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IDENTIFICATION OF DIFFERENCES IN CHEMICAL COMPOSITION AMONG WHOLE STICK AND SLICED NITRAN SALAMIS TROUGH PRINCIPAL COMPONENT ANALYSIS

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ABSTRACT

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The subject of this work was to examine differences in chemical composition of sliced and whole stick Nitran salamis, purchased from various manufacturers. Nitran salamis are traditional dry fermented meat products of Slovak origin. Taking into account variations in raw materials, production process and potential adulteration, differences in chemical composition within one brand of salami from different manufacturers might be expected. Ten salamis were determined for basic chemical composition attributes and Principal Component Analysis was applied on data matrix to identify anomalous ones. It has been shown that six attributes, namely: protein without collagen of total protein, total meat, total fat, collagen of total protein and NaCl, were the most important for salamis as first two Principal Components together explained 70.16% of variance among them. Nitran D was found to be the most anomalous salami, as had the lowest value of protein without collagen of total protein (14.14% ±0.26%), total protein (18.83% ±0.50%) and NaCl (9.55% ±1.93%), when compared to its whole stick variant Nitran C and other samples. In addition to collagen of total protein content, Nitran D together with Nitran A, F and H did not satisfied the legislatively determined criterion, which is $\leq 16\%$. This suggested that extra connective tissues were added to intermediate products, which resulted in high variability and inferior quality of final products. It is a common practice in the meat industry to increase the protein content or water binding properties of meat products.

Keywords: PCA; Nitran salami; quality; protein; collagen

INTRODUCTION

Salamis are dry fermented meat products that are popular across most of European countries (Fabbri and Cevoli, 2015). Such countries or their geographic regions produce characteristic salamis trough traditional manufacturing processes. In brief, meat (pork and beef) and fat are minced and mixed with salt, curing agents (nitrate and nitrite), spices, herbs, sugar, starter cultures and other additives such as non-meat proteins (Fongaro et al., 2015; Cevoli et al., 2014). The mixture is stuffed into natural or artificial casing and then subjected to fermentation and drying (ripening) stage (Fongaro et al., 2015). During these phases, physical, chemical and microbiological transformations take place in salami (Jerković et al., 2010; Martín-Sanchéz et al., 2011), gradually giving a product with characteristic colour, flavour, taste and texture (Papavergou et al., 2012). The degree of changes and the final quality of salami depend on product formulation, the variations in raw meat used, the starter culture and processing conditions (Marino et al., 2015; Van Schalkwyk et al., 2011, Zajác et al., 2015).

After ripening, when the desired characteristics are reached, the product can leave the ripening room and is ready to be placed in market (Fongaro et al., 2015). However, the physical and biochemical activities inside the salamis are not stopped at this phase and proceed at a rate depending on several factors, mainly temperature. In

particular, further water lost can be avoided by using of modified packaging atmosphere (**Tabanelli et al., 2013**). Taking into account variations in raw materials, production process and potential adulteration, differences in chemical composition within one brand of salami from different manufacturers might be expected.

The subject of study was to identify the differences in chemical composition of traditional Slovak Nitran salamis in relation to the manufacturer and variant (i.e. either whole sticks or slices packaged in modified atmosphere) using Principal Component Analysis (PCA).

The PCA is multivariate statistical method used for the identification of the most important directions of variability in a multivariate data matrix and presenting the results graphically. This technique has already been used by **Bianchi et al.**, (2007) who discriminated between the two kinds of Italian salamis according to profile of volatile compounds. Herranz et al., (2008) applied the PCA on the fatty acid profile in order to separate Milano-type salamis into different groups. Van Schalkwyk et al., (2011) performed PCA on chemical composition of salamis from game meat, in order to examine differences and consumer acceptability. Corral et al., (2013) used this technique to examine the relationship among reduction of salat content and textural parameters, chemical composition and physical properties of Italian salamis.

