PE5.07 Development and Application of Analytical Methods for Certain Food Additives Determination in Meat Products with the Aim of Consumers' Protection 411.00

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Abstract—Analytical methods for determination of three food additives used in meat products are developed: method for determination of carrageenan (E 407) and colours – cochineal (carminic acid E 120) and ponceau 4R (E 124). The application of these methods has been verified on control and experimental samples of meat products. Developed methods are HPLC with UV/Vis detection for determination of E 120 and E 124 and FTIR ATR method for determination of E 407. HPLC system was WATERS ALLIANCE with UV/Vis detector and for the infrared spectroscopy, BRUKER FTIR spectrophotometer TENSOR 27 with MVP Pro Diamond ATR accessory. Recovery for the E 120 was in the from 86.48% to 107.49% in the concentration range from 1mg/kg to 100 mg/kg, for E 124, recovery was 45.62% to 102.55% in the concentrations interval from 5 mg/kg to 100 mg/kg while recovery of E407 was from 80.83% to 139.43% for the concentrations range from 0.2% to 2%. Testing of analytical methods on model samples showed their applicability in determination of three food additives

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Index Terms—Food aditives, Carrageenan, Cochineal, Ponceau 4R.

I. INTRODUCTION

Large number of papers and scientific studies deals with the area of food additives determination in foodstuffs, alcoholic and non-alcoholic beverages. However, few papers that cover the field of determination of E 120, E 124 and E 407 in meat products are available. Improvement of sensory properties of meat products, primarily their appearance by addition of colors as well as achieving water binding and more favorable texture by adding carrageenan became common practice in meat products manufacturing. Therefore, the need for development of new, or modification of existing analytical methods for the determination of these food additives in meat products appeared. In accordance with all this, and in the interest of consumers' protection, we developed and verified on model samples, analytical methods for determination of three food additives in cooked sausages of frankfurter type, finely chopped cooked sausages in 75 mm casing diameter, finely chopped cooked sausages of «Parisian» type, chopped cooked sausages of «Tirol» type and chopped meat cans The quality of meat products, besides its sensory properties and basic chemical composition, consists also of the microbiological status and safety parameters, i.e. the presence of absence of environmental contaminants, toxic elements, pesticides and residues of veterinary drugs. Also, the inspection of meat products' quality consists of sensory analysis and declaration inspection as well as determination of basic chemical parameters and food additives. Food additives, among the other, influence on appearance and sensory properties of meat products. It is not uncommon that nutritive properties of the product are not in accordance with general appearance and high sensory grades. Colors can mask some cheaper filling components such as skins. In that case, the consumer can have the wrong impression on the product's quality, mislead by its desirable appearance and color, which is often the decisive factor when buying the product. Carrageenan improves the texture of meat products. However, since this food additive is also a hydrocolloid, it increases the incorporation of water into the filling of the meat products. The aim of this paper is to develop reliable analytical methods for rapid and efficient determination of three food additives commonly used in meat products: carrageenan, E 407 and colors – cochineal (carminic acid), E 120 and ponceau 4R, E 124 and to verify the applicability of developed methods on specified meat products.

II. MATERIALS AND METHODS

Material Chemicals were purchased from the following manufacturers: ethanol, 96%, Zorka Pharma, Serbia; methanol, HPLC grade, J. T.

Baker, the Netherlands; water, HPLC grade, Sigma – Aldrich, Switzerland; hydrochloric acid 37-38% p.a., J. T. Baker, the Netherlands; ammonium hydroxide 25% p.a., Zorka Pharma, Serbia and sodium hydroxide, p.a., Centrohem, Serbia. Analytical standard of carminic acid ≥96.0%, Fluka, Sigma – Aldrich, Switzerland; analytical standard of ponceau 4R, Sigma – Aldrich, USA, carrageenan of commercial purity, type I, Sigma – Aldrich, USA. For the preparation of standards stock solutions, 10mg of each color was weigh and transferred in separated 100 mL volumetric flasks. The standards were dissolved in water. Stock solution concentration was 0.1 mg/mL. These solutions were used for working standards preparation. Carrageenan was determined in powdered form.

Manufacture of meat products Meat products were manufactured in semi-industrial conditions. The following types of products were produced: cooked sausages of frankfurter type, finely chopped cooked sausages in 75 mm casing diameter, finely chopped cooked sausages of «Parisian» type, chopped cooked sausages of «Tirol» type and chopped meat cans.

The experimental group of products was manufactured of beef and pork, fatty tissue, ice, additives (except investigated colors and E 407), while control group was manufactured of beef, pork, mutton and chicken meat, ice, fatty tissue, additives, spices and soy proteins (soybean flour and soy isolates, depending on the product), carrageenan and E 120.

