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Study of the Changes in Protein Fractions and Amino Acids of an Unfermented South African Dried Sausage

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ABSTRACT

Changes of the proteins in food, brought about by processing conditions, affect texture and taste. Many studies have been conducted on the changes of the proteins in fermented sausages, which is aided by the proteolytic enzymes or lactic acid producing organisms. Information on the changes brought about by the meat enzymes and drying process as such is still unknown. We report results in this respect as observed for South African dried sausage. Results show insolubilization of certain proteins and protein hydrolysis similar to ripening conditions. Most changes in proteins were observed in the smaller proteins with molecular mass below 10 000 Da with consecutive release of amino acids. Drying at different temperatures were shown to affect the observed changes in

proteins.

Key words: Protein, unfermented sausage, South Africa, proteolytic enzyme, processing conditions

INTRODUCTION

South African dried sausage is a traditional and popular meat product that can be classified as an intermediary moisture meat product. It can be distinguished from European types of dried sausages in that it is not cured nor fermented, but preservation is obtained by an artificial drop in pH and subsequent drop in water activity. Many studies have been reported on the changes of proteins during ripening of meat (Sharp, 1963, Penny & Ferguson-Pryce, 1978 and Ouali et al, 1987) and fermented dry sausages (Johansson *et al.*, 1994, Roca & Incze, 1990, Shackelford *et al.*, 1990, and Garcia de Fernando & Fox, 1991), eg. salami, saucisson (Astasiaran *et al.*, 1990 and Beriain *et al.*, 1993), chorizo (Lois *et al.*, 1987), etc. In the fermented sausages the preservation is brought about by firstly a drop in pH due to the fermentation of carbohydrates by lactic acid forming microorganisms, and secondly by a drop in water activity. The changes in the proteins in these products are aided by the proteolytic enzymes of the lactic acid producing microorganisms. However, no information is available on the changes brought about by the meat enzymes, and drying process as such. It is in fact not possible to study these effects in the fermented sausages, due to the major contribution of the microorganisms. The South African dried sausage is therefore the ideal model for such an investigation. In this study, the changes in protein fractions and amino acids during different drying conditions are reported as evidence of the role of the meat enzymes and the conditions during drying.

MATERIALS AND METHODS

Sausage manufacture

There are many adaptations of the recipe for dried sausage. A traditional recipe was chosen for this initial study. One batch of 10 kg raw sausage was premixed by mincing the meat and fat through a 13 mm plate. The resulting premix is then mixed with a salt containing spice pack (Dry wors complete pack 802340, Freddy Hirsch, South Africa) (The spice pack contains no nitrates and nitrites nor proteolytic agents. It contains sodium diacetate) and minced through a 4.5 mm plate. The formulation is summarized in [Table 1](#). Use of the spice pack resulted in a 1.5% salt content and 0.93% sodium acetate (equivalent to 0.78%, acetic acid) of the raw meat mixture. The mixture was stuffed in 19 mm collagen casings. Five kg of sausage was dried in a cold room at 12°C to simulate

the traditional drying process (dry sausage was traditionally manufactured during winter months in South Africa at ambient temperature with no controlled temperature or humidity). The other 5 kg was dried in a small commercial drying cabinet at 28°C to demonstrate the effect of accelerated drying.

Compositional analysis

Sausage was sampled after stuffing and after drying to the desired moisture content. Due to the low moisture content of the end products, the pH could not be determined directly, but was carried out by preparing an aqueous suspension of the sausage. Moisture was determined by drying at 100°C to constant weight, and protein by macro Kjeldahl analysis.

