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Comparison of extraction conditions for milk and hen's egg allergens

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1 Comparison of extraction conditions for milk and hen's egg 2 allergens

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15 University of Hamburg, Institute of Food Chemistry, Grindelallee 117, 20146 Germany;
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17

18 19 **Abstract**

20
21 The evaluation of recovery rates by extracting dried milk and egg powder using eleven
22 different extractants gave approximately similar results for both foods. Compared to the other
23 extraction solutions investigated, "1% Tween 20 and 0.4% Triton X-100" and "4 % SDS" are
24 the most suitable extractants to isolate proteins of hen's egg or milk. Comparing calculated
25 protein recovery rates of egg and milk powder extracts the results clearly indicated that the
26 choice of a suitable extractant is of particular importance. Qualitative investigation of the
27 extracts via LDS - PAGE followed by silver staining as well as immunoblotting confirmed the
28 results of protein quantification. Hence, the immunoblots showed that the extraction agents
29 had no negative influence on the antigenicity of the extracted allergenic proteins. In this
30 study, variation of extraction temperature neither led to any benefit in extraction quality nor to
31 degradation. Changing pH did not reveal any trends, but progressive protein hydrolysis under
32 strong alkaline conditions. Evaluation of recovery rates as well as results of unspecific and
33 specific staining of the extracts showed that an extraction time of one hour is sufficient for an
34 appropriate sample preparation. For investigations with and without food matrix different
35 results were obtained. In summary, wheat starch did not influence the extraction quality
36 within all examined materials and different extractants. In contrast, using fat powder and dry
37 cake mix, respectively, led to different results in the extraction procedure. When fat powder
38 and dry cake mix were used as food matrices, some protein recovery rates decreased and
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3 27 some increased depending on the allergen material. These results highlight the fact that the
4
5 28 suitability of the extractant not only depends on the properties of the allergen but furthermore
6
7 29 on the type of matrix containing the allergen.
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12 32 **Keywords:** Food allergy, hidden allergens, extraction of allergens, hen's egg, cow's milk
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21 35 **Introduction**

22 36
23
24 37 Cow's milk and hen's egg as well as their products belong to eight food or food groups which
25
26 38 are responsible for about 90 % of allergic reactions (Hefle and Taylor, 2004). The
27
28 39 consumption of small amounts can already cause life-threatening or lethal reactions (Vieluf et
29
30 40 al., 2002). Nevertheless, allergic persons can unintentionally consume allergenic material if
31
32 41 these are unexpectedly part of a food product e.g. because of food contamination / cross
33
34 42 contact within the manufacturing process (Hefle and Taylor, 2004). The threshold doses for
35
36 43 triggering symptoms after ingestion of the offending food are strongly dependent on the
37
38 44 patient's individual susceptibility and the allergic potential of the particular food. Clinical data
39
40 45 shows that exposure to even about 1 mg of the allergen can elicit adverse reactions up to
41
42 46 anaphylactic shocks in individuals with IgE-mediated food allergies (Besler et al., 2001;
43
44 47 Moneret-Vautrin and Kanny, 2004). The legislation on food labelling only concerns allergenic
45
46 48 ingredients that are knowingly and deliberately added to food products.
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52 50 The analytical proof of the presence of small amounts of allergens, must use a technique
53
54 51 sensitive enough to specifically detect the allergens in those amounts that might trigger
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56 52 allergic reactions in sensitized individuals. The determining step in allergen detection is the
57
58 53 isolation of the allergens from the food which can be influenced by numerous factors. The
59
60 54 diversity of foods in terms of composition and texture as well may require appropriate

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3 55 extraction methods. The extraction solution must provide efficient isolation of the allergenic
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5 56 proteins from the sample into a liquid phase and minimize background effects due to non-
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7 57 specific binding. In food many ingredients are available to participate in complex physical and
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10 58 chemical reactions with the allergenic proteins (Davis et al., 2001). Especially during the
11
12 59 manufacturing process the matrix might cause covalent modification of proteins through food
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14 60 processing. As well as antigenicity, the extractability of allergens can be decreased in these
15
16 61 reactions, they might be unchanged or even increased (Besler et al., 2001).
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20 63 The aim of this study was to evaluate and compare extraction solutions for the isolation of
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22 64 milk and egg allergens. For this purpose extractability of allergenic proteins of dried milk
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24 65 powder, sweet whey, acid whey, whole egg, egg yolk and egg white with eleven different
25
26 66 extraction agents was examined. Extractants were selected by collecting recipes already
27
28 67 described in literature to isolate allergens. In addition, chosen extraction solutions represent
29
30 68 a huge diversity in pH, ionic strength, and detergent content. As extraction quality describing
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32 69 parameters protein yield, the composition of the proteins as well as the antigen patterns were
33
34 70 determined. To evaluate, if food matrix influences the extractability of proteins, wheat starch,
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36 71 fat powder and an experimental dry cake mix were employed in the extraction experiments.
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41 42 73 **Materials and Methods**

43 44 74 *Reagents and materials*

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46 75 Milk powder, dried sweet whey, dried acid whey, whole egg powder, powdered egg yolk and
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48 76 powdered egg white were obtained from Kampffmeyer Food Innovation GmbH (Hamburg,
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50 77 Germany). Powdered egg white was purchased from Sigma-Aldrich Chemie GmbH (Munich,
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52 78 Germany). To obtain powdered egg yolk, liquid whole egg was separated mechanically and
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54 79 lyophilized referring to homogenization. Commercially available wheat starch (protein-free,
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56 80 Heinrich Klenk GmbH & Co. KG, Schwebheim, Germany), fat powder (protein-free coconut
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58 81 oil, Kampffmeyer Food Innovation GmbH, Hamburg, Germany) and an experimental dry cake
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60 82 mix were used as matrix. The dry cake mix contained sucrose (70.5 %, Kampffmeyer Food

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3 83 Innovation GmbH, Hamburg, Germany), fat powder (17.7 %), wheat starch (9.8 %), sodium
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5 84 hydrogen carbonate (1.6 %, Merck Chemicals, Darmstadt, Germany) and sodium chloride
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7 85 (0.4 %, Merck Chemicals, Darmstadt, Germany).
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12 87 Sheep anti-casein serum and biotinylated rabbit anti-sheep serum were purchased from
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14 88 antibodies-online GmbH (Aachen, Germany). Rabbit anti-whey and rabbit anti-ovalbumin
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16 89 sera were both acquired from Sigma-Aldrich Chemie GmbH (Munich, Germany). Biotinylated
17
18 90 goat anti-rabbit antibodies were bought from Rockland (Gilbertsville, PA, USA) and
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20 91 streptavidin-horseradish peroxidase was obtained from Bio-Rad Laboratories GmbH
21
22 92 (Munich, Germany). All other reagents were of analytical grade and purchased from Sigma-
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24 93 Aldrich Chemie GmbH (Munich, Germany), Fluka (Buchs, Switzerland) and Serva
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26 94 Electrophoresis GmbH (Heidelberg, Germany).
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31 96 *Composition of extractants*

