Expo 2015

Falsification of food

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Contents

Introduction

Recently, detection of falsified food and protection of consumer interests are being more and more frequently discussed. The main aim of falsification is an effort of producers to defraud consumers and thereby gain an economic advantage over their competitors. Food falsification has been taking attention of the public, media and also government authorities. All over the world there are many institutions that are engaged in falsifying or in the development of technologies for detection of falsification. In the Czech Republic, the main state authority is Czech Agriculture and Food Inspection Authority.

Although falsification has become a much discussed topic only in the last few years, it has already existed in the Middle Ages. In Greece and Rome, wine was colored and flavored. In Great Britain, the most frequently falsified foodstuffs were coffee and tea. Nowadays, falsification involves all kinds of food and is widespread throughout the world. The most commonly falsified commodities include luxury food (spirits, wine, coffee and spices) or foodstuffs sold in larger quantities (meat and dairy products, plant oils, fruit juices, pasta).

1 Theoretical part

1.1 Falsification of food

Falsification of food means an addition of unhealthy, unsafe or undeclared food substances. The main methods of falsification are:

1. Deliberate addition of another, either cheaper or more accessible substance in food that partially or completely replaces original (often expensive) raw materials (addition of soybean oil to olive, addition of sugar to honey, substitute of meat in meat products, addition of coffee hulls or cereals into coffee)

2. Deliberate addition of additives in order to disguise the real quality or origin of the food (coloring white wine red, coloring or flavoring of lesser-quality products)

3. Misuse of well-known brands of food (replacement of vintages of wines, use of packaging, labels and names commemorating well-known brand name)

4. Use of undeclared production technology

5. Deliberate misleading labeling of foods on its composition, origin, age, variety or a business name

A clear classification is sometimes difficult, because among the listed types of falsification there are many combinations.

1.2 Health hazards

Although in most cases falsification involves "only" selling less quality product for a higher price, sometimes it may have serious consequences for consumers. Often, toxic substances are used as substitutes e.g. diethylene glycol in order to highlight the taste of wine or mineral oil for vegetable oil dilution. Well known is the affair of 2008, when poisoning by falsified powdered milk containing melamine[1] affected 300 000 Chinese children, of whom at least six children died and hundreds of them have long-term or life-long consequences. 2 years ago in the Czech Republic, there was another incident, so called methanol affair, which caused 48 deaths. For some consumer groups, such as young children or allergic people, might be dangerous e.g. undeclared presence of cheaper cow's milk in soy drinks or peanuts in ice cream or chocolate.

1.3 Used methods

This chapter describes 2 methods that are used in the practical part of this work.

1.3.1 Liquid chromatography

The most important method of liquid chromatography is HPLC. The abbreviation is formed from two possible names of this technique: high-performance liquid chromatography or high-pressure liquid chromatography. HPLC is used to separate components of the sample in order to determine their presence and concentration. The analysis is performed on the device which is called liquid chromatograph.

During the chromatography a liquid (mobile phase) is pumped through a bed of particles (stationary phase). The stationary phase is a film of relevant substance deposited on the support surface or solid adsorbent. Each component in the sample interacts slightly differently with the stationary phase. This causes different flow rates for the different components and the samples are separated.

The result of HPLC analysis is a chromatogram. If the analyzed mixture is well distributed, then each component corresponds to one peak.

Position of the peak, which is defined by retention time, specifies substance (qualitative analysis), the peak area determines concentration of the substance in the mixture (quantitative analysis).

[1] Melamine = organic compound that is used in production of plastics and fertilizers

Liquid chromatography can be used for resolution of types of fruit, wheat species or types of meat and for detection of falsification of honey or additions of milk powder to pasteurized milk.

1.3.2 ELISA

ELISA (Enzyme-Linked Immunosorbent Assay) is one of the most widely used immunological methods for detection of antibodies. It is a technique based on a specific binding reaction between an antigen[2] and an antibody. The most widely used method for the determination of antigens is a noncompetitive ELISA (in other words, the sandwich technique). In this technique, two antibodies – enzyme-labeled and immobilized – are attached to the detected antigen and create a sandwich. Noncompetitive ELISA consists of several steps.

