

Research paper

Novel ELISA for the detection of raw and processed egg using extraction buffer containing a surfactant and a reducing agent

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Abstract

Enzyme-linked immunosorbent assay (ELISA) has been considered extremely useful for the detection of markers of allergenic substances in food, because it is simple, offers a suitable sensitivity, and is useful in providing quantitative results. Allergenic protein present in processed food can be denatured or altered, hindering therefore their possibility to be extracted and detected. This paper reports the development of an ELISA method that can be used for the determination of allergenic proteins in buffer solutions containing SDS, a surfactant, and 2-mercaptoethanol, a reducing agent. Measurement by ELISA in solutions containing 1% SDS and 7% 2-mercaptoethanol has been made possible by using an antibody prepared through immunization with an antigen denatured with SDS and 2-mercaptoethanol. This ELISA technique can be used to measure proteins in food that have been denatured by various manufacturing processes. An example is egg white albumin, which is susceptible to heat denaturation and has been difficult to recover from food in the past. Its recovery was improved 10- to 100-fold by the new ELISA method as compared with previous methods. This means that allergenic substances in food can now be detected quantitatively. This method can be very useful in allergy prevention and control strategies.

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1. Introduction

The immune system, which is the self-defense system of the body, sometimes responds in a way that is harmful to the body. Some of these responses are referred to as allergic reactions environment of food. Allergies caused in part by the surrounding are increasing in incidence and have recently become a societal problem. The number of children suffering from allergic reactions is also reported to have increased. Allergic

Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; 2-ME, 2-mercaptoethanol; OVA, egg white albumin; POD, horseradish peroxidase; SDS, sodium dodecyl sulphate; TMB, 3,3',5,5'-tetramethylbenzidine; Tween 20, polyoxyethylene-sorbitan monolaurate.

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symptoms are diverse and vary according to each individual, some of whom show a severe symptom called ‘anaphylactic shock’, which is life-threatening. In order to prevent the inducement of allergic symptoms, it is important to avoid consuming foods with unknown ingredients some of which can be known allergens. In order to keep the diets of allergy patients safe, research is under way to determine the threshold intake doses of allergenic foods (Taylor et al., 2002). Subsequently regulations have made necessary to detect and quantify allergenic substances in food as accurately as possible.

Detection methods for allergenic substances in food include enzyme-linked immunosorbent assay (ELISA) (Engvall and Perlman, 1972), immunochromatography and Western blotting, which target proteins, and polymerase chain reaction (PCR), which targets genes. Of these methods, ELISA is considered extremely useful for the detection of allergenic substances in food, because it is simple, high in detection sensitivity, and excellent in terms of quantification.

Food products can be subjected to processing treatments such as heating, pressurization and sterilization, during which proteins contained in food are often denatured in various manners. When an antibody generated against a native protein is used to detect such denatured proteins, its reactivity is often decreased as compared with its reactivity towards the native protein, because the denatured protein has undergone a change in 3D structure. In addition, it is necessary to extract the protein from the food commodity before enabling its detection check on the presence of allergenic substances by ELISA. Denatured or altered proteins are often less water-soluble than native proteins, which sometimes renders their extraction from food difficult. Surfactants and denaturants can be used to solubilize and extract insoluble proteins. However, measurement by ELISA with an extraction solution containing a denaturant cannot be made accurately because the antibody is affected by the denaturant. Furthermore, dilution of the extract to the extent that the denaturant does not influence the antibody sometimes makes detection of the extracted protein difficult.

In order to avoid this problem, investigations were carried out where a plate is coated with a protein treated with a surfactant and then an antigen–antibody reaction is enabled in a buffer system without the surfactant (Lechtzner et al., 2002). However, to our knowledge, no study has referred a method for quantification by an antigen–

antibody reaction in a buffer system containing surfactants or reducing agents. This paper describes a new ELISA that has been developed with the aim of solving the above-mentioned problems. This study focuses on the detection of egg protein that can be difficult to detect by ordinary ELISA after processing treatments such as heating. With this new approach, it is possible to extract these proteins more efficiently from food, carry out an antigen–antibody reaction, and detect the egg protein with a high recovery rate in a buffer system containing a surfactant and a reducing agent. This method is applicable to proteins in other food products.