32
33 97 In this investigation, eleven different extractants were used to extract protein from dairy and
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35 98 egg products: A: 0.01 M phosphate-buffered saline containing 10 mM Na₂HPO₄, 1 mM
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37 99 KH₂PO₄, 3 mM KCl and 140 mM NaCl, pH 7.4 (Schubert, 2003); B: 0.01 M phosphate-
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39 100 buffered saline containing 2 mM NaH₂PO₄, 8 mM Na₂HPO₄ and 150 mM NaCl, pH 7.4 (Hefle
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41 101 and Lambrecht, 2004); C: 0.01 M phosphate-buffered saline containing 150 mM NaCl, 10
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43 102 mM Na₂HPO₄, pH 7.2 (Leduc et al., 1999); D: physiological saline containing 150 mM NaCl,
44
45 103 pH 6.6 (Langeland, 1982); E: 1 % Tween 20[®], pH 6.0 (Fernandez et al., 1999); F : 8 M urea,
46
47 104 pH 7.7 (Natarajan et al., 2005); G: 100 mM acetate buffer, pH 3.8 (Hirose et al., 2004); H: 86
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49 105 mM NaCl, 30 mM NaHCO₃, 0.4 % phenol, pH 8.2 (Coca, 1922); I: 1 % Tween 20[®], 0.4 %
50
51 106 Triton X-100[®], 280 mM NaCl, 40 mM NaH₂PO₄, pH 7.4 (Yeung et al., 2000); J: 4 % Sodium
52
53 107 dodecyl sulphate, pH 8.5 (Kato et al., 2001a); K bidistilled water. All extraction solutions
54
55 108 were prepared using bidistilled water and were stabilized with 0.02 % NaN₃. For
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57 109 investigations with the aim to optimize pH-value of selected extractants, J (pH 8.5) and I (pH
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3 110 7.4) were additionally prepared with pH-values adjusted to 10.5 and 12.5 and 9.4 and 11.4,
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5 111 respectively. Adjustment of pH was carried out with 0.1 M NaOH.
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10 113 *Protein isolation*

11 114 Principally, extraction procedure was carried out as follows. According to the protein content
12
13 115 measured via Kjeldahl method, 30 mg milk powder, 100 mg sweet whey, 100 mg acid whey,
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15 116 25 mg whole egg powder, 15 mg dried egg white and 30 mg dried egg yolk, respectively,
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17 117 were weighed into a 50 mL polypropylene centrifuge tube (Nunc GmbH & Co. KG,
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19 118 Wiesbaden, Germany). In the case of matrix addition, 1 g matrix (wheat starch, fat powder,
20
21 119 dry cake mix) was also weight in the centrifuge tube and the dry material was mixed 5 sec
22
23 120 with a vortex mixer (VWR international, Darmstadt, Germany; mixing frequency 2,500 rpm)
24
25 121 before extractant was added. Protein extraction was carried out by suspending sample
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27 122 material with 20 mL of extraction solution. The homogenate was extracted over night on a
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29 123 laboratory shaker (Bühler, Tübingen, Germany; shaking frequency 150 rpm) at room
30
31 124 temperature and centrifuged afterwards 30 min at 10,000 rpm (Sigma-Aldrich Chemie
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33 125 GmbH, Munich, Germany). Solids were removed by filtration (Macherey-Nagel GmbH & Co.
34
35 126 KG, Düren, Germany; filter paper grade MN 616). Investigations with the aim to optimize
36
37 127 extraction temperature were additionally carried out at 4 °C on a laboratory shaker in a
38
39 128 standard refrigerator (Linde, München, Germany) and at 40 °C using a laboratory incubator
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41 129 (IRC-1-U Clim-o-shake, Adolf Kühner AG, Birsfelden, Swiss; shaking frequency 150 rpm).
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43 130 For examination of extraction duration, protein isolation as described above was interrupted
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45 131 after 0.5, 1, 2, 4 and 8 hours. Each extraction process was performed in triplicate.
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53 133 *Protein quantification*

54 134 Absolute protein content of used dry materials was determined via Kjeldahl method analysing
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56 135 the nitrogen content. The conversion factors from nitrogen to protein were as recommended
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58 136 6.38 (dairy products) and 6.25 (egg products), respectively. The protein concentration of the
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60 137 extracts was measured photometrically according to the method of Lowry (1951) using

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3 138 bovine serum albumin (BSA) and ovalbumin respectively as standards. Photometrical
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5 139 measurement of protein content was favoured over Kjeldahl method because in comparison
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7 140 the latter is of high expenditure of time and technical effort. The Folin's reagent used in the
8
9 141 Lowry method consists amongst others of phosphomolybdic / phosphotungstic acid. A
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11 142 standard curve was produced for each run using a 500 mg / L protein solution prepared
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13 143 using the appropriate extractant. The protein content of the extracts prepared with "Coca's
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15 144 solution" (containing phenol) was measured by the method of Bradford (1976) because
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17 145 phenol interferes with the Lowry method. Bradford reagent consists of Coomassie Brilliant
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19 146 Blue G-250 and phosphoric acid. Quantitative assessments of extracts and standards were
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21 147 performed by measuring optical density at 720 nm (Lowry method) and 590 nm (Bradford
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23 148 method) using a spectrometer (MRX Plate Reader, Dynex Technologies, Frankfurt,
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25 149 Germany).

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151 *Lithium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (LDS - PAGE)*

152 Proteins were separated with precast NuPAGE® Bis-Tris 12 % gels (Invitrogen GmbH,
153 Karlsruhe, Germany) under denaturing conditions at 200 V for 55 min using NuPAGE®
154 MOPS [3-(N-morpholino) propanesulfonic acid] SDS running buffer (Invitrogen GmbH,
155 Karlsruhe, Germany). Following the manufacturers instructions obtained extracts were
156 diluted 1:1 in LDS (lithium dodecyl sulphate) sample buffer (Invitrogen GmbH, Karlsruhe,
157 Germany) containing dithiothreitol for reduction at room temperature. Sample load was 20 µL
158 per lane. Proteins were further silver stained or transferred by blotting method onto a
159 nitrocellulose membrane.

160

161 *Silver staining*

162 The proteins were detected by standard silver staining procedure according to (Heukeshoven
163 and Dernick, 1986) following LDS - PAGE. After fixing the gel (30 % ethanol, 10 % acetic
164 acid in water; 30 min) it was sensitized in 0.1 % sodium thiosulfate (0.1 % Na₂S₂O₃, 30 %
165 ethanol, 6.8 % sodium acetate in water). After washing with water the gel was incubated for
166 20 min in 0.2% silver nitrate solution (0.2 % AgNO₃, 0.02% formaldehyde in water).