1. On the surface of sample well, antibodies specials for the target antigen are adsorbed.

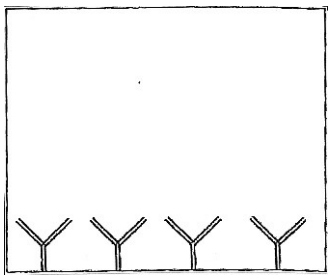

Figure 1: Step 1

2. A sample of food is added into the sample well. Antigens begin to bind to specific antibodies. Sample residues and the unbound antigens are removed by washing in water.

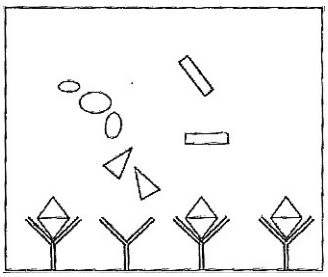

Figure 2: Step 2

3. An enzyme-labeled antibody is added into the sample well. It binds to the antigen and creates the so called "sandwich". Unbound labeled antibodies are removed by washing in water.

[2]Antigen = a substance which evokes production of one or more antibodies. Each antibody binds to a specific antigen through the interaction of engagement similar to fit between a lock and a key.

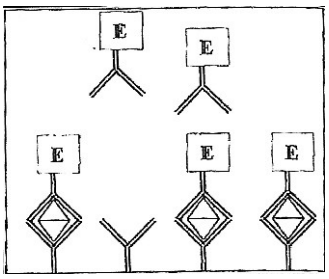

Figure 3: Step 3

4. A substrate is added into the sample well. The enzyme causes conversion of the substrate into a colored product.

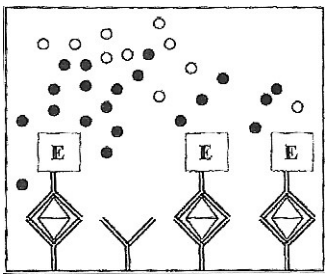

Figure 4: Step 4

Evaluation of the results is carried out visually or spectrophotometrically.

In detection of falsification, ELISA is used for identification of soybean proteins in dairy and meat products, determination of specific components in cereals or generic distinction of meat.

2 Aim

The aim of my work was to summarize the history, ways of falsification and methods used for detection of falsified food. Specifically I focused on meat products because they are among the most commonly falsified commodities. However, the main goal of this work was to verify the quality or to detect falsification of products currently available on the Czech market and to compare the results with falsification cases recorded last year. Another objective was to test and optimize methods for detecting of falsified food for laboratories at the Mendel University in Brno.

3 Practical part

3.1 Determination of net muscle protein

3.1.1 Method

Determination of net muscle protein is based on the analysis of a sample prepared by acid hydrolysis on a liquid chromatograph. The result of separation of the sample is the determination of concentration of creatinine, which is a nitrogen-containing organic substance, whose quantity in the body is directly proportional to the amount of muscle tissue. Out of this concentration, net muscle protein content can be subsequently calculated. In this part of work, 25 meat products bought in common retail chains in the Czech Republic were verified. The products came from eight manufacturers, whose names were replaced by numbers in order to protect their privacy.

3.1.2 Procedure

Samples were cut and homogenized on a disintegrant. A mixture of $2\,g$ of the product with $30\,ml$ 30% H_2SO_4 was placed in a flask in an oven at 105 °C and overnight, acid hydrolysis occurred. Next day, the flask content was cooled and after addition of demineralized water filtered. To $2,5\,ml$ of the filtrate $10\,ml$ of phosphate buffer were added and the solution was neutralized to pH 5–8 using $10M\ NaOH$. It was cooled again, refilled with phosphate buffer, refiltered and placed in a small vial. Then the mobile phase ($0,35\,g$ of sodium 1-octane sulfonate monohydrate with $500\,ml$ of phosphate buffer), concentrated standard and calibration line of creatinine were prepared. The solutions in closed vials were analyzed on liquid chromatograph (HPLC) with UV / VIS detector.