MATERIAL AND METHODOLOGY

Samples

Ten Nitran salamis from five different manufacturers were purchased from local supermarkets in Nitra, Slovakia. From each manufacturer, Nitran salami was purchased as whole stick and slices packaged in modified atmosphere. Salamis were labelled and assigned by codes according to variant (W = whole stick, S = slices) and manufacturer (number 1-5) (Table 1).

Chemical analysis

Analysis of chemical composition was accomplished at Department of Food Hygiene and Safety, SUA, Nitra, Slovakia. Determined attributes were as follows: the content of water (W), ash (A) and NaCl according to ISO 1442:1997, ISO 937:1998 and ISO 1841-1:1996, respectively; content total fat (TF) by acid hydrolysis and ether extraction according to AOAC 991.36; content of total protein (TP) by Kjeldahl method according to AOAC 2011.11 (content of nitrogen multiplied by factor 6.25) and content of hydroxyproline (H) according to ISO 3496:1994. The content of collagen (C) was calculated by multiplying of H with factor 8. The TP was used to calculate the protein without collagen as percentage of total protein (P-CTP), the collagen as percentage of total protein (CTP). Apparent total meat content (TM) was calculated according to McLean (1999). Each determination was performed in triplicate and results represent mean values with standard deviations (SDs).

Statistical analysis

The means and SDs of numeric data were computed using Microsoft Office Excel 2010. The PCA analysis was then performed on mean values of numeric data for 9

Salamilabel Variant Manufacturer										
Salamiaber	variant	Manufacturer								
Nitran A	W	1								
Nitran B	S	1								
Nitran C	W	2								
Nitran D	S	2								
Nitran E	W	3								
Nitran F	S	3								
Nitran G	W	4								
Nitran H	S	4								
Nitran I	W	5								
Nitran J	S	5								

attributes (without H) using the TANAGRA 1.4.50 software. In order to enchance the interpretation of principal components (PCs), both the CTR coefficients (contributions of points to dimensions) and the correlation coefficients among attributes were calculated within the PCA.

RESULTS AND DISCUSSION

Table 2 summarises the means and SDs of the measurements.

The correlation coefficients among attributes are shown in Table 3. There existed several strong correlations among some attributes. Besides the positive and moderate correlation with P-CTP, TM and A, TP correlated with 3 attributes (NaCl, CTP and TF) negatively and 2 attributes (C and W) slightly. The weak correlation among TPrelated attributes and W was expected, as salamis are basically dried and should have low content of water after drying and ripening stage (**Corral et al.**, (**2013**).

 Table 2 Means and standard deviations of measurements for salamis attributes.

