NON VOLATILE AMINES IN DRY SAUSAGE.

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The concentration of some important amines was determined in dry fermented sausages. Total amine content ranged from 23.8 mg to 195.2 mg per 100 g of dry matter. Maximal amounts of 28.6 mg of histamine, 39.6 mg of putrescine and 150.6 mg of tyramine per 100 g of dry matter were found. γ-amino butyricacid, decarboxylation product of glutamic acid, was found in concentration up to 207.1 mg per 100 g of dry matter. The formation of putrescine from arginine was suggested. A large variation in the amine content and composition was found, possibly related to different methods of fabrication and to the specific flora of the sausages. In view of possible harmful effects of these amines, it is stated that concentrations reported in this note deserve careful reflection and further investigation.

AMINES NON VOLATILS DANS LE SAUCISSON SEC.

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On a déterminé la concentration en amines dans 30 différentes marques de saucisson sec. La concentration totale varie entre 23,8 mg et 195,2 mg per 100 g de matière sèche. On a trouvé jusqu'à 28,6 mg d'histamine, 39,6 mg de putrescéine et 150,6 mg de tyramine par 100 g de matière sèche. Acide γ-amino butyrique, produit de décarboxylation de l'acide glutamique atteint des concentrations de 207,1 mg par 100 g de matière sèche. La formation de putrescéine à partir de l'arginine est suggérée. Il y a des grandes variations dans la concentration et la composition des amines ce qui s'explique peut-être par les différents modes de fabrication et les flores diverses des saucissons. Tenu compte de l'action eventuellement nocive de ces amines, les concentrations mentionnées dans ce travail méritent la plus grande attention.

G2:2

NICHT-FLUCHTIGE AMINEN IN ROHWURST.

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Die voliegende Untersuchung betrifft 30 verschiedene Rohwurstmarken. Die Gesamtkonzentration an aminen zwankte von 23,8 mg bis zu 195,2 mg pro 100 g Trockensubstanz. Maximalkozentrationen von 28,6 mg histamine, 39,6 mg putrescine und 150,6 mg tyramine pro 100 g T.S. werden gefunden. γ-NH_2-Buttersäure, decarboxylierungsprodukt von Glutaminsäure erreicht bis zu 207,1 mg pro 100 g T.S. Die Bildung von Putresceine aus Arginine wird suggeriert. Die Ergebnisse deuten auf eine grosse Schwankung in Amineart und - gehalt, was möglicherweise zusammenhängt mit den verschiedene Herstellungsverfahren und Keimgruppe. Unserer Ansicht nach wird man den Problem des Auftretens dieser unerwünschter Amine in Rohwurst vermehrte Beachtung schenken müssen.

НЕЛЕТУЧИЕ АМИНЫ В СУХОКОПЧЕНОЙ КОЛБАСЕ

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Настоящее исследование относится к 30 сортам сухокопченой колбасы. Определялась концентрация важных аминов. Общая концентрация аминов колеблется между 23,8 мг и 195,2 мг на 100 г сухого вещества. Определено максимальную концентрацию 28,6 мг гистамина, 39,6 мг путресцина и 150,6 мг тирамина на 100 г сухого вещества. γ-NH_2 - масляной кислоты, продукта декарбоксилирования глутаминовой кислоты, найдено 207,1 мг на 100 г сухого вещества. Предполагается, что происходит образование путресцина из агринина. Результаты указывают на большие колебания в видах и содержании аминов, что, возможно, связано с различными способами продукции и группами микрофлоры в колбасе.
Имея ввиду нежелательные эффекты, вызываемые указанными аминами, было установлено, что приведенные в этом сообщении концентрации аминов заслуживают дальнейшего внимания и исследования.