Calculation of equation of the calibration line and the calculation of concentrations of samples were conducted in the ChemStation program. Calculation of final concentration was performed according to the following formula:

$$cm(\tfrac{mg}{100g}) = \tfrac{c(\mu g/ml)\cdot V(ml)}{m(g)}$$

cm - final concentration of creatinine, c - creatinine concentration in the sample solution calculated from ChemStation program calibration graph, V - volume of measuring cup, m - weigh of sample

Calculation of net muscle protein (NMP) was performed according to the following formula:

$$NMP(\tfrac{g}{100g}) = \tfrac{cm(\frac{mg}{100g})}{20}$$

Number 20 here presents an empirical constant.

3.2 Determination of presence of poultry meat by the ELISA method

3.2.1 Method

To perform the ELISA method, Biokits FAST Immunostick Meat Species Screening Kit from the company Neogen Corporation were used. Determination of presence of poultry was carried out on 24 meat products bought again in common stores. Products came from eight manufacturers, in three cases manufacturers were not listed.

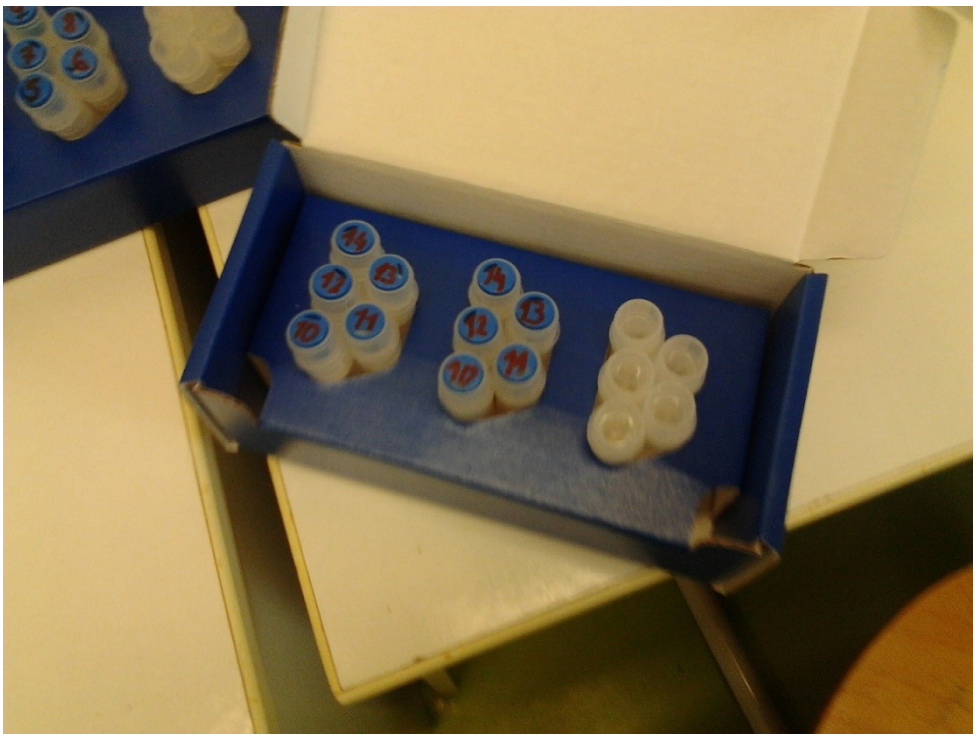

Figure 5: ELISA Kit

3.2.2 Procedure

10 grams of a sample were placed in a plastic bag with $10\,ml$ of cold water and homogenized. Several drops of this mixture were placed in immunotubes. After 10 minutes, the sample residue and unbound antigens were removed by washing in water. Then, enzyme labeled antibodies were added to the immunotubes. After binding, these antibodies formed the aforementioned sandwich. The washing step was repeated again. A substrate (CDR solution) was added and by enzymatic action, it converted into a colored product. Eventually, STOP solution was used to stop color reaction. The results were interpreted on the basis of the color change.

