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Determination of yolk:white ratio of egg using SDS-PAGE

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Abstract The aim of present study was to determine liquid egg adulteration based on yolk:white ratio of egg using sodium dodecyl sulphate-polyacrylamide gel electrophoresis and by measuring Brix. To construct a calibration model, liquid egg samples were prepared by mixing egg yolk and white (yolk:white) at different concentrations. A high coefficient of determination value ($R^2 = 0.992$) was obtained. Limit of detection and limit of quantification were estimated as 62 g/kg and 187 g/kg. The accuracy of the model was tested using eleven LWE samples, and the yolk ratios of 90.9% of these samples were predicted successfully. Liquid whole egg (LWE) containing additional egg white up to 30% was also predicted with a low relative error of less than 10%. Yolk:white ratio of LWE samples and authentication of the components of LWE (containing extra white or water) can be determined using proposed method.

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Introduction

The importance of food quality and safety have been pronounced more frequently in recent years, and it seems that they will maintain to be a significant issue in food industry and in public, as well as in researches (Grunert, 2005). Egg is one of the good raw materials which is comprehensively used in the food industry as a main ingredient thanks to its exceptional functional properties and techno-unique features (Dev et al., 2008; Huopalahti et al., 2007).

Food producers commonly prefer to use liquid egg instead of shell eggs due to its ease of use and microbial reliability (Ahn et al., 1997; Rossi et al., 2010). It is critical for producers to have information about liquid egg composition before it processing. If producers control the liquid egg composition, they will be able to overcome further problems such as adulteration with water that may originate from liquid egg in subsequent steps. Adulteration can be performed by fraudsters in the composition of egg products (Gossner et al., 2009; Sharma and Paradakar, 2010; Zhu et al., 2009) or egg-food products such as egg pastas and egg tarts (Agulló and Gelós, 1996; Sharma and Paradakar, 2010) by means of adding non-egg protein or lipid to compensate for the lack of yolk or white (albumen). Therefore, it is critical for determination of egg adulteration and quality to ensure the accuracy of egg products.

Egg internal quality is determined by measuring physical and chemical quality parameters (Roberts, 2004). Conventionally, quantitative analyses of egg components (protein, lipid or water) have been performed by using reference methods such as Kjeldahl method and other official methods proposed by the Association of Analytical Communities (AOAC) (Horwitz, 1980; Willians, 1984). However, these analyses are highly complicated and time consuming. SDS-PAGE, ion-exchange chromatography, capillary electrophoresis, coupled capillary isotachophoresis with capillary zone electrophoreses are also some of the major techniques used in qualitative analysis of egg components (Kvasnička, 2003; Mc Bee and Cotterill, 1979; Raikos et al., 2006; Uysal et al., 2017). Other techniques such as infrared transmission spectroscopy, attenuated total reflectance Fourier transformation infrared (ATR-FTIR) spectroscopy, visible transmission spectroscopy, differential scanning calorimetry, and high-performance liquid chromatography (HPLC) have been also used to analyze egg composition and quality (Awadé and Efstathiou, 1999; Kemps et al., 2006; Laca et al., 2010; Osborne and Barrett, 1984; Uysal et al., 2019). On the other hand, the methods have been used for determining egg yolk and white content in foods based on the detection of some characteristic egg yolk constituents such as cholesterol, phosphorus, phospholipids, fatty acids, or egg white constituents such as specific egg proteins (Cizkova et al., 2004; Sajdok et al., 1990). Actually, the yolk:white ratio of liquid egg can be determined using one of the above-mentioned techniques by determining the amounts of total protein and lipid in liquid egg. However, liquid egg adulteration may mislead in the analysis of total amount of protein and lipid due to the addition of substitute protein, lipid or other ingredients to compensate for the lack of yolk or white constituents. Therefore, a more specific technique, which analyze a more particular component of both egg white and yolk rather than total quantification, is needed. The most appropriate method to determine this fraud would be utilizing the specific protein bands since proteins are the basic units of egg white and yolk. For that reason, SDS-PAGE technique was preferred to reveal the true yolk:white ratio and adulteration of liquid egg.

In this study, a new approach based on SDS-PAGE technique was proposed to determine the yolk:white ratio of liquid egg based on protein component, which makes it possible to reveal the adulteration in liquid egg composition. Measurements of both yolk:white ratio and Brix value of liquid egg were taken to determine the liquid egg composition including extra water or egg white. To the best of our knowledge, the proposed approach is the first study to use SDS-PAGE for determination of yolk:white ratio of liquid egg that enable detection of liquid egg adulteration.

Materials and methods

Chemicals

Reagent-grade glycine (≥ 99.7%), tris (99.8–100.1%), glycerol (99.5%), TCA (≥ 99.7%), and potassium hydroxide (KOH, ≥ 90.0%) were purchased from Merck Co. (Darmstadt, Germany). Reagent-grade SDS (98%) was supplied from Sigma (St. Louis, MO, USA). Reagent-grade methanol (99.8%) was purchased from Sigma Aldrich (Steinheim, Germany), and reagent-grade 2-mercaptoethanol (99.0%), bisacrylamide (99.0%), ammonium persulfate (AP, 99.0%), and TEMED (99.0%), were purchased from AppliChem (Darmstadt, Germany).

