Electrophoretic Method For Edible Eggs Species Identification

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The present paper describes a simple and efficient electrophoretic method to identify the species provenience of edible eggs in food products. The proteic pattern of egg yolk and egg white was described using polyacryl amide gel electrophoresis under denaturating conditions (PAGE-SDS) separately for the egg yolk and for the egg white proteins from five edible egg species, as follows: hen, goose, duck, turkey and quail. The molecular weight of each protein strip was calculated using a molecular weight standard curve. Separately, an electrophoretic protein pattern of all the mentioned samples was done using polyacryl amide gel electrophoresis under undenaturating conditions (native PAGE). The results show clearly distinct patterns in electrophoregrams resulted both in denaturating and undenaturating conditions for each species. These methods could be useful tools for egg species routine identification in various food industrial mixtures.

Keywords: Egg species detection, egg yolk proteins, egg white proteins, polyacryl amide gel electrophoresis

One of the highest quality and inexpensive source of proteins in foodstuff is represented by egg proteins. Due to their functional properties such as gel and foam formation, egg white proteins are extensively used as ingredients in processed foods [1-4]. Common industrial food products contain either egg extensively heated (pastry products containing egg such as cakes, waffles, muffins, pancakes, egg noodles, egg pasta) or lesser extensively heated egg (fresh mayonnaise).

Attempts to optimise the quantification method for egg white proteins in industrial food mixtures are still made [5, 6]. Not the same are the facts concerning the identification of egg provenience.

Electrophoresis was selected due to its specificity and simplicity for proteins separation. In clinic, serum protein electrophoresis is recommended as a diagnostic technique for increasing the accuracy of the diagnosis in cases of immunodeficiency, liver disease, nephrotic syndrome and acute, subacute and chronic inflammatory diseases [7].

Allergic reactions to poultry's egg proteins represent one of the most frequent primary food allergies affecting around 1.6% of children below the age of three, but are in the majority of cases outgrown before school age [8-10]. Major allergens originate primarily from egg-white, and include ovomucoid and ovalbumin, which constitute 10% and 50% of egg white proteins respectively [11]. As we know, the duck and goose eggs are bigger and heavier than the hen ones. On the other hand, those eggs contains proteins with higher allergic potential than hen's ones. This fact, lead us to the necessity to precisely determine the possible frauds concerning the egg species in the food.

Protein structure is genetically determined and phylogenetically more conservative than most characters used in taxonomy. The electrophoretic profiles of egg proteins can provide the phylogenetic relationships, being a useful tool for species determination.

The hen egg white protein composition has not yet been fully defined till recent years. Seven of the major known hen egg white proteins were identified using electrophoretic, chromatographic or combined techniques [12-15]. Also, a simplex polymerase chain reaction (PCR) has been applied for the specific detection of hen, duck, turkey, and guinea fowl in egg products using species-specific primers targeting the mitochondrial cytochrome b genes [16]. Nevertheless the last proposed method is a potentially reliable technique but not so applicable in routine food analysis for the research of fraudulent species mixture practices due to their expensive reagents and rather sophisticated technique and equipment.

The egg yolk proteins, which are studied in lesser extent, contain also important proteins, almost all the antibody in egg, such as immunoglobulin Y [17].

All the quoted works emphasise on the theoretical studies of egg proteins but there is no one upon our knowledge proposing simple and easy applications for fraud detection concerning the egg species in industrial food mixtures.

The present work, point out a possible simple and reproducible method to identify the species of the egg proteins in industrial food, both for egg yolk and egg white ones.

Experimental part

Chemicals and Materials

Acrylamide, bis-acrylamide, ammonium persulfate, Tris base, Tris-glycine buffer *p*H = 6.8, *N,N,N2 ,N2* -tetra-methyl-ethylenediamine (TEMED), Coomassie brilliant blue R250, glycerol, sodium dodecyl sulphate (SDS), 2-mercapto-ethanol, were purchased from Sigma. Molecular weight markers were Sigma Marker wide range, mol weight 6,500-200,000 Da.

