Antioxidant effectiveness of differently sized oil-in-water emulsions loaded with rosemary extract in salami

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Abstract – The antioxidant effectiveness of differently sized oil-in-water emulsions (10 % (w/w) Miglyol 812N, 2 % (w/w) Tween 80, pH 5) loaded with rosemary extract in raw fermented sausages was investigated. Differently sized oil-in-water emulsions (d_{43} = 160 – 7200 nm) containing 100,000 ppm rosemary extract in the oil phase or without added antioxidant were prepared using a high shear blender and/or high-pressure homogenizer. Optical microscopy and static light scattering measurements indicated that the emulsions were physically stable during the course of the experiment, except for the slight aggregation of the emulsion without added antioxidant with a mean droplet size d_{43} of 7200 nm. Incorporation of 0.48 % oil-in-water emulsion loaded with rosemary extract into raw fermented sausages retarded lipid oxidation significantly in comparison to sausages without added antioxidants. The analyses of the primary-(peroxide value) and secondary-(hexanal) oxidation products yielded the unexpected result that emulsions with larger droplet sizes were more effective than smaller ones. These findings might be caused by the physical location of the antioxidant compounds in the oil-water interface of the emulsions.

Key Words – droplet size, lipid oxidation, oil-in-water emulsion, rosemary extract, salami

I. INTRODUCTION

Raw fermented sausages are traditional products, and their production traces back centuries. Due to the combination of growth hurdles, such as low pH and low water activity (aw), it is traditionally considered as safe and can be stored for long periods of time (up to two years) without cooling [1]. However, conditions of high temperature and illumination may promote oxidative alterations in raw fermented sausages [2]. Antioxidants are known to delay these oxidative alterations in early stages of processing. Among natural antioxidants, extracts produced from the leaves of rosemary (*Rosmarinus officinalis L.*) are one of the most common plant extracts used in the food industry. However, the addition of plant extracts to foods is still challenging due to the fact that many antioxidative compounds have a low water solubility, as it is the case with carnosic acid and carnosol [3]. Therefore, it is necessary to increase the water solubility of these compounds. In addition, a homogenous distribution of small amounts (i.e. parts per million: ppm) of antioxidants in meat products is another challenge. Consequently, oil-in-water emulsions have been shown to be a promising carrier system to incorporate lipophilic ingredients into food matrices [4].

Nanoemulsions (mean diameter ≤ 200 nm) have gained recent interest among food manufacturers and scientists as novel delivery and encapsulation systems for lipophilic bioactives due to their beneficial properties [5]. Moreover, several studies have demonstrated that the bioavailability of encapsulated nonpolar components is higher in nanoemulsions than in conventional emulsions because of the high surface-to-volume ratio and small particle size [5, 6].

Therefore, the aim of this study was to establish a better understanding of the effect of droplet size on the antioxidant activity of oil-in-water emulsions loaded with rosemary extract in raw fermented sausages. We hypothesized that the antioxidant activity of oil-in-water emulsions loaded with rosemary extract may increase when the droplet size decreases. To test this hypothesis, the antioxidant activity of a series of differently sized oil-in-water emulsions containing rosemary extract was evaluated by measuring their impact on lipid oxidation in raw fermented sausages.

II. MATERIALS AND METHODS

Materials

Miglyol 812N was purchased from Sasol Germany GmbH (Hamburg, Germany). Rosemary extract donated by Flavex (Rehlingen, Germany) was analyzed by high-performance liquid chromatography (HPLC) to contain 33.9 % carnosic acid and 4.7 % carnosol [7]. Polysorbate 80 (Tween 80) was obtained from Carl Roth (Karlsruhe, Germany). Chemicals for buffer solutions, peroxide determination, and standards for gas chromatography (GC) and HPLC were all purchased from Sigma-Aldrich (Steinheim, Germany). All solvents were of analytical grade and obtained from VWR International GmbH (Darmstadt, Germany). Lean pork meat and fat were obtained from a local supplier (Faerber, Kulmbach, Germany).

Emulsion preparation

Emulsions were prepared by homogenizing 10 % (w/w) oil phase (Miglyol 812N, with or without added rosemary extract) with 90 % (w/w) emulsifier solution (2 % (w/w) Tween 80 in 10 mM sodium phosphate buffer, pH 5.0) with a high shear blender (T10 basic Ultra-Turrax, IKA, Staufen, Germany) at high speed (24,000 min^{-1}) for 5 min to form a coarse pre-emulsion. This procedure created (i) (unloaded) Miglyol oil-in-water emulsions with a particle size of 7200 nm ($o/w_{control}$), and (ii) Miglyol oil-in-water emulsions containing 100,000 ppm of rosemary extract in the oil phase ($o/w_{d=4600}$). Afterwards, the pre-emulsion loaded with rosemary extract was passed five times through a high-pressure homogenizer (EmulsiFlex-C3, Avestin, Ottawa, Canada) at 1500 bar to generate finely dispersed emulsions (iii) with a particle size of 160 nm ($o/w_{d=160}$).