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3 167 Afterwards the gel was developed in 2.5 % sodium carbonate (2.5 % Na₂CO₃, 0.01%
4 168 formaldehyde in water). Staining was terminated using 1.8 % EDTA-solution (1.8 %
5 169 ethylenediaminetetraacetate in water).
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10 171 *Immunoblotting*

12 172 The isolated proteins separated by LDS - PAGE were transferred electrophoretically from the
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14 173 gel onto the surface of a nitrocellulose membrane (2 µm, Whatman, Kent, UK) using semidry
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16 174 blotting method. In brief, dried membranes were blocked with blocking solution (containing
17
18 175 0.5 % Tween 20[®], 0.9 % sodium chloride and 0.6 % tris base) and incubated overnight with
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20 176 sheep anti-casein (1 : 400), rabbit anti-whey (1 : 200000) and rabbit anti-ovalbumin
21
22 177 (1 : 200000) antibodies, respectively, at room temperature. Membranes were then incubated
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24 178 consecutively with biotinylated rabbit anti-sheep serum (1 : 100000; milk powder) or
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26 179 biotinylated goat anti-rabbit serum (1 : 7500; powdered sweet whey, powdered acid whey,
27
28 180 whole egg powder, powdered egg yolk, powdered egg white) and streptavidin - horseradish
29
30 181 peroxidase. Staining solution containing TMB and DONS (0.06 % 3, 3', 5, 5' -
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32 182 Tetramethylbenzidine, 0.2 % dioctylsodiumsulphosuccinate, 25 % ethanol, 0.7 % citric acid,
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34 183 1 % Na₂HPO₄ in water) was used for staining.
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38 184

40 185 **Results**

42 186 *Protein quantification*

44 187 Protein recovery rates in the extracts were estimated by relating theoretical calculated
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46 188 protein content measured via the Kjeldahl method to total protein yield of the extract
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48 189 measured with Lowry or Bradford method (data not shown). The estimated recovery rates
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50 190 are presented in Table 1 and Table 2. Extracting milk proteins from milk powder, recovery
51
52 191 rates range from 69 to 109 % except for solution G yielding 16 % protein. Highest recoveries
53
54 192 in protein isolation were achieved using solution I and J as extractants. Estimated recovery
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56 193 rates for extraction of milk proteins from milk powder in dry cake mix ranged from 40 to 87 %
57
58 194 except for solution G recovering 14 % protein. Within these extracts, extractants B, D and J
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60 195 gave the highest protein recoveries. Extracting proteins from milk powder in wheat starch

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3 196 and fat powder, the highest protein contents were obtained in the extracts prepared using
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5 197 solution I, J and C or I and D, respectively (Table 1).
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10 199 For all the egg materials which were examined, the extraction agents I and J aqueous solution
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12 200 gave the highest protein yields (Table 2). In contrast to the extractants mentioned above, the
13
14 201 protein contents of the extracts prepared using saline solutions (A, B, C, D), G or H to extract
15
16 202 egg proteins from egg material led to recovery rates lower than 30 %. Where egg proteins
17
18 203 were isolated from egg powder in dry cake mix, solutions I and J gave the highest protein
19
20 204 recovery rates. Extracting proteins from egg material in wheat starch and fat powder as food
21
22 205 matrix, the highest protein contents were measured in the extracts prepared using solutions I
23
24 206 and J. But also E achieved high recovery of protein from egg material in wheat starch (Table
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26 207 2).
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31 209 In this study, optimization of pH-value of extractants as well as extraction temperature was
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33 210 carried out. Therefore, solution I (pH 7.4) and J (pH 8.5) were additionally prepared having a
34
35 211 pH of 9.4 and 11.4 (I) as well as 10.5 and 12.5 (J), respectively. Extraction procedures were
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37 212 simultaneously performed at three different extraction temperatures (4 °C, RT = room
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39 213 temperature and 40 °C). The estimated recovery rates are presented in Tables 3 and 4.
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41 214 Comparing the protein yields there is not much of a difference. The deviation of the obtained
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43 215 recovery rates is of the order of the calculated standard deviation.
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49 217 With the aim to investigate optimum extraction duration, extraction procedures were
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51 218 performed at different extraction temperatures using solution I (pH 7.4) and J (pH 8.5) as
52
53 219 extractants to isolate milk and egg proteins from milk powder and whole egg powder and
54
55 220 from wheat starch and cake mix, respectively. Extraction procedures were stopped after
56
57 221 30 min, 1 h, 2 h, 4 h and 8 h and protein contents were measured subsequently. Resulting
58
59 222 recovery rates are shown in Tables 5 and 6. Regardless of extractant and extraction
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3 223 temperature there is not much of a difference comparing protein recoveries. The extracts
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5 224 prepared of solution J have slightly higher protein contents than those made of by solution I.

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10 226 *Lithium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (LDS - PAGE)*

11 227 The composition of the isolated protein extracts were analyzed by electrophoretic separation
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13
14 228 and silver staining. Comparison of (i) the number of protein bands and (ii) the intensities of
15
16 229 characteristic protein bands illustrates considerable protein diversity between the examined
17
18 230 extracts. LDS - PAGE and silver staining patterns of proteins extracted from egg powder and
19
20 231 milk powder using different extractants are shown in Figure 1. Those extracts prepared from
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22 232 egg powder using solution I, J and E gave particularly strong stained bands of conalbumine,
23
24 233 ovalbumine and ovomucoid. Extracts prepared using solution G or H gave weakly stained
25
26 234 bands of characteristic egg proteins. Similar results were obtained extracting powdered dairy
27
28 235 products. The extractants I, J and E gave strongly stained bands of characteristic milk
29
30 236 proteins α -lactalbumin, β -lactoglobulin and casein, whilst solution G gave weakly detected
31
32 237 bands representing characteristic milk proteins. Analysis of protein patterns of extracts
33
34 238 obtained extracting allergen material out of food matrix did not reveal wide differences in
35
36 239 band intensity of characteristic milk and egg allergens.

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41
42 241 The influence of the pH-value of the extractants was investigated.. Solution I was prepared
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44 242 having pH-values of 7.4, 9.4 and 11.4, respectively. The pH-value of solution J was adjusted
45
46 243 to 8.5, 10.5 and 12.5, respectively. Detection of the electrophoretically separated extracts via
47
48 244 silver staining demonstrated that extracted proteins out of egg products and milk products led
49
50 245 to similar results. Figure 2 shows the LDS - PAGE and silver staining patterns of proteins,
51
52 246 adjusted to different pH-values and used for extraction at 4 °C, room temperature and 40 °C.
53
54 247 Comparing the band intensities of the characteristic proteins in the extracts prepared with
55
56 248 solution I pH 7.4 and pH 9.4 no differences appeared. Likewise, the band intensities of the
57
58 249 characteristic proteins in the extracts obtained using solution J pH 8.5 were as strong as
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60 250 those prepared at pH 10.5. Any characteristic proteins could be visualized in the extracts

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3 251 made of solution J with a pH of 12.5. Conversely, any characteristic proteins detected after
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5 252 electrophoretic separation of the extracts made from solution I with a pH of 11.4 or the
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7 253 detection of the characteristic proteins was much weaker.
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10 254
11 255 The band intensities obtained via silver staining of milk and egg proteins isolated at different
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13 256 temperatures were almost identical. Extracts obtained at the end of varied extraction
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15 257 durations using solution I (pH 7.4) and J (pH 8.5) were electrophoretically separated and
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17 258 proteins were detected via silver staining. Regardless of extractant and extracted material,
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19 259 band intensities of characteristic proteins did not differ in terms of extraction duration (data
20
21 260 not shown).
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27 262 *Immunoblotting*
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29 263 Whether the estimated extractants ensure comprehensive isolation of intact allergens
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31 264 specific immunochemical staining on nitrocellulose membrane after LDS - PAGE and
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33 265 Western Blotting was performed (Figures 3 and 4). The extracts derived with solution I, J and
34
35 266 E present most strongly stained bands of ovalbumine compared to solutions G and H in the
36
37 267 immunoblots. The results of unspecific staining procedure as well as the determination of the
38
39 268 protein concentration are reflected in the results of immunochemical staining. Rabbit anti-
40
41 269 ovalbumin antibody used for detection of albumin also detects conalbumin (76 kDa) as
42
43 270 characteristic egg protein. Quite similar results were obtained by immunochemical detection
44
45 271 of milk proteins. Band intensities of milk proteins of purchased extracts looked rather similar
46
47 272 to the extracts described in this paper and Immunoblots showed only slight variations in
48
49 273 protein recovery rates. Anti-casein antibody used for detection of casein also detects bovine
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51 274 serum albumin (BSA; 76 kDa) as characteristic milk protein.
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57 276 The influence of wheat starch and dry cake mix on extraction efficiency and quality was
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59 277 investigated by comparing the extract properties prepared with matrix addition and without as
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278 described above. Addition of 8 M urea as extractant to wheat starch or dry cake mix