2. Materials and methods

2.1. Materials

2-Mercaptoethanol (2-ME), sodium dodecyl sulfate (SDS), polyoxyethylene-sorbitan monolaurate (Tween 20), and methanol special grade were supplied by Nakarai Co., Ltd. 10× Tris/glycine buffer (pH, 8.3) and 10× Tris/glycine/SDS buffer (pH, 8.3) were provided by Bio-Rad Laboratories, Inc. Bovine serum albumin RIA grade (BSA), horseradish peroxidase (POD), 3,3',5,5'-tetramethylbenzidine (TMB), and Hybond-P (PVDF membrane) were from Wako Pure Chemical Industries Co., Ltd., Toyobo Co. Ltd., Moss, Inc., and Amersham Biosciences Co., Ltd., respectively. Freeze-dried whole egg powder was prepared from fresh egg of white leghorn.

In addition, the following buffers were prepared for the experiment: 120 mM Tris/HCl (pH 7.4) containing 0.1% BSA and 0.05% Tween 20 (Buffer A), and 20 mM Tris/HCl buffer (pH 7.4) containing 150 mM NaCl and 0.05% Tween 20 (Buffer B).

2.2. Methods

2.2.1. Preparation and purification of antibodies for specificity

2.2.1.1. Preparation of a specific anti-OVA antibody (anti-native-OVA antibody). For immunization, an adjuvant containing about 0.2 mg of OVA (egg albumin, 5× cryst., supplied by Seikagaku Corp.) per injection was hypodermically injected as an antigen about five times (~1 mg per rabbit) at appropriate

intervals (1 week) (Coligan et al., 1995). Whole blood was collected and the serum was separated for purification. An affinity column prepared by the fixation of OVA to a HiTrap NHS-activated HP column (Pharmacia Biosciences Co., Ltd.) was used to purify the OVA-specific antibody. The serum was applied to the column equilibrated with 20 mM Tris/HCl buffer (pH 7.4) containing 150 mM NaCl, and the antibody that eluted with 0.1 M glycine/HCl buffer (pH 2.3) was taken to be a specific anti-OVA antibody (anti-native-OVA antibody).

2.2.2. Preparation of a specific antibody against OVA denatured with SDS and 2-ME (anti-SDS-OVA antibody)

For immunization, 5 mg/mL of OVA in PBS was added 1% SDS and undiluted 2-ME at final concentrations of 1% and 0.07%, respectively, for over 12 h. Then 1% SDS and 0.07% 2-ME exposed antigen was added adjuvant to inject rabbits (~1 mg per rabbit) in several applications as described above. The serum obtained from whole blood was purified for the denatured-OVA-specific antibody with a HiTrap column to which denatured OVA was fixed. The antibody obtained through this purification was taken to be a specific anti-SDS-OVA antibody.

2.3. Standard and sample extraction

2.3.1. Homogenization of samples

A Millser IFN-700G homogenizer (Iwatani International Corp.) was used to homogenize samples. Samples were treated with the Millser a few times for 30 s for homogeneity.

2.3.2. System without SDS and 2-ME

Thirty-eight milliliters of Buffer A (see Materials) was added to 2 g of a homogenized sample, which was then treated with the Millser three times for 30 s for extraction. After confirming the pH was around 6.0–8.0 with pH paper, the sample was centrifuged at 3000×g for 20 min and the supernatant was filtered through 5A filter paper (Advantec Tokyo Kaisha, Ltd.) to obtain the extract.

