# Changes in non-protein nitrogen compounds during dry sausage ripening

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## Introduction.

It is well known, that the concentration of water soluble nitrogen compounds in dry Sausage increases during ripening and can reach values up to 25 % of the total Nitrogen (Maillet and Henry, 1960) (Pezacki and Duda, 1962) (Mihalyi and Körmendy, 1967). The composition and concentration of several groups of these compounds, such as free amino-acids, peptides, nucleotides and nucleosides determine to a large extent the final aroma of dry sausage (Dahl, 1970) The availability of automated analysis has recently intensified research into free amino acid production in dry sausage (Reuter and Langner, 1968) (Langner, 1969) which is at least partly due to bacterial protease activity (Pohja and Niinivaara, 1960) (Sajber et al, 1971). Also, Cantoni (1968) stated that the major nucleotide present initially in sausage is Inosinic Acid (IMP) formed by deamination of Adenylic Acid, soon after rigor mortis. During ripening, phosphomonoesterase and nucleosidase activity produce Inosine nucleoside and hypoxanthine respectively.

Langner (1972) reports an average ammonia concentration of 75 mg/100 g of D.M., as determined on twelve different brands of dry sausage, whereas Niinivaara et al (1961), Körmendy and Gantner (1962) and Stanculescu et al (1970) report values for total free amino acid $\alpha$-nitrogen ($\alpha$ - N) in dry sausages.

We are not aware however of any work, describing the quantitative contribution of different compounds to the total non protein nitrogen fraction (N.P.N. fraction) at various stages of dry Sausage ripening.

In this paper, we report chages in different groups of N.P.N. compounds, including ammonia, free amino-acids, peptides, nucleotides, nucleosides and amines, during dry sausage ripening, as influenced by the presence of a "Starter culture".

Materials and Methods.

- Preparation of Sausages : Two batches of sausages, prepared in a local factory were used, and referred to as expt. 2 and expt. 3 respectively. Their composition and preparation is described in a preceding paper (De Ketelaere et al, 1973) and they differed by the addition in expt. 3 of a starter culture (Duploferment). Samples used, were those obtained in other work reporting changes in D.M., pH and carbohydrate metabolism.(De Ketelaere et al, 1973). Before analysis, they were treated as described by Demeyer et al (1973).

- Analytical Methods:

Total N.P.N. compounds were extracted by homogenisation of 5 g sample in 25 ml of 0.6 N $HClO_4$. After filtration, neutralisation and dilution to known volume, total N.P.N. was determined by the Kjeldahl method. Samples of the extract were used for determination of $NH_3$ (Conway, 1962), total $\alpha$-N using leucine as standard (Rosen, 1957), total peptide bound $\alpha$-N after acid hydrolysis (Weidner and Eggum, 1966), total nucleotides (as IMP-equivalents) and total nucleosides + bases (as hypoxanthine equivalents) (Macy et al, 1970). Free amino-acids and weak amines were extracted from a second sausage sample with picric acid (Stein and Moore, 1954) and quantitated using a Technicon "Auto Analyzer" and norleucine as Internal Standard (Spackman et al, 1958). Part of the highly basic amines were extracted from a third sample of sausage (Hill et al, 1970) and separated using the Technicon "Auto Analyzer" as described by Vandekerckhove and Henderickx (1973).

Results and Discussion.

Table 1 shows the concentration of the different N.P.N. compounds investigated, expressed as mg N / 100 g of D.M., at various stages of the ripening process.

Table 1. Concentration of N.P.N. compounds at various stages of ripening (mg N/100 g of D.M.).

| Stage of Ripening | Expt. 2 | | | | | | Expt.3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (days) | 0 | 3 | 9 | 15 | 22 | 36 | 0 | 3 | 9 | 15 | 22 | 36 |
| $NH_3$ | 24 | 30 | 40 | 58 | 62 | 76 | 25 | 27 | 43 | 61 | 57 | 73 |
| $\alpha$ - N | 141 | 188 | 204 | 234 | 243 | 255 | 155 | 200 | 225 | 230 | 255 | 302 |
| Peptide-N | 161 | 195 | 209 | 152 | 147 | 145 | 225 | 235 | 204 | 168 | 171 | 113 |
| Nucleot. - N | 34 | 33 | 15 | 13 | 12 | 12 | 37 | 21 | 17 | 16 | 13 | 14 |
| Nucleos. - N | 33 | 41 | 54 | 78 | 83 | 83 | 31 | 42 | 51 | 75 | 89 | 89 |
| Total N.P.N. | | | | | | | | | | | | |
| Determinded | 537 | 775 | 790 | 789 | 803 | 820 | 544 | 706 | 805 | 802 | 806 | 889 |
| Calculated[a] | 494 | 615 | 660 | 664 | 677 | 704 | 600 | 670 | 683 | 683 | 727 | 730 |
| % recovery | 92.2 | 79.3 | 83.5 | 84.1 | 84.3 | 82.0 | 110.2 | 94.9 | 84.8 | 85.1 | 90.1 | 82.1 |