Particle size analysis

Droplet size distributions were determined by static light scattering (Horiba LA-950, Retsch Technology, Haan, Germany). Emulsions were diluted to a droplet concentration of approximately 0.005 % (w/v) using an appropriate buffer solution (10 mM sodium phosphate buffer, pH 5.0) to avoid multiple scattering effects.

Optical microscopy

All emulsion samples were examined microscopically. Images were taken with a Canon Powershot G10 digital camera (Canon, Tokyo, Japan) attached to an Axio Scope optical microscope (A1, Carl Zeiss Microimaging, Göttingen, Germany) at a magnification of the objective 40×.

Preparation of raw fermented sausages

Sausages were prepared without added spices, except for black pepper, to avoid interactions with the rosemary extract (Table 1). Lean meat and fat, curing salt, black pepper powder, glucose and starter cultures were homogenized in a bowl chopper (K+G Wetter 45 C, Biedenkopf-Breidenstein, Germany) as follows: Firstly, the frozen meat was minced at low speed for 2 bowl chopper rotations, followed by 40 rotations at high speed. After that, fat was added and homogenized for 10 rotations at high speed. Then, the curing salt, black pepper powder, glucose and starter cultures were added and homogenized for 8 rotations at high speed. Finally, different oil-in-water emulsions were added to the meat batter at the end of the homogenization process and mixed for 15 rotations at low speed. The final meat batter was filled into permeable collagen casings (Naturin R2 60 mm × 40 cm, Viscofan, Tajonar-Navarra, Spain).

Table 1 Composition of raw material used in the preparation of salami in each treatment

Raw material	Proportion of raw material (%)
Frozen lean pork	62.35
Frozen back fat	33.57
Curing salt[a]	2.69
Black pepper powder	0.29
Glucose	0.58
Starter culture[b]	0.04
Emulsion[c]	0.48

[a]Contained 95.5 % sodium chloride and 0.5 % nitrite (Südsalz GmbH, Heilbronn, Germany); [b]B-LC-007 contained *Debaryomyces hansenii*, *Lactobacillis sakei*, *Pediococcus acidilactic*, *Peciococcus pentosaceus*, *Staphylococcus carnosus and Staphylococcus xylosus* (Chr. Hansen, Hørsholm, Denmark); [c]with and without 100,000 ppm rosemary extract in the oil phase

Maturation and storage of s

The dry-cured sausages were fermented and matured for 21 days at 22 and 15 °C in a climate chamber (Allround System Rondair, MC 3.2, Maurer AG, Reichenau, Germany) at 93 % to 85 % relative humidity. After maturation, sausages were sliced into 2 mm slices (SE 12, Bizerba, Balingen, Germany). Slices were stored in sealed trays with 60 % O_2, 25 % CO_2 and 15 % N_2 at 7 °C in the

dark for a period of 49 days to accelerate the oxidation process.

Determination of lipid oxidation products

The POV of the raw fermented sausages was quantified according to a method by Bluechel and Honikel (2006) [8], with some modifications. An amount of 30 g minced meat was mixed with 70 mL dichloromethane using a shaker plate for 8 min. The mixture was subsequently filtered through a pleated filter. A volume of 20 mL of the organic extract was evaporated with nitrogen and stored at 105 °C for 30 min to determine the dry residue. Another 20 mL of the organic extract was mixed with 20 mL acetic acid followed by the addition of 0.5 mL of a saturated potassium iodide solution. The reaction was stopped after 60 s with 30 mL double distilled water. Subsequently, 1 mL of a freshly prepared starch solution was added. Finally, the mixture was titrated from dark blue to colorless with a sodium thiosulfate solution (0.01 – 0.1 N).

Hexanal was determined by placing 2 g of the meat samples into 20 mL headspace vials sealed with PTFE/silicone septa (Agilent Technologies, Santa Clara, CA, USA). Hexanal was detected using an Agilent 5975c MSD chromatograph system connected to a G1888 headspace autosampler equipped with ChemStation software (Agilent Technologies, Santa Clara, CA, USA). Separation was carried out on a 30 m × 0.25 mm HP-5MS column with a 0.25 µm film thickness (Agilent Technologies, Santa Clara, CA, USA). Samples were heated to 60 °C for 10 min in the autosampler heating oven. The sample split ratio was 7. The following oven program was used: starting from 40 °C for 2.0 min and increasing to 110 °C at a rate of 8 °C/min. Subsequently, temperature was raised from 110 to 160 °C at a rate of 15 °C/min. Helium was used as the carrier gas with a flow rate of 1.5 mL/min. A triple axis mass spectroscopy detector (MSD) was used. Hexanal concentrations were determined from peak areas using a standard curve mad form hexanal.

