P-02-24

Effect of production process parameters on the distribution of bacteria in the fermented sausage matrix (#366)

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Introduction

In the production of fermented sausages lactic acid bacteria play an important role as starter cultures ensuring the development of the characteristic flavor and aroma as well as the safety of the product. In the fermentation process, the starter organisms must be able to establish themselves in the meat matrix and prevail over the autochthonous flora as well as possible contaminants. Only then can the safety and a reproducible sensory profile be warrantied. The homogenous distribution of the starter culture is one of the main factors affecting its pervasiveness in the sausage matrix. The raw sausage mass is a solid matrix consisting of muscle protein and fat particles of various sizes. The meat comminuting process in the bowl chopper used to produce the meat batter also serves the effective distribution of the starter culture in the meat batter. An insufficient distribution of the starter organisms in the batter, may lead to its underrepresentation in large areas of the sausage. This in turn may allow the growth of contaminating organisms and autochthonous flora with negative consequences in the safety and sensory profile of the product.

In artisanal and small-scale production of sausages, the selection of bowl-chopping process parameters may be intuitive and dependent on the preferences of each producer. The effects of the different process parameters in the spatial distribution of the starter culture in the meat batter matrix have not yet been studied. The aim of this work was to investigate the effects that some basic bowl-chopping process parameters have on the distribution of a culture in the meat batter. The factors investigated were, the relative mass of the processed meat batter compared to the size of the bowl-chopper, the duration of the high-speed chopping and the rate of addition of liquid culture in the meat batter.

Methods

E. coli K12 JM109 carrying plasmid pTM30gfp was used for the expression of Green Fluorescent Protein under the *lac* promoter and induction with IPTG. This strain was used as an alternative to starter cultures for the evaluation of the spatial distribution of culture cells in the sausage matrix and was added to a final concentration of 4×10^6 CFU/g. The sausage batter was prepared with S VIII grade 20% pork back fat and 80% grade S II thick shoulder pieces. Chopping of the meat and mixing of the ingredients was done in a benchtop bowl-chopper. The number of revolutions of the bowl at high speed (16 or 28), the mass of the batter in kg (2 or 4) and the number of bowl revolutions during which the liquid culture was added to the batter (0 or 2) were systematically varied according to a full factorial setup with 3 factors in two levels (2^3) and supplemented with center points. Each experiment was repeated 3 times (42 runs in total). The meat batter was filled in plastic casings (600g) and frozen (-18 °C) until use. Confocal fluorescence microscopy was used to visualize the positions of the fluorescent cells in 81 fields of 0.11 mm^2 each per sausage. Twenty pictures in depth were taken from each field in steps of 5 µm to a total depth of 100 µm. In total more than 68000 pictures were taken and analyzed. The number of fluorescent cells appearing in focus in every picture was noted. The distribution of the cells was evaluated using the Dispersion Index (DI) as calculated for each sausage from the ratio of variance to the mean of fluorescent cells observed in each picture (DI_p) or in each field (DI_f). The DI was used as the response variable in the factorial regression analysis of the results using Minitab® 18.

Results

According to the DI values the distribution of the cells indicated clumping, irrespective of processing conditions. The DI_f ranged 7.89 – 29.61 and the DI_p 1.84 to 6.0. The Factor "Number of revolutions at high speed" significantly affected DI_p ($p = 0.029$). A greater number of revolutions at high speed led to lower DI_p values indicating an improvement in the homogeneity of distribution of the cells. An increase of the mass of the batter also had a positive influence on the DI_p although not statistically significant ($p = 0.533$). Pictures taken from the sausage matrix with phase contrast microscopy in combination with fluorescence microscopy showed that the fluorescent cells were located only in the peripheral area of tightly packed fat particles and of packs of muscle cells. Further processing of microscopic images gave indications that longer duration of high speed bowl-chopping influenced the size of fat particles and muscle cells thereby affecting the homogeneity of cell distribution in the sausage matrix.

Conclusion

Altering the processing conditions of meat comminution and mixing for the production of meat batter is able to increase the homogeneity of distribution of cells in the sausage matrix. High-speed bowl-chopping for extended duration homogenizes the cell distribution probably by reducing the particle size of the different sausage components.

Notes