[a] Obtained by addition, after correction of $\alpha$-N for 25 % non - $\alpha$ - N

Addition of individual values for each stage, results in values lower than the total N.P.N. determined. This discrepancy is related to differences in colour and colour intensity of the ninhydrin reaction product between different amino acids, the presence of non -$\alpha$- N in the free amino acids (approximately 25 % of total amino acid N) and the expression of all nucleotides as IMP and of all nucleosides as hypoxanthine. Indeed, besides nucleoside monophosphates, di- and tri-phosphates may be present, whereas nucleosides are present besides hypoxanthine. However addition, after correction of $\alpha$-N for the presence of 25 % non -$\alpha$-N, results in an average recovery of 91.3 $\pm$ 4.2 % (expt. 3) and 83.6 $\pm$ 1.8 % (expt. 2) of determined N.P.N. (table 1).

From the data in table 1, the concentration changes (as mg N/100 g D.M.) for the different compounds during various stages of ripening were calculated and presented in table 2. These data show that during the first 3 days of ripening, the rate of $\alpha$- N production is maximal and exceeds the rates of peptide production and $NH_3$ production. During this period intensive carbohydrate metbolism and bacterial growth also takes place (De Ketelaere et al, 1973).

In the following periods, the rate of ammonia production increases, but remains inferior to the rate of $\alpha$-N production, whereas concentration of peptide - N decreases. These results indicate, that free amino acids are produced at a faster rate than ammonia and peptides: % $\alpha$-N in total N.P.N. increases from ca. 35 % to 50 % at the end of the ripening period. Nucleotides decrease in concentration, whereas nucleosides and bases increase in concentration, the changes being most intensive the first 3 days of ripening. The lack of stoichiometry between nucleotide disappearance and nucleoside formation is probably related to the expression of results as IMP and hypoxanthine, as explained earlier.

Table 2. Concentration changes of N.P.N. compounds during various periods of ripening (mg N/100 g D.M.)

| Period | Expt. 2 | | | | Expt. 3 | | | |
|---|---|---|---|---|---|---|---|---|
| (days) | 0-3 | 3-15 | 15-36 | 0-36 | 0-3 | 3-15 | 15-36 | 0-36 |
| $NH_3$ | 6 | 28 | 18 | 52 | 2 | 34 | 12 | 48 |
| α-N | 47 | 46 | 21 | 114 | 45 | 30 | 72 | 147 |
| peptide-N | 34 | -43 | -7 | -16 | 10 | -67 | -55 | -112 |
| nucleot.-N | -1 | -20 | -1 | -22 | -16 | -5 | -2 | -23 |
| nucleos.-N | 8 | 37 | 5 | 50 | 11 | 33 | 14 | 58 |

The presence of a starter culture produced no striking differences, except for a higher final concentration of $\alpha$-N in expt. 3, coupled to a lower concentration of peptide - N. These findings suggest a higher exopeptidase activity in expt. 3. In both experiments, the most significant increase was observed for $\alpha$-N (total free amino acids). In order to determine the individual amino acids responsible for the increase, amino-acid analyses were carried out on samples obtained after 0, 15 and 36 days of ripening. The results are presented in table 3. They show that glutamic acid is the predominant amino acid in the initial samples, because of its addition as an additive. At the end of the ripening period, it is replaced by alanine. Concentration changes for individual amino acids were calculated between final and initial samples, and presented in fig. 1. They show that the major amino acids responsible for the increase in total $\alpha$-N are alanine, leucine, valine, serine, glycine and proline (increase $>$ 5 mg $\alpha$-N/100 g D.M.), followed by phenylalanine, aspartic acid, lysine, $\gamma$-amino butyrate, isoleucine and methionine ($0 <$ increase $\leq$ 5mg $\alpha$-N/100 g D.M.). Threonine shows the largest increase in expt. 2, but not in expt. 3. For most amino acids, increases observed are larger in expt. 3, confirming the data for total $\alpha$-N. In both experiments, a considerable part of the added glutamate disappears, and is at least partly decarboxylated to $\gamma$-amino-butyric acid, confirming results obtained by Langner (1969)(1972). Because of these results the use of glutamate as a flavor additive in dry sausage may be questioned. Other amino acids may be decarboxylated during dry sausage ripening, as indicated by the disappearance of histidine, tyrosine and ornithine.

