FREE AMINO ACIDS IN AIR-DRIED SAUSAGES.

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SUMMARY

The objective of this study was to investigate the effect of temperature and ingredients on the levels of free amino acids in air-dried fermented sausages. Sausages added *Staphylococcus xylosus* were fermented at different temperatures and added various amounts of salt, glucose, nitrite, nitrate and *Pediococcus pentosaceus* in accordance with a six factor fractional design. After 8 weeks of ripening, the fermentation temperature at the beginning of the ripening and the addition of nitrate have significant effects on the levels of free amino acids. A high fermentation temperature and a low nitrate level favours the concentration of free amino acids at the end of ripening. The levels of individual amino acids are influenced at various degrees by both factors.

Introduction:

During ripening of fermented sausage, protein is slowly degraded by proteolytic enzymes of endogenous and microbial origin. This mostly leads to an increase of the concentration of water soluble proteinous material like peptides and free amino acids (Naes et al., 1992). Amino acids are among the most reactive of the major food components, and during food processing they can react with sugars, fats and their oxidation products. These reactions influence the organoleptic properties of the food.

Although protein changes could be an important source of flavour compounds in fermented dry sausage, through the involvement of amino acids in e.g. Maillard reactions, little scientific information is available on the modifications taking place during ripening. The variations of FAA patterens can also reflect differences in the metabolic activities of the fermenting microflora during dry sausage manufacture (DeMasi et al. 1990). Furthermore, the distinct conditions of fermentation may be involved in the differences observed.

The purpose of this study, was to determine free amino acid concentration changes in fermented sausages as influenced by fermentation temperature and ingredients as a possible indication of the processes leading toward the formation of sausage flavour. The results presented here are related to the end of ripening.

Materials and methods:

Sausage production: The sausage formulations included 80% pork, 17,5% pork back fat, 1g/kg *Staphylococcus xylosus* (FloraCarn SX (freezedried), Chr. Hansens's Lab. A/S, Denmark) and salt, nitrite, nitrate, glucose and *Pediococcus pentosaceus* (Floracarn P-1 (freeze-dried, Chr. Hansen's Lab. A/S) according to table 1. The sausages were fermented at either 15 or 25°C for 48 hours and dried at 15°C for 54 days. For further details see Stahnke (1994).

Experimental design and statistics: The six factors used in this study included fermentation temperature, nitrite, nitrate, glucose, salt and *Pediococcus pentosaceus*. The values of each factor level were selected to cover af wide spectrum of ingredient concentrations in sausage types from all over Europe (Ockermann, 1989). The effect of each factor on the level of individual free amino acids were determined simultaneously using a six factor fractional design at two levels with resolution IV ($¼x2^6$) augmented by 2 central points as described by Box et al (1978). The factor levels are shown in table 1.

Statistical analyses of data were computed using multiple linear regression and analysis of variance (MODDE version 2.0 (1992), UMETRI AB, Umeaa, Sweden).

Preparation of free amino acid extract. The applied procedure was modified after Aristoy and Toldrá (1991). 5 g of fermented sausage was diluted 1:30 with 0.1 N cold hydrochloric acid. Samples were then

homogenized at 0-5°C in a Stomacher homogenizer (Seward Laboratory) for 6 minutes and centrifuged at 13.000 g for 25 minutes at 4°C. Supernatants were filtered through Whatmans No. 4 filter paper and diluted tenfold. The diluted filtrate was ultrafiltered through polyethersulfone membranes, 10kDa cutoff (10 ml stirred cell, Filtron Technology). The ultrafiltrate was directly used for HPLC-analysis.

Analysis of free amino acids by reverse phase HPLC: *Apparatus.* The HPLC system (WATERS) consisted of two Waters M510 high pressure pumps, a Waters WISP M712 autosampler, Waters TCM column oven, a Waters M420 AC fluorescence detector with 334-nm bandpass excitation filter and 425-nm long-pass emisson filter and af Waters SIM A/D interface module. The system was further equipped with a Water data and chromatography control station with Baseline version 3.30 chromatographic software package.