Equipment

The spectral measurements were made on UV/V-650 Jasco spectrophotometer (Tokyo, Japan) using standard 1.00 cm quartz cells.

The concentration gradient in separating gel was done using a Bio-Rad gradient casting device.

The electrophoresis was run in a Bio-Rad electrophoresis device with a PROTEAN® II xi cell.

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Sample preparation

The samples consisted in hen, quail, turkey, duck and goose fresh eggs purchased from Romanian market. For each species it was taken five eggs to make a common sample.

Egg yolk samples from each species (from five eggs each) were separated and mixed, then stirred whit demineralised water at a ratio 2 g sample: 20 mL water to make an emulsion. The solution was kept for 10 min at -19°C, for lipid separation. The watery phase was separate after centrifugation at 6000 rpm for 5 min. The obtained solutions were determined concerning the protein content using Lowry method [18], and eventually diluted at a protein concentration of about 1.33 mg/mL and further processed according to the PAGE-SDS and undenatured PAGE protocols. The processing method for egg yolk protein extraction was adapted from Dong Uk & col [17].

Egg white samples from each species (from five eggs each) were separated and mixed, then stirred with demineralised water at the ratio 1 g sample/ 10 mL demineralised water for 15 minutes. After that, the content in soluble protein in each sample was determined using Lowry method [18] and eventually diluted at a protein concentration of about 1.33 mg/mL. The diluted samples were submitted to the further processing according to the PAGE-SDS and undenatured PAGE protocols.

SDS PAGE

SDS gel electrophoresis was performed according to Laemmli [19], using 4% acrylamide in stacking gels and separating gels consisting in a linear concentration gradient gel (5-15% acrylamide). The SDS-containing sample buffer (2% SDS, 20% glycerol, 5% 2-mercaptoethanol, and 0.5% bromophenol blue in 62 mM Tris-HCl buffer, *p*H 6.8) was used for protein denaturation. The diluted samples were maintained for three min at 100°C. The migration buffer used was 25 mM Tris, 192 mM glycine, and 0.1% SDS. Electrophoresis was carried out at 75 V in the stacking gel and at 150 V in the separating gel for 1 h 30 min. After migration the gel was stained with 0.05% Coomassie Blue R250, 49.95% water, 40% ethanol and 10% acetic acid for 1 hour and subsequently distained with 50% water, 40% ethanol and 10% acetic acid.

Native PAGE

Native polyacrylamide gel electrophoresis was performed according to Walker [20], in 0.5 M Tris-HCl buffer, *p*H 6.8, for stacking gel (4% acrylamide) and 1.5 M Tris-HCl buffer, *p*H 8.8, for separating gel (7.5% acrylamide). Samples were diluted in 62 mM Tris-HCl buffer, *p*H 8.8, 10% glycerol, and 0.5% bromophenol blue. Migration was performed in the same conditions as SDS PAGE in 24 mM Tris glycine buffer, *p*H 8.8.

Image Analysis

Molecular weights were calibrated by migrating Sigma markers on identical gels. The calibration curve obtained by expressing the R_f of the markers versus log M_w (R^2 = 0.9812) was used to calculate the molecular weight of the isolated protein fractions in egg yolk and egg white electrophoregrams.

Results and discussions

Table 1 and figure 1 show the results obtained in PAGE-SDS of egg white proteins from the five species. One can see that there are significant differences between the protein mass in the egg white for each species, even between those which are quite close genetically related (e. g. hen and quail or duck and goose).

Qualitative differences were observed among the individual proteins of the different species investigated. The proteic pattern show specific characteristics (e.g. different proteic strip numbers and different molecular weight) which could be easily detected. In the case of some egg white major proteins such as ovalbumin (50 kDa), ovomucoid (36 kDa), they could be identified according to their specific molecular weight. Our results are in agreement with the ones reported by Miguel & col [15]. On the other hand, different staining techniques (such as silver staining) could modify the results, as we can see in the Desert & col. work [12].