Table 3. Concentrations of Free Amino Acids[a] at stages of Ripening (mg α-N/100 g D.M.)

| Stage of Ripening (days) | Expt. 2 | | | Expt. 3 | | |
|---|---|---|---|---|---|---|
| | 0 | 15 | 36 | 0 | 15 | 36 |
| Asp | 0.74 | 1.90 | 5.30 | 0 | 3.79 | 7.25 |
| Thr | 0.82 | 3.30 | 25.20 | 0 | 4.95 | 6.94 |
| Ser | 1.73 | 5.50 | 9.10 | 0 | 7.20 | 9.85 |
| Glu | 19.00 | 7.20 | 5.60 | 25.40 | 24.30 | 18.30 |
| Pro | 0 | 3.40 | 5.50 | 0 | 5.72 | 6.45 |
| Gly | 3.00 | 6.15 | 8.80 | 0 | 7.65 | 14.20 |
| Ala | 10.20 | 20.90 | 25.20 | 3.92 | 23.10 | 22.30 |
| Val | 1.44 | 6.35 | 8.85 | 0 | 7.41 | 11.95 |
| Met | 0.56 | 2.72 | 3.84 | 1.60 | 3.38 | 5.26 |
| Ileu | 1.60 | 3.74 | 5.45 | 2.24 | 4.50 | 8.10 |
| Leu | 1.06 | 11.50 | 13.30 | 8.30 | 13.20 | 17.60 |
| Phe | 0.93 | 4.50 | 5.25 | 2.98 | 5.00 | 7.15 |
| Lys | 2.07 | 4.67 | 6.35 | 2.30 | 3.94 | 6.46 |
| His | 0.73 | 1.46 | 0.01 | 2.77 | 2.20 | 0 |
| Tyr | 0.77 | 0 | 0 | 0 | 0 | 0.30 |
| γ-N B.A[b] | 0 | 2.72 | 4.07 | 1.22 | 7.89 | 12.50 |
| Orn[c] | 1.16 | 0 | 0 | 1.98 | 0 | 0.83 |

[a] Shorthand notation

[b] γ-amino butyric acid (γ-N calculated as α-N)

