The use of hops in meat production

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Abstract

The effect of hops (*Humulus lupulus*) on the quality of meat products was studied in connection with current demands for the wider usage of natural compounds in the food industry. Various additions of hop extracts were tested in heat-treated meat products under industrial conditions. The following technological properties were measured: colour (reflectance spectrophotometry), pH, water activity, lipid oxidation (TBARS) and texture (Warner-Bratzler shear force test). The meat products were also subjected to sensory analysis. The results suggest that hop preparations can positively affect the oxidative stability and organoleptic properties of the final product.

Hops, meat products, natural compounds

Hops are used as one of the basic ingredients in beer production, but in meat products? Nevertheless, the use of hop preparations as secondary ingredients or additives in meat products can and does make sense. Constituents of hops may act as natural preservatives or antioxidants.

The possibility of use natural additives and their advantages and disadvantages was described (Pipek et al. 2016). Their great advantage is that people trust them. People often mistakenly believe, however, that natural additives are healthier than additives with E-numbers.

Numerous herbs, fruits, spices and their extracts are described in the literature. Those used most frequently are rosemary, sage, cranberries and other berries of the genus *Vaccinium*, tea, pomegranates and various spices.

Tests are being conducted on plant extracts and fermentation products that may serve as antioxidants or antimicrobial agents and contribute to product safety and the preservation or improvement of their technological and organoleptic properties and, in particular, their shelf life (Pipek et al. 2016). The crop-plants that researchers are testing include some that are rather exotic to us, though generally only ones, those are common in the places where they occur.

Even our famous crop-plant hops, for which our brewing industry is renowned, is a natural additive with good prospects for use. Hops and preparations made from hops have been tested in certain foods (Villalobos-Delgado et al. 2015). Hops and their derivatives have been stated to have a number of therapeutic effects beneficial to the health (Karabín et al. 2016).

The common hop (*Humulus lupulus* L.) is the most widespread type of hop and exists in a number of varieties that differ in terms of the colouring of the hop vine (red and green) and their composition, particularly the composition of hop resins and essential oils. Only unfertilised female heads, which contain the greatest quantity of chemically active compounds, are important from the technological viewpoint (Briggs et al. 2004).

In addition to water, hop cones also contain saccharides, lipids, proteins and other nitrogenous substances (primarily nitrates), minerals and a large quantity of technologically important compounds that are generally divided into hop resins, essential oils and polyphenols (Kocourková et al. 2014).

Hop resins, produced in the lupulin glands, are non-polar substances that make up approximately 15 to 30% of the total weight of dried hops and are responsible for their bitter taste (Basařová et al. 2010). They are differentiated into hard and soft resins which are further divided into α-bitter acids and β-bitter acids.

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The *α*-bitter acids are comprised of a mixture of humulone analogues and are difficult to dissociate and therefore show poor solubility in aqueous solutions and are chemically unstable (Basařová et al. 2010). The *α*-bitter acids have a slight bacteriostatic effect (Kramer et al. 2015); their antimicrobial activity is weakened by the presence of divalent cations, while the addition of monovalent cations has the opposite effect (Almaguer et al. 2014).

The *β*-bitter acids make up approximately 3 to 5% of dried hops by weight and show poor solubility in water. They are a mixture of lupulone and other compounds (Basařová et al. 2010). They are one of the main antimicrobial substances in hops and also have an antioxidant effect (Kramer et al. 2015 and Van Cleemput et al. 2009).

The essential oils in hops are the volatile constituents of hops responsible for their typical aroma. Dried hops contain 0.5 – 3% essential oils by weight (Kocourkova et al. 2014). Their composition depends on technological conditions – they are a complex mixture of compounds (Karabín et al. 2016 and Basařová et al. 2010). Plant essential oils are generally considered antimicrobial agents, and similar properties can also be expected from hop essential oils. Strong antioxidant effects, neutral behaviour and pro-oxidation functions of the constituents contained in hop essential oils have been observed (Karabín et al. 2016). Hop essential oils are, along with resins, the principal substances responsible for the antibacterial effects of hops (Roj et al. 2015); antimicrobial effects of essential oils against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella* spp. and *Candida albicans* have been confirmed (Karabín et al. 2016).