Statistical analysis

Each experiment was performed on at least three samples. Results are reported as averages and standard deviations using Excel (Microsoft, Redmond, WA, USA). Statistical analysis was performed using one-way ANOVA by JMP 11.0 (SAS Institute, Cary, NC, USA). Normality and equality of variances were tested (p for rejects 0.05) as assumptions. In addition, samples not normally distributed and/or of unequal variance were tested using a non-parametric Kruskal-Wallis test. Differences at $p < 0.05$ were considered to be significant

III. RESULTS AND DISCUSSION

Characterization of base emulsions

The volume-weighted mean particle diameter (d_{43}) measured of all the emulsions did not change during the course of the experiment (data not shown), thus indicating that the emulsions were stable regarding droplet coalescence and Ostwald ripening. Optical microscope images confirmed these findings (data not shown). In general, oil-in-water emulsions stabilized by Tween 80 tend to only have low magnitudes of ζ-potential, as was the case for the unloaded oil-in-water emulsion $o/w_{control}$ [9]. However, ζ-potentials of the emulsions $o/w_{d=4600}$ and $o/w_{d=160}$ become more negatively charged due to the addition of rosemary extract (data not shown). This change in ζ-potential suggests that components of the rosemary extract were located at the droplet surface.

Oxidative stability of raw fermented sausages

As expected, the raw fermented sausages containing rosemary extract ($S_{RE\ 4600}$ and $S_{RE\ 160}$) exhibited significantly ($p < 0.001$) lower concentrations of primary and secondary oxidation products after 49 days of storage compared to the sausages without added antioxidant ($S_{Control}$) at + 7 °C (Fig. 1). The peroxide value decreased by more than 79 % and the formation of hexanal was also decreased by more than 90 % at 7 °C after 49 days of storage. However, contrary to our hypothesis stated previously, the nanoencapsulated rosemary extract had a significantly lower antioxidant effectiveness compared to the macroemulsions containing antioxidants during the course of the experiment (Fig. 1). These findings might be caused by the physical location of the antioxidant compounds within the base emulsions. Several studies have shown that many of the variations in the antioxidant behavior of compounds that differ in polarity can be explained

by their tendency to distribute onto the surface of emulsified oils [10]. The fact that lipophilic antioxidants (e.g. carnosol and carnosic acid) can accumulate at the oil-in-water interface may explain the findings shown in Fig. 1. In our study, the interfacial area was increased by a factor of 29 in the 160 nm emulsions compared to the 4600 nm emulsions [11]. Consequently, more of the antioxidant compounds (e.g. carnosol) were accumulated at the oil-water interface in the emulsion o/$w_{d=160}$ and could not migrate into the fat of the raw fermented sausage where lipid oxidation occurred.

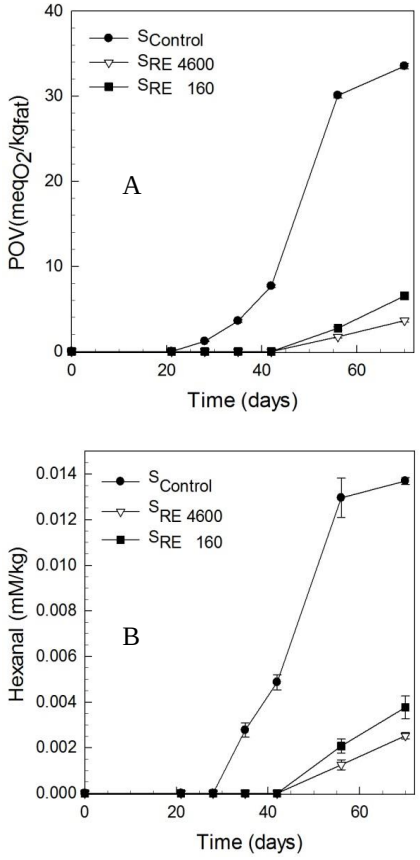

Figure 1. Peroxide value (A) and formation of hexanal (B) in raw fermented sausages according to treatment at 7 °C during storage in the dark.

IV. CONCLUSION

Results suggest that the antioxidant activity of an oil-in-water emulsion depends on the physicochemical properties of the compounds used, the microstructure of the system and the specific application. Therefore, statements that nanoemulsions would always be "better" or more "functional" should, thus, be taken with caution.

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