The dispersion was then cooled to a temperature of 20 °C and stored for 12 h at room temperature prior to use.

2.2.2. Manufacture of emulsion type sausages

Emulsion type sausages were prepared in the meat pilot plant facilities at the University of Hohenheim. Meat and fat were stored at 2 °C prior to production. Meat (pork shoulder) and fat (pork back fat) were

[1] Hydrophilicity is the property of a molecule to preferentially interact with polar solvents, such as water, and therefore molecules having this attribute are predominately present in the aqueous phase of a system. (McNaught, 1997).

[2] Lipophilicity denotes the property of a component to preferentially associate with nonpolar molecules leading often to an accumulation of such compounds in lipid phases.

[3] Amphiphilic molecules are molecules that are part hydrophilic and part hydrophobic. An example of such compounds are small molecule emulsifiers that are composed of a polar head (e.g. a polyethyleneoxide group) and a non-polar tail (e.g. a fatty acid group). They tend to accumulate at oil–water interfaces, a property which is known as surface activity.