4 Results

4.1 Determination of net muscle protein

The aim was to determine whether meat products comply the statutory limit for net muscle protein. The following table provides a description of each of the tested products and the legally required minimum content of net muscle protein.

Name of product	Description of product	Minimum content of NMP (%)
Vysočina	durable heat-treated salami (beef and pork, pork fat, pepper)	13
Herkules	durable heat-untreated (fermented) smoked salami (beef and pork, pork fat, salt, spices)	14
Paprikáš	durable heat-untreated (fermented) salami (beef and pork, pork fat, pork skin, salt, spices, paprika)	14
Poličan	durable heat-untreated salami (fermented) smoked salami (beef and pork, pork fat, salt, spices)	16
Lovecký salám	durable heat-untreated salami (fermented) smoked salami (beef and pork, pork fat, salt, spices, beetroot)	15
Standardní šunka	standard ham	10
Výběrová šunka	selected ham	13

Figure 6: Description of tested products

From figure 7 it is apparent that three products did not comply with the decree (in the table in red). These were samples number 10 (vysočina), 23 (poličan) and 28 (lovecký salám). All of these products came from producer C[3] . To all samples, an unexpected uncertainty was added in order to ensure at least 95% reliability.

In addition, 2 products (8 – vysočina, 25 – poličan, in the table in blue) from producer B failed to comply the limit after deducting the expanded uncertainty. And one sample - again from producer C (18 – paprikáš, in the table in green) – complied with the decree after adding the expanded uncertainty. Although these three are below or just over limit, by using the expanded uncertainty they cannot be regarded as unsatisfactory.

The best quality product was a salami from producer G, which contained 5 % more NMP than the limit requires. The limit also fulfilled tested products of companies D, E and F.

[3]Names of producers could not be mentioned for the protection of privacy, the list of producers is in the annexes - Annex A

sample	producer	weight	concentration (µg/ml)	R	concentration (mg/100 g)	NMP	average (mg/100g)	stand. deviation (mg/100g)	RSD		expanded uncertainty (mg/100g)
8a - vysočina	B	2,0005	20,57100	5	257,07	12,85	13,04	0,19	0,01432	±	0,37
8b - vysočina	B	2,1676	22,93669	5	264,54	13,23					
10a - vysočina	C	2,0292	20,49081	5	252,45	12,62	12,56	0,07	0,00518	±	0,13
10b - vysočina	C	2,1615	21,60192	5	249,85	12,49					
18a - paprikáš	C	2,0292	21,79400	5	268,50	13,43	13,82	0,40	0,02863	±	0,79
18b - paprikáš	C	2,0295	23,08230	5	284,33	14,22					
23a - poličan	C	2,2115	25,75315	5	291,13	14,56	14,48	0,08	0,00542	±	0,16
23b - poličan	C	2,1701	24,99869	5	287,99	14,40					
25a - poličan	B	2,0897	27,08715	5	324,06	16,20	16,00	0,20	0,01244	±	0,40
25b - poličan	B	2,1186	26,78694	5	316,09	15,80					
28a - lovecký	C	2,1216	22,01924	5	259,47	12,97	13,10	0,13	0,00983	±	0,26
28b - lovecký	C	2,1009	22,23744	5	264,62	13,23					

Figure 7: Part of the resulting table of determination of NMP (the whole table is in the annexes - Annex C)

4.2 Determination of presence of poultry meat

The aim was to determine whether some meat products contain poultry meat, whose presence is not declared on the label. The following table provides a description of each of the tested products.

Name of product	Description of product
Šunkový salám	salami very similar to ham, it contains large pieces of pork (pork, beef)
Gothajský salám	soft salami (pork, beef, pork fat, spices, salt)
Vídeňské párky	Vienna sausages
Špekáčky	sausages made from a mixture of pork, beef, pork fat, pepper, garlic and paprika
Kabanos	long heat-treated smoked salami (pork and beef, garlic, spices)
Ostravská klobása	sausage made from minced pork large pieces of pork
Jemné párky	soft sausages (pork, beef, pork fat, pork skin)
Debrecínské párky	sausages (pork, beef, garlic, paprika, marjoram)
Salám Junior	soft salami with a typical pink colour (pork, beef, veal, paprika)

Figure 8: Description of tested products

This method was firstly tested on a standard - chicken ham (see Figure 9). From a total of 24 tested products[4] , samples 1 (šunkový salám), 5 (gothajský salám), 6 (gothajský salám) and 8 (Vídeňské párky) did not comply with the decree.

