PARSLEY EXTRACT AS AN ALTERNATIVE FOR CURING SALT IN MORTADELLA-TYPE SAUSAGES – IMPACT ON THE GROWTH OF *LISTERIA MONOCYTOGENES*

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Abstract – The market of alternatively cured meat products expands rapidly owing to the increasing awareness of health for consumers. Vegetable extracts may be used as natural nitrate sources in processed meat. Due to the absence of curing salt, these meat products might be more susceptible to pathogenic bacterial growth. The objective of this study was therefore to investigate the growth of *Listeria monocytogenes (L .m.)* in mortadella-type sausages, manufactured with 1.07 (V3), 2.14 (V4) and 4.29 (V5) g parsley extract/kg sausage meat. The parsley variants were compared to an uncured (V2) and a traditional nitrite-cured control (V1). Over storage time (28 days), bacterial counts of V1, V2, V3 and V4 increased by approx. 6 $\log_{10}$ units until the end of storage time. A smaller increase (approx. 5 $\log_{10}$ units) and a significantly lower content of *L. m.* compared to all other variants were found for V5. Besides, an ingoing amount of 4.29 g parsley extract/kg sausage meat reduced the residual nitrite content by 40% (6.82 ± 0,86 mg nitrite/kg) compared to the traditionally cured control (11.02 ± 1.27 mg nitrite/kg). Finally, this study demonstrates the inhibiting potential of parsley extract on *L. m.* with special regard to significantly reduced residual nitrite contents in mortadella-type sausages.

Key Words – alternative curing, microbiological safety, natural nitrate sources

I. INTRODUCTION

Nitrite has an inhibitory effect on the growth of microorganisms, such as *Listeria monocytogenes* (*L.m.*) [1,2] and therefore ensures microbiological safety and shelf life in cured meat products [3]. Furthermore, it contributes to the typical red color and the flavor development of processed meat. However, there are risks associated with the use of nitrite: nitrosamine formation can occur in the production process, during preparation by consumers or the digestion step in the stomach [4]. Nitrosamines are considered to be carcinogenic [5,6]. The International Agency For Research Cancer (IARC) classified processed meat products in group 1 (carcinogenic to humans) and recommended the reduction of intake of cured meat products as far as possible [7]. In the USA and some European countries, natural sources of nitrate, such as celery or swiss chard, are already used in the manufacturing process of meat products. In combination with a nitrate-reducing starter culture, lower residual nitrite contents can be detected compared with traditionally cured products [8,9]. Nevertheless, there are still concerns about the microbiological safety of those products. Some studies confirm a lack of bacterial inhibition in these products [9,10], as a result of the use of lower nitrite levels. The objective of this study was to investigate the effect of different concentrations of parsley stem extract powder on the growth of *L. m.*, a common contamination germ of cured and heat-treated ready-to-eat meat products like sliced and pre-packaged mortadella-type sausages [2,11,12].

II. MATERIALS AND METHODS

Traditional nitrite-cured (V1) and uncured (V2) mortadella-type sausages, as well as sausages with different ingoing concentrations of parsley extract powder (V3: 1.07 g, V4: 2.14 g and V5: 4.29 g parsley extract/kg sausage meat) were produced in three independent trials. The quantities of parsley extract used corresponded to an amount of 30 (V3), 60 (V4) and 120 ppm (V5) nitrate, respectively. For the parsley variants, a nitrate-reducing starter culture consisting of *Staphylococcus carnosus* was added. After a holding time (90 min, 40 °C) and cooking (core temperature 72 °C), the sausages were cooled overnight (4 °C). One day after production, sausages were cut into slices of about 25 g (1 ± 0.2 cm). The slices were put in polypropylene trays and inoculated with a suspension of *L. m.*: one colony of *L .m.* was transferred to Brain Heart Infusion (Merck KGaA, Darmstadt, Germany) and incubated for 24 hours at 37 °C. Thus, bacterial counts of about 10^8 CFU/mL were reached. The bacterial suspension was diluted to a calculated amount of 10^4 CFU/mL, and 0.1 mL of the inoculum was equally spread on each slice. Thus, a bacterial count of 5×10^2 CFU/g sausage was expected. The trays were sealed, gas-flushed (70 % N_2, 30 % CO_2) and stored at 7 ± 0.2 °C for further analysis on days 1, 7, 14, 21 and 28 of storage.