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3 279 containing samples caused a strong enhancement of extract viscosity. Those solutions were
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5 280 unemployable for further analysis. Band intensities referring to silver and immunochemical
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7 281 staining do not differ noteworthy comparing the extracts made with matrix addition or without
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10 282 (Figures 3 and 4). Considering dilution factors, extracts of solution I, J as well as E present
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12 283 most strongly stained bands of ovalbumine compared to solutions G and H in the
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14 284 immunoblots. Purchased extracts showed similar band intensities. The immunoblots affirm
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16 285 the slight variation in protein recovery rates obtained for extracts made of dairy products.
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20 287 Comparing the band intensities of the characteristic proteins in the extracts prepared with
21
22 288 solution I (pH 7.4 and pH 9.4) and J (pH 8.5 and 10.5), respectively, no differences
23
24 289 appeared. Any characteristic proteins could be visualized in the extracts made of solution J
25
26 290 pH 12.5 whilst the detection of the characteristic proteins extracted via solution I" pH 11.4
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28
29 291 was much weaker.
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33 293 The band intensities obtained via immunochemical staining of milk and egg proteins isolated
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35 294 at different temperatures were almost identical. Regardless of extractant and extracted
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37 295 material, the band intensity of the characteristic proteins did not differ in terms of extraction
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39 296 duration (data not shown).
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44 298 **Discussion**
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46 299 The ultimate prerequisite for sensitive analytical allergen detection is an efficient and reliable
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48 300 extraction method.
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50 301 *Protein recovery rates*

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52 302 Protein recovery rates were calculated by comparing the total protein concentration of the
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54 303 food sample with the protein yields of the extracts. The photometric detection, which was
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56 304 much more suitable for the screening of the extracts than the Kjeldahl procedure, only
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58 305 provides a relative but not an absolute quantification of the proteins. Nevertheless, the
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60 306 relative quantification was sufficient to assess the efficiency of the different extractants.

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5 308 *Ranking the best extractant*
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7 309 The evaluation of recovery rates of extracted milk and egg material using eleven different
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9 310 solutions led to similar results. The solutions I and J achieved the highest protein yields.

11 311 In the case of milk samples, the extractants had only minor impact on the extraction
12
13 312 efficiency. On the other hand, the extraction efficiency of egg material was much more
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15 313 sensitive to the solution used for the protein extraction. The major difference between milk
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17 314 and egg is the fat content. Because of the higher fat content of egg material, extractants
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19 315 containing a detergent yielded higher protein recovery rates as compared to those without.
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21 316 Emulsifying properties of egg-lecithin alone are not sufficient to extract egg powder properly.
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23 317 These results clearly indicate that the composition of the extractant is of particular
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25 318 importance for the efficiency and quality of the extract.
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31 320 *Quality of the protein extracts*

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33 321 Analysis of the extracts by LDS - PAGE followed by silver staining as well as immunoblotting
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35 322 confirmed the results of protein quantification. Solution I as well as J led to the strongest
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37 323 bands of characteristic milk and egg proteins, respectively. However, it has to be considered,
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39 324 that silver staining generally possesses a lower sensitivity than photometrical protein
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41 325 quantification. Slight variations concerning protein content in the extracts cannot be
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43 326 discriminated after electrophoretical separation.
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49 328 Detection of selected proteins by western blotting clearly demonstrated that both extraction
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51 329 solutions had no negative influence onto the antigenicity of the extracted allergenic proteins.
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53 330 Solution I and J are the most qualified extractants to isolate proteins of milk. In case of hen's
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55 331 eggs, they are the only suitable solutions for the efficient extraction of proteins. Hence, hen's
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57 332 egg is more sensitive to the extractant than milk.
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334 *Optimization of the extraction procedure: temperature and pH*

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3 335 For the elucidation of the optimal temperature range, extractions were carried out at 4 °C,
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5 336 room temperature and 40 °C. According to Howard et al. (1988) solubility of proteins can be
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7 337 increased at 40 °C compared to lower temperatures. In contrast, thermal treatment of
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10 338 allergens can influence conformational epitopes, which might lose their binding capacity to
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12 339 specific antibodies (Besler et al., 2001). In the worst case, false-negative results are
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14 340 obtained. In this study, variation of extraction temperature neither led to any benefit in
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16 341 extraction quality nor to degradation. Comparison of recovery rates obtained via extraction at
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18 342 different temperatures resulted in essentially similar results. Also unspecific staining as well
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20 343 as immunoblots did not show any differences in band intensity comparing the extracts
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22 344 prepared at different extraction temperature. The influence of pH-values of extractants was
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24 345 investigated using solution I and J prepared having different pH-values. Solubility of proteins
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26 346 strongly depends on the pH-value of the medium. Proteins have minimum solubility in water
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28 347 or salt solutions at the pH which corresponds to their isoelectric point (pI). Because milk
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30 348 proteins as well as egg proteins have pI lower than 7 (except lysozyme), solution I (pH 7.4)
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32 349 was additionally prepared having a pH of 9.4 and 11.4 and solution J (pH 8.5) was prepared
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34 350 having pH-values of 10.5 and 12.5. In this study, evaluation of the influence of the protein
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36 351 content in the extracts changing the pH did not show any tendencies. In contrast, analysis of
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38 352 unspecific staining of the electrophoretically separated extracts demonstrated, that solution I
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40 353 with a pH of 11.4 caused a partial hydrolysis of the proteins, whilst solution J with a pH of
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42 354 12.5 brought the proteins about to a total hydrolysis. Alkaline hydrolysis of proteins causes
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44 355 progressive disintegration up to small peptides so that they are not detectable neither via
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46 356 unspecific nor specific staining procedure. As a consequence, these extractants are not
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48 357 applicable for further analysis damaging allergens antigenicity, although, solution I pH 7.4
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50 358 and 9.4 as well as J pH 8.5 and 10.5 did not show this hydrolysis effect in the immunoblots.
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57 360 *Optimization of the extraction duration*

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59 361 To establish an extraction procedure in routine analyses, sample preparation should not be
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362 too time-consuming. Otherwise, extraction duration has to guarantee maximum recovery of

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3 363 proteins. Evaluation of recovery rates, results of unspecific and specific staining of the
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5 364 extracts showed that an extraction time of 1 hour is sufficient for an appropriate sample
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7 365 preparation.
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11 367 *Food matrix*