In conclusion, PAGE-SDS followed by Coomassie Blue staining of proteins manages to separate the egg white proteins, and qualitative and quantitative differences were observed between studied species.

The same fact can be seen in the electrophoregrams of the egg yolk proteins of the five studied species (table 2 and fig. 2). Also in this case, the proteic patterns have clearly distinct features for each species.

SDS-PAGE is a common way for analysing proteins. It should be stressed that this method separates denatured protein. Moreover, many of the egg white proteins are highly

Table 1

SDS–PAGE ANALYSIS OF EGG WHITE PROTEIN FRACTIONS FROM THE FIVE STUDIED SPECIES ON POLYACRYLAMIDE GEL LINEAR GRADIENT 5-15% STAINED WITH COOMASSIE BLUE R250

Hen egg white		Quail egg white		Turkey egg white		Duck egg white		Goose egg white	
R_f	Molecular weight (kD)	R_f	Molecular weight (kD)	R_f	Molecular weight (kD)	R_f	Molecular weight (kD)	R_f	Molecular weight (kD)
0.19	296.16	0.21	274.93	0.19	296.16	0.19	296.16	0.17	319.03
0.34	169.53	0.33	175.96	0.22	264.89	0.25	236.93	0.25	236.93
0.6	64.46	0.45	112.61	0.33	175.96	0.33	175.96	0.32	182.62
0.76	35.55	0.63	57.66	0.62	59.84	0.37	151.63	0.35	163.34
0.79	31.80	0.74	38.30	0.76	35.55	0.45	112.61	0.61	62.11
		0.79	31.80	0.81	29.52	0.62	59.84	0.77	34.25
		0.86	24.51	0.87	23.61	0.79	31.80	0.85	25.44
		0.92	19.61			0.93	18.89	0.87	23.61

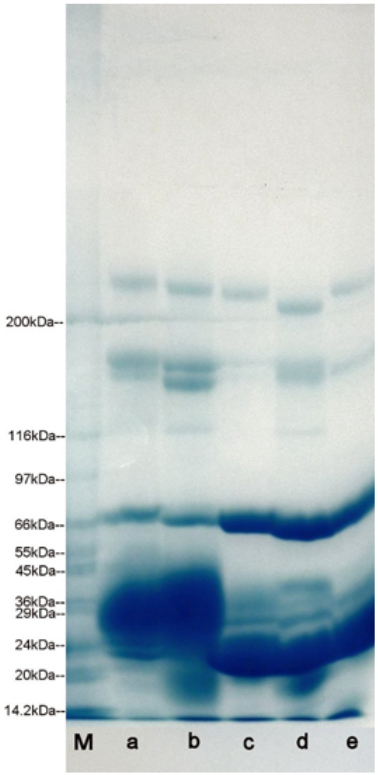

Fig. 1. PAGE-SDS of egg white proteins in the five studied species: M (weight markers), (a) hen; b) quail; c) turkey; d) duck; e) goose

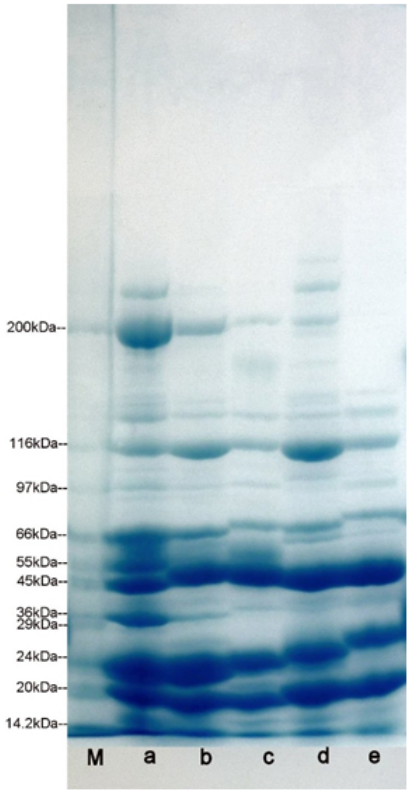

Fig. 2. PAGE-SDS of egg yolk proteins in the five studied species: M (weight markers), (a) hen; b) quail; c) turkey; d) duck; e) goose