2.3.3. System containing SDS and 2-ME

Nineteen milliliters of Buffer A containing 1% SDS and 7% 2-ME was added to 1 g of a homoge-

nized sample. The sample was then shaken back and forth at 90–110 rpm (moving distance, 3 cm) overnight (over 12 h at room temperature) for extraction. The subsequent centrifugation and filtration steps to obtain the extract were the same as those described for the system without SDS and 2-ME.

2.4. Protein assay

The extracts obtained were analyzed with a 2D Quant Protein Assay Kit (Pharmacia Biosciences Co., Ltd.).

2.5. ELISA

2.5.1. ELISA in the system without SDS and 2-ME

A solution of egg extract was used as a standard and adjusted to make up the following concentration gradient by means of a protein assay: 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0 ng/mL as a blank (Buffer A only). These standards, plus the sample, which was diluted stepwise with Buffer A, were applied to an ELISA plate coated with the anti-native-OVA antibody at 100 µL/well and allowed to stand at 25 °C for 1 h for the primary reaction. Buffer B, 300 µL/well, was used to wash the plates, and the washing was repeated six times. An anti-native-OVA antibody labeled with POD was added to the ELISA plate at 100 µL/well and allowed to stand for 30 min for the secondary reaction. After a second washing, TMB, 100 µL/well, was added and the enzyme reaction was allowed to run at 25 °C for exactly 10 min. The reaction was stopped by the addition of 100 µL/well of 1 N H₂SO₄. Absorbance was measured at 450 nm, the dominant wavelength, and at 630 nm, the subdominant wavelength. The amount of egg protein was calculated from the absorbance of the sample by using the standard curve obtained.

2.5.2. ELISA in the system containing SDS and 2-ME

A solution of egg extract denatured with SDS and 2-ME was used as a standard and adjusted to a concentration of 1 µg/mL by means of a protein assay. It was diluted 20-fold to 50 ng/mL with Buffer A and then serially diluted 2-fold to make up a concentration gradient of 25, 12.5, 6.25, 3.125, 1.56, 0.78, and a blank (0 ng/mL). The concentrations of SDS and 2-ME were set at 0.05% SDS and 0.35% 2-ME, respectively, for the

extraction. In addition, the sample extract was diluted 20-fold with Buffer A so that its SDS and 2-ME concentrations were made the same as those in the standards. The standards and sample extracts, 100 μ L/well each, were applied to the ELISA plate, coated with the anti-SDS-OVA antibody to enable the primary reaction to occur. An anti-SDS-OVA antibody labeled with the POD was used as the enzyme-labeled antibody (Yoshitake et al., 1982) and diluted with Buffer B containing 1% BSA. The conditions and the reaction times for the washing and secondary reaction steps were the same as those described above for the ELISA in a system without SDS and 2-ME.

2.5.3. ELISA to SDS and 2-ME untreated OVA and treated OVA

The reaction of anti-OVA antibody to SDS and 2-ME treated OVA was performed using the freeze-dried egg powder dissolved in a solution containing 1% SDS and 7% 2-ME. Then it was left for 0, 1, 2, 4, and 6 h and then exposed to first reaction buffer containing SDS and 2-ME. The solution was subsequently subjected to ELISA as described above. Two antibodies were used: an anti-native-OVA antibody, specific for native OVA; and an anti-SDS-OVA antibody, specific for SDS-treated OVA.

2.6. Western blotting

The egg extract obtained from the assay where SDS and 2-ME were used and an OVA sample denatured with SDS and 2-ME, as described in the above methods, were mixed with a loading buffer (a mixture of Laemmli Sample Buffer [Bio-Rad Laboratories, Inc.] and 2-ME at 19:1) at a ratio of 1:2. After boiling in a boiling water bath for 5 min, the samples were cooled on ice and used for electrophoresis. SDS-PAGE (Laemmli, 1970) was performed at a constant electric current of 30 mA/gel by applying 20 μ L/lane of the egg extract sample and the OVA extract sample to a 15% separation gel (TEFCO, Ltd.). A kaleidoscope standard (Bio-Rad Laboratories, Inc.) was used as a molecular weight marker. The proteins were blotted onto PVDF membranes in a transblot SD cell (Bio-Rad Laboratories, Inc.) at a constant electric current of 120 mA (2 mA/cm) for 1 h. The membranes were shaken in a blocking buffer (Buffer B containing a final concentration of 0.1% BSA) for 1 h for blocking treatment.