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14 368 Comparing the results of qualitative and quantitative investigations of extraction of milk and
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16 369 egg allergens out of certain dried milk and egg products with and without the presence of
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18 370 several other food matrices, different findings were obtained. In summary, wheat starch
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20 371 influences the extraction yield and the extraction quality with regard to the protein and
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22 372 antigen pattern for all materials which were tested and for different extractants. In contrast,
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24 373 using fat powder and dry cake mix, respectively, different extraction procedures led to
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26 374 different results. Whilst fat powder did not influence recovery rates of milk powder, for sweet
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28 375 whey and whole egg powder, up to twice as much protein was isolated from powdered egg
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30 376 yolk. However, isolation of egg proteins from egg yolk combined with dry cake mix resulted
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32 377 in protein recovery rates quite similar as those obtained for extracts without any matrix. On
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34 378 the other hand extraction of proteins from whey as well as powdered egg white blended with
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36 379 fat powder and dry cake mix, respectively, for some extractants lower protein recovery rates
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38 380 were found. Detection of electrophoretically separated proteins confirmed the results of
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40 381 protein quantification in giving weaker stained bands. These results highlight the fact that the
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42 382 suitability of extractant not only depends on allergen properties but furthermore on the
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44 383 surrounding food matrix.
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51 385 *What is the best procedure?*

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53 386 In this study, in the case of isolating proteins out of milk material "1% Tween 20 and 0.4%
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55 387 Triton X-100" pH 7.4 was the most suitable extraction agent. To extract egg proteins out of
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57 388 egg material the best results were obtained using 4 % SDS aqueous solution at pH 8.5. If the
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59 389 allergens are to be extracted out of complex food matrices containing sugars, fat and
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390 proteins, the extractant mentioned above are still suitable, but partly less quantitative. In the

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3 391 case of egg allergens in a starch and fat containing matrix Tween 20[®] as extractant gave
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5 392 high protein recoveries. Regardless of allergen material and food matrix, carrying out the
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7 393 extraction procedure for 1 hour at room temperature guarantees high protein yields without
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10 394 any negative influence on the allergens antigenicity.

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12 396 **Acknowledgments**

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19
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21 400

22 401 **References**

23
24
25
26 402 Besler M, Steinhart H, Paschke A. 2001. Stability of food allergens and allergenicity of
27
28 403 processed foods. J Chromatogr B Biomed Sci Appl. 756: 207-228.

29
30 404 Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram
31
32 405 quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:
33
34 406 248-54.

35
36
37 407 Coca AF. 1922. Studies in specific hypersensitiveness. V. The preparation of fluid extracts
38
39 408 and solutions for use in the diagnosis and treatment of the allergies with notes on the
40
41 409 collection of pollens. J Immunol. 7: 163-78.

42
43
44 410 Davis PJ, Smales CM, James DC. 2001. How can thermal processing modify the antigenicity
45
46 411 of proteins? Allergy. 56 Suppl 67: 56-60.

47
48 412 Fernandez SS, Padilla AP, Mucciarelli S. 1999. Protein extraction from Atriplex lampa
49
50 413 leaves: potential use as forage for animals used for human diets. Plant Foods Hum
51
52 414 Nutr. 54: 251-259.

53
54
55 415 Hefle SL, Taylor SL. 2004. Food allergy and the food industry. Curr Allergy Asthma Rep. 4:
56
57 416 55-9.

- 1
2
3 417 Hefle SL, Lambrecht DM. 2004. Validated sandwich enzyme-linked immunosorbent assay for
4
5 418 casein and its application to retail and milk-allergic complaint foods. *J Food Prot.* 67:
6
7 419 1933-1938.
8
9
10 420 Heukeshoven J, Dernick R. 1986. Neue Ergebnisse zum Mechanismus der Silberfärbung.
11
12 421 Paper presented at: Electrophoresis Forum '86; Technische Universität München.
13
14 422 Hildebrandt S, Steinhart H, Paschke A. 2008. Comparison of different extraction solutions for
15
16 423 the analysis of allergens in hen's egg. *Food Chem.* 108: 1088-1093.
17
18 424 Hirose J, Kitabatake N, Kimura A, Narita H. 2004. Recognition of native and/or thermally
19
20 425 induced denatured forms of the major food allergen, ovomucoid, by human IgE and
21
22 426 mouse monoclonal IgG antibodies. *Biosci Biotechnol Biochem.* 68: 2490-2497.
23
24
25 427 Howard SB, Twigg P, Baird JK, Meehan EJ. 1988. The solubility of hen egg-white lysozyme.
26
27 428 *J Cryst Growth.* 90: 94-104.
28
29 429 Kato Y, Oozawa E, Matsuda T. 2001a. Decrease in antigenic and allergenic potentials of
30
31 430 ovomucoid by heating in the presence of wheat flour: dependence on wheat variety
32
33 431 and intermolecular disulfide bridges. *J Agric Food Chem.* 49: 3661-3665.
34
35 432 Kato Y, Suginozawa K, Fujiwara M. 2001b. A novel and simple method of insolubilization of
36
37 433 ovomucoid in cookies prepared from batter containing egg white. *Food Sci Technol*
38
39 434 *Res.* 7: 35-38.
40
41
42 435 Langeland T. 1982. A clinical and immunological study of allergy to hen's egg white. II.
43
44 436 Antigens in hen's egg white studied by crossed immunoelectrophoresis (CIE). *Allergy.*
45
46 437 37: 323-33.
47
48
49 438 Leduc V, Demeulemester C, Polack B, Guizard C, Le Guern L, Peltre G. 1999.
50
51 439 Immunochemical detection of egg-white antigens and allergens in meat products.
52
53 440 *Allergy (Copenhagen).* 54: 464-472.
54
55 441 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin
56
57 442 phenol reagent. *J Biol Chem.* 193: 265-75.
58
59
60

- 1
2
3 443 Moneret-Vautrin DA, Kanny G. 2004. Update on threshold doses of food allergens:
4
5 444 Implications for patients and the food industry. *Curr Opin Allergy Clin Immunol.* 4:
6
7 445 215-219.
8
9
10 446 Natarajan S, Xu C, Caperna TJ, Garrett WM. 2005. Comparison of protein solubilization
11
12 447 methods suitable for proteomic analysis of soybean seed proteins. *Anal Biochem.*
13
14 448 342: 214-220.
15
16 449 Schubert S. 2003. Charakterisierung und Isolierung von Allergenen der Tomate
17
18 450 (*Lycopersicon lycopersicum* (L.) Karst. ex Farw.) [dissertation]. [Institut für Biochemie
19
20 451 und Lebensmittelchemie]: Universität Hamburg.
21
22 452 Vieluf I, Besler M, Paschke A, Steinhart H, Vieluf D. 2002. Practical approach to adverse
23
24 453 food reactions. *New Trends in Allergy V*, 5th, Davos, Switzerland, Sept. 15-17, 2000.
25
26 454 190-202.
27
28
29 455 Yeung JM, Newsome WH, Abbott MA. 2000. Determination of egg proteins in food products
30
31 456 by enzyme immunoassay. *J AOAC Int.* 83: 139-143.
32
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34 457
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36 458
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Table 1: Protein recovery rates (%) estimated using different extractants to isolate milk proteins out of milk material or out of milk material in matrix

(WM = without matrix; WS = wheat starch; FP = fat powder; CM = cake mix)