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AMINES IN DRY FERMENTED SAUSAGE

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INTRODUCTION

We already mentioned the occurence of highly basic amines in dry fermented sausages (Dierick et al, 1974). Recently, Rice et al (1975) reported the presence of histamine (concentrations lower than 1 mg per 100 g) and of tyramine (up to 123.7 mg per 100 g in a Genova salami) in meat products. Amine contents of processed pork bellies has also been investigated (Spinelli et al, 1976). Concentrations per 100 g of lean tissue ranged from 0.03 mg for cadaverine to 8.1 mg for spermine, while type of processing did not significantly affect amine contents.
It is well known that high levels of these amines constitute a possible danger to patients treated with inhibitors of monoamineoxydase (MAO) since the pathway for inactivation of amines after ingestion is blocked (Walker., 1965). Furthermore, some amines are suspected to precipitate migraine attacks in susceptible subjects, e.g. tyramine and 2-phenylethylamine (Hanington., 1967; Sandler., 1974). Recently, Warthesen (1975) showed that the diamines putrescine and cadaverine can react with nitrite to form heterocyclic carcinogenic nitrosamines respectively nitrosopyrrolidine and nitrosopiperidine.
This paper deals with the amine content of commercial Belgian dry sausages as part of a study on the nonprotein nitrogen compounds in this type of meat product. (Vandekerckhove, 1976)

EXPERIMENTAL

Amines were separated using a Technicon amino acid analyzer.
As the resin we used in our previous work (Vandekerckhove and Henderickx., 1973) was no longer available, a new method was developped using another resin (Technicon Chromobeads C 2) and sodium buffers (Vandekerckhove., 1976). Amines were extracted from dry sausage as follows : 5 g of mixed sausage was extracted twice with 1 N $HClO_4$ (50 ml) by homogenisation (3 min) in a Virtis apparatus (Gardiner, N.Y.). After neutralisation with KOH, filtration and evaporation under vacuum, the residu was redissolved in 10 ml 0.1 % HCl. An aliquot of this was placed on the column. The method used, allows quantitative separation of arginine, histamine, cadaverine, putrescine, tyramine, 2-phenylethylamine, agmatine and tryptamine.

RESULTS AND DISCUSSION

Table 1 shows the amine content of 24 different brands of Belgian dry fermented sausage, expressed as mg/100 g of dry matter. Tyramine and putrescine, the respective decarboxylation products of tyrosine and ornithine, were the prevailing amines found. The occurence of high concentrations of tyramine and histamine corresponds with the disappearance of tyrosine and histidine during dry sausage ripening (Dierick et al, 1974). The presence of putrescine is probably related to the fact that only low amounts of free arginine were detected. Indeed, arginine is a well known precursor in the formation of putrescine by a number of pathways summarized in fig. 1 (Prins., 1975). Pathways 1 and(or) 2 were the most likely to occur, because small amounts of ornithine were found in the free amino acid fraction of dry sausage (Dierick et al,

1974), whereas we never detected the presence of agmatine. Reasons for the large variation in amine concentrations found (table 1) are not clear. There was no apparent relationship between amine content and concentrations of other components of the sausage (ammonia, α-NH_2-N, protein content, pH) (Vandekerckhove., 1976). However, three separate samples of French air dried sausages referred to as "pur porc", showed a different amine pattern, mainly due to an increased content of cadaverine, the decarboxylation product of lysine (Table 2). Concentrations of cadaverine and histamine were comparable to the concentration of putrescine in these samples, in contrast to data presented in table 1. This striking difference in amine composition is certainly not related to any difference in amino acid composition of the meat used. Indeed pork and beef amino acid composition are similar (Antila and Antila., 1967; Gruhn., 1965). In a separate experiment, we determined the amount of free glutamic acid and its decarboxylation product, γ-amino butyric acid (Table 3). The fact that glutamic acid is frequently used as a flavor additive in Belgian dry sausage explains the very high levels often found. The table also illustrates that a large part of the glutamic acid liberated by proteolysis during the ripening process (or added) can be decarboxylated by the bacterial flora. Because of the repulsive taste of γ-amino butyric acid, its production certainly has a negative effect on the dry sausage flavor. The factors underlying the variation in the amounts of decarboxylation products present must probably be sought in the use of different ripening conditions and(or) of specific bacteria in the preparation of the sausages. We plan to further investigate these factors. In view of the possible harmfull effects of some amines, as already mentioned (Walker., 1965; Hanington., 1967; Sandler., 1974; Warthesen., 1975) amine concentrations reported in this note deserve attention and further investigation. In this respect we plan to analyse the same commercial dry sausages for the possible occurence of nitrosopyrrolidine and -piperidine.

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TABLE 1 : FREE ARGININE AND AMINES IN DRY FERMENTED SAUSAGE
(mg/100 g D.M.)