For the primary immunostaining reaction, the anti-SDS-OVA antibody at a concentration of 0.5 μ g/mL was shaken with the membranes for 1 h. Buffer B (see Materials) was used for washing by shaking (three times for 5 min). For the secondary reaction, the membranes were shaken in the presence of a biotin-conjugated anti-rabbit-IgG antibody for 30 min using a VECTASTAIN ABC-AP Rabbit IgG kit (Vector Laboratories, Ltd.). After a second washing step, the membranes were shaken for a tertiary reaction with an avidin-biotin complex (an ABC solution) labeled with alkaline phosphatase (AP), which was included in the VECTASTAIN ABC IgG kit (Vector Laboratories, Ltd.). After additional washing step, the membranes were shaken with 100 mM Tris/HCl (pH 9.5) for 15 min. An Alkaline Phosphatase Substrate Kit IV (BCIP/BNT) (Vector Laboratories, Ltd.) was used for detection. Color was developed by shaking for about 5 min.

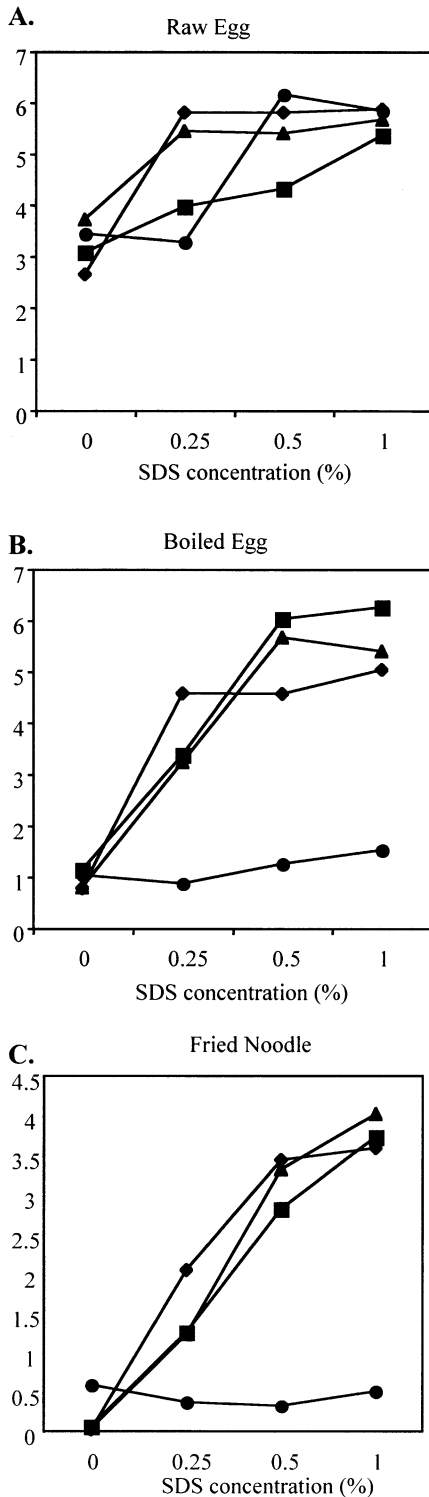
2.7. Preparation of model processed foods

Freeze-dried whole egg powder was mixed with raw foods to prepare processed food items containing the egg protein at two different concentrations: 10 μ g/g and 50 μ g/g. The following were prepared as model processed foods: orange juice (main raw ingredients: orange juice concentrate, sugar, and citric acid), biscuit (main raw ingredients: wheat flour, shortening, sugar, and salt), and strawberry jam (main raw ingredients: strawberry, sugars, and pectin).