Polyphenols are secondary metabolites of hops comprising 2 to 5% of the total weight of dried hop heads (Kunze 2004). They are relatively reactive and largely hydrophilic compounds that easily undergo oxidation-reduction changes; the reducing power of polyphenols is important to the use of hops in the food industry as they have a strong antioxidant effect and are capable of providing protection against oxidation (Karabín et al. 2016 and Almaguer et al. 2014). Their antioxidant effect can be divided according to the mechanism of action; they are capable of binding polyvalent ions of metals that would act as catalysts during oxidation reactions; they may also inhibit enzymes that catalyse oxidation and react with free radicals and reactive oxygen and nitrogen species (Karabín et al. 2016 and Falowo et al. 2014).

It is stated in the literature that hops can, in view of their antioxidant and antimicrobial properties, be used in foods such as meat and meat products, dairy products and salads, etc. (Kramer et al. 2015 and Karabín et al. 2016) depending on the form in which hops are applied to the foodstuff. Dried hop heads can be used themselves, though this would entail the risk of microbial and chemical contamination (residues of pesticides) and a non-homogenous non-standard ingredient, for which reason hop preparations are applied which significantly facilitate handling, are chemically more stable and allow the use of the individual hop fractions.

Hops preparations are made by the mechanical treatment of hops (powders, pellets) or by extraction (with water, ethanol or carbon dioxide) (Basařová et al. 2010 and Kunze 2004).

The majority of studies concerning the application of hops in the food industry consider their antimicrobial effects. Their main preservative constituents are *β*-bitter acids which have an inhibitory effect only on gram-positive species of bacteria (the genera *Listeria, Lactobacillus, Streptococcus, Staphylococcus, Micrococcus* and *Bacillus*). Gram-negative species are evidently resistant thanks to their lipopolysaccharide layer (Van Cleemput et al. 2009 and Abram et al. 2015).

The antilisterial effects of hops have been observed on slices of pork meat and ham and on sausages dipped in a solution of *β*-bitter acids. The surface treatment of meat products caused an immediate reduction in the number of cells and extended the lag phase

of *L. monocytogenes*, while having no effect on organoleptic properties or colour (Shen et al. 2009; Abram et al. 2015; Kramer et al. 2015; Wang and Shen 2015 and Wang et al. 2016).

The antioxidant properties of hops, for which polyphenols are primarily responsible, are no less significant. The greatest content of polyphenols is obtained from hop heads by extraction with water or a mixture of ethanol and water (Önder et al. 2013). What's more, a combination of an extract from hop heads with an extract of bilberry leaves (*Vaccinium myrtillus*) can expand the spectrum of chemical compounds and thereby increase the antioxidant properties (Bubueanu et al. 2015).

The effect of the addition of hop dispersion (hop pellets mixed with deionised water) and a hop extract (hop pellets boiled in deionised water and filtered) on lipid and protein oxidation and the colour and organoleptic properties of lamb burgers has been studied. The addition of hops significantly restricted lipid and protein oxidation during the refrigerated and frozen storage of cooked and raw burgers; the results were similar as in the case of the widely used ascorbate. Hop extract did not affect the taste of the burgers, in fact it suppressed the formation of a rancid taste. In contrast, the addition of hop dispersion changed the taste of the burgers slightly and reduced their acceptability, although it had better antioxidant effects (Villalobos-Delgado et al. 2015).

Extracts of hop leaves also have an antioxidant effect, though these effects are much weaker than those of hop heads. They could, nevertheless, be used as this is an extremely cheap material (Abram et al. 2015).

Thanks to the positive properties mentioned above, hop preparations could be a suitable additive to increase the safety and extend the shelf life of meat products (Kramer et al. 2015).

Materials and Methods

We conducted our own experiments to test various hop preparations in heat-treated meat products which were subsequently analysed as follows:

The first laboratory trial experiments focused on the selection of a suitable type of hop preparation. We tested hop pellets and debittered pellets which were mixed into a sausage batter in a mixer and heat treated, with the model samples prepared in this way then being subjected to sensory evaluation. Formulations for production tests were prepared on the basis of this evaluation.

Two production tests were conducted in which the same doses of debittered hop pellets were added to the basic batter for Lyoner type ("Junior") sausages under industrial conditions. The batter was filled in plastic casings and heat treated in the standard way. Following production, samples of the sausages were taken to the laboratory at the University of Chemistry and Technology where they were stored at 2 °C for a period of two months and regularly analysed; organoleptic properties, fat oxidation (TBARS), colour, pH, water activity, texture, residual nitrite content and the growth of microorganisms were evaluated.