Milk material	Matrix	Extractant										
		A	B	C	D	E	F	G	H	I	J	K
milk powder	WM	88 ± 4	85 ± 3	90 ± 2	89 ± 3	85 ± 2	86 ± 3	16 ± 1	69 ± 4	109 ± 4	94 ± 4	86 ± 8
	WS	87 ± 1	91 ± 4	92 ± 4	88 ± 4	79 ± 7	-	10 ± 0	69 ± 4	116 ± 6	92 ± 5	89 ± 2
	FP	86 ± 3	79 ± 5	83 ± 5	98 ± 5	44 ± 0	-	27 ± 5	78 ± 2	106 ± 4	85 ± 5	88 ± 2
	CM	71 ± 4	82 ± 9	72 ± 13	87 ± 13	40 ± 3	-	14 ± 0	81 ± 3	74 ± 5	82 ± 8	74 ± 4
Sweet whey	WM	81 ± 1	85 ± 3	91 ± 3	92 ± 3	85 ± 3	97 ± 4	53 ± 1	37 ± 1	138 ± 12	112 ± 3	85 ± 4
	WS	81 ± 4	89 ± 7	90 ± 1	93 ± 1	89 ± 6	-	45 ± 3	36 ± 1	142 ± 4	105 ± 2	91 ± 8
	FP	80 ± 1	84 ± 5	80 ± 2	101 ± 2	47 ± 3	-	65 ± 11	42 ± 1	87 ± 1	92 ± 3	95 ± 2
	CM	83 ± 4	84 ± 3	63 ± 10	110 ± 10	47 ± 2	-	77 ± 4	32 ± 3	75 ± 2	86 ± 6	89 ± 5
Acid whey	WM	99 ± 10	95 ± 1	102 ± 3	79 ± 3	79 ± 2	93 ± 4	41 ± 2	46 ± 0	140 ± 6	112 ± 5	60 ± 3
	WS	94 ± 5	107 ± 9	106 ± 7	59 ± 7	83 ± 3	-	31 ± 2	55 ± 3	137 ± 9	113 ± 8	30 ± 1
	FP	81 ± 2	82 ± 1	79 ± 6	96 ± 6	41 ± 2	-	71 ± 2	43 ± 2	96 ± 3	91 ± 2	30 ± 2
	CM	81 ± 1	81 ± 3	82 ± 12	101 ± 12	47 ± 2	-	97 ± 1	44 ± 1	87 ± 2	91 ± 5	66 ± 5

Table 2: Protein recovery rates (%) estimated using different extractants to isolate egg proteins out of egg material or out of egg material in matrix

(WM = without matrix; WS = wheat starch; FP = fat powder; CM = cake mix)

		1	2	3	4	5	6	7	8	9	10	11	12
Whole egg	WM	27 ± 2	26 ± 2	20 ± 1	23 ± 2	108 ± 3	74 ± 5	26 ± 1	22 ± 1	119 ± 11	112 ± 3	64 ± 2	87
	WS	22 ± 1	22 ± 2	22 ± 2	21 ± 1	108 ± 10	-	21 ± 2	27 ± 2	100 ± 4	117 ± 4	70 ± 0	84
	FP	20 ± 1	22 ± 1	18 ± 2	41 ± 2	75 ± 1	-	22 ± 2	30 ± 2	125 ± 0	134 ± 5	64 ± 6	97
	CM	29 ± 2	25 ± 1	21 ± 4	28 ± 3	78 ± 1	-	27 ± 3	30 ± 3	100 ± 2	124 ± 4	45 ± 2	112
Egg yolk	WM	37 ± 2	36 ± 1	38 ± 0	30 ± 2	86 ± 0	63 ± 4	79 ± 2	49 ± 2	129 ± 6	113 ± 5	16 ± 0	71
	WS	38 ± 1	38 ± 2	39 ± 4	30 ± 2	83 ± 4	-	62 ± 1	45 ± 1	111 ± 9	108 ± 2	20 ± 1	73
	FP	81 ± 6	70 ± 4	70 ± 11	75 ± 4	77 ± 2	-	86 ± 6	33 ± 2	151 ± 9	139 ± 4	94 ± 7	90
	CM	38 ± 2	38 ± 1	50 ± 3	45 ± 4	85 ± 5	-	107 ± 8	35 ± 3	113 ± 12	133 ± 6	51 ± 1	83
Egg white	WM	76 ± 2	76 ± 7	61 ± 11	53 ± 1	121 ± 3	116 ± 8	37 ± 2	20 ± 0	138 ± 16	123 ± 5	77 ± 2	86
	WS	76 ± 3	79 ± 1	71 ± 1	69 ± 1	120 ± 3	-	39 ± 1	21 ± 2	135 ± 9	132 ± 2	88 ± 2	95
	FP	15 ± 1	14 ± 0	19 ± 0	16 ± 0	90 ± 1	-	39 ± 2	32 ± 1	130 ± 10	145 ± 4	24 ± 2	111
	CM	16 ± 2	19 ± 2	22 ± 8	26 ± 4	78 ± 0	-	52 ± 6	44 ± 1	120 ± 4	141 ± 9	41 ± 2	102

Table 3: Protein recovery rates (%) using solution I and J, respectively, at different pH-values to isolate milk proteins from milk material and from milk material in certain matrices at different temperatures. (WM = without matrix; WS = wheat starch; CM = cake mix)

Dry milk material	Extractant	Matrix	pH	Recovery rates [%]				
				4 °C	RT	40 °C		
Milk powder	I	WM	7.4	95 ± 6	103 ± 5	96 ± 4		
			9.4	72 ± 7	82 ± 9	89 ± 7		
			11.4	90 ± 4	91 ± 2	95 ± 3		
		WS	7.4	95 ± 2	71 ± 4	85 ± 6		
			9.4	63 ± 4	59 ± 2	68 ± 6		
			7.4	97 ± 2	89 ± 1	89 ± 3		
		CM	9.4	60 ± 3	62 ± 6	57 ± 5		
			J	WM	8.5	73 ± 3	75 ± 5	74 ± 5
					10.5	83 ± 2	83 ± 3	78 ± 3
	12.5	99 ± 2			93 ± 4	90 ± 5		
	WS	8.5	90 ± 1	96 ± 6	96 ± 5			
		10.5	92 ± 4	91 ± 5	92 ± 1			
		CM	8.5	98 ± 3	97 ± 5	99 ± 6		
	10.5		112 ± 7	108 ± 2	115 ± 6			
	Sweet whey		I	WM	7.4	85 ± 2	87 ± 3	100 ± 3
9.4		82 ± 1			90 ± 3	79 ± 6		
11.4		111 ± 4			107 ± 1	105 ± 2		
WS		7.4		80 ± 5	70 ± 4	80 ± 3		
		9.4		76 ± 6	87 ± 5	81 ± 3		
		CM		7.4	89 ± 3	83 ± 3	87 ± 1	
9.4			76 ± 3	70 ± 1	75 ± 6			
J			WM	8.5	67 ± 3	72 ± 1	77 ± 4	
		10.5		88 ± 0	91 ± 1	89 ± 4		
	12.5	129 ± 11		118 ± 11	110 ± 2			
WS	8.5	103 ± 5	102 ± 3	109 ± 7				
	10.5	101 ± 1	100 ± 1	101 ± 1				
	CM	8.5	113 ± 4	111 ± 1	114 ± 1			
10.5		118 ± 2	113 ± 2	111 ± 1				
Acid whey		I	WM	7.4	81 ± 5	95 ± 2	96 ± 1	
	9.4			91 ± 1	91 ± 10	89 ± 4		
	11.4			109 ± 6	128 ± 12	109 ± 8		
	WS		7.4	84 ± 3	87 ± 4	87 ± 3		
			9.4	81 ± 2	49 ± 5	88 ± 7		
			CM	7.4	80 ± 7	97 ± 4	95 ± 3	
	9.4	87 ± 7		85 ± 4	86 ± 2			
	J	WM		8.5	71 ± 2	75 ± 4	76 ± 1	
			10.5	87 ± 2	98 ± 6	83 ± 2		
12.5			113 ± 2	113 ± 3	101 ± 4			
WS	8.5	100 ± 2	102 ± 2	101 ± 3				
	10.5	96 ± 3	100 ± 5	101 ± 1				
	CM	8.5	118 ± 12	115 ± 4	111 ± 3			
10.5		108 ± 2	119 ± 7	108 ± 11				