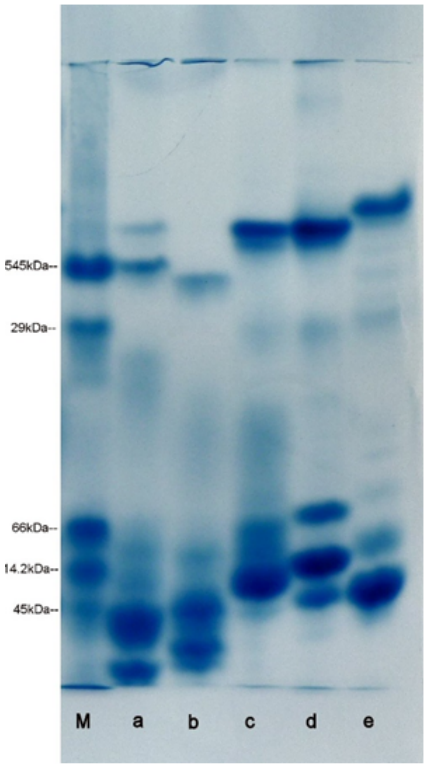

Fig. 3. Undenaturating PAGE of egg white proteins in the five studied species: M (weight markers), (a) hen; b) quail; c) turkey; d) duck; e) goose

Hen egg yolk		Quail egg yolk		Turkey egg yolk		Duck egg yolk		Goose egg yolk	
R_f	Molecular weight (kD)	R_f	Molecular weight (kD)	R_f	Molecular weight (kD)	R_f	Molecular weight (kD)	R_f	Molecular weight (kD)
0.17	319.03	0.25	236.93	0.23	255.22	0.12	384.22	0.37	151.63
0.25	236.93	0.37	151.63	0.31	189.54	0.16	331.11	0.4	135.63
0.34	169.53	0.41	130.67	0.4	135.63	0.22	264.89	0.45	112.61
0.37	151.63	0.46	108.50	0.46	108.50	0.3	196.72	0.53	83.63
0.41	130.67	0.54	80.58	0.53	83.63	0.37	151.63	0.59	66.90
0.46	108.50	0.62	59.84	0.61	62.11	0.4	135.63	0.62	59.84
0.5	93.50	0.7	44.44	0.66	51.57	0.46	108.50	0.7	44.44
0.54	80.58	0.78	33.00	0.7	44.44	0.51	90.09	0.74	38.30
0.56	74.80	0.82	28.44	0.76	35.55	0.61	62.11	0.81	29.52
0.62	59.84	0.87	23.61	0.85	25.44	0.63	57.66	0.9	21.12
0.68	47.87	0.92	19.61	0.9	21.12	0.74	38.30		
0.71	42.82			0.93	18.89	0.75	36.90		
0.78	33.00			0.96	16.90	0.83	27.40		
0.87	23.61					0.91	20.35		
0.91	20.35					0.95	17.54		
0.96	16.90								

Table 2
SDS–PAGE ANALYSIS OF EGG YOLK PROTEIN FRACTIONS FROM THE FIVE STUDIED SPECIES ON POLYACRYLAMIDE GEL LINEAR GRADIENT 5-15% STAINED WITH COOMASSIE BLUE R250

glycosylated, which constitutes a major limit for their detection and visualization by this technique.

Sometimes one needs to analyze native, nondenatured proteins. On such occasions it is necessary to use a nondenaturing system (native PAGE). The highlighting of protein complexes, naturally present in the egg white and in the egg yolk could be made possible by applying this technique.