The colour of the meat products was measured with a Minolta CM 5 reflectance spectrophotometer. The resultant values of lightness (L^*) and coordinates for red (a^*) and yellow (b^*) colour in the CIELab colour system with SCI reflection were recorded by the computer program Spectra Magic.

The TBARS value was used as the criterion of lipid oxidation. Following distillation with water vapour, 0.02M 2-thiobarbituric acid in 90% acetic acid was added to an aliquot of distillate. After heating (35 minutes) the mixture was cooled and absorbance at 538 nm was measured on an Evolution 60 spectrophotometer. The result was expressed as the concentration of malondialdehyde (in $mg \cdot kg^{-1}$).

Texture was measured as the Warner-Bratzler shear force using an Instron 5544 instrument. Cuboids of a size of 25 x 25 x 100 mm were sliced from the sausages; the crosshead speed was 80 $mm \cdot min^{-1}$. The maximum values of force [N] were recorded by the program Series IX and processed with the exclusion of outlier values by the statistical program STATISTICA 10.

The pH values of the samples were measured with a Seven Go SG2-B pH-meter with an InLab®Solids needle-tipped glass combination electrode. The values obtained were statistically evaluated by an F-test and t-test.

Water activity (a_w) was measured on an AquaLab 4TEV instrument. The values obtained were statistically evaluated by an F-test and a t-test.

The residual nitrite content was determined by a modified spectrophotometric method according to Griess (1879). The values obtained were statistically evaluated by an F-test and a t-test.

Sensory evaluation was performed at the sensory laboratory at the University of Chemistry and Technology in Prague. Evaluators of various age categories were presented with anonymous samples and the members of the panel evaluated a number of taste descriptors, recording their intensity on a 100-mm line scale.

A microbiological investigation was performed at the State Veterinary Institute, Olomouc. Total viable counts and the occurrence of enterobacteria, *Listeria monocytogenes*, *Salmonella* spp. and *Clostridium perfringens* were evaluated.

Results and Discussion

The production test checked the effect of the addition of hop extracts on the properties of "Junior" sausages made in the standard way. The addition of hop extracts led to the limitation of oxidation, evidence of which is provided by changes to the TBARS value during storage (Plate I, Fig. 1). Statistical comparison of the values obtained on the samples with the control at a level of significance of $\alpha = 0.05$ and higher show that debittered hop pellets restrict lipid oxidation during the storage of meat products most effectively at a quantity of 0.05% by weight and 0.10% by weight.

Colour was slightly affected by the addition of debittered hop pellets; the small differences seen were evidently the result of a combination of a number of factors. The relatively short period of cutting and mixing following the addition of the hop preparation may have caused the uneven dispersion of the added colouring in the batter that was evident from a visual inspection. As can be seen from Table 1, there was a slight fall in lightness L^* and the coordinate for red colour a^*. The addition of hop extracts in a solution, which enabled more even distribution in the batter, was selected for further experiments.

Table 1. The effect of added debittered hop tablets on colour in the first production test

Addition [% weight]	L^*	a^*	b^*
0	70.48 ± 0.31	12.55 ± 0.52	10.26 ± 0.44
0.05	70.70 ± 0.48*	12.03 ± 0.41**	9.71 ± 0.34**
0.10	69.86 ± 0.25**	12.28 ± 0.39*	10.35 ± 0.58
0.15	69.45 ± 0.38**	12.32 ± 0.36*	10.47 ± 0.48*
0.20	69.60 ± 0.42**	12.11 ± 0.33**	10.37 ± 0.44

* Statistically not identical to the control (at a level of significance of $\alpha = 0.05$)

** Statistically not identical to the control (at a level of significance of $\alpha = 0.001$)

As expected, the values of pH and water activity were not affected by the addition of debittered hop pellets which is in accordance with the available data in the literature (Abram et al. 2015).

The texture of the sausages was also not affected by the addition of hop preparation (Plate I, Fig. 2). There is no statistically significant difference ($\alpha = 0.001$) between the control and the samples containing 0.05 – 0.15% debittered hop pellets by weight. Lower values of shear force were measured only in the sample with a 0.20% addition of debittered pellets.