3. Results

3.1. Improvement in protein extraction with SDS and 2-ME

Surfactants and reducing agents are useful for the solubilization of insoluble proteins. We extracted proteins from food with buffer solutions containing various concentrations of SDS and 2-ME and examined the relationship between the amount of protein extracted and the concentrations of SDS and 2-ME (Fig. 1). A raw egg (Fig. 1A), a boiled egg (Fig. 1B), and egg-fried noodles (Fig. 1C) were used to determine the amount of protein that could be extracted from food samples, which had been processed in various manners. All



three samples showed an increase in the amount of protein extracted as the concentrations of SDS and 2-ME increased. Furthermore, the amount of protein almost reached a plateau in extract solutions containing 0.5% SDS and 2% 2-ME.

In addition, other food types were also tested for differences between the amount of protein extracted with a buffer (Buffer A) without SDS and 2-ME and the amount of protein extracted with a buffer containing 1% SDS and 7% 2-ME (Table 1). It was evident that the amount of protein extracted from all types of food increased markedly when the solution contained SDS and 2-ME. From these results, it was found that the amount of protein extracted increased by 10- to 100-fold when the extracting solution contained sufficient amounts of SDS and 2-ME.

3.2. Development of ELISA in solutions containing SDS and 2-ME

An antigen–antibody reaction must take place in the presence of SDS and 2-ME in order to perform ELISA for proteins extracted with solutions containing SDS and 2-ME. We have attempted such development for the determination of egg albumin (OVA) by ELISA in presence of SDS and 2-ME. The occurrence of binding between of the anti-OVA antibody and the OVA in the presence of SDS and 2-ME was assessed through the experimental procedure. SDS and 2-ME treated OVA was dispensed to the wells coated anti-native-OVA antibody or anti-SDS-OVA antibody. The anti-native-OVA antibody showed a reaction with native OVA that still remained at time 0, but did not react with denatured or altered OVA that had been exposed to SDS and 2-ME for 1 h (Fig. 2). By contrast, it was shown that the anti-SDS-OVA antibody was able to detect the denatured and altered OVA sample even in the presence of high concentrations of SDS and 2-ME, and the measured values were not affected even after 6 h of exposure to SDS and 2-

Fig. 1. Improvement in protein extraction with SDS and 2-ME. A raw egg (A), a boiled egg (B), and egg-fried noodles (C) were extracted with buffer solutions containing SDS at the concentrations indicated in the figure and 2-ME at concentrations of 0 (solid circle), 1% (solid square), 2% (solid triangle), and 7% (solid diamond). The extraction method is described in Materials and methods. Duplicate measurements were taken and the graph shows the average values.

Table 1
Improvement of protein extraction with SDS and 2-ME

Food sample	SDS 0%, 2-ME 0%	SDS 1%, 2-ME 7%
	Protein concentration (mg/ml)	
Whole barley	0.1	10
Pepper	0.02	3.4
Corn	0.09	11
Soybean	2.6	27
Rice	0.06	5

Samples were extracted with a solution without SDS and 2-ME and a solution containing them. Extracted proteins were measured by a protein assay, as described in Materials and methods. Results are representative of three independent experiments.

ME. We also confirmed that anti-SDS-OVA antibody recognizes untreated OVA.

With treated a cross reactivity state reaching 35% (considering OVA as a reference). Such cross reactivity could be attributed to the polyclonal nature of the antibody, which is able to recognize various epitopes including those of non-denatured egg protein. In addition, OVA was subjected to Western blotting using the anti-SDS-OVA antibody in order to confirm whether or not the anti-SDS-OVA antibody recognized OVA (Fig. 3). Fig. 3 confirmed such specific recognition. The above results corroborate the specific reaction of

