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Antimicrobial efficacy of a spice ferment in emulsion type sausages and restructured ham

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ABSTRACT

The antimicrobial efficacy of a fermented spice preparation was assessed in emulsion type sausages and restructured hams and compared to that of two commercially-used antimicrobials; sodium lactate and lauric arginate. Restructured hams and emulsion type sausages were formulated with either sodium lactate (15×10^3 µg/g), lauric arginate (N^{α}-lauroyl-L-arginine ethyl ester; LAE; 0.2×10^3 µg/g) or a fermented spice preparation (20×10^3 µg/g), and effect on microbial growth and sensory properties determined. The spice ferment retarded the growth of *Listeria innocua* on the surface of emulsion type sausages by about 16 days, while sodium lactate and lauric arginate retarded the growth for 6 and less than 1 days, respectively. On restructured hams, antimicrobial efficacy was lower with growth retardations being 10, 4 and 1 days for the spice ferment, sodium lactate and lauric arginate, respectively. Little activity of all three antimicrobials was found against contamination with *Lactobacillus curvatus*. No significant deviation in the sensory properties occurred upon addition of antimicrobials to either sausages or hams. Considering that growth of *Listeria* is one of the key problems in ready to eat meat products, the results are quite promising. Moreover, results suggest that consumers' demands for products without chemical additives may be addressed by exchanging lactate or acetate with fermented spices.

1. Introduction

Emulsion type or Lyoner-style sausages and restructured hams are microbiologically sensitive products. A high pH of 6.2 and a_w values >0.97 are insufficient hurdles to significantly inhibit microbial growth (Blom et al., 1997; Lautenschläger & Troeger, 2007). The growth of microorganisms in these two product classes depends mainly on the processing steps that follow the thermal treatment, e.g. cooling, slicing, packaging and storage. This is because products are exposed to temperatures of >70 °C during manufacture thereby inactivating the majority of vegetative cells (Borch, Kant-Muermans, & Blixt, 1996; Comi & Iacumin, 2012; Reij & Den Aantrekker, 2004). Hence, post–thermal recontamination, particularly with psychotropic lactic acid bacteria growing during cold-storage in the low oxygen environment of vacuum packages, determines shelf life.

Water-soluble antimicrobials such as sodium lactate or sodium acetate have proven to be particularly suitable in meats (Davidson, Sofos, & Branen, 2005). A water-soluble fatty acid derivative of arginine; lauric arginate (LAE) has also been found to be effective, particularly for the surface decontamination of meats (Luchansky et al., 2005). The compound has recently been approved for use in processed meats in Europe (Byrne, 2014; European Food Safety Agency (EFSA), 2012).

Although antimicrobials have been used throughout the history of food production by humankind to counteract microbial contaminations, a negative consumer attitude towards "preservatives" has arisen, especially against synthetically-derived compounds. As such there is a quest underway to find alternative substances that may be obtained from natural sources (Burt, 2004; Davidson & Harrison, 2002; Ricke, Kundinger, Miller, & Keeton, 2005). Such substances may for example be extracted from plants and microbial

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sources, and include organic acids and phenolic compounds such as acetic or lactic acid, terpenes, coumarins, or flavonoids (Davidson & Harrison, 2002; Gaysinsky & Weiss, 2007; Perumalla et al., 2013; Wang et al., 2012). The compounds may affect microorganisms by a variety of mechanisms. For example, they may permeate the bacterial membrane to then alter intracellular pH, or they may insert themselves into the membrane to then cause either pore formation or disrupt the functionality of membrane bound protein complexes that are essential to generating ATP (Additives Worldwide (AWW) GmbH, 2012; Wang et al., 2012).

The use of naturally-derived antimicrobials in foods with complex structures such as meats or dairy products is however limited. The addition of these compounds to foods can cause undesirable organoleptic and structural changes and thus compatibility with the food matrix is of uttermost importance (Burt, 2004; Wang et al., 2012). For example, Gyawali and coauthors concluded in their review that the use of natural antimicrobials remained low because of their effect on flavor and aroma. Nevertheless, some naturally-occurring antimicrobial are currently being used in food products, and there remain many as yet unexplored sources (Gyawali & Ibrahim, 2014). For instance, the antioxidant and antimicrobial activity of an extract of *Kitaibelia vitifolia* as alternative to the added nitrite in fermented dry sausage was recently investigated. There, authors reported that an ethanolic extract of *K. vitifolia* preserved fermented dry sausages during storage, with a high efficacy against *E. coli* (i.e. an MIC of 15 μg/mL) (Kurćubić et al., 2014).

A new approach to generate potentially potent antimicrobial formulations from natural sources is to ferment the raw materials that are currently used as sources for the above listed naturally-occurring antimicrobials. Fermentation of carbohydrate and proteinaceous substrates under defined conditions yields secondary metabolites that may possess antimicrobial properties or that may enhance activity of antimicrobials (Davidson & Harrison, 2002; Rhodia Inc., 2003).

