

## Summary for the final report

### Stabilization of sausage products and boiled ham with natural hop extracts (Hop Sausage)

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The objective of the research project “Hop Sausage” was to take advantage of the antimicrobial properties of a beta acid rich hop extract (BRHE, 40 % beta acids) for applications in meat products, in order to increase food safety or to achieve a shelf life prolongation. A high antimicrobial effect of beta-acids from the hop plant has been demonstrated against various gram-positive bacteria including *Listeria monocytogenes*, lactic acid bacteria or Clostridia. These bacteria play an important role with regard to food safety or the microbial spoilage of meat products. Within the first part of the project, various growth inhibition tests with relevant microorganisms were carried out in-vitro, in order to characterize the antimicrobial properties of the applied hop extract. Based on these findings, the hop extract was subsequently applied in a standard recipe of cured boiled sausage as a natural preservative. The antimicrobial efficiency of the hop extract in bologna was investigated within various storage trials using *Listeria monocytogenes* and *Lactobacillus sakei* as test microorganisms. *Listeria monocytogenes*, due to its ubiquitous distribution and its unique properties, is of high relevance when it comes to the safety of packaged, ready-to-eat meat products. This is mainly due to the pronounced tolerance to nitrite salt and protective gas as well as the psychrotrophic character with rapid growth even at refrigerator temperatures. Lactic acid bacteria such as *Lactobacillus sakei* are usually responsible for the microbial spoilage of bologna and ham. Therefore, the work in this project was primarily focused on the investigation of the growth inhibition potential of the hop extract BRHE regarding *Listeria monocytogenes* and *Lactobacillus sakei*.

#### **Antimicrobial efficiency of hop extract in-vitro**

Within the first part of the project, the antimicrobial efficiency of the hop extract BRHE (Hopsteiner) was investigated in-vitro. For this purpose, conditions which are relevant in case of cured meat products were simulated within various checkerboard assays. The hop extract was combined with sodium nitrite and tested for its growth inhibition against selected bacteria under variable conditions. According to the results, the hop extract BRHE can be regarded as an additional component of the hurdle concept. This is due to its increased antimicrobial effect when combined with a reduced water activity, a lowered pH value, storage under refrigeration or the use of a modified gas atmosphere. Combining the hop extract with these different hurdles led to a stepwise decrease of the minimum inhibitory concentration of BRHE. It was shown that under conditions that are relevant for packaged boiled sausages (pH 6;  $a_w$  0.97; use of protective gas; storage at 7 ° C; addition of nitrite), a concentration of only 0.2 µg BRHE/ ml is sufficient to achieve a complete growth inhibition of *Listeria monocytogenes* over several weeks. Under ideal growth conditions, a 16-fold higher concentration of the hop extract was necessary to prevent the growth of *L. monocytogenes* in tryptic soy broth. Accordingly, under certain conditions, even a very small concentration of the hop extract can be sufficient for microbiological stabilization. *Lactobacillus sakei* showed a slightly higher resistance towards the hop extract BRHE compared to *L. monocytogenes*. A concentration between 25 µg/ml and 50 µg/ml was necessary to achieve a complete growth

inhibition. The growth of *Clostridium sporogenes* was inhibited at a concentration of less than 1.6 µg/ml BRHE.

The impact of an oil-containing matrix on the antimicrobial effect of BRHE was also assessed in-vitro. The addition of 1 % dispersed sunflower oil to the culture media led to an increase of the minimum inhibitory concentration by the factor 32-125 in case of *L. monocytogenes*. Due to this severe reduction of the antimicrobial efficiency of BRHE in presence of oil, it could be expected that a fat containing food matrix would likewise quench the antimicrobial effect considerably. Therefore, it was assumed that significantly higher concentrations of hop extract are likely required in order to achieve a similar antimicrobial effect on a fat containing sausage. In addition to the growth inhibition tests, a potential antioxidant effect was investigated in-vitro as well. However, a pronounced antioxidant effect was not found for the extract BRHE, so an oxidative stabilization of meat products was not expected.