The results obtained by native PAGE of the egg white proteins of the five studied species are shown in table 3 and figure 3. One can see on the figure net differences

Table 3

NATIVE PAGE ANALYSIS OF EGG YOLK PROTEIN FRACTIONS FROM THE FIVE STUDIED SPECIES ON 7.5% POLYACRYLAMIDE GEL STAINED WITH COOMASSIE BLUE R250

Hen egg yolk proteins R_f	Quail egg yolk proteins R_f	Turkey egg yolk proteins R_f	Duck egg yolk proteins R_f	Goose egg yolk proteins R_f
0.17	0.25	0.23	0.12	0.37
0.25	0.37	0.31	0.16	0.4
0.34	0.41	0.4	0.22	0.45
0.37	0.46	0.46	0.3	0.53
0.41	0.54	0.53	0.37	0.59
0.46	0.62	0.61	0.4	0.62
0.5	0.7	0.66	0.46	0.7
0.54	0.78	0.7	0.51	0.74
0.56	0.82	0.76	0.61	0.81
0.62	0.87	0.85	0.63	0.9
0.68	0.92	0.9	0.74	
0.71		0.93	0.75	
0.78		0.96	0.83	
0.87			0.91	
0.91			0.95	
0.96				

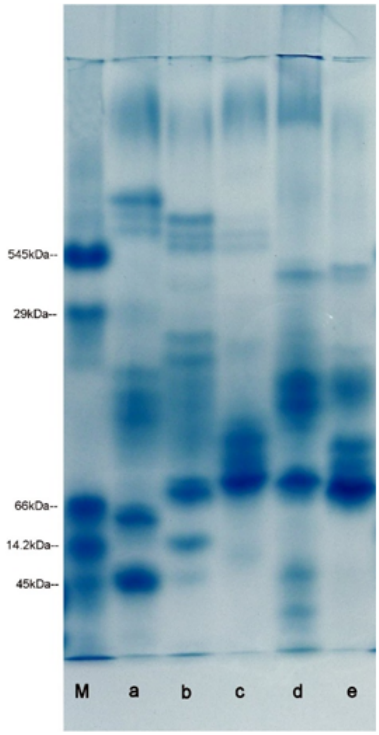

Fig. 4. Undenaturating PAGE of egg yolk proteins in the five studied species: M (weight markers), (a) hen; b) quail; c) turkey; d) duck; e) goose

Hen egg white proteins R_f	Quail egg white proteins R_f	Turkey egg white proteins R_f	Duck egg white proteins R_f	Goose egg white proteins R_f
0.19	0.21	0.19	0.19	0.17
0.34	0.33	0.22	0.25	0.25
0.6	0.45	0.33	0.33	0.32
0.76	0.63	0.62	0.37	0.35
0.79	0.74	0.76	0.45	0.61
	0.79	0.81	0.62	0.77
	0.86	0.87	0.79	0.85
	0.92		0.93	0.87

Table 4

NATIVE PAGE ANALYSIS OF EGG WHITE PROTEIN FRACTIONS FROM THE FIVE STUDIED SPECIES ON 7.5% POLYACRYLAMIDE GEL STAINED WITH COOMASSIE BLUE R250

between the proteic patterns belonging to each of the studied species.

The same observations could be noted in the native PAGE of egg yolk proteins (table 4 and fig. 4). It should be stress that this technique is more simple and inexpensive than the PAGE-SDS. Those recommend it as a very good method to identify the egg species in raw egg mixtures, taking into account that the technique uses raw proteic material, without thermal treatment.

Given to the PCR technique proposed by Nau & col. [16], the two proposed electrophoretic techniques are much more simple and inexpensive.

Conclusions

The paper describes two electrophoretic methods for edible egg species determination in food products.

PAGE-SDS reveals egg white major proteins as well as other ones less known and characterized. The method could be adapted for egg species determination in processed food mixtures.

Native PAGE is also a useful technique to identify the egg species in thermal unprocessed food mixtures, being more simple and efficient.

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Manuscript received: 23.09.2016