Reagents: The chemicals used were of analytical grade and the solvents of chromatographic grade. All chemicals were purchased from Merck, with the exception of the amino acid standard and the OPA reagent solution, which were obtained from Sigma. The solvents were obtained from Merck.

Derivatization: Using *o*-phthaldialdehyde (OPA), the amino acid was submitted to an automated program of precolumn derivatization in the autosampler. OPA in the presence of 2-mercaptoethanol reacts rapidly with primary amino acids to form highly fluorescent, thio-substituted isoindoles (Jones and Gilligan, 1983). For reasons of simplicity the details of derivatization steps are not discussed.

Chromatographic system: The column was a Waters Nova-Pak C18 cartridge column: 3,9mm x 150mm (4µm particle size). The column oven was set at 45°C, 10 microliters of sample or standards and 90 microliters of OPA was injected into the system.
The solvent system consisted of two eluents: (A): Tetrahydrofuran:methanol:0.1 M sodium acetate (pH=7.8) (-2,5%(v/v):9,5%(v/v)%:88%(v/v));(B): Methanol. To achieve separation of the amino acid derivatives the gradient in table 2 was performed:

Free amino acids were identified and quantified by co-injection and comparison of their retention times with those of authentic standards. Two replicates with duplicate analyses were made. All concentrations were calculated as mg/g protein to take the water loss during ripening into account.

Results and discussion:

The most important feature of Table 3 is that the levels of free amino acids are influenced by temperature and nitrate. No effect of glucose and nitrite observed, nor any no two-factor interactions appeared.

As the regression coefficients for the effect of temperature are positive, this implies that a higher fermentation temperature increases the formation of free amino acids (FAA). This was not unexpected due to the temperature dependence of chemical and enzymatic reactions. It is less is evident from table 3, that the level of individual amino acids are influenced to a different degree by temperature. This has also been demonstrated by (Spicher and Nierle, 1984) although this was in sourdough.

Table 3 also shows, that the addition of nitrate seems to decrease the level of individual amino acids. Nitrate can maybe decrease the FAA level by inhibiting the microorganisms partly involved in the productions of the FAA. Another explanation may be, that the FAA can be converted to α-hydroxy acids by way of the VanSlyke reaction, in which the amino group may interact with nitrous acid formed from sodium nitrite which can originate from added nitrate by nitrate reductase activity. A similar effect was also demonstrated in cured ham by (Piotrowski et al., 1970). The chemical analyses (not shown) show that residual nitrite is significantly ($p<0.5$) favoured by nitrate addition.

One explanation for that only the effect of nitrate and temperature is seen after 8 weeks is the low water-content (23-26%) and high salt-content (10.2-14.2% (salt-in-water)). This results in an extremely inhibiting environment that slows down the enzymatical reactions that may be influenced by the other factors.

One may speculate as to why the initial level of salt, with two exceptions, have no effect on the amino acid levels. There may be two explanations to this. One possibility is, that at this stage the salt concentrations are so high (10.2-14.2% salt-in-water), that they are equally inhibitory to any proteolytic activity. However, Córdoba et al. (1994), showed that in dry cured ham salt concentrations in the range 3-12% salt-in-water had no marked effect on proteolytic activity. A similar effect could be possible in this case.

From the numerical values of the regression coefficients it may be concluded, that temperature has more effect (2-3 times) than nitrate on the concentration level of free amino acids.

Glutamic acid, threonine, methionine and tryptophan tended to be sligthy, but not significantly ($p<0.1$) influenced by addition of *P. pentosaceus*. The effect of *P. pentosaceus* may be blurred because other lactic acid bacteria present in the sausage may display a similar metabolic pattern.

The results from the other sampling times (not presented here) show a much more complex effect from the investigated factors on the level of free amino acids. At each stage of ripening the amino acid profile has its own characteristic pattern. This pattern may result from the enzymatic degradation of peptides by various microorganisms and also from amino acid interconversion, excretion and degradation.

Conclusion:

The fermentation temperature at the beginning of the ripening and the addition of nitrate have significant effects on the levels of free amino acids after 8 weeks of ripening. A high fermentation temperature and a low nitrate level favours the concentration of free amino acids at the end of ripening.

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