8:3 The influence of multiextraction on the level of the residual nitrite determination in model, scalded, comminuted meat products.

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Introduction

A new approach regarding the problem of quantitative analysis and toxicity of nitrite was suggested by Frouin and Thenot /1976/ and Noel, Rougie and Goutefongea /1981/, which is based on a research findings focussed on the re-distribution of NO linked to protein and not protein fractions of the meat tissue and the observed fate of nitrite in cured meat products during processing and storage. Many other authors are indicating the existance of the labile and strongly bounded nitrite. Woolford /1976/, Miwa et.al./1978/, Cassens et.al./1976/, Emi-Miwa et.al./1976/, Woolford et.al./1976/, Rougie and Goutefongea /1976/, Duda and Szmańko /1981/, Okayama and Itoh /1983/, Cassens et.al./1979/.

The purpose of the present work was to evaluate the influence of the multiextraction on the amount of free nitrite determined in model, comminuted, scalded sausage-type meat products, processed from either beef or pork meat.

Materials and methods

Model sausage were processed separately from de-fatted and de-sinewed pork m. semimembranosus and/or beef m. semitendinosus, dissected from carcasses after 24 hours of chilling at 4°C, ground through plate with 2 mm hole and thereafter, in order to simulate chopping, meat was homogenized for 16 min. with 30 percent of water, 200 ppm of nitrite and 200 ppm of sodium ascorbate without adding fat and salt. Model sausage mince was stuffed into a 150 ml glass beaker and after 2 hours of curing at 4°C was heated in 85°C thermocontrolled water bath until 80°C was reached in a geometrical centre and these temp. was kept for 5 min. After thermal treatment the model sausage were cooled to 20°C in running tap water for 5 min. The residual nitrite was determined 24 and 72 hours after completion of the processing and refrigerated storage at 4°C.

The experimental material, after removing from glass container, was twice minced through plate with 2 mm hole in laboratory mincer, thermal drip was added and ground mince was very well mixed.

Extraction technique: two separate, grounded 50 g samples was blended with 25 ml of saturated borax solution for 3 min. at 3000 rev/min. in a laboratory blender and then, using 75 ml of distilled water, transferred into 200 ml volumetric flask, pH was measured and the content was heated for 30 min. in a boiling water bath with occasional mixing. Upon cooling in running tap water to 20°C, the suspension was de-proteinazed for 30 min. at a room temp. by adding 5 ml. of potassium ferrocyanate and 5 ml. of zinc acetate /both reagent - saturated solutions/ and thereafter quantitavely transferred into centrifuge containers and centrifuged at 3000 rev/min. for 10 min. The supernatant was filtered under vacuum through filter paper into volumetric flask and after adjustment to 200 ml. by adding distilled water and mixing, two 10 ml volumes of the extract was taken and the nitrite was determined using modified according to Hildrum /1976/ Griesse reagent and measuring the absorbance at 540 nm./ The modified Griesse reagent used: 0,5 g of sulfanilic acid was dissolved in 20 ml of hot water /distilled/ and mixed with 150 ml 15 percent of acetic acid; 0.1 g of N - naftyletylendiamine was dissolved in 150 ml of 15 percent acetic acid solution. Both reagents were mixed 1:1 just before $NaNO_2$ determination. The residue after first extraction and centrifugation, was re-extracted for four times exactly as described above i.e. the sample was extracted 5 times in total and nitrite was determined in each consecutive eluants./Fig.1./

Results and Discussion

The results of the investigation are presented in Table 1 and show that 24 and 72 hours after completion of the model sausage manufacturing from both pork and beef and then after it's storage at chilling temp., only approx.47 - 52 percent of the estimated total amount of the residual nitrite eluted during 5 consecutive extractions could be determined by single i.e. one extraction, while as much as approx.77 - 80 percent after two repeated elutions. Third and fourth extractions were significantly contributing to the total determined quantity of the residual nitrite, although at much

more less quantitative extend than the first and second elutions, as after four extractions it was possible to determine approx.97 percent of the total quantity of the residual nitrite estimated during five consecutive extractions.