All products were subjected to sensory evaluation. Basic chemical composition was determined by accredited analytical methods (water content, total proteins content, relative content of connective tissue proteins in total proteins, fat content and ash, pH and aw value). Also, the presence of proteins of animal origin, presence and content of soy proteins, content of E 120 and presence of E 407 were determined. Model meat product was produced in laboratory conditions from beef, pork, fatty tissue, ice and additives, with the addition of E 124 color (control sample was made without the color addition).

After the thermical treatment, pasteurisation and sterilisation, content of E 124 was determined. Samples without the label are control samples, experimental samples were labeled as follows: A and A1 (cooked sausages of frankfurter type); B and B1 (finely chopped cooked sausages in 75 mm casing diameter); C and C1 (finely chopped cooked sausages of «Parisian» type; D and D1 (coarsely chopped cooked sausages in «Tirol» type and E and E1 (chopped meat cans). Sample preparation E 120 - Weigh 1g of sample, add 10mL of 96% ethanol and 1mL 0,1mol/dm3 HCl. Homogenise on the ultra-turrax for 5min. Centrifugate for 10min at 4000 rpm. Decant the supernatant into the 100mL round bottom flask and evaporate on rotary evaporator at 55oC up to the volume of about 1mL.

Transfer the supernatant to polyamide SPE cartridges. Wash the cartridges with 1 mL of deionised water and 1mL of methanol. Elute with 1mL of 0,1% TFA in methanol 1:1.[1] Filter the extract into the HPLC vials trough 0.45µm nylon syringe filters. E 124 - Weigh 1g of sample, add 10mL of 96% ethanol and 1mL of 25% ammonia. Homogenise on the ultra-turrax for 5min. Centrifugate for 10min at 4000 rpm. Decant the supernatant into the 100mL round bottom flask and evaporate on rotary evaporator at 55oC up to the volume of about 1mL. Transfer the supernatant to polyamide SPE cartridges. Wash the cartridges with 1mL of deionised water and 1mL of methanol. Elute with 1mL of 0.5% ammonia:methanol 1:1.[2] Filter the extract into the HPLC vials trough 0.45µm nylon syringe filters. E 407 - Weigh 10g of sample, add 150mL of 0,1mol/dm3 sodium hydroxide solution in the mixture 96% ethanol:water 30:70 v/v. Homogenise on the ultra-turrax for 5min..

Heat the sample in microwave oven up to the boiling point (2-3 min). Stir and leave over night. Decant into the 100mL centrifuge tubes. Centrifugate for 10 min at 4000rpm. Discard the supernatant. Transfer the residue into the 100mL round bottom flask. Evaporate on rotary evaporator to dryness at 60oC. Scratch the small amount of film from the flask wall using spatula and record the infrared ATR spectrum. HPLC Chromatographic system for determination of E 120 and E 124 consisted of quaternary pump with degasser, autosampler and column heater (Alliance separation module 2695, Waters, Milford, MA, USA) and UV detector (2487 Dual λ absorbance detector, Waters). Chromatographic separation was carried out on Phenomenex Luna C18(2) column, 150 x 3 mm 5 µm with C18 guard column from the same manufacturer.

For the determination of E 120, the following gradient elution was performed: 0-5min

0,1% TFA in methanol:0,1%TFA 10:90 v/v 5-10min 0,1% TFA in methanol:0,1%TFA 60:40 v/v 10-15min 0,1% TFA in methanol:0,1%TFA 10:90 v/v. Column temperature was maintained at 35oC at the flow rate of 1mL/min.

Total run time was 15 minutes, injection volume 10µl. E 120 was detected at 494nm. Chromatographic conditions for determination of E 124 were the following: flow 1,00mL/min, column temperature 30oC. Elution was isocratic, the mobile phase was 0,2% ammonia in methanol: 0,2% ammonia 10:90 v/v. Run time was 7 min. Injection volume was 10µl. Detector wavelenght was 510nm. Empower Pro software was used for system control, data acquisition and processing. FTIR-ATR spectrophotometry

For the determination of E 407, FTIR spectrophotometer (Tensor 27, Bruker Optics, Germany) with ATR accessory (MVP Pro Diamond, Harrick Scientific, USA) was used. The following recording conditions were applied : recording interval was 1500-500cm-1, number of scans was 128 at the resolution of 2cm-1[3]. Quantitation was achieved by measuring peak intensity in the range of 1110 – 950cm-1. OPUS software was used for instrument control, spectra recording, processing and quantification. Sensory evaluation Sensory evaluation was carried out by 6 experienced auditors by applying the simple descriptive test. The evaluated parameters were graded on the scale from 1 to 10. Descriptive statistics was applied for the processing of the results. Using Microsoft Excel software. Comparison of the results obtained from different groups of products was carried out using analysis of variance (ANOVA: single factor) and t-test (two sample assuming unequal variance).