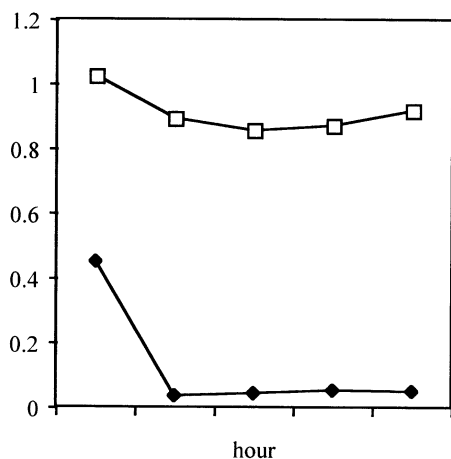


Fig. 2. Antigen–antibody reactions in the presence of SDS and 2-ME. Egg protein, 1.28 $\mu\text{g}/\text{mL}$, was dissolved in a solution containing 1% SDS and 7% 2-ME and the solution was allowed to stand for the durations indicated in the figure. The treated egg protein was detected by ELISA using an anti-native-OVA antibody (solid diamond) and an anti-SDS-OVA antibody (open square). The ELISA procedure is described in Materials and methods. Triplicate measurements were taken and the graph shows the average values.

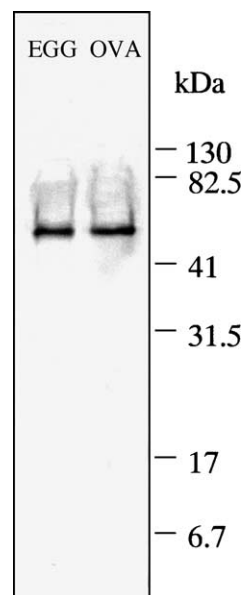


Fig. 3. Immunoblots of egg protein with an antibody against OVA treated with SDS. Egg and egg white albumin were treated with a solution containing SDS and 2-ME and then prepared for SDS-PAGE as described in Materials and methods. After electrophoresis, the anti-SDS-OVA antibody was used for immunoblot analysis. Immunostaining was done as described in Materials and methods. The immunoblots shown are representatives of two independent experiments with similar results.

the anti-SDS-OVA antibody with both denatured and altered OVA in the presence of SDS and 2-ME.

Next, a study was undertaken to examine how high the concentrations of SDS and 2-ME could be used without inhibiting the reactions involved in a sandwich ELISA using the anti-SDS-OVA antibody. The ELISA was performed in buffer solutions (Buffer A) containing SDS and 2-ME at various concentrations and standard curves were prepared (Fig. 4). The result showed that standard curves could be drawn up to an SDS concentration of 1% and a 2-ME concentration of 7%, making it possible to perform ELISA in solutions containing high concentrations of SDS and 2-ME.

3.3. Rates of protein recovery from model processed foods and measurements of proteins extracted from commercially available foods by ELISA

Model processed foods, to which known amounts of egg protein was added, were prepared in order to examine how accurately the ELISA could detect the

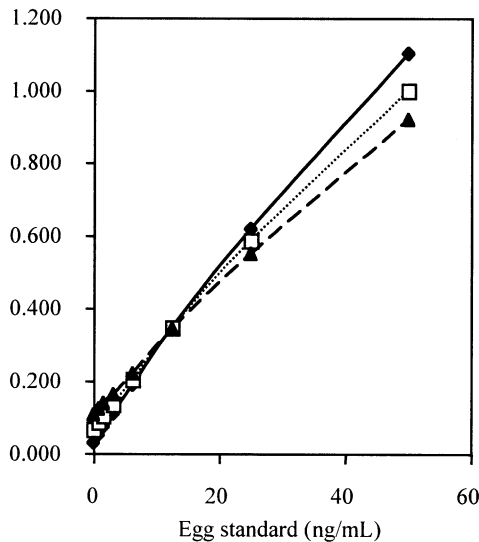


Fig. 4. Standard curves generated in the presence of various concentrations of SDS and 2-ME. Egg protein, 50 ng/mL, was used as a standard and the following 2-fold dilution series was prepared using Buffer A containing SDS and 2-ME: blank, 0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50 ng/mL. ELISA was performed with a system in which an anti-native-OVA antibody was used in a buffer without SDS and 2-ME (solid diamond), a system in which an anti-SDS-OVA antibody was used in a buffer containing 0.25% SDS and 1% 2-ME (open square), and a system in which the anti-SDS-OVA antibody was used in a buffer containing 1% SDS and 7% 2-ME (solid triangle). The ELISA procedure was done as described in Materials and methods. The graph shows the average values of triplicate measurements.