Sample	NaCl (%)	A (%)	W (%)	TF (%)	H (%)	C (%)	TP (%)	P-CTP (%)	CTP (%)	TM (%)
	3.99	5.32	35.06	36.62	0.52	4.16	23.19	19.03	17.94	148.12
Nitran A	±1.19	± 0.04	± 0.55	± 1.33	± 0.08	± 0.97	± 0.18	±0.21	± 0.44	± 2.49
Nº4 D	3.44	4.39	31.33	43.12	0.37	2.96	20.84	17.88	14.20	142.31
Nitran B	± 0.75	± 0.18	± 0.91	± 1.13	± 0.05	± 0.17	± 0.10	± 0.97	± 0.97	± 1.78
Nitnon C	1.19	5.02	31.82	39.29	0.31	2.48	23.10	20.62	10.74	149.05
Mitrail C	± 0.58	± 0.27	± 1.07	± 0.82	± 0.02	± 0.22	± 0.58	± 0.58	± 0.82	± 1.85
Nitron D	9.55	4.68	28.09	50.85	0.41	3.28	17.42	14.14	18.83	120.29
	± 1.93	± 0.05	± 0.51	± 0.95	± 0.12	± 0.41	± 0.44	± 0.26	± 0.50	± 0.98
Nitron F	3.30	4.88	34.87	38.19	0.44	3.52	24.94	21.40	14.13	159.51
	± 1.33	± 0.17	±0.16	± 0.42	± 0.10	± 0.24	± 0.15	± 0.74	± 1.21	± 1.59
Nitron F	3.61	4.40	35.44	36.46	0.48	3.84	22.12	18.28	17.36	141.13
	± 1.80	± 0.11	± 1.27	± 0.74	± 0.06	± 0.45	± 0.62	± 0.23	± 1.32	± 0.71
Nitran G	1.25	4.89	33.82	39.52	0.41	3.28	21.75	18.47	15.08	131.26
Initian G	± 0.36	± 0.20	± 0.46	± 0.60	± 0.22	±0.22	± 0.34	± 0.34	± 0.71	± 0.57
Nitron H	3.85	4.70	22.80	49.79	0.51	4.08	24.62	20.54	16.57	162.01
	± 1.17	± 0.30	± 1.50	± 0.31	± 0.03	± 0.70	± 0.83	± 0.63	± 0.94	± 1.14
Nitron I	3.65	4.58	33.37	39.44	0.38	3.04	20.81	17.77	14.61	133.00
	± 1.05	± 0.09	± 0.46	± 1.04	± 0.02	± 0.56	± 0.70	± 0.50	± 1.24	± 1.82
Nitron I	4.42	5.45	23.29	47.95	0.44	3.48	22.25	18.77	15.64	144.50
	± 0.86	± 0.13	± 0.38	± 0.42	± 0.05	± 0.28	± 0.51	± 0.22	± 0.35	± 0.66

Table 3 Correlation coefficients among attributes of chemical composition of Nitran salamis.										
	P-CTP	ТР	NaCl	TM	СТР	TF	С	W	Α	
P-CTP	1.00									
TP	0.97	1.00								
NaCl	-0.75	-0.65	1.00							
TM	0.90	0.93	-0.46	1.00						
СТР	-0.57	-0.35	0.71	-0.31	1.00					
TF	-0.39	-0.35	0.62	-0.14	0.31	1.00				
С	0.12	0.36	0.22	0.34	0.75	0.04	1.00			
W	0.07	0.04	-0.35	-0.14	-0.14	-0.91	-0.11	1.00		
Α	0.29	0.32	-0.11	0.23	-0.05	0.02	0.16	-0.22	1.00	

Table 4 Results from the PCA analysis for the first five PCs.

Principal component	Eigen value	Proportion of variance explained (%)	Cumulative variance explained (%)
1	4.02	44.91	44.91
2	2.27	25.25	70.16
3	1.55	17.24	87.40
4	0.84	9.36	96.76
5	0.24	2.75	99.51

The results of the PCA analysis are presented in Table 4. Four PCs were extracted that accounted for 96.76% of the total variation. The first 3 of these PCs explain together 87.40% of total variation. In other words, these PCs are the most important, because 87.40% of total variance for Nitran salamis, in the 9 considered attributes, can be condensed into three new attributes (PCs). The eigen value of PC correspond with its importance.

For example, when Bianchi et al., (2007) performed the PCA on the class of aldehydes, the first two PCs accounting for the 68.00% of the variance, allowed to group the salamis according to their kind. Herranz et al., (2008) analysed nutritional indices in Milano salamis using 4 attributes and found that first two PCs for salamis explained 76.50% of the total variation. Van Schalkwyk et al., (2011) found the first two PCs analysing variables of sensory, microbiological, textural and physicochemical, from matured game salamis explained 86.74% of the total variability of those measurements. In Italian salamis, Corral et al., (2013) reported that 57.87% of total variation is explained by the first two PCs with measurements using number of parameters including fat, protein and water content.

Table 5 shows that all attributes of salamis had similar proportion (correlation value) in the 1st PC except for C, W and A. After P-CTP, the most important attributes for the 1st PC were TP, TM, TF, CTP and NaCl. So, the 1st PC is mainly defined by these attributes, while the 2^{nd} one is mainly described by C, TF, TM and W. The 3rd PC the best describes differences in TF, CTP, W and C among the samples. The 4th PC is predominantly defined by A, as that had little importance in the previous PCs. Ultimately, the last 5th PC explains the smallest proportional variance among attributes.