The residual nitrite content is interesting. As can be seen in Table 2, there was an increase in the residual nitrite content in meat products with the increasing addition of debittered

Table 2. The effect of added debittered hop pellets on the residual nitrite content

Addition [% by weight]	0	0.05	0.10	0.15	0.20
c [mg·kg^{-1}]	27.26 ± 0.66	29.21 ± 0.60*	32.47 ± 0.14**	34.45 ± 0.04**	34.44 ± 0.10**

* Statistically identical to the control (at a level of significance of $\alpha = 0.001$)

** Statistically not identical to the control (at a level of significance of $\alpha = 0.05$)

hop pellets. A possible explanation for this is the transformation of nitrogenous substances in hops into nitrates and their subsequent reduction in the presence of nitrate-reducing microorganisms. A second explanation may be the antioxidant effect of debittered hop pellets, or perhaps a combination of both the given causes of the increase in the residual nitrite content. The legislative limit of 150 $mg \cdot kg^{-1}$ for the maximum quantity that may be added during the course of production was not, however, exceeded in any sample (Regulation No. 1333/2008).

The results of the microbiological analysis are given in Table 3. No significant microbial growth was seen over the course of 45 days of storage. Insignificant positive findings for the TVC in samples with hop preparations can be put down to merely slightly increased initial contamination, probably from hop pellets. No significant antimicrobial effects were expected of the hop preparation because the antimicrobial effects of hop constituents are caused primarily by *β*-bitter acids which were removed from debittered hop pellets. Enterobacteria, salmonella, *Listeria monocytogenes* and *Clostridium perfringens* were not found either at the beginning nor the end of storage.

Table 3. The effect of individual additions of debittered hop pellets on the number of microorganisms at the beginning and end of storage in the first production test

Addition [% weight]		0.00	0.05	0.10	0.15	0.20
TVC	Beginning	$< 1.10^2$	3.10^2	1.10^2	$< 1.10^2$	1.10^2
[$CFU \cdot g^{-1}$]	End	$< 1.10^2$	1.10^2	2.10^2	1.10^2	4.10^2

The sensory evaluation of products in the first production test did not reveal any significant differences between the individual samples (Plate II, Fig. 3) and none of the samples was definitively considered either unacceptable or the best by the evaluators. In spite of the addition of a debittered preparation, the intensity of bitter taste in the meat products corresponded to the increasing concentration of hop additive. The evaluators also had the task of defining any unfamiliar taste they detected. The tastes they stated most frequently were spicy and peppery tastes, followed by a burning or astringent taste or the taste of garlic, parsley and lemon. None of the evaluators, however, identified the addition of hops. The addition of debittered hop pellets can, therefore, be thought on the basis of the results of the sensory evaluation not to have a negative effect on the taste of the meat products or their acceptability to consumers.

The antioxidant properties of debittered hop pellets in meat products were, therefore, confirmed in the first production test, with the best antioxidant effects being obtained by the addition of 0.05% and 0.10% by weight.

The repeat experiment

The “Junior” sausages were again produced for the repeat production test with the same additions of hop preparation, though the method of their application was changed. While in the previous experiment debittered hop pellets were added, which presented a risk in relation to microbial contamination and which were visible on the slice even when extremely fine mincing was performed, the addition of a hop extract obtaining using the process according to Villalobos-Delgado et al. (2015) and Önder et al. (2013) was tried in the repeat experiment. The same quantities as in the first experiment were monitored during the course of storage in the samples prepared in this way.

Lipid oxidation (evaluated by the TBARS value) was, as in the previous experiment, limited by the addition of hop extracts (Plate II, Fig. 4), with the exception of the 0.20%

addition which resulted in a reversal from an antioxidant to a pro-oxidant action, evidently as a result of overdosing. The spread of TBARS values during the course of storage was associated both with the irregularity of composition of real samples of meat products and evidently with the differing speed of formation of primary and secondary products of lipid oxidation. Statistically significant (α = 0.05 and higher) differences in TBARS values between samples with the addition of extracts and the control did not appear until after 16 days of storage.