Fractionation of sausage nitrogen

Sausage nitrogen was fractionated and analysed as reported by Garcia and Fox (1991) with minor modifications. Briefly, the sausage was homogenized in distilled water (1/5,w/v) with an Ultra Turrax, centrifuged at 16 000 g at 5°C for 10 minutes, and the pellet re-extracted three times. Supernatants, water soluble nitrogen (WSN), were pooled and ultrafiltrated through regenerated cellulose membranes with a nominal molecular weight cut-off of 10 000 Da (Millipore). A part of the permeate was freeze dried, redissolved in water and used for chromatography analysis on Sephadex G-25F (0.9 x 68) cm at a flow speed of 7.1 ml per hour. Chromatography fractions were collected at 10 minute intervals. The absorbancy of each fraction was measured at 280 nm and 204 nm. Eluting peaks were subjected to amino acid analysis both before and after hydrolysis. Freeze dried material was hydrolysed in 6 M HCl at 110°C for 24 hours. (Amino acid analyses were carried out by the Department of Biochemistry, University of Pretoria, Pretoria).

The rest of the permeate was furthermore made to 5% phosphotungstic acid (PTA) and held at 4°C over night. The precipitate was sedimented at 5000 g for 5 minutes. The precipitate was dispersed in water and solubilised by addition of NaOH to pH 7.0 and freeze dried. The supernatant was also adjusted to pH 7.0 and freeze dried. The concentration of free amino acids in the PTA-soluble fractions was determined as described by Doi *et al.* (1981).

The water-insoluble material was homogenized in 0.6 M NaCl (1/5,w/v) with an Ultra Turrax, centrifuged at 16 000 g at 5°C for 10 minutes, and the pellet re-extracted twice. The supernatants, salt soluble nitrogen (SSN), were pooled and ultrafiltrated as for WSN. A part of the permeate was freeze dried, redissolved in 0.6 M NaCl and used for chromatography analysis as described for WSN, but eluting with 0.6 M NaCl and determining absorbancy at

280 nm, 230 nm and 204 nm. The rest of the permeate was made to 2% trichloroacetic acid (TCA) and held at 4°C over night. This mixture was then filtered through Whatman No. 1 paper, the pH adjusted to pH 7.0 with NaOH and freeze dried.

The salt-insoluble material was delipidized by extraction with chloroform/methanol (2/1, v/v) and homogenization with an Ultra Turrax and then filtered under vacuum through Whatman No.1 paper. Extraction was repeated three times. Nitrogen was determined in the raw and dried sausages, soluble fractions, ultrafiltration permeates and PTA and TCA soluble fractions by the macro-Kjeldahl method.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the water and saltinsoluble fraction and UF-retentates, using a modification of the Laemmli (1970) procedure, according to the Hoefer Scientific Instruments instruction manual. The sample buffer for the WSN was as described by this manual. For the SSN the sample buffer was used as described by Greaser et al (1983). The buffer contained 8 M urea, 2 M thiourea, 0.05 M Tris (pH 6.8), 0.7 M β -mercaptoethanol and 3% SDS (w/ v). The same sample buffer was used for the insoluble fractions, but with sonification (Branson Sonifier and Cell Disrupt B-30) in sample buffer prior to addition of SDS. Sample concentrations of 3.33 mg.ml⁻¹ in sample buffer were used for the WSN and SSN fractions, and 4 mg.ml⁻¹ of insoluble fractions. Electrophoresis was performed on 5 or 15 μ l of a prepared sample with 15% resolving gels and 4% stacking gels, using the μ Mighty Small[®] miniature slab gel electrophoresis unit SE 260 (Hoefer Scientific Instruments). An acrylamide to methylenebisacrylamide ratio of 200:1 were used. The following standards were used as molecular mass references (Boehringer Mannheim): lysozyme (14 307 Da), trypsin inhibitor (20 100 Da), trios phosphate isomerase (26 626 Da), aldolase (39 212 Da), glutamate dehydrogenate (55 562 Da), fructose-6-phosphate kinase (85 204 Da), β -Galactosidase (116 000 Da) and b2-Macroglobulin (340 000 Da).