Our experimental data are confirming the observations made by Noel Rougie and Goutefongea /1981/, whom's are indicating that new concept comes into play with regard to the residual nitrite determination, namely, that a kind of balance between free nitrite and bound nitrite exists. According to the above mantioned authors these is due to the fact that when previously present free nitrite is estimated, the next part of the bound nitrite is realeased and becomes measurable as free nitrite. Data in Table 2 show that practically after second extraction the pH of the eluates is stabilized at the some level and therefore can not be considered as being influencing the release of the bound nitrite during next three extractions. These findings are also indicating that some fraction of the added nitrite is relatively labile bounded to the potential substrates and could easily be available as a free nitrite.

However, in general, considering determined absolute quantity of the residual nitrite in relation to the amount initialy added i.e. 200 ppm, it should be underlined and stated that nitrite is very strongly bounded to the sausage mince substrates as only 9 - 12 percent of the added nitrite is released during five consecutive extractions.

CONCLUSIONS

1. The present laboratory practice of the residual nitrite determination in cured meat products, based on the one extraction only does not allow to determine the real amount of free nitrite in cured meat commodities and therefore appropriate amendmends to the common present obligatory analytical procedure should be urgently considered.
2. In order to determine the effective quantity of the residual nitrite in cured meat products no less than two consecutive extractions of the analytical sample is recommended.

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Table 1

Release of the residual nitrite during multiextraction of model sausage.

CHARACTERISTIC	BEEF MODEL SAUSAGE											
	$NaNO_2$ determined ofter 24h of storage at 4°C						$NaNO_2$ determined after 72h of storage at 4°C					
	Extraction No.						Extraction No.					
	1	2	3	4	5	1 + 5	1	2	3	4	5	1 + 5
$NaNO_2$/ppm/n=20	11,81	7,59^x	3,21^x	1,61^x	0,54	24,76	10,65	5,56^x	2,03^x	1,66$^-$	0,40	20,30
Sd	1,15	0,94	0,89	0,62	0,55		1,20	0,66	0,75	0,37	0,28	
S^2	1,3[illegible]	0,88	0,79	0,38	0,30		1,44	0,43	0,56	0,14	0,08	
ppu ±	0,55	0,45	0,43	0,29	0,27		0,50	0,32	0,36	0,18	0,13	
Σ Ext.No. 1-2;2-5/ppm/		19,40^x		5,36^x				16,21^x		4,09^x		
Sd		1,40		1,09				0,59		0,77		
S^2		1,96		1,19				0,35		0,59		
ppu ±		0,67		0,52				0,44		0,39		
% TE	47,70	30,65	12,96	6,50	2,18	99,99	52,46	27,39	10,00	8,18	1,97	100,00
Σ % TE.Ext. No.1-2;3-5		75,35		21,64		99,99		79,85		20,15		
% I.A./ppm/	5,30	3,79	1,60	0,80	0,27	12,36	5,32	2,78	1,05	0,83	0,20	10,18
Σ % IA.Ext. No.1-2;3-5/ppm/		9,69		2,67		12,36		8,10	2,08			10,18

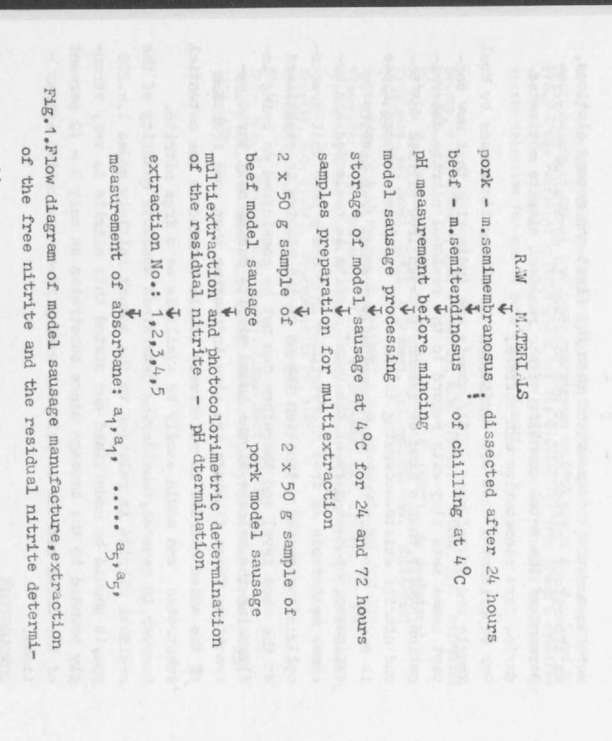

Fig.1.Flow diagram of model sausage manufacture,extraction of the free nitrite and the residual nitrite determination.