Table 4. The effect of added hop extracts on the colour of samples in the repeat production tes

Addition [% weight]	*L**	*a**	*b**
0.00	72.67 ± 0.47	12.55 ± 0.46	8.97 ± 0.19
0.05	72.85 ± 0.22*	12.61 ± 0.39	8.93 ± 0.31
0.10	72.65 ± 0.40 *	12.54 ± 0.44	8.89 ± 0.29*
0.15	72.79 ± 0.39	12.44 ± 0.33	9.22 ± 0.39**
0.20	72.22 ± 0.22**	12.67 ± 0.37	9.04 ± 0.30

* Statistically not identical to the control (at a level of significance of α = 0.05)

** Statistically not identical to the control (at a level of significance of α = 0.001)

Colour quantities *L**, *a** and *b** were not significantly affected by the addition of hop extracts (Table 4). Furthermore, particles of hops of differing colour did not appear as they did in the first experiment.

The values of pH and water activity were not affected by the addition of hop extracts, as was also true in the first production test. The slight differences in the values fell within the range of experimental error.

Texture evaluated on the basis of shear force according to Warner and Bratzler (Plate III, Fig. 5) was not statistically significantly (α = 0.001) affected by hop extracts up to a 0.15% addition. Statistically significantly (α = 0.05) lower values of shear force were measured in the sample with a 0.20% addition of extract, as in the first experiment. The reason for this fall may have been the restriction of protein oxidation which generally increases the hardness of meat products. It is also stated in the literature (Villalobos-Delgado et al. 2015) that hop products restrict protein oxidation, and it can, therefore, be expected that this is the reason for the softer texture in sausage containing 0.20% by weight hop extract and debittered hop pellets.

Additions of hop extracts did not significantly affect the residual nitrite content in meat products. The measured values given in Table V are the same in the individual samples as in the control at a level of significance of α = 0.001.

Table 5. The effect of added hop extracts on the residual nitrite content in the repeat production test

Addition [% weight]	0	0.05	0.10	0.15	0.20
c [mg·kg^{-1}]	17.56 ± 1.24	20.55 ± 0.63	19.45 ± 0.52	20.84 ± 0.65	19.06 ± 0.56

The effect of the addition of extracts on the residual nitrite content in the repeat experiment is less significant than in the first experiment. It is also clear that this content is also lower in the control sample which is logical in view of the fact that samples from two production series cannot be identical under real conditions. The contents are, however, significantly lower than the legislative limit of 100 mg·kg^{-1} in all samples. The smaller effect of hop

extracts on the residual nitrite content can be sought in the reduction to the nitrate content during their preparation, which is obviously a positive thing.

The total viable counts of microorganisms (Table 4) were not affected by the addition of hop extracts and were extremely low. This is associated with the overall high standard of hygiene and the high value of thermal pasteurisation. Enterobacteria, salmonella, *Listeria monocytogenes* and *Clostridium perfringens* were not found either at the beginning or the end of storage.

The sensory evaluation in the repeat production test (Plate III, Fig. 6) was organised differently than in the first experiment. The evaluators were first presented with a control with which they subsequently compared unknown samples. Statistically significant differences were attained in the evaluation of the intensity of meaty and rancid taste. At quantities of 0.15 – 0.20% by weight, the hop extract demonstrably limited the formation of a rancid taste, and at lower concentrations reduced the intensity of the meaty taste. Hop extract would not seem to have had a demonstrable effect on the intensity of salty taste, though it did reduce the intensity of bitter taste.

Table 6. The effect of added hop extracts on the total numbers of microorganisms in the repeat production test

Addition [% weight]		0	0.05	0.10	0.15	0.20
TVC	Beginning	2 x 102	3 x 102	1 x 102	1 x 102	4 x 102
[CFU·g^{-1}]	End	< 102	2 x 102	< 102	< 102	7.3 x 102

Finally, two samples were made on a production scale – a control sample and a sample with the addition of hop extract (0.15% by weight). These samples were presented to a large number of evaluators at the symposium New Directions in the Production and Evaluation of Foods in Skalský Dvůr. The results can be seen in (Plate IV, Fig. 7). No significant differences were found. It is interesting that the sample with hop preparation received a slightly better overall evaluation.

Conclusions

The application of hop extract in meat products seems to be appropriate. At the appropriate dosage lipid oxidation is restricted and the final products obtain a positive sensory evaluation. Work is continuing on making dosaging more precise and on the modification of the composition of hop preparations.

Acknowledgments

This research was conducted with the financial support of the project NAZV QJ1610202.

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