RESULTS AND DISCUSSION

The composition of the raw and dried sausages is given in [Table 2](#). The final moisture contents were 10.7% and 12.7%, and the water activities 0.534 and 0.625 for the sausages dried at 28°C and 12°C respectively. At the higher drying temperature of 28°C a water content of less than 28% ([Figure 1](#)) and a water activity below 0.8 ([Figure 2](#)) was reached within 48 hours. At 12°C these properties were obtained only after 168 hours (7 days). The pH did not change perceptibly during drying. With the pH at 5.69, the latter drying conditions would therefore still permit changes in proteins by enzymatic hydrolysis by cathepsins (Penny and Dransfield, 1979), as it is generally accepted that enzyme activity may be effective at water activity values as low as 0.5.

The effect of prolonged storage time on the changes in nitrogen content of the water-soluble fractions are shown in [Table 3](#). WSN decreased from 17.77% by 2.94% after drying at 28°C and by 2.52% at 12°C. However, where the WSN permeate, peptides smaller than 10 000 Daltons, showed a decrease of 0.86% from 8.65% after drying at 12°C, it increased slightly by 0.1% for the 28°C drying temperature. In contrast, the PTA soluble fraction increased at the lower drying temperature from 1.74% by 0.14% and stayed virtually constant after the higher drying temperature by decreasing by only 0.07%. According to the analysis by the Cd-ninhydrin method, the content of free amino acids did not change much; from 0.139% to 0.114% and 0.141% after drying at 28°C and 12°C respectively. It has to be noted that these determined amounts of amino acids may, however, not be correct, since the Cd-ninhydrin technique may also react positively with short oligo peptides (Doi *et al.*, 1981). By amino acid analysis, however, a different tendency was observed, which is discussed below.

Chromatography of WSN-permeate of raw and dried sausages are shown in [Figures 3a - 3c](#). The fractions pooled were selected according to absorbancy peaks observed at both 280 nm and 204 nm, and are shown by bands above the curves. Very obvious is the high absorbancy at 204 nm for Peak 3 with a very low absorbancy at 280 nm. Almost the same absorbancy at 204 nm was observed for Peak 4, but with a high absorbancy at 280 nm.

A quick glance at the chromatography profiles would suggest that the only changes observable is a decrease of peak 5 and an increase of peak 6 after drying at 12°C. Comparisons of peaks 1-4 of the sausage samples at the respective wavelengths of absorbancy also indicate changes in peak areas relatively to the other peaks, however smaller. No conclusion should be drawn from the absorbancies at these wavelengths alone, as they may give misleading information. The peaks may contain non-peptide compounds such as salts and pigments, since the WSN-permeate could not be dialysed without a resulting loss of peptide material. The nonprotein material would contribute to absorbancy, hence the difference in absorbancy at the selected wave lengths mentioned above. It is therefore necessary to discuss the observations with the aid of the amino acid analyses (Tables [4](#), [5](#), [6](#), [7](#))

Free amino acids were mainly found in Peak 3 of each sausage fraction. For the raw sample, 80%, of the free amino acids were found in this fraction, and for both dried samples, 98% ([Table 4](#)). When subtracted from the total amino acids, the free amino acids make up 2.25%, 4.32%, and 5.31% of the total amino acids of the corresponding peaks 3. The analyses for the Peak 3 fractions are shown in [Table 3](#). Of the small amounts of free amino acids found in the other peaks, most consisted of Gly, and these amounts did not increase after drying. The free amino acids only made up 1.79% of the WSN permeates of the raw sample, whereas in the dried sausages they increased to 4.88% and 5.71% when dried at 28°C and 12°C respectively ([Table 4](#)). This is calculated to 0.318%, 0.744%

and 0.847% of the total Nitrogen respectively ([Table 3](#)).

This increase in free amino acids is not in accordance with the analysis by the Cd-ninhydrin method, where limited changes in the content of free amino acids were determined. As mentioned above, the Cd-ninhydrin test may not be accurate as a result of insensitivity and low specificity. The data obtained by the amino acid analysis are therefore accepted as being more accurate.