Figure 1, Figure 2 and Figure 3 display the correlation scatterplot of attributes on first four PCs. The attributes are interpreted according to the correlations among each other (Table 3) and each PC (Table 5). Thus, attributes close to each other are positively correlated, attributes separated 180° are negatively correlated, whereas if they are separated by 90° they are independent.

The Figure 1 displays that P-CTP is the most positively correlated with TP and TM. On the other hand, this attribute group is negatively correlated with TF, CTP and NaCl, which are, by contrary, positively correlated to each other. The 2nd PC is the best characterized by C and W because they are placed farthest from its origin. The 3rd PC shows that TF is in the highest negative correlation with C (Figure 2). The Figure 3 indicates the independence of A from other attributes.

The most valuable asset of the CTR coefficients (Table 6) to the PCA consists in their utility, when finding the samples that contributed to the particular PC markedly is

Table 5 Correlation coefficients in the eigen vectors (loadings) for the five first PCs, with percent and total percent contributions to explained variance.

Attributo	_	PC 1		PC 2		PC 3		PC 4		PC 5
Attribute	ρ	% (tot.%)								
P-CTP	0.96	93 (93)	0.22	5 (98)	0.07	1 (99)	-0.07	1 (99)	0.04	0 (99)
TP	0.91	83 (83)	0.38	15 (98)	-0.09	1 (99)	-0.08	1 (100)	0.01	0 (100)
NaCl	-0.86	74 (74)	0.30	9 (84)	-0.08	1 (84)	-0.01	0 (84)	0.39	16 (100)
TM	0.79	63 (63)	0.50	26 (89)	0.02	0 (89)	-0.24	6 (95)	0.19	4 (99)
CTP	-0.64	41 (41)	0.46	22 (63)	-0.60	37 (99)	0.01	0 (99)	-0.07	1 (100)
TF	-0.58	34 (34)	0.56	32 (66)	0.54	30 (96)	-0.15	3 (99)	-0.02	0 (99)
С	0.01	0 (0)	0.71	52 (52)	-0.68	46 (98)	-0.05	0 (98)	-0.12	2 (100)
W	0.28	8 (8)	-0.70	50 (58)	-0.61	38 (96)	0.08	1 (97)	0.15	3 (99)
А	0.26	7 (7)	0.41	18 (24)	0.13	2 (26)	0.85	73 (100)	0.04	0 (100)
Variation explained	4.04	45 (45)	2.27	25 (70)	1.55	17 (87)	0.84	9 (97)	0.24	3 (100)

Salami	CTR to PC1	CTR to PC2	CTR to PC3	CTR to PC4	CTR to PC5
Nitran A	1.37	4.02	29.53	22.63	1.27
Nitran B	0.54	6.57	3.34	14.07	0.00
Nitran C	13.68	9.97	23.87	2.54	3.60
Nitran D	66.15	0.05	0.37	0.24	10.97
Nitran E	15.51	0.23	1.84	0.89	33.41
Nitran F	0.02	1.93	25.84	9.15	2.34
Nitran G	0.23	6.90	0.28	4.65	41.04
Nitran H	1.40	41.73	1.85	25.59	4.84
Nitran I	0.78	12.16	0.01	0.45	0.23
Nitran J	0.33	16.45	13.06	19.78	2.31

Table 6 CTR coefficients of samples to each PC.

needed, i.e. to uncover the anomalous parameters of the samples in which they differ in each other (Table 5).

According to the CTR coefficients for 1^{st} PC it can be noted that variance in P-CTP, TP, TM, TF, CTP and NaCl is mainly given by opposition between Nitran D (CTR = 66.15) and remaining samples of Nitran salamis (CTR <16.00) (Table 6). Numeric data confirm this, as Nitran D had the lowest content of P-CTP, TP and TM



Figure 1 Correlation scatterplot – PC1 vs. PC2.