[4]The list of producers is in the annexes - Annex B

Figure 9: Result of the standartd

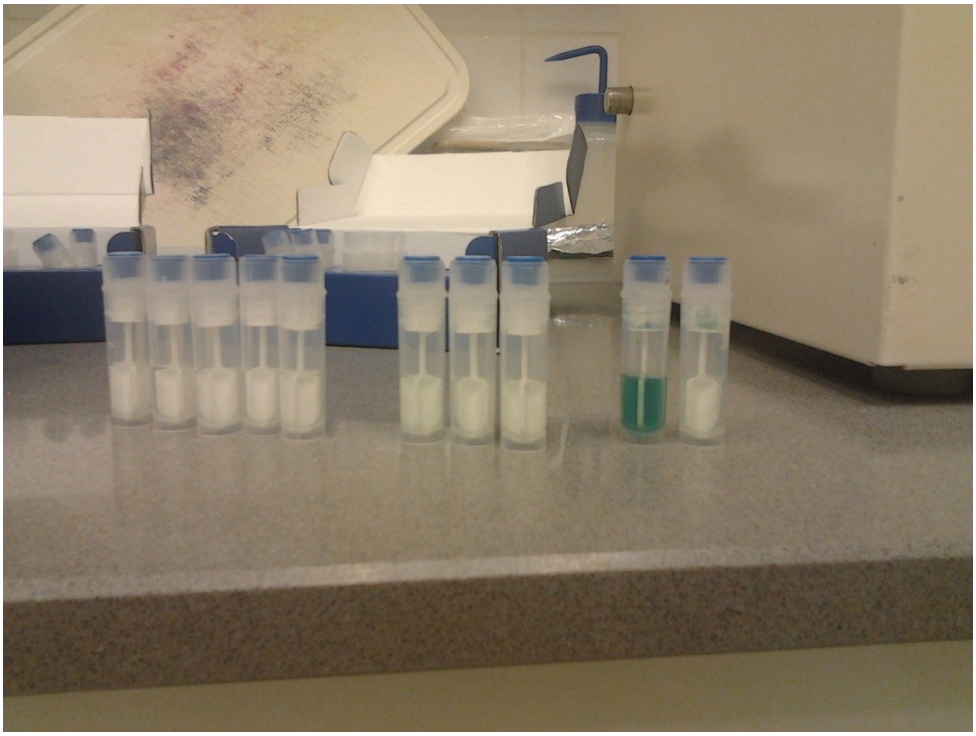

Figure 10: ELISA test results (negative samples on the left, positive samples on the right and in the middle, the second sample from the right is the standard)

5 Conclusion

The theoretical part of this work summarizes the most common ways of food falsification and health risks to consumers. Furthermore, it describes two common methods used to detect poor quality food. In the practical part, the quality of meat products commonly available in the Czech Republic was verified using these methods. Falsification was generally proved in 9 of the 70 products.

Subsequently, these results were compared with the website Food pillory which last year managed in collaboration with Czech Agriculture and Food Inspection Authority and the Ministry of Agriculture to detect twenty-nine cases of falsification of meat products. Surprisingly, none of these products corresponded with those, which were revealed by this work. These results bring several following facts.

Detection of high amount of falsified food may be unsettling, but on the other hand, such a result was expected. Not all sellers are honest, and therefore the food should be chosen very carefully. A consumer should pay particular attention to the seller, verified brand and country of origin. It is also important to check the expiration date every time. On the other hand, very positive finding is that none of the products of poor quality was noticed earlier. It is obvious that either the competent authorities or the producers themselves quickly react and try to eliminate low-quality products from sale.