In [Table 4](#) it is shown that Gly is the most abundant free amino acid in the raw sausage, with less free Glu, Ser, Thr and Ala and none of the other amino acids. After drying, the amount of free Gly increased, but its contribution to the total amount of free amino acids decreased as more of the other amino acids were released. The amounts of free Glu, Ser, Thr and Ala also increased drastically, with the addition of Asp, Val, Ile and Leu. Large differences in concentration of free amino acids between the sausages dried at 28°C and 12°C can be seen for Asp, Glu, Gly, Thr and Ala.

In [Tables 5, 6, 7](#), the amino acid content of peptides in the chromatography fractions is given, which was calculated by subtraction of free amino acids in each fraction from the total amino acid content obtained after hydrolysis, and presented as mol%.

As mentioned, comparison of the chromatography profiles ([Figure 3](#)) only shows changes in peaks 5 and 6, especially for the sausage dried at 12°C. However, when the amino acid analyses of the chromatography fractions are taken into consideration, it can be deduced that the peptide composition of these fractions were also changed. Comparison of the amino acid analyses of peptides from the chromatography peaks show that small changes (a difference in at least 1 mol%) are observed for some amino acids and drastic changes were observed for others after drying. The peptides from Peak 1, for instance show small changes for Asp, Ser, Gly, Thr, and Lys, but a sharp decrease in molar contribution of Pro from 20.30%, to slightly above 4%, after drying. No great difference can be observed in the amino acid content of the peptides in Peak 1 for both dried sausages. Peaks 2-6 showed similar small changes for some amino acids, but large changes (a difference in more than 2 mol%) for more than one amino acid; Asp, His, Thr, Ala, Leu and Lys in Peak 2; Glu, His, Arg, Thr, Ala, Pro, and Lys in Peak 3; Glu, Gly, His, Thr, Ala, Pro, Tyr, Val and Met in Peak 4; Gly, Ala, Tyr, Phe and Lys in Peak 5 and Gly and Lys in Peak 6. In general, Peaks 2-5 show less differences between the changes in the mentioned amino acids in both dried sausages than observed for the raw sample. However, there are also large differences where this generalization does not occur, but definite differences between amino acid amounts in all three sausage samples can be seen. This can be indicated by comparison of His and Lys of Peak 2, Glu, His, Arg, Pro, of Peak 3, Glu, Gly, Ala and Val of

Peak 4, and Gly, Ala, Tyr and Lys of Peak 5. The amino acid content or peptides in Peak 6 does not differ after drying at different temperatures. Only the amounts seem to differ according to the chromatography profile. Since this size exclusion chromatography would only separate mixtures of peptides of different molecular sizes, implicating that each peak would consist of a mixture of peptides, these amino acid analysis data indicate that for Peak 1 and especially Peak 6, the drying conditions affect the amount of peptides observed, but not the type of peptides found within these peaks. Within each of the peaks 2-5, however, not only the amounts of peptides are therefore shown to be different, but also the constituting amino acid composition of peptides. One reason for these differences could be insolubilisation of some peptides due to denaturation during drying, with a resulting absence in the fractions, which will be explained below in the comparison of electrophoretograms. The formation of new peptides due to hydrolysis can, however, not be ruled out, since an increase in free amino acids, as possible hydrolysis products, were observed.

The phenomenon that peptides, which have a larger molecular size than free amino acids, were eluted later, needs to be discussed. Peaks 1-3 are eluted before the total volume, and Peak 4 with the total volume, whereas peaks 5 and 6 thereafter. One reason why elution of peptides may deviate from the normal size exclusion rules, is a high hydrophobicity (Determann, 1969). This is supported by the amino acid compositions (**Tables 5, 6, 7**) of peptides in Peaks 4 - 6, where it is shown that there are relatively less charged and hydrophilic amino acids than hydrophobic ones, compared to the peptides in Peaks 1-3. The differences in amounts of peptide material and also their amino acid compositions, observed for peaks 5 and 6, after drying at the two different temperatures, would therefore also have sensory implications, since it was shown by other researchers that peptides with a molecular weight of less than 6000 and a hydrophobic character, contribute to bitter taste (Ney, 1971).