III. RESULTS AND DISCUSSION

Determination of E 120 Calibration was performed in the concentrations range from 1mg/l (mg/kg) to 100mg/l (mg/kg). Good linearity was achieved, correlation coefficient square (R2) was 0.9948. Recovery for each determined concentration is shown in Table 1.

Table 1. Recovery of E 120

Concentration of added E120 (mg/kg)	Mean concentration E120 (n = 9) (mg/kg)	Recovery (%)	STD
1	0,96	95,81	0,12
25	21,62	86,48	1,02
50	53,74	107,49	7,42
75	71,57	95,42	3,59
100	91,29	91,29	6,57

Determination of E 124 Method for determination of E 124 (Ponceau 4R) was carried out in the concentrations range from 5mg/l (mg/kg) to 100mg/l (mg/kg). Good linearity was achieved, correlation coefficient square (R2) was 0.9945. Recovery for each determined concentration is shown in Table 2.

Table 2. Recovery of E 124

Concentration of added E124 (mg/kg)	Mean concentration E124 (n = 9) (mg/kg)	Recovery (%)	STD
5	2,28	45,62	0,17
10	5,44	54,39	0,21
25	16,07	64,29	0,57
50	38,78	77,56	1,43
75	65,37	87,16	2,04
100	102,55	102,55	3,53

Determination of E 407 Method for determination of E 407 (carrageenan) was primarily developed for qualitative investigation of E 407 presence in meat products. By further modification, method was extended to quantitative determination of this additive. Investigated range of concentrations in the samples was from 0.2 to 2%.

Table 3. Recovery of E 407

Quantity of added E 407 (%)	Experimenatlly determined quantity of E 407 (%)	Recovery (%)
0.20	0.28	139.43
0.50	0.51	102.07
0.80	0.65	80.83
1.00	0.94	94.17
1.50	1.36	90.74
2.00	2.11	105.35

Table 3 shows the method recovery Verification of developed methods in determination of meat products quality Control and experimental samples were subjected to sensory evaluation.

Table 4. Mean values of sensory evaluation grades of meat products

	A	A1	B	B1	C	C1	D	D1	E	E1
Appearance of the cutting surface*	7	6.5	6	5	6	6	7.5	7.5	8,5	6.5
Filling color**	8	6.5	7.5	3.5	7.5	4	8	7	8.5	6.5
Homogeneity of cutting surface color*	8.5	8	8.5	5	8	7	8.5	8	8.5	7
Color persistance 10 min after the cutting*	9	8	8.5	6	8.5	7.5	8.5	8	9	7.5
Odour****	9	6	8	6	7.5	5	8.5	7	8	6.5
Texture**	7.5	5.5	7	5.5	7	5	8	7	8.5	6
Juiciness**	8.5	6	7.5	5.5	7.5	5.5	7.5	7	8	6
Taste before the cooking test***	9	7	7.5	6	7.5	5	8	7	9	5.5
Taste after the cooking test****	9	6.5								
Overall impression***	8.5	6.5	7.5	5	7.5	5	8	7	8.5	6

* differences are not statistically significant
** differences are statistically significant $p<0.05$
*** differences are statistically very significant $p<0.01$
**** differences are statistically highly significant $p<0.001$

Table 4 shows mean values of products grades. The results of the verification of developed methods for the determination of E 120, E 124 and E 407 are shown in Tables 5, 6 and 7.

Table 5. The results of determination of presence and quantity of E 120 in the samples

Sample	Quantity of E 120 (mg /kg)
A	-
A1	95
B	-
B1	80
C	-
C1	85
D	-
D1	95
E	-
E1	70

Table 6. The results of determination of presence and quantity of E 124 in the samples

Model meat product	Added E 124 (mg/kg)	Added E 124, (mg/kg)
After the pasteurisation	45	41.65
After the sterilisation	45	46.78

Table 7. The results of determination of presence and quantity of E 407 in the samples

Sample	Quantity of E 407 (%)
A	-
A1	0.9633
B	-
B1	0.9411
C	-
C1	0.6341
D	-
D1	1.242
E	-
E1	0.594

IV. CONCLUSION

The obtained results showed that these analytical methods can be sucessfully applied for determination of three food additives in meat products – cooked sausages, coarsely and finely chopped cooked sausages and chopped meat cans. The processes of pasteurisation and sterilisation showed no significant influence on the content of additives and their concentrations in model meat products.

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