Although the tested products only came from the Czech Republic, this work may have use for other countries as well. The practical part describes the exact process by which the quality of the food can be easily verified. These procedures were validated and optimized and may be readily used. In addition, thanks to my work, non-competitive ELISA method was implemented and optimized at the Faculty of Agronomy of Mendel University in Brno.

Acknowledgements

I would like to express my sincere gratitude to my external consultant MVDr. Olga Cwiková, Ph.D., and internal consultant RNDr. Kateřina Cibulková for valuable advice, thought-provoking comments on my work and great patience. I am also grateful to RNDr. Alena Ansorgová for assistance in the laboratory. Last but not least, I would like to thank the South Moravian Centre for International Mobility for financial support of my work.

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Figure 5 - 10: author - Markéta Pitrová

List of Annexes

Annexes

sample	producer
7 - vysočina	A
8 - vysočina	B
9 - vysočina	B
10 - vysočina	C
11 - vysočina	D
12 - herkules	E
13 - herkules	C
14 - herkules	F
15 - herkules	B
16 - herkules	G
17 -paprikáš	G
18 -paprikáš	C
19 -paprikáš	B
20 -paprikáš	F
21 - poličan	G
22 - poličan	F
23 - poličan	C
24 - poličan	B
25 - poličan	B
26 - lovecký salám	G
27 - lovecký salám	B
28 - lovecký salám	C
29 - lovecký salám	B
30 - standardní šunka	H
31 - výběrová šunka	F

Annex A: List of meat products tested by HPLC

sample	producer
1 - šunkový salám	A
2 - šunkový salám	*not listed*
3 - šunkový salám	B
4 - gothajský salám	*not listed*
5 - gothajský salám	*not listed*
6 - gothajský salám	C
7 - vídeňské párky	D
8 - vídeňské párky	C
9 - vídeňské párky	E
10 - špekáček	F
11 - špekáček	E
12 - špekáček	G
13 - kabanos	C
14 - kabanos	C
15 - ostravská klobása	H
16 - ostravská klobása	B
17 - ostravská klobása	C
18 - jemné párky	E
19 - jemné párky	A
20 - debrecínské párky	C
21 - debrecínské párky	D
22 - debrecínské párky	C
23 - junior	B
24 -junior	C