The changes in Nitrogen content of the salt soluble fractions are shown in **Table 3**. In general, all the SSN fractions increased after drying, with a higher increase when dried at 12°C than at 28°C. SSN increased from 11.71% by 0.46% and 1.95% after drying at 28°C and 12°C respectively, SSN-permeate from 0.32 by 0.40% and 0.65%, and TCA-soluble material from 0.24% by 0.41%, and 0-64%. The drop in WSN and increase in SSN would immediately suggest insolubilisation due to denaturation of proteins, however, more protein material, approximately 80% more, became insoluble than became salt soluble, which is supported by the electrophoretograms and discussed below.

Chromatography of SSN-permeate of raw and dried sausages are shown in **Figures 4a - 4c**. Absorbancy of these peptides at 280 nm was very low, and is consequently not shown. Absorbancy was therefore also determined at 230 nm, the peptide bond absorbency, and 204 nm. In the SSN more drastic changes in peptide composition seem

to have occurred than observed for the WSN. The peak between fractions 24 - 28 increased after drying, and also to a much greater extent after drying at 12°C. This observation may confirm the increase in the SSN and SSN permeate shown in [Table 3](#). A small increase of absorbancy by these peptides at 280 nm (results not shown) was observed after drying, suggesting an increase in peptides containing aromatic amino acids after drying, again with the largest change observed for the material from the sausage dried at 12°C. The small peaks, or shoulders, between fractions 20 - 24 only increased slightly after drying, also with an increase in absorbancy at 280 nm. Due to the high salt content in these fractions, which cannot be removed without loss of peptide material, amino acid analyses could not be performed to provide more concluding evidence for changes in salt soluble peptide composition.

Electrophoretograms of the UFretentates of WSN and SSN and insoluble proteins are shown in [Figure 5](#). Insolubilisation of some proteins occurred. At least the myomesin heavy chain and 130 kDa protein became less soluble, although an increase in the insoluble fraction is not clearly visible. The 30 kDa protein became less salt soluble, and appeared as stronger bands in the insoluble fraction. Furthermore a 18 kDa protein became insoluble, however it is not certain whether it is troponin C or alkaline light chain 2. Inspection of the soluble fractions shows that a 80 kDa protein became more salt soluble, and less water soluble, at least for the sausage dried at 28°C. In general, insolubilisation appears to be greater in the sample dried at 28°C, which gives some explanation for the above observation by nitrogen content ([Table 3](#)) that more protein material became insoluble than became salt soluble. Decreases in solubility of proteins in dried sausages and model systems was reported by several researchers, some of whom have ascribed it to denaturation (Klement et al, 1973 and Mihalyi and Kormendy, 1967).

Inspection of the salt soluble and water soluble electrophoretic bands show that hydrolysis of proteins occurred similar to that shown for ripened meat (Sharp, 1963, Penny & Ferguson-Pryce, 1978 and Ouali *et al.*, 1987). Hydrolysis products are already observed in the raw mixture, which might be due to ageing, since the meat for the sausages was bought from a butcher, and could have been two to three days post slaughtering. Nevertheless, at least, the troponins T, 1 and C, and tropomyosin seem to be affected and their bands became less prominent. The 27 kDa protein, on the other hand, showed increased amounts. These data may therefore confirm that the conditions during drying are still favourable for hydrolysis by proteolytic enzymes, such as the cathepsins, especially when slower drying conditions are allowed.

Alpha actinin and a 50 kDa protein became more water soluble after drying. The bands for tropomyosin and myosin alkaline chain 2 show a similar tendency. Higher water solubility seemed to be more explicit in the sausage

dried at 12 °C, hence confirming the difference in WSN between the two dried samples discussed above. From the electrophoretic data, the increase in salt solubility as shown in [Table 3](#), could however not be shown.

