

FREE AMINO ACIDS MODIFICATIONS DURING DRIED SAUCISSON CURING PROCESS

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BACKGROUND

The modifications that affect the meat proteins during the curing process of saucisson are reflected in the apparition of the non-protein fraction (peptides and free amino acids) related to the organoleptic quality of the final product (Kato et al. 1989).

The objective of the present study was the analysis of free amino acids in saucisson during the dry-curing process.

MATERIAL AND METHODS

Materials: The saucisson used in this study was prepared in a pilot plant. The ingredients used were the following: Lean pork (60%), beef meat (20%), pork back fat (20%), common salt (20 g/Kg), sugar (45 g/Kg), nitrate (0.1 g/Kg), nitrite (0.2 g/Kg), polyphosphates (sodium pyrophosphate and potassium metaphosphate) (0.2 g/Kg), sodium ascorbate (0.3 g/Kg) and black pepper (4 g/Kg). The saucisson was manufactured as follows: Frozen pieces of pork and beef were mixed with salts and spices in a cutter. Frozen pork fat was added after being minced in a 3-mm diameter mincer. The mixture was vacuum minced and stored at 4°C for 20 hours. It was then stuffed in 60-mm diameter saucisson cellulose casing, fermented for 3 days (25°C, 90%R.H.) and dried for 4 weeks (15-18°C, 75-80%R.H.).

The saucisson was analyzed in the following manufacturing stages: after mincing, after fermentation, half-way through the drying period and the final product. Four saucissons were analyzed at each stage and each analysis was carried out in duplicate.

Methods: The preparation of amino acid extracts was made according to the method described by Aristoy and Toldrá (1991). 4g of saucisson were diluted 1:5 with 0.1N HCl, and homogenized in a stomacher homogenizer. Supernatant was filtered, deproteinized by adding 2.5 volumes of acetonitrile and centrifuged (15000 rpm, 5 min). Free amino acids were derivatized with phenylisothiocyanate using the Waters PICO-TAG method. Samples were filtered through a 0.45 µm membrane (Millipore) before analysis. Methionine sulfone was used as internal standard.

Samples were analyzed by reverse phase separation in a Waters High Performance Liquid Chromatograph (HPLC). The temperature was controlled to 46°C, and phenylthiocarbamyl derivatives were determined at 254nm. A gradient with two solvents were used: a) 70mM sodium acetate adjusted to pH 6.55 with acetic acid and added with 5% acetonitrile, and b) 45% acetonitrile 40% water and 15% methanol. The amino acid standard solution (Sigma Chemical Co.) was used to determine the R.F. of each amino acid. All the solvents used were HPLC grade.

RESULTS AND CONCLUSIONS

Table 1 gives the mean values of the free amino acids at each stage. Levels of the amino acids: glutamic acid, alanine, carnosine, valine, leucine, isoleucine and taurine made up 73.18 % of the total in the final product. The major amino acids in the minced mix were glutamic acid, taurine, alanine, carnosine, cystine and leucine. There was an increase in the amino acid content along the curing process of saucisson but some of them decreased in the 15 last days.

There was a high positive correlation ($R^2 > 0.75$) between the concentration of aspartic acid, glutamic acid, serine, glycine, anserine, threonine and the total amino acids with the curing time. Table 2 sets out the regression equations and the coefficient of determination (R^2) for these variables. The concentration of these amino acids can be estimated approximately based on the time of curing.

When multifactor components analysis was applied to the samples, the variance explained by the first two principal factors was 83.2% of the total. Fig. 1 presents the plot of the samples in the plane defined by these two principal rotated factors. Proline, alpha amino butyric, tyrosine, valine, methionine, isoleucine and leucine are strongly correlated with the first factor which explained 52.4% of the total variance. These amino acids are the responsible for the differences found among the first stages of saucisson. Glutamic acid, carnosine, tyrosine, anserine threonine, serine, glycine and taurine contributed more strongly to the second factor, and they contributed to set the difference between final product and the rest of the stages.

REFERENCES

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Table 1. Changes in Free amino acids concentration (mg/100 g d.m.) Along the curing process of saucisson

	MINCED MIX	FERMENTED	DRYING (14 days)	FINAL PRODUCT	Signification
Aspartic acid	6.32 ^a	8.53 ^a	16.70 ^b	22.97 ^c	***
Glutamic acid	374.51 ^a	493.90 ^b	530.13 ^b	886.85 ^c	***
Serine	8.27 ^a	13.02 ^a	16.24 ^a	45.41 ^b	***
Anserine	—	4.56 ^a	7.33 ^a	66.80 ^b	***
Glycine	18.16 ^a	25.99 ^a	41.16 ^b	69.59 ^c	***
Taurine	43.50 ^{a,b}	20.09 ^a	15.08 ^a	81.92 ^b	***
Threonine	4.93 ^a	11.07 ^a	14.08 ^a	34.11 ^b	***
Alanine	48.09 ^a	148.06 ^b	190.18 ^c	216.35 ^d	***
Carnosine	63.18 ^{a,b}	13.95 ^a	13.17 ^a	206.26 ^b	***
Proline	10.00 ^a	55.23 ^b	68.80 ^c	53.50 ^b	***
3-methyl Histidine	—	—	26.01 ^a	23.60 ^a	***
alpha amino butyric	—	10.73 ^a	20.30 ^b	16.26 ^{b,c}	***
Tyrosine	18.50 ^a	39.81 ^b	71.10 ^c	38.14 ^b	***
Valine	22.38 ^a	125.58 ^b	198.80 ^c	158.45 ^d	***
Methionine	9.65 ^a	43.29 ^b	95.07 ^c	60.12 ^d	***
Cysteine	88.09 ^{a,b}	67.37 ^a	133.97 ^b	45.54 ^a	**
Isoleucine	18.84 ^a	97.31 ^b	169.59 ^c	132.66 ^d	***
Leucine	39.43 ^a	221.47 ^b	392.44 ^c	305.41 ^b	***
Phenylalanine	21.46 ^{a,b}	21.30 ^b	57.76 ^c	45.49 ^{a,c}	***
Tryptophan	8.64	25.04	37.47	43.58	ns
Lysine	6.64 ^{a,b}	6.29 ^b	10.73 ^{a,b,c}	34.04 ^{a,d}	**
Total amino acids (mg/g d.m.)	8.09 ^a	14.47 ^b	21.22 ^c	24.43 ^d	***

* The same superscript mean no significant differences ($P > 0.05$)

Table 2. Results of lineal regression between some free amino acids and the time of saucisson curing. Amino acid (mg/100 g dry matter) = $a + b \times \text{time (days)}$

Amino acid	a	b	R ²
Aspartic acid	8.98	0.47	0.89
Glutamic acid	430	7.57	0.78
Serine	6.32	1.08	0.87
Glycine	20.45	1.45	0.95
Anserine	7.32	2.03	0.83
Threonine	6.06	0.79	0.89
Total aa (mg/g d.m.)	12.87	0.38	0.75

R² = determination coefficient

Fig.1 Graphic representation of the different curing process stages in the space delimited by the two first rotated factors