### **Antimicrobial efficiency of hop extract in cured boiled sausage**

Based on the results of the in-vitro tests, the hop extract BRHE was added to a standard recipe for a cured boiled sausage (bologna). After an initial decrease of the beta acid concentration in the sausage right after production, the concentration of lupulones remained constant throughout storage of the sausage. Within various series of experiments, different conditions with regard to sausage production and subsequent storage were taken into account. Due to matrix effects arising mainly from the high fat content of the sausage, considerably higher concentrations of the hop extract were necessary to inhibit the growth of *Listeria monocytogenes* on the packaged scalded sausage compared to the in-vitro tests. While concentrations of BRHE between 0.2 and 3.2 µg/ml led to a complete growth inhibition of *Listeria monocytogenes* in liquid media, concentrations of 0.4 - 0.8 g BRHE /kg sausage were necessary to achieve a similar effect on the sausage surface. The following general findings were made within the different storage trials with inoculated sausage.

- The hop extract can be added to the sausage meat during the cutting process as a stock suspension using deionized water for dilution.
- The time at which the hop extract is added during cutting does not have an impact on its antimicrobial effectiveness. It can be added at the beginning or at the end of the cutting process.
- The all-in process as well as the lean meat process are both equally suitable for the production of sausage with hop extract.
- The water activity, which is affected by the fat content of the meat, has a high impact on the antimicrobial efficiency of the hop extract in the sausage. For a given standard recipe of cured boiled sausage, a higher fat content goes along with a lowered water activity, which in turn leads to a pronounced growth inhibition effect of the hop extract in the sausage.
- As long as the water activity is controlled by other measures like water binding additives, a lower fat content of the sausage is favourable for the antimicrobial effect of the hop extract. A lower fat content reduces the enrichment of lipophilic hop beta

acids in the fatty phase of the sausage, which otherwise quenches the antimicrobial effect.

- The conditions under which the cut boiled sausage is stored has a decisive influence on the effectiveness of the hop extract against *Listeria monocytogenes*. While only a slight growth retardation can be achieved in a vacuum packaging, the use of a modified gas atmosphere (20% CO<sub>2</sub> / 80% N<sub>2</sub>) significantly reduced microbial growth with 0.4 g BRHE / kg sausage and completely inhibited the growth of *Listeria monocytogenes* with 0.8 g BRHE / kg sausage. Accordingly, the use of hop extract in the sausage combined with modified atmosphere packaging leads to a synergistic antimicrobial effect. Due to the common use of protective gas for the packaging of cut boiled sausage, this finding is of great practical relevance.
- The antimicrobial effect of the BRHE extract was also demonstrated against the widespread spoilage bacterium *Lactobacillus sakei*. In this case, 0.8 g BRHE / kg sausage were necessary for a significant growth inhibition.

### **Impact of hop extract on the quality of bologna**

With regard to potential quality changes of the manufactured sausages due to the addition of hop extract, colour measurements and sensory tests were particularly carried out. A concentration of up to 0.8 g hop extract / kg sausage did not affect the colour of the sliced bologna either when stored in the dark or when exposed to daylight. Overall, the sensory evaluation showed that the taste perception of the hop extract in the scalded sausage was very variable among the panelists. While the hop extract was clearly noticed by some individuals due to its bitter note, it was not perceivable by others at the concentrations used. The sensory tests also revealed that the extract, if noticed, was not necessarily perceived negatively. Overall, up to a concentration of 0.8 g / kg, no statistically significant difference of taste was found between the reference sausage without hop extract and the bologna with hop extract. However, due to the variable perception of the hop extract described above, the differences were very closely insignificant in each case.

### **Conclusion**

Overall, the results of this project show that the use of hop extract represents a promising approach to increase the microbiological safety of foods such as meat products. As an antimicrobial plant substance, hop extract could be used in a consumer-friendly manner in the sense of "clean labelling", which follows the current trend with regard to a sustainable use of natural ingredients instead of chemical preservatives. Besides *Listeria monocytogenes* and *Lactobacillus sakei*, beta acids may also inhibit the growth of other gram-positive lactic acid bacteria or clostridia in meat products. However, the hop extract is currently only approved as a spice ingredient in form of an aroma component and not as a preservative. With regard to the use of the hop extract in boiled sausage, its use as a flavoring substance seems possible, as long as a significant impact on sensory properties of the sausage is ensured. According to the current legislation in Germany, the addition as a preservative is not possible. For this purpose, approval of the hop extract as a designated preservative would be required.

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