To conclude, the data show that in South African unfermented dry sausage, certain proteins become insoluble due to the drying conditions, and that proteins are hydrolysed similar to ripening conditions. Most changes in proteins, however, occur in the smaller proteins with molecular mass below 10 000 Da with consecutive release of amino acids. The effect of insolubilisation was less, and the hydrolysis higher in the sausage dried slower at 12°C where water activity conditions were more favourable than for the sample dried quickly at 28°C. It would therefore be interesting, were it possible, to investigate the changes in proteins in fermented sausages brought about by the inherent meat enzymes and drying conditions separately from those effected by the fermentation organisms. The present study might perhaps provide some information.

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Figure 1. Changes in moisture content during drying of sausages at 28°C ad 12°C

Figure 2. Changes in water activity during drying of sausages at 28°C and 12°C

Figure 3. Chromatographs of the WSN-permeates of raw sausage mixture (a), and sausages dried at 28°C (b) and 12°C (c) on Sephadex G25F (0.9 x 67 cm, 7.1m l.h⁻¹)

Figure 4. Chromatographs of the SSN-permeates of raw sausage mixture (a), and sausages dried at 28°C (b) and 12°C (c) on Sephadex G25F (0.9 x 67 cm, 7.1 ml.h⁻¹)

Figure 5. Electrophoretograms of standard, WSN, SSN (a) and standards and insoluble proteins (b). Material from raw sausage mixture are in lanes a, d, g; sausages dried at 28°C in lane b, e, h; and sausages dried at 12°C in lanes c, f, i

Table 1. Formulation of raw sausage mixture

Ingredient	Inclusion Level (%)
Lean Beef (90/10)	86.0
Sheep caudal fat	10.8
Spice pack	3.2
Total	100.0

Table 2. Compositional analysis of raw and dried sausages

Parameter	Raw Sausage	Sausage Dried Mixture at 28°C	Sausage Dried at 12°C
Water activity	0.965	0.534	0. 625
pH	5.505	5.610	5. 690
Moisture content	60.159	10.700	12.708
% Dry matter	39.841	89.300	87.292
% Nitrogen	3.891	8.462	8.589
% Protein	24.319	52.890	53.681
% Nitrogen in dry matter	9.766	9.476	9.839
% Protein in dry matter	61.040	59.227	61.494

Table 3. Nitrogen content of protein fractions (Expressed as % of total N)

Parameter	Raw Mixture	Dried at 28°C	Dried at 12°C
WSN	17.17	14.84	15.25
WSN Permeate	8. 65	8.77	7.78
PTA-soluble	1.74	1.67	1. 88
Amino acids (Cd-ninhydrin)	0.139	0. 141	1. 114
Amino acids (Analysis)	0.318	0. 847	0. 744
SSN	11.71	12.16	13.66
SSN Permeate	0. 32	0.72	0. 97
TCA-soluble	0. 24	0.65	0. 88

Table 4. Analysis of free amino acids in Chromatography peaks 3 of WSN from sausage samples

Sample	Raw		28°C		12°C	
Amino Acid	nmol	Mol %	nmol	Mol %	nmol	Mol%
Asp	0.00	0.00	84.81	4.08	37.92	1.89
Glu	21.20	4.87	760.42	36.58	628.69	31.36
Ser	12.32	2.63	43.76	2.11	52.35	2.61
Gly	302.08	69.40	312.81	15.04	289.01	14.42
His	0.00	0.00	0.00	0.00	0.00	0.00
Arg	0.00	0.00	0.00	0.00	0.00	0.00
Thr	32.96	7.57	103.65	4.99	128.24	6.40
Ala	66.72	15.33	559.41	26.91	663.12	33.08
Pro	0.00	0.00	0.00	0.00	0.00	0.00
Tyr	0.00	0.00	0.00	0.00	0.00	0.00
Val	0.00	0.00	118.93	5.72	119.47	5.96
Met	0.00	0.00	0.00	0.00	0.00	0.00
Ile	0.00	0.00	35.38	1.70	27.97	1.40
Leu	0.00	0.00	59.76	2.88	58.11	2.90
Phe	0.00	0.00	0.00	0.00	0.00	0.00
Lys	0.00	0.00	0.00	0.00	0.00	0.00
Total	435.28	100.00	2978.8	100.00	2004.89	100.00
% of Peak 3	2.52		43.20		53.14	