Figure 2 Correlation scatterplot – PC1 vs. PC3.



Figure 3 Correlation scatterplot – PC1 vs. PC4.

(14.14%, 17.42% and 120.29%, respectively) and the highest one of TF, CTP and NaCl (50.85%, 18.83% and 9.55%, respectively).

Collagen content is used as an index of the quality for fermented and dried meat products (da Silva et al., 2015). However, the total content is limited by regulatory agencies (Sentandreu and Sentandreu, 2014). According to Decree of the Ministry of Agriculture of the Slovak Republic and the Ministry of Health of the Slovak Republic no. 1895/2004-100 establishing a chapter of the Food Codex of the Slovak Republic regulating meat products (2005), fermented and dried meat products have to contain maximally 16% CTP. Thus, the values above this limit indicate extra addition of collagen or its hydrolysates, which is a common practice in the meat industry to increase the protein content or water binding properties of meat products (Sentandreu and Sentandreu, 2014). Nitran D was not the most in accordance with this criterion among the salamis (CTP = 18.83%). On the contrary, Nitran E, C and H belonged to group of the TP-rich salamis, when compared to Nitran D, though Nitran H did not satisfy CTP content.

The positions of labels on the loading plot also correspond with this observation (Figure 4). Nitran E, C, H and A are clustered together on the right side of the scatterpplot, because of similarities in attributes explained by 1^{st} PC. However, Nitran D, due to its unlikeness, is separated from other salamis, on the left opposite side of the scatterplot.

The CTR coefficients indicated that Nitran H, J, I and C also marcantly contributed to variance in C, TF, TM and W, described by 2^{nd} PC. Nitran H and A were those with the highest amount of C (4.08% and 4.16%, respectively), whereas Nitran C contained the lowest one (2.48%). But in turn, Nitran H and J had the lowest content of W (22.80% and 23.29%, respectively) and the highest one of TF (49.79% and 47.95%, respectively). Salami is one of the meat products that contain high fat content, usually up to 30% (**Pramualkijja et al., 2015**).

The CTR values for 3^{rd} PC showed that Nitran A was the poorest in TF (36.62%), but on the other hand the richest in C and CTP (4.16% and 17.94%, respectively) (Figure 5).

Nitran H, A, J and B contributed to variance in A, which was explained by 4^{th} PC. Nitran H and B belonged to the group of low content of A, while Nitran A and J belonged to that one with the highest one (5.32% and 5.45%, respectively) (Figure 6).



Figure 4 Loading plot - PC1 vs. PC2.



Figure 5 Loading plot - PC1 vs. PC3.



Figure 6 Loading plot - PC1 vs. PC4.

CONCLUSION

It can be concluded that the PCA has shown how chemical attributes of salamis are grouped in the independent sets. In both the 1st and the 2nd PCs, the P-CTP, TP, TM, TF, CTP and NaCl attributes had the highest loadings. In other words, these attributes explained the large part of observed variation in chemical composition among Nitran salamis, which make these attributes as a main predictor of salamis quality. The most

distinct differences in these attributes were observed within a pair of Nitran salamis from manufacturer 2 (Nitran C and D). The sliced variant (Nitran D) had the lowest value of TP-associated attributes and the highest ones of TF, CTP and NaCl, even within all the salamis. Besides Nitran D, Nitran A, F and H also did not satisfy CTP content specified in the Decree of MASR and MHSR no. 1895/2004-100, which might indicate extra addition of connective tissue. The CTP content of Nitran B, C, E, G, I and J was in accordance with the decree, whereas Nitran C was that sample with the lowest one (10.74%). Some differences in other attributes were also observed within and among the all couples of salamis, which confirmed the uniqueness of each one. These differences were notable, but not so relevant compared to those described by 1st PC.

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