target protein in food that had undergone processing such as heating and pressurization. Proteins were extracted with Buffer A without SDS and 2-ME and Buffer A containing 1% SDS and 7% 2-ME for ELISA. The anti-native-OVA antibody was used for the former extract and the anti-SDS-OVA antibody was used for the latter extract. The extracts were diluted so that the measured values fell within ranges quantification of the standard curves. Recovery rates were determined by comparing the values obtained by ELISA measurement with the added amount of protein. The model-processed foods were prepared by adding egg protein at 10 µg/g and 50 µg/g to orange juice, biscuit, and jam. It was confirmed that none of the raw food items was contaminated with egg protein. The recovery rate of protein from the extracts containing SDS and 2-ME ranged from 75% to 93% for orange juice, from 36% to 47% for biscuit, and

Table 2

Improvement of protein recovery rate measured by ELISA in the presence of SDS and 2-ME

Processed food	Recovery			
	SDS, 2-ME negative		SDS, 2-ME positive	
	10 ppm	50 ppm	10 ppm	50 ppm
Orange juice	1.23	1.22	92.8	75.8
Biscuit	1.48	0.82	47.2	36.2
Jam	0.65	0.52	46.9	38.4

Processed food samples, to which 10 µg/g or 50 µg/g of egg protein was added, were prepared. The samples were extracted with a solution without SDS and 2-ME, and the extracts were subjected to ELISA using an anti-native-OVA antibody (SDS, 2-ME negative). In addition, the samples were extracted with a solution containing SDS and 2-ME for ELISA using an anti-SDS-OVA antibody (SDS, 2-ME positive). The extraction and ELISA procedures are described in Materials and methods. Results are representative of three independent experiments.

from 38% to 47% for jam. These recovery rates were considerably higher than those determined for the extracts prepared without SDS and 2-ME (Table 2).

In addition, ELISA was performed on commercially available food items, in which egg was used as a raw ingredient, by extracting them using Buffer A with and without SDS and 2-ME. As shown in Table 3, all of the samples gave values of egg protein that were 10- to 100-fold higher when the extracts contained SDS and 2-ME. These results confirm the efficiency of the ELISA to accurately detect and quantify egg protein when extracted with a solution containing SDS and 2-ME.

Table 3

Increase caused by SDS and 2-ME in values measured by ELISA

Processed food	SDS, 2-ME negative	SDS, 2-ME positive
Hamburg steak	–	21
Rice gruel	–	2496
Sandwiches	27.6	2255
Biscuit	2000	15 000
Bread	–	5.6
Cookies	290	13 000
Fried noodle	–	170

Commercially available food products were extracted with a solution without SDS and 2-ME and the extracts were subjected to ELISA using an anti-native-OVA antibody (SDS, 2-ME negative). The food samples were extracted with a solution containing SDS and 2-ME for ELISA using an anti-SDS-OVA antibody (SDS, 2-ME positive). The extraction and ELISA procedures are described in Materials and methods. Results are representative of three independent experiments.