Annex B: List of meat products tested by ELISA

sample	producer	weight (g)	c (µg/ml)	R	c (mg/100 g)	NMP	average (mg/100g)	stand. deviation (mg/100g)	RSD	%		expanded uncertainty (mg/100g)
7a - vysočina	A	2,0412	23,90402	5	292,77	14,64	**14,75**	0,12	0,00787	0,79	±	**0,23**
7b - vysočina	A	2,0496	24,38340	5	297,42	14,87						
8a - vysočina	B	2,0005	20,57100	5	257,07	12,85	**13,04**	0,19	0,01432	1,43	±	**0,37**
8b - vysočina	B	2,1676	22,93669	5	264,54	13,23						
9a - vysočina	B	2,1231	23,36604	5	275,14	13,76	**13,90**	0,14	0,01023	1,02	±	**0,28**
9b - vysočina	B	2,0973	23,55917	5	280,83	14,04						
10a - vysočina	C	2,0292	20,49081	5	252,45	12,62	**12,56**	0,07	0,00518	0,52	±	**0,13**
10b - vysočina	C	2,1615	21,60192	5	249,85	12,49						
11a - vysočina	D	2,1298	32,45478	5	380,96	19,05	**18,73**	0,32	0,01687	1,69	±	**0,63**
11b - vysočina	D	2,1135	31,13774	5	368,32	18,42						
12a - herkules	E	2,0549	28,82797	5	350,72	17,54	**17,86**	0,32	0,01800	1,80	±	**0,64**
12b - herkules	E	2,0399	29,66669	5	363,58	18,18						
13a - herkules	C	2,0295	24,28584	5	299,16	14,96	**15,22**	0,27	0,01747	1,75	±	**0,53**
13b - herkules	C	2,0421	25,30584	5	309,80	15,49						
14a - herkules	F	2,1173	25,57818	5	302,01	15,10	**15,57**	0,47	0,03011	3,01	±	**0,94**
14b - herkules	F	2,0924	26,84690	5	320,77	16,04						
15a - herkules	B	2,0969	27,14389	5	323,62	16,18	**16,30**	0,11	0,00701	0,70	±	**0,23**
15b - herkules	B	2,1147	27,76098	5	328,19	16,41						
16a - herkules	G	2,1561	20,50812	5	237,79	*poor sample*	**14,73**	0,00	0,00000	0,00	±	**0,00**
16b - herkules	G	2,0377	24,01619	5	294,65	14,73						
17a - paprikáš	G	2,0833	30,56799	5	366,82	18,34	**18,14**	0,20	0,01083	1,08	±	**0,39**
17b - paprikáš	G	2,0685	29,70070	5	358,96	17,95						
18a - paprikáš	C	2,0292	21,79400	5	268,50	13,43	**13,82**	0,40	0,02863	2,86	±	**0,79**
18b - paprikáš	C	2,0295	23,08230	5	284,33	14,22						
19a - paprikáš	B	2,0240	27,02533	5	333,81	16,69	**16,62**	0,07	0,00398	0,40	±	**0,13**
19b - paprikáš	B	2,1151	28,01758	5	331,16	16,56						
20a - paprikáš	F	2,0624	24,47470	5	296,68	14,83	**14,70**	0,14	0,00931	0,93	±	**0,27**
20b - paprikáš	F	2,0569	23,95918	5	291,20	14,56						
21a - poličan	G	2,0844	30,06125	5	360,55	18,03	**18,08**	0,05	0,00272	0,27	±	**0,10**
21b - poličan	G	2,1097	30,59239	5	362,52	18,13						
22a - poličan	F	2,0776	29,86775	5	359,40	17,97	**18,11**	0,14	0,00792	0,79	±	**0,29**
22b - poličan	F	2,1640	31,60670	5	365,14	18,26						
23a - poličan	C	2,2115	25,75315	5	291,13	14,56	**14,48**	0,08	0,00542	0,54	±	**0,16**
23b - poličan	C	2,1701	24,99869	5	287,99	14,40						
24a - poličan	B	2,0285	27,92095	5	344,11	17,21	**17,43**	0,23	0,01301	1,30	±	**0,45**
24b - poličan	B	2,1706	30,66433	5	353,18	17,66						
25a - poličan	B	2,0897	27,08715	5	324,06	16,20	**16,00**	0,20	0,01244	1,24	±	**0,40**
25b - poličan	B	2,1186	26,78694	5	316,09	15,80						
26a - lovecký	G	2,1199	31,50896	5	371,59	18,58	**20,00**	1,42	0,07098	7,10	±	**2,84**
26b - lovecký	G	2,0770	35,58888	5	428,37	21,42						
27a - lovecký	B	2,0836	28,37020	5	340,40	17,02	**16,58**	0,44	0,02661	2,66	±	**0,88**
27b - lovecký	B	2,1925	28,30530	5	322,75	16,14						
28a - lovecký	C	2,1216	22,01924	5	259,47	12,97	**13,10**	0,13	0,00983	0,98	±	**0,26**
28b - lovecký	C	2,1009	22,23744	5	264,62	13,23						
29a - lovecký	B	2,1430	36,82944	5	429,65	21,48	**25,21**	3,73	0,14799	14,80	±	**7,46**
29b - lovecký	B	2,0574	47,64161	5	578,91	28,95						
30a - šunka	H	2,0749	16,81540	5	202,60	10,13	**10,13**	0,0018	0,00018	0,02	±	**0,00**
30b - šunka	H	2,0186	16,36500	5	202,68	10,13						
31a - šunka vyb.	F	2,1327	26,45619	5	310,13	15,51	**15,63**	0,12	0,00767	0,77	±	**0,24**
31b - šunka vyb.	F	2,0564	25,90397	5	314,92	15,75						

Annex C: The resulting table of determination of NMP