For quantitative analysis of *L. m.* according to ISO 11290-2:2005, one slice (~25 g) of each package was transferred into a stomacher bag together with 225 mL ½ fraser broth (Oxoid GmbH, Wesel, Germany). After homogenization (200 rpm / 2 min.), samples were diluted in a 10 fold dilution series and plated in duplicate on the respective selective agar (OCLA Agar, Oxoid GmbH, Wesel, Germany) and then incubated at 37 °C for 24 hours. Subsequently, colonies were identified and counted. Nitrite contents in the sausages were determined according to DIN EN 12014-3. On days 1, 7 and 14, samples from each variant of sausage were investigated. For statistical analysis, SAS Enterprise Guide 5.1 (Statistic Analyzing Systems, SAS Institute Inc., Cary, NC, USA) was used. P values lower than 0.05 were considered as significant.

III. RESULTS AND DISCUSSION

Based on a mean bacterial count of 4.6×10^2 CFU *L. m.*/g sausage in all sausage variants on day 1, V2 (uncured) and V3 (30 ppm nitrate from parsley extract) exhibited the greatest growth of *L. m.* with an increase of approx. 6 $\log_{10}$ units, resulting in bacterial counts of 8.91 ± 0.14 (V2) and 8.68 ± 0.27 (V3), respectively, on day 28 of storage. Similar growth ($P > 0.05$, approx. 6 $\log_{10}$ units) of *L. m.* was found for V1 and V4 until the end of storage time and no significant differences were noted between the bacterial counts of V1, V2, V3 and V4 on day 28.

Though, the lowest bacterial counts were determined for V5 on days 7, 14, 21 and 28 of investigations ($P < 0.05$ on days 7 and 28). In this study, bacterial loads for V1 were continually lower than for the uncured sausages (V2). This is in accordance with several studies describing the inhibitory effect of nitrite on the growth of *L. m.* [1,2].

Residual nitrite for V1 was higher ($P < 0.05$) than for V2, V3, V4 and V5, concerning all examination days. These results correlate with the prevailing opinion that alternative processed meat products show lower residual nitrite contents [8,9] than normally-cured meat products. Although V3, V4 and V5 were produced without the addition of curing salt, nitrite was formed from nitrate due to the addition of the nitrate-reducing starter culture in these variants. Thus, residual nitrite in the sausages containing parsley extract may have an effect on bacterial growth in these variants. Lower ingoing nitrate concentrations from parsley extract resulted in lower residual nitrite contents in our study. These observations are attributable to a lower conversion from nitrate to nitrite during manufacture in the parsley extract sausages with 60 ppm (V4) and 30 ppm (V3), respectively.

It is remarkable, that V5 showed the lowest growth of *L. m.* with approx. 5 $\log_{10}$ units until the end of storage. The bacterial counts of V5 were lower than for all the other sausage variants on days 7, 14, 21 and 28 ($P < 0.05$ on day 28). We have to point out, that this also applies to the traditionally cured variant (V1). In addition, the residual nitrite contents for V5 were significantly lower compared to V1. After production with an ingoing amount of 4.29 g parsley extract/kg sausage meat, residual nitrite content of V5 was reduced by 40 % compared to the traditionally cured control (V1). This leads to the assumption, that the inhibiting effect of nitrite may not be the only reason for listerial growth reduction in our study. Beside pH, aw and heating temperature [3], parsley extract may itself have an inhibiting effect on *L.m.*. Wong et al. [13] investigated the antibacterial effect of parsley and found phenolic compounds being responsible for its antimicrobial activities.

IV. CONCLUSION

Parsley extract is suitable as a nitrate source for the alternative production of mortadella-type sausages without the addition of traditionally used curing salt. Concerning bacterial inhibition, parsley extract powder in higher concentrations seems to be comparable with the potential of traditionally-cured sausages. At the same time, reduction of residual nitrite levels can be achieved. Consequently, consumers` intake of nitrite may be reduced by marketing alternatively cured meat products. Future studies will be necessary to evaluate the effect of parsley extract in various meat products and its impact on physico-chemical and sensory parameters. Additionally, challenge studies are necessary to confirm the inhibitory effect of parsley extract on several microorganisms and to investigate the causal mechanism of its inhibition on *L. m.*

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