Table 4: Protein recovery rates (%) using solution I and J, respectively, at different pH-values to isolate egg proteins from egg material and from egg material in certain matrices at different temperatures. (WM = without matrix; WS = wheat starch; CM = cake mix)

Dry egg material	Extractant	Matrix	pH	Recovery rates [%]		
				4 °C	RT	40 °C
Whole egg	I	WM	7.4	94 ± 1	89 ± 7	99 ± 5
			9.4	102 ± 4	98 ± 8	101 ± 2
			11.4	110 ± 3	103 ± 4	94 ± 6
		WS	7.4	93 ± 4	93 ± 1	92 ± 4
			9.4	102 ± 3	101 ± 1	101 ± 8
			7.4	100 ± 3	105 ± 9	105 ± 2
	J	WM	9.4	105 ± 1	105 ± 2	106 ± 3
			8.5	114 ± 3	120 ± 2	126 ± 4
			10.5	109 ± 2	117 ± 3	114 ± 2
		WS	12.5	110 ± 3	111 ± 7	111 ± 6
			8.5	111 ± 5	117 ± 7	114 ± 9
			10.5	102 ± 6	108 ± 4	113 ± 5
CM	8.5	111 ± 7	113 ± 5	106 ± 1		
	10.5	92 ± 4	91 ± 4	100 ± 2		
	7.4	114 ± 8	109 ± 9	117 ± 3		
Egg white	I	WM	9.4	116 ± 4	115 ± 3	127 ± 6
			11.4	126 ± 6	123 ± 7	118 ± 3
			7.4	107 ± 6	115 ± 5	116 ± 5
		WS	9.4	123 ± 7	123 ± 9	126 ± 1
			7.4	114 ± 2	114 ± 4	121 ± 7
			9.4	118 ± 5	120 ± 4	127 ± 10
	J	WM	8.5	102 ± 4	122 ± 2	131 ± 2
			10.5	119 ± 7	116 ± 1	126 ± 6
			12.5	118 ± 6	124 ± 6	120 ± 7
		WS	8.5	124 ± 8	119 ± 8	127 ± 2
			10.5	126 ± 4	116 ± 5	119 ± 3
			8.5	123 ± 11	122 ± 3	118 ± 5
CM	10.5	105 ± 6	109 ± 6	114 ± 4		
	7.4	121 ± 2	121 ± 2	132 ± 11		
	WM	9.4	125 ± 10	111 ± 6	103 ± 5	
		11.4	116 ± 1	111 ± 7	112 ± 7	
		7.4	106 ± 1	101 ± 0	103 ± 7	
	J	WS	9.4	118 ± 7	109 ± 5	114 ± 8
7.4			105 ± 3	106 ± 3	105 ± 7	
9.4			115 ± 3	121 ± 7	113 ± 2	
CM		8.5	108 ± 1	110 ± 10	121 ± 8	
		10.5	117 ± 2	113 ± 1	117 ± 6	
		12.5	103 ± 6	109 ± 7	105 ± 3	
	8.5	115 ± 4	112 ± 3	114 ± 5		
	WS	10.5	111 ± 1	110 ± 2	111 ± 4	
		8.5	113 ± 4	113 ± 10	111 ± 5	
CM	10.5	92 ± 6	87 ± 3	96 ± 6		

Table 5: Protein recovery rates obtained by varied extraction durations at three temperatures using solution I (pH 7.4) and J (pH 8.5) to isolate milk proteins from skim milk (WM = without matrix; CM = cake mix).

Extractant	Matrix	Temp.	Extraction duration [h]					
			0	0.5	1	2	4	8
J (pH 8.5)	WM	4 °C	81 ± 1	82 ± 0	90 ± 1	85 ± 2	85 ± 6	82 ± 2
		RT		81 ± 2	97 ± 1	89 ± 3	80 ± 7	85 ± 4
		40 °C		82 ± 3	89 ± 3	78 ± 1	82 ± 3	84 ± 3
	CM	4 °C	80 ± 1	89 ± 8	98 ± 3	92 ± 7	99 ± 2	88 ± 5
		RT		80 ± 6	90 ± 9	98 ± 5	75 ± 7	86 ± 0
		40 °C		82 ± 4	88 ± 7	87 ± 1	96 ± 2	91 ± 7
I (pH 7.4)	WM	4 °C	96 ± 1	88 ± 1	101 ± 9	84 ± 2	86 ± 1	82 ± 0
		RT		94 ± 2	102 ± 4	86 ± 0	114 ± 9	86 ± 1
		40 °C		84 ± 7	112 ± 8	83 ± 1	87 ± 1	88 ± 8
	CM	4 °C	78 ± 4	90 ± 4	83 ± 1	80 ± 5	86 ± 2	83 ± 1
		RT		80 ± 0	90 ± 6	83 ± 4	80 ± 6	77 ± 7
		40 °C		83 ± 3	92 ± 5	76 ± 7	93 ± 9	78 ± 3

Table 6: Protein recovery rates obtained by varied extraction durations at three temperatures using solution I and J to isolate egg proteins from whole egg powder (WM = without matrix; CM = cake mix).

Extractant	Matrix	Temp.	Extraction duration [h]					
			0	0.5	1	2	4	8
J (pH 8.5)	WM	4 °C	107 ± 4	106 ± 5	105 ± 1	104 ± 4	106 ± 3	104 ± 4
		RT		110 ± 3	108 ± 3	104 ± 2	104 ± 1	109 ± 2
		40 °C		110 ± 0	109 ± 2	109 ± 4	106 ± 3	107 ± 1
	CM	4 °C	115 ± 6	116 ± 6	105 ± 3	112 ± 1	105 ± 4	113 ± 8
		RT		119 ± 12	117 ± 5	112 ± 4	111 ± 3	112 ± 6
		40 °C		116 ± 0	122 ± 3	120 ± 2	123 ± 0	111 ± 6
I (pH 7.4)	WM	4 °C	94 ± 6	96 ± 2	99 ± 2	99 ± 5	104 ± 2	104 ± 3
		RT		96 ± 4	93 ± 3	98 ± 2	94 ± 3	96 ± 6
		40 °C		99 ± 3	103 ± 3	95 ± 2	101 ± 0	101 ± 3
	CM	4 °C	91 ± 2	92 ± 1	93 ± 1	92 ± 1	89 ± 6	91 ± 0
		RT		90 ± 0	99 ± 1	93 ± 2	94 ± 4	93 ± 5
		40 °C		112 ± 3	112 ± 6	117 ± 3	117 ± 2	108 ± 0