Table 2.

pH of the consecutive eluents during multiextraction of model sausage.

Initial pH n= 5	BEEF MODEL SAUSAGE											
	pH determined after 24 h of storage at 4°C						pH determined after 72h of storage at 4°C					
	Extraction No.						Extraction No.					
	0^x	1	2	3	4	5	0^x	1	2	3	4	5
	n = 10						n = 10					
$\bar{x}$ 5,67	8,01	6,09	6,54	6,83	7,01	6,89	8,16	6,12	6,35	6,84	7,00	7,00
Sd 0,42	0,31	0,65	0,33	0,53	0,42	0,39	0,33	0,69	0,62	0,45	0,35	0,30
s^2 0,18	0,10	0,42	0,11	0,28	0,18	0,15	0,11	0,48	0,38	0,20	0,12	0,09
ppu ±	0,24	0,11	0,25	0,40	0,32	0,29	0,25	0,52	0,47	0,34	0,26	0,23
	PORK MODEL SAUSAGE											
$\bar{x}$ 5,61	8,01	6,12	6,94	6,94	6,94	6,93	8,17	6,10	6,25	6,80	6,95	7,00
Sd 0,46	0,35	0,65	0,30	0,46	0,54	0,39	0,60	0,67	0,45	0,50	0,36	0,32
s^2 0,21	0,12	0,42	0,09	0,21	0,29	0,15	0,36	0,45	0,20	0,25	0,13	0,10
ppu ±	0,26	0,11	0,23	0,34	0,40	0,29	0,45	0,50	0,15	0,38	0,27	0,24

0^x - pH ofter homogenization with borax saturated solution

Cont.Table 1.

	PORK MODEL SAUSAGE											
$NaNO_2$/ppm/n=20	11,27	7,07^x	3,30^x	1,70^x	0,51	23,85	8,80	5,58^x	1,77^x	1,43$^-$	0,42	18,00
Sd	1,54	1,04	1,02	0,94	0,51		1,99	1,19	0,70	0,33	0,33	
S^2	2,37	1,08	1,04	0,88	0,26		3,96	1,42	0,49	0,11	0,11	
ppu ±	0,73	0,50	0,49	0,45	0,25		0,91	0,57	0,37	0,16	0,16	
Σ Ext.No.1-2;3-5 /ppm/		18,34^x		5,51^x				14,38^x		3,62^x		
Sd		1,81		1,37				2,21		0,31		
S^2		3,28		1,88				4,88		0,10		
ppu ±		0,87		0,66				1,07		0,31		
% T.E.	47,25	29,64	13,84	7,13	2,14	100,00	48,89	31,00	9,83	7,94	2,33	99,99
Σ % T.E.Ext. No.1-2;3-5		76,89		23,11		100,00		79,89		20,10		99,99
% I.A./ppm/	5,63	3,53	1,65	0,85	0,25	11,91	4,40	2,79	0,88	0,71	0,21	8,99
Σ % I.A.Ext. No.1-2;3-5		9,16		2,75		11,91		7,19		1,80		8,99

Σ Ext.No.1-2;3-5 =Total amount of the residual nitrite determined during first and second and third, fourth and fifth elutions,ppm.

% T.E. = Percent of the residual nitrite amount determined during each elution in relation to the total quantity estimated during five extractions.

Σ % T.E.Ext.No.1-2;3-5 = As above but expressed as percent extracted during first two and last three elutions.

% I.A./ppm/ = The amount of the residual nitrite determined during each elution in relation to the initial quantity added in ppm.

Σ % I.A.Ext.No.1-2;3-5= As above but expressed as determined during first two and last three extractions in ppm.

x = differences significant /P 0,05/ - differences not significant / P 0,05/.