Sample	arg.	hist.	put.	cad.	tyr.	2-ph. eth.	Tot. am.
1	2.83	6.54	7.67	1.01	10.18	-	25.40
2	1.71	5.86	8.42	0.54	12.51	-	27.33
3	10.19	12.32	5.96	1.10	16.17	0.71	36.26
4	2.28	1.27	3.13	2.44	16.94	-	23.78
5	4.73	tr	8.73	2.29	17.84	tr	28.86
6	14.69	tr	4.96	2.61	21.73	-	29.30
7	19.98	1.81	14.54	1.26	33.92	2.32	53.85
8	13.21	0.91	4.56	0.99	19.58	-	26.04
9	2.13	2.60	17.21	4.88	28.59	1.19	54.47
10	10.57	tr	8.57	5.02	34.01	1.00	48.60
11	4.24	tr	24.64	3.34	32.91	1.06	61.95
12	5.89	tr	9.18	2.61	26.46	0.90	39.15
14	0.97	5.82	27.73	5.57	54.95	0.62	94.69
15	1.02	9.13	18.82	5.33	43.49	3.46	80.23
16	0.49	3.44	9.19	4.04	31.93	tr	48.60
17	2.52	tr	26.38	4.47	46.14	-	76.99
18	tr[d]	8.27	21.68	2.53	31.47	0.55	64.50
19	1.40	tr	17.02	1.35	40.48	1.13	59.98
20	tr	tr	11.29	tr	25.68	-	36.97
21	2.72	2.34	39.57	0.95	150.63	1.70	195.19
22	2.05	1.50	17.60	0.67	69.14	0.65	89.56
23	1.90	19.74	36.53	2.32	63.97	6.06	128.62
24	11.16	15.01	7.46	2.71	29.61	tr	54.79
26	1.22	1.89	10.71	0.91	25.42	1.22	40.15
$\bar{X}$[a]	4.91	4.10	15.06	2.46	36.82	0.94	59.39
min[b]	tr	tr	3.13	tr	10.18	-	23.78
max[c]	19.98	19.74	39.57	5.57	150.63	6.06	195.19

a : mean value; b : minimum amount found; c : maximum amount found;
d : traces.
arg. = arginine; hist. = histamine; put. = putrescine; cad. = cadaverine;
tyr. = tyramine; 2-ph. eth. = 2-phenylethylamine; Tot. am. = total amines.

TABLE 2 : AMINES IN FRENCH AIR DRIED SAUSAGES "PUR PORC" (mg/100 g D.M.)

sample	arginine	histamine	putrescine	cadaverine	tyramine	2-phenyl-ethylamine	Total amines
13	4.42	2.65	19.82	29.84	58.62	0.39	111.32
25	5.17	28.59	29.64	16.16	78.71	1.31	154.47
31	1.21	11.38	7.49	6.12	31.92	tr	56.91
$\bar{x}^a$	3.60	14.21	18.98	17.37	56.42	0.57	107.57

a : mean value

TABLE 3 : FREE GLUTAMIC ACID AND γ-AMINO BUTYRIC ACID IN DRY FERMENTED SAUSAGE (mg/100 g D.M.)

sample	glut.acid	γ-N BA[a]	sample	glut. acid	γ-N BA[a]
1	86.41	31.87	18	427.98	37.61
2	285.91	14.13	19	241.50	24.14
3	17.85	148.23	20	442.68	79.56
4	190.26	3.24	21	27.93	159.93
5	179.34	59.76	22	193.20	27.08
6	83.26	141.83	23	248.85	46.81
7	66.67	62.41	24	268.06	12.22
8	511.24	4.78	25	289.69	11.04
9	362.67	13.91	26	166.11	12.14
10	217.03	tr[e]	27	30.24	207.18
11	179.76	24.29	28	189.00	102.08
12	73.60	64.55	29	165.16	55.13
13	246.22	34.66	30	249.58	6.03
14	210.00	13.17	$\bar{x}^d$	216.25	47.30
15	311.95	6.92	min.[b]	17.85	tr[e]
16	305.44	12.36	max.[c]	511.24	207.18
17	219.87	1.91			

a : γ-amino butyric acid; b : minimum amount found; c : maximum amount found; d : mean value; e : traces.

FIG. 1 : PATHWAYS OF ARGININE CATABOLISM BY BACTERIA.