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3 1 Fig. 1: LDS - PAGE and silver staining patterns of proteins extracted from egg powder (left)
4 and milk powder (right), respectively, using different extractants. MW: molecular weight
5 marker; CON: Conalbumin (76 kDa); OVA: Ovalbumin (44.5 kDa); OVO: Ovomuroid (28
6 kDa); LYS: Lysozyme (14.3 kDa); BSA: Bovine serum albumin (66.3 kDa); CAS: Caseins
7 (20.6 -25.2 kDa); LG: β -lactoglobulin (18.3 kDa); LA: α -lactalbumin (14.2 kDa).
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7 Fig. 2: LDS - PAGE and silver staining patterns of proteins extracted from egg powder using
8 solution J (left) and I (right), respectively, adjusted to different pH-values and used for
9 extraction at 4 °C, room temperature (RT) and 40 °C. MW: molecular weight marker; CON:
10 Conalbumin (76 kDa); OVA: Ovalbumin (44.5 kDa); OVO: Ovomuroid (28 kDa); LYS:
11 Lysozyme (14.3 kDa).
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13 Fig. 3: Left: LDS - PAGE and Immunoblotting of ovalbumin extracted from egg powder using
14 different extractants and detection via anti-ovalbumin antibody. Right: LDS - PAGE and
15 Immunoblotting of casein extracted from milk powder using different extractants and
16 detection via anti-casein antibody. MW: molecular weight marker; CON: Conalbumin (76
17 kDa); OVA: Ovalbumin (44.5 kDa); OVO: Ovomuroid (28 kDa); LYS: Lysozyme (14.3 kDa);
18 BSA: Bovine serum albumin (66.3 kDa); CAS: Casein (20.6 -25.2 kDa).
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20 Fig. 4: Left: LDS - PAGE and Immunoblotting of ovalbumin extracted from egg powder out of
21 dry cake mix as matrix using different extractants and detection via anti-ovalbumin antibody.
22 Right: LDS - PAGE and Immunoblotting of casein extracted from milk powder out of dry cake
23 mix as matrix using different extractants and detection via anti-casein antibody. MW:
24 molecular weight marker; CON: Conalbumin (76 kDa); OVA: Ovalbumin (44.5 kDa); OVO:
25 Ovomuroid (28 kDa); LYS: Lysozyme (14.3 kDa); BSA: Bovine serum albumin (66.3 kDa);
26 CAS: Casein (20.6 -25.3 kDa).
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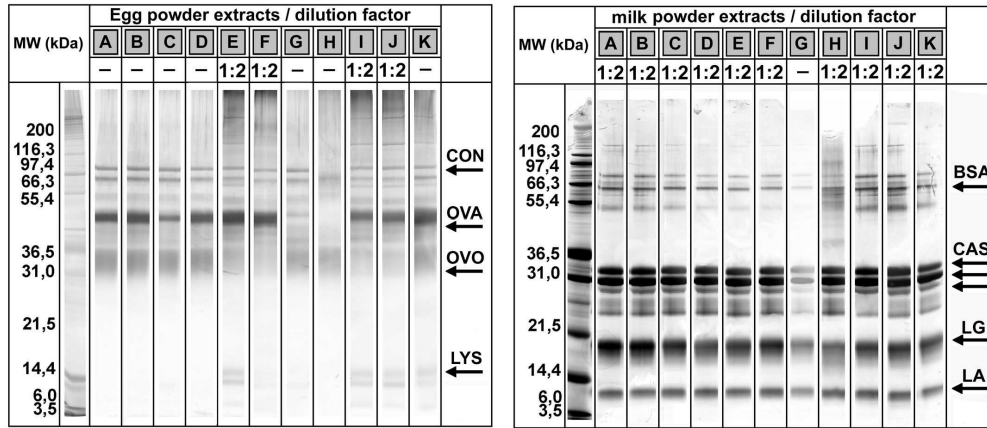


Figure 1
299x129mm (183 x 183 DPI)

Peer Review Only

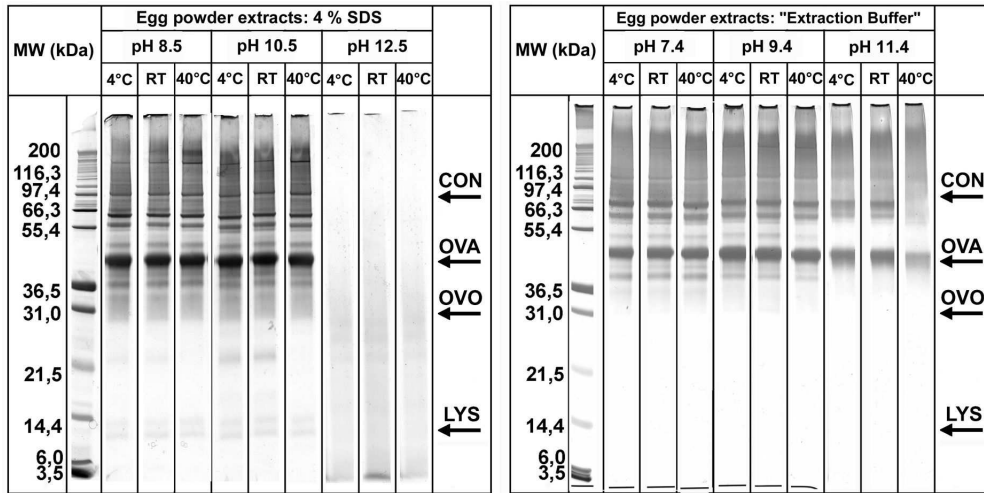


Figure 2
259x129mm (183 x 183 DPI)

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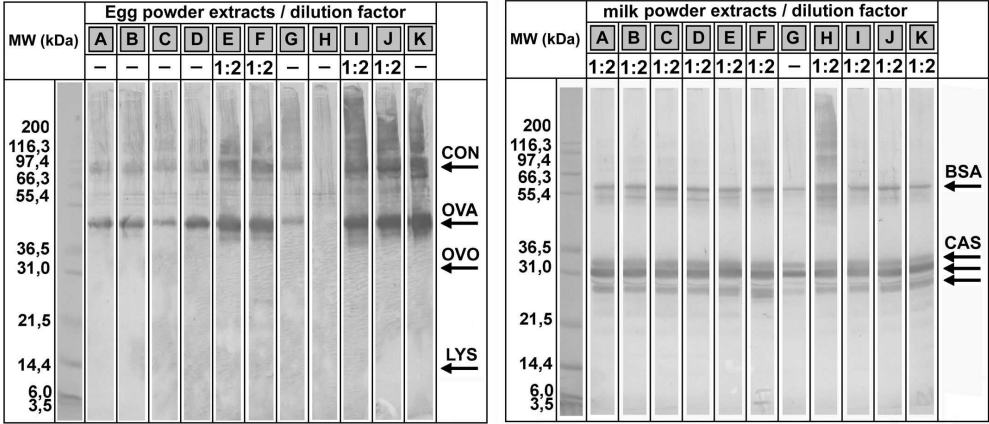


Figure 3
299x129mm (183 x 183 DPI)