4. Discussion

The addition of SDS and 2-ME to the extraction buffer is very useful for the extraction of proteins from raw eggs, boiled eggs, and fried noodles (Fig. 1A–C). 2-ME cleaves intermolecular and intramolecular S–S bonds formed between cysteine residues of proteins, and SDS helps solubilize proteins by disrupting most of their non-covalent bonds. The amount of protein extraction from the boiled egg and fried noodles with an extraction solution containing more than 1% 2-ME increased as the SDS concentration increased. However, the amount of protein increased little in extracts prepared without 2-ME, even when the SDS concentration was raised. It can be considered that SDS has a sufficient effect when coupled with the action of 2-ME, which cleaves the S–S bonds formed during protein alteration, denaturation, and insolubilization, which can be caused by the heat treatment. Furthermore, a 2-ME concentration of 1% can be considered sufficient for cleaving the intermolecular S–S bonds resulting from processing in the boiled egg and fried noodle samples. As long as the 2-ME concentration was sufficiently high (2% or more), the amount of protein extracted from the raw and boiled eggs was constant and did not change even as the SDS concentration increased; thus, the protein was inferred to have been completely extracted (Fig. 1A,B). On the other hand, the amount of protein extracted from the fried noodles increased with the increase in SDS concentration (Fig. 1C), and it is conjectured that it would keep increasing with further rises in the SDS concentration. Because the fried noodles contain wheat and many wheat proteins are hydrophobic, SDS concentrations higher than those used for the raw and boiled eggs were deemed necessary to increase protein extraction from the fried noodles. It can be concluded that the SDS concentration necessary for maximal protein extraction varies according to the food, because the hydrophobic protein content (including denatured or altered proteins) varies.

As shown by the examples in Table 1, the amount of protein extracted from various raw food ingredients was increased markedly by the addition of SDS and 2-ME to the extracting solution. Thus, SDS and 2-ME are effective for increasing the extraction of protein even from non-processed, raw food ingredients.

ELISA for egg protein treated with SDS and 2-ME must be performed in a system in which an anti-SDS-OVA antibody is used instead of an anti-native-OVA antibody (Fig. 2). The complete lack of a reaction between the anti-native-OVA antibody and the egg protein treated with SDS and 2-ME was deemed to be due to a complete loss of the reactive sites on egg white albumin caused by the SDS and 2-ME treatments. By contrast, the reactivity was sufficiently maintained regardless of treatment time when the anti-SDS-OVA antibody was used (Fig. 2). This shows that the coating antibody did not lose its binding ability by denaturation or alteration with SDS and 2-ME. Furthermore, the anti-SDS-OVA antibody showed a strong, specific reactivity with egg white albumin denatured or altered with SDS and 2-ME. The result of the Western blotting confirmed that the anti-SDS-OVA antibody has specific reactivity only towards egg white albumin (Fig. 3). From these results, it can be concluded that the use of an anti-SDS-OVA antibody has made measurement by ELISA possible in a system containing SDS and 2-ME in the primary reaction.

It is necessary to use sufficient amounts of SDS and 2-ME for protein extraction. The minimum requirements of SDS and 2-ME for protein extraction may vary according to the type of processed food. Color development, dependent on egg protein concentration, was observed even under the conditions of high SDS and 2-ME concentrations at 1% and 7%, respectively, when the anti-SDS-OVA antibody was used (Fig. 4). Thus, it is clear that measurement by ELISA is possible in a system containing high concentrations of SDS and 2-ME in the primary reaction.

The amount of egg protein measured in both the model processed foods and the commercially available food items showed clear differences between the system using extraction solutions without SDS and 2-ME and the anti-native OVA antibody, and the system using extract solutions containing SDS and 2-ME and the anti-SDS-OVA antibody (Tables 2 and 3). SDS and 2-ME have considerably improved dissolution in the denatured or altered OVA, especially because these foods were all heat-treated. This study has demonstrated that the determination of an egg protein content in processed foods is considerably improved by the development of a new ELISA method for egg proteins with a focus on OVA. Processed foods could be measured for other proteins present in milk, wheat,

peanuts, and buckwheat in addition to egg (unpublished date). In conclusion, this method offers a new perspective for the determination of allergenic proteins in processed food, and is expected to be extremely useful in food allergy prevention.

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