An example is the fermentation of skim milk, whereby a variety of fermentation products are produced, with organic acids (lactic and acetic acid) being the dominant compounds (Additives Worldwide (AWW) GmbH, 2012; Rhodia Inc., 2003).

The object of this study, i.e. the use of the fermented seasoning "SuperSpice Arom" is an exemplary result of such a fermentation. The ferment is derived from onions and glucose syrup by a two-step fermentation process (Additives Worldwide (AWW) GmbH, 2011, 2012; Dai, Normand, Weiss, & Peleg, 2010). Since onions and syrup are also often used as ingredients in hams and/or sausages and have a high compatibility with these products, we hypothesized that the above mentioned ferment may be similarly suitable for hams and sausages but possess noticeable antimicrobial activities.

To this purpose, we determined the antimicrobial efficacy of the spice ferment to inhibit the growth of *Lactobacillus curvatus* and *Listeria innocua* at 7 °C after a recontamination of emulsion type sausages and restructured hams. We compared its efficacy to two other commercially-used, water soluble antimicrobials, e.g. sodium lactate and lauric arginate. A sensory evaluation of products was carried out to determine potential organoleptic deviations from controls.

2. Materials and methods

2.1. Materials

2.1.1. Antimicrobials

Sodium DL-lactate solution (50 wt% in H_2O) was obtained from Sigma Aldrich® (Steinheim, Germany). Mirenat® NSM (85.5 wt% maltodextrin, 14.5 wt% lauric arginate) and the spice ferment "SuperSpice Arom" were purchased from Meat Cracks Technologie GmbH (Mühlen, Germany). The amounts added to meat products and the concentrations of antimicrobials used are shown in Table 1. The concentrations chosen were based on regulatory approved and/or recommended use levels. The ferment was produced in a two-step fermentation process with lactic and acetic acid fermenting cultures. Details can be found elsewhere (Additives Worldwide (AWW) GmbH, 2011, 2012).

2.1.2. Emulsion type sausages and restructured hams

Pork meat and pork fat were purchased from MEGA (Stuttgart, Germany), curing salt (0.4–0.5 g/100 g sodium nitrite, 2.5 mg/100 g potassium nitrite) from ZENTRAG (Frankfurt am Main, Germany), sodium diphosphate from fibrisol-MUSCALLA GmbH (Viernheim, Germany), ascorbic acid, di-phosphate Bullifos LL and the spice "Meisterklasse-S" from FRUTAROM Savory Solutions GmbH (Korntal-Münchingen, Germany), and the brine Golden PCR10 from Meat Cracks Technologie GmbH (Mühlen, Deutschland).

2.1.3. Bacterial strains and microbial test media

L. curvatus LTH 683 and *L. innocua* LTH 3096 (strain collection of the Department of Food Microbiology, University of Hohenheim, Stuttgart, Germany) were used to assess the antimicrobial efficacy of antimicrobials in meat products. Standard I nutrient broth, MRS broth, MRS agar, anaerobic jars and Anaerocult® A were obtained from Merck KG (Darmstadt, Germany). Peptone water (buffered) and agar–agar was purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany).

2.1.4. Analytical chemicals

Organic acids in spice ferment, in emulsion type sausages, and in restructured hams were determined by HPLC analysis using the following chemicals: potassium di-hydrogen phosphate and acetonitrile from Carl Roth GmbH & Co. KG (Karlsruhe, Germany), o-phosphoric acid (85%) from Th. Geyer GmbH & Co. KG (Renningen, Deutschland), L (+)-lactic acid monolithium salt and sodium acetate from Merck Co. KG, (Darmstadt, Germany); anhydrous Carrez I (potassium hexacyano (II) ferrate tri-hydrate) from Fluka, Sigma–Aldrich (Steinheim, Germany); and Carrez II (zinc sulfate 7 hydrate) from J. T. Baker, (Deventen, Netherlands). All chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Emulsion type sausages

Sausages were manufactured from 50 wt% pork meat, 25 wt% pork fat and 25 wt% water. Meat and fat were separately passed through a meat grinder (type W114T82487-1, Seydelmann, Stuttgart, Germany). Meat was added to a bowl chopper (type K64 VA-K, Seydelmann KG, Stuttgart, Germany) and half the water (as ice), 1.8 wt% curing salt and 0.1 wt% sodium diphosphate were added. The mix was processed in the bowl chopper until a temperature of 2 °C was reached. Minced fat, 0.05 wt% ascorbic acid and 0.5 wt% of a spice blend was added

Table 1
Concentrations in, and total amount of antimicrobials added to, emulsion type sausages and restructured ham. The added amount of antimicrobial was weight based on the weight of the batch and desired concentration.

Antimicrobial	Concentration (10^3 μg/g)	Total amount added to batch (g)
None (control)	0	0
Lauric arginate	0.2	0.4
Spice ferment	20	40
Sodium lactate	15	30