Table 5. Amino acid analysis of Chromatography fractions of WSN from raw sausage

Fraction	Mol % Amino Acid					
	1	2	3	4	5	6
Amino Acid						
Asp	3.65	3.80	4.09	4.77	2.82	0.91
Glu	26.58	27.31	11.52	6.56	3.56	1.53
Ser	1.76	1.90	2.91	3.12	2.63	1.35
Gly	15.51	6.95	8.20	11.43	64.14	85.26
His	13.61	30.63	38.04	1.92	0.69	0.31
Arg	1.06	1.34	2.79	2.36	1.84	0.34
Thr	3.77	8.07	6.80	3.02	2.68	1.46
Ala	4.11	5.86	7.78	22.41	4.55	1.43
Pro	20.30	3.68	2.54	10.62	2.07	0.55
Tyr	0.45	0.29	0.94	8.58	1.38	1.14
Val	1.86	2.13	3.36	11.92	2.48	0.70
Met	0.45	0.29	0.94	8.58	1.38	1.14
Ile	1.34	1.08	1.75	2.38	2.06	0.86
Leu	2.12	2.02	3.54	4.23	3.45	0.83
Phe	1.07	0.82	1.65	3.00	1.93	0.44
Lys	2.38	3.75	3.38	3.19	3.04	2.65

Table 6. Amino acid analysis of Chromatography fractions of WSN from sausage dried at 28°C

Fraction Amino Acid	Mol % Amino Acid					
	1	2	3	4	5	6
Asp	7.47	6.23	2.26	2.46	2.13	1.02
Glu	27.12	26.10	17.30	25.17	5.00	1.25
Ser	3.86	2.66	2.55	5.10	1.51	0.61
Gly	17.16	8.92	5.68	22.38	57.02	91.76
His	14.01	13.01	42.62	3.51	2.59	0.61
Arg	1.67	1.98	5.88	4.04	0.93	0.22
Thr	4.93	5.20	1.90	3.05	1.84	0.42
Ala	1.67	1.98	0.05	4.04	0.93	0.22
Pro	4.93	5.20	4.70	3.05	1.84	0.42
Tyr	0.72	1.36	1.58	2.73	6.83	0.24
Val	2.74	3.47	2.60	2.73	1.70	0.42
Met	0.74	2.06	1.09	0.63	1.67	0.11
Ile	2.07	3.79	1.89	1.87	2.56	0.61
Leu	2.54	4.15	3.14	3.79	2.75	0.71
Phe	1.06	2.25	1.62	2.58	4.32	0.45

Table 7. Amino acid analysis of Chromatography fractions of WSN from sausage dried at 12°C

Fraction Amino Acid	Mol % Amino Acid					
	1	2	3	4	5	6
Asp	7.25	5.46	2.65	4.71	2.30	0.85
Glu	26.32	28.13	16.15	9.34	4.48	1.11
Ser	3.46	2.60	2.60	4.99	2.41	0.47
Gly	18.03	9.39	5.41	28.04	71.94	91.76
His	14.80	17.65	44.51	4.38	1.83	0.60
Arg	1.84	1.97	6.65	4.32	1.45	0.35
Thr	5.00	5.98	1.98	5.98	1.99	0.47
Ala	5.15	5.74	0.05	14.21	1.82	1.01
Pro	4.31	5.31	3.81	3.17	2.06	0.44
Tyr	0.84	0.71	1.72	2.22	0.83	0.28
Val	2.69	3.06	2.65	4.16	1.73	0.49
Met	0.71	1.29	1.16	1.09	0.61	0.15
Ile	1.93	2.17	1.69	2.25	1.42	0.58
Leu	2.81	3.02	2.81	4.54	2.26	0.76
Phe	1.32	1.47	1.61	2.72	1.19	0.34
Lys	3.56	6.06	4.58	3.89	1.70	0.34