[c] Ornithine

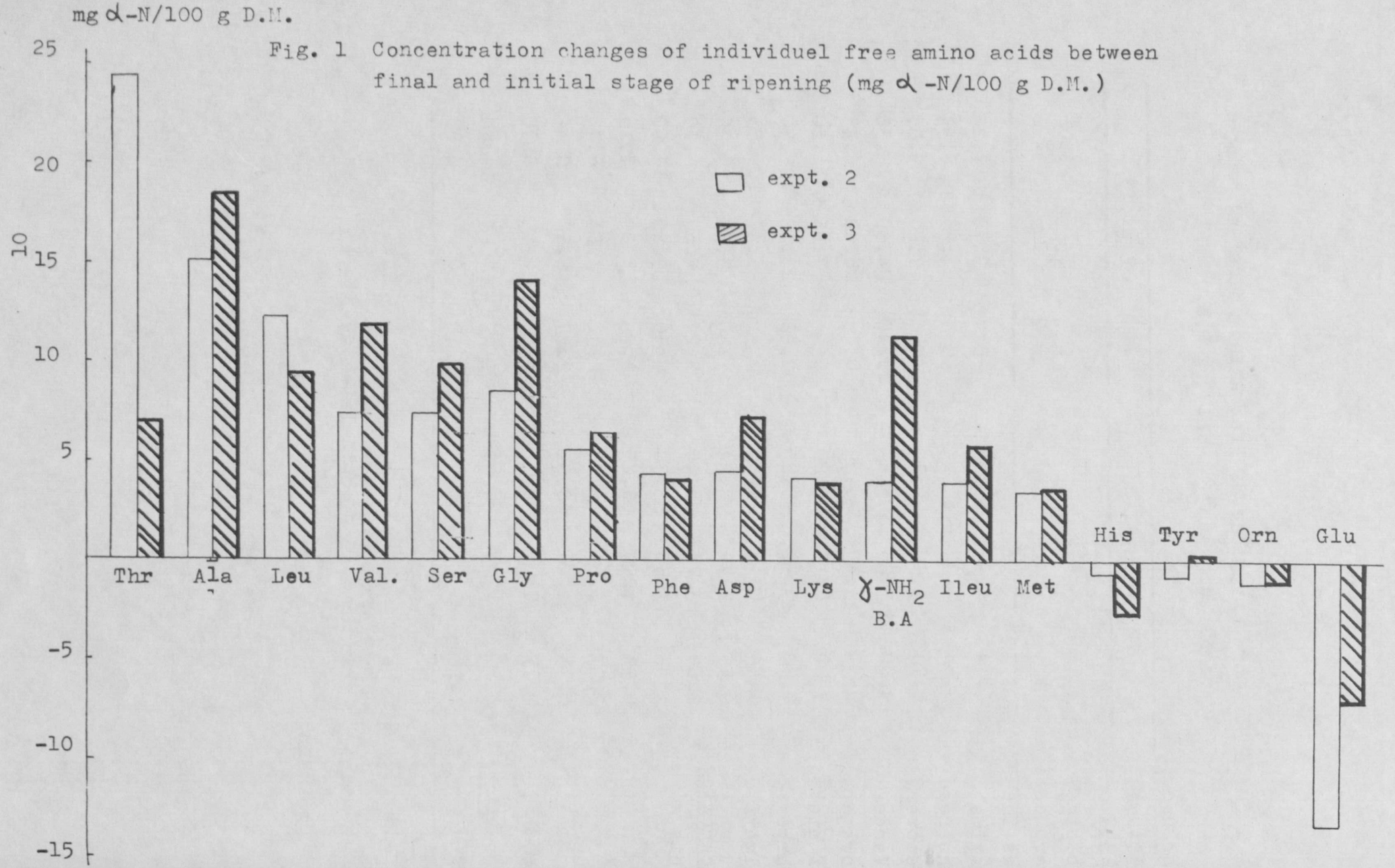


Fig. 1 Concentration changes of individuel free amino acids between final and initial stage of ripening (mg α -N/100 g D.M.)

The decarboxylation products of these amino acids are histamine, tyramine and putresceine respectively. Analysis of highly basic amines was carried out on samples obtained from expt. 3. Although very small amounts only were detected, the concentration of the latter three amines was increased at least tenfold, the rate of increase being maximal, during the first three days of ripening (fig.2). The results obtained are of relative value only, as the recovery of the amine extraction procedure is very low (Vandekerckhove and Henderickx, 1973). They are in line however with the observed decrease in the concentration of histidine, tyrosine and ornithine. Cadaverine, decarboxylation product of lysine, was also detected in significant amounts.

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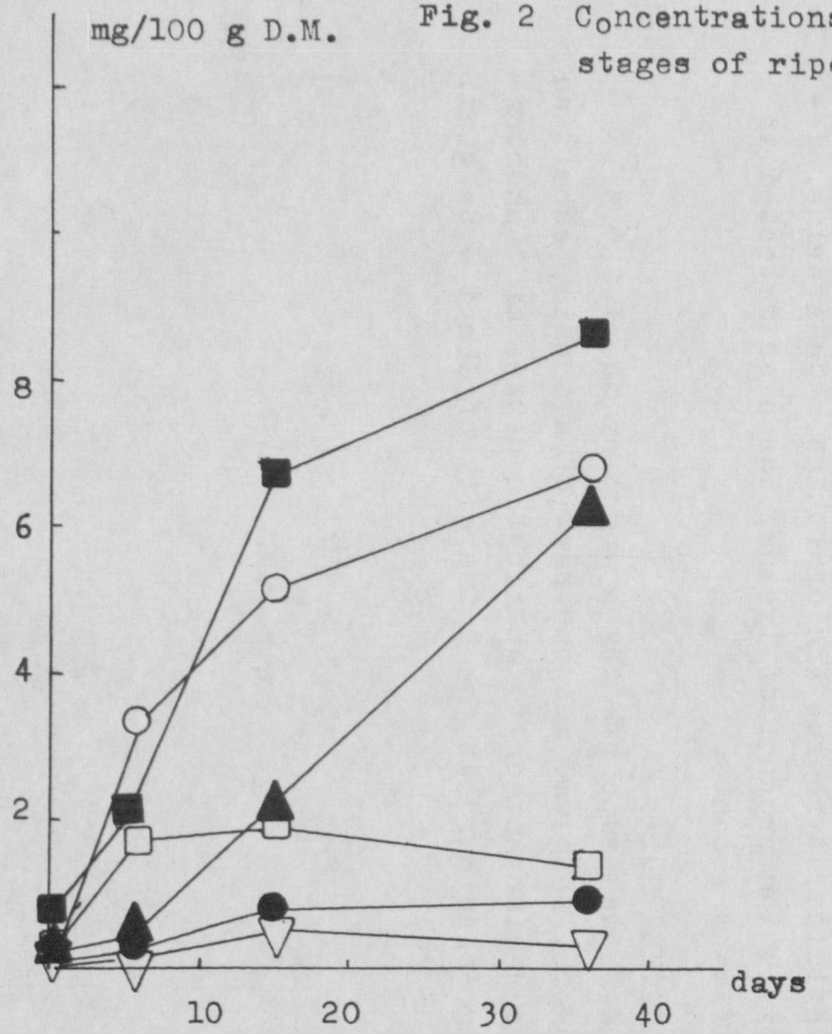


Fig. 2 Concentrations of some amines at various stages of ripening in expt. 3 (mg/100 g D.M.)

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