Preparation of liquid egg samples

The experiment was carried out using different shell eggs (n = 14), including grade A-large and medium eggs obtained from the commercial channel. Three of these shell eggs were used to form a calibration curve. A shell egg was manually broken and separated as liquid egg yolk (LEY) and liquid egg white (LEW). In order to construct the calibration curve, liquid egg samples were prepared by mixing LEY and LEW at different concentrations ranging from 0 to 1000 g/kg, with an interval of 100 g/kg (a total of ten points). This sample preparation was separately implemented for three shell eggs. The rest of the shell eggs (n = 11) was used for validation. In addition, liquid whole egg (LWE) sample and LWE containing extra egg white or water were prepared. LWE sample was prepared by breaking shell egg as a whole which composed of 30%:70% (w/w) yolk:white ratio, respectively. Samples of LWE containing extra egg white or water were also prepared by the addition of egg white or water in LWE instead of egg yolk content (30%). 65%, 55%, 40%, 30%, 20%, 10%, and 5% (w/w) of egg yolk was replaced with egg white or water.

Brix measurements

Brix values (total soluble solid content) of the samples of LWE and LWE containing extra egg white and water were measured with an automatic, optical refractometer (RFM 330, Bellingham+Stanley Ltd., Kent, UK) by the method of Bian et al. (2006) with small changes. A 1 mL sample was poured into the refractometer glass prism, and the measurements were taken at 25 °C. The observed data was recorded as Brix value of the sample.

SDS-PAGE analysis

The yolk:white ratio and water addition in liquid egg were determined using SDS-PAGE (Bio-Rad, Hercules, CA, USA) technique, where a similar procedure was proposed by Laemmli (Laemmli, 1970). Stacking (5%) and separation (12.5%) gels were prepared from a stock solution of 30 g/kg by weight of acrylamide: *N*,*N*'-methylenebisacrylamide (29:1).

For SDS-PAGE analysis, all liquid egg samples were diluted twenty times with deionized water. The diluted samples were mixed with the sample buffer and kept in a boiling water bath for 5 min. Electrophoresis was employed arranging a current of 30 mA per gel until the bromophenol blue marker reached the bottom of the gel. Following electrophoresis, gels were kept in a fixation solution overnight in order to fix proteins. These gels were stained with the dye solution for about 3 h in order to detect proteins, and then the coomassie-stained gels were washed with water.

Analysis of protein bands

Stained protein bands in gels were analyzed by using image analysis software (Phoretix 1D Pro, Totallab Inc., Warwickshire, UK). Protein band intensity was measured by removing the background intensity. The calibration curve was formed using two protein bands having the highest intensity values at 45 and 120 kDa molecular weight. The calibration curve was improved using different concentrations of egg yolk versus to the ratio of band volume (120/45). SDS-PAGE gel analysis was performed in triplicate (using three different shell egg samples) for the calibration. To obtain calibration curve, an average value of the yolk concentrations obtained from the gel analyses (for three egg samples) was used and standard deviation of these values were calculated. Standard deviation (±) of the values was represented using the error bars. The yolk concentration of the validation egg samples (the rest of the shell eggs, n = 11) were predicted by improved model. In addition, the yolk content of the liquid egg samples (containing additional egg white or water) were also predicted using the model. Accuracy of the model was evaluated by coefficient of determination value (R^2). Validation of the model was performed calculating relative error (RE, %) value defined by Eq. 1 (Goni et al., 2008).

$$RE(\%) = 100 \times \frac{(actual - predicted)}{actual}. \qquad (1)$$

The term of 'actual' refers to the real amount of the selected data, whereas the term 'predicted' refers to a value computed by the model. Limit of detection (LOD) and limit of quantification (LOQ) of values were also calculated for the model. LOD was estimated according to the expression $3.3\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. LOQ was calculated by using the expression $10\sigma/S$ (Pellati et al., 2004).

Results and discussion

The starting point of this research was to determine whether the liquid egg adulterated (with water) or not. This question was based on a rather simple analytical approach that analyze whether the liquid egg has the desired yolk:white ratio or not. When extra egg white is added to LWE, yolk concentration decreases, which also leads to a change in the ratio of yolk:white and Brix value. However, although addition of water causes a decrease in the yolk and egg white concentrations and °Brix value, it does not result in any change in the ratio of yolk:white.

Determination of yolk:white ratio in LWE

An SDS-PAGE analysis was performed using liquid egg samples including yolk:white at different concentrations ranging from 0 to 1000 g/kg with an interval of 100 g/kg (in a total ten points). The SDS-PAGE gel image of protein bands of the samples is presented in Fig. 1. Molecular weights of the proteins of the egg white and the yolk were determined using marker proteins (lane 15). Both the egg white and the yolk proteins were sorted according to their molecular weights from top to the bottom. Ovalbumin and conalbumin in the albumen (lane 14) were determined at molecular weights of 45 kDa and 79 kDa, respectively. The measured molecular weights were found to be

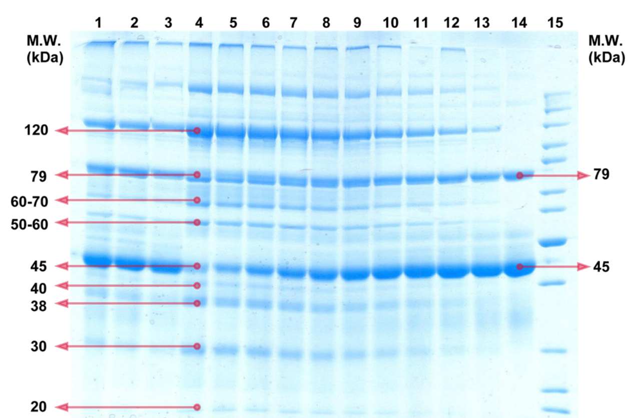

Fig. 1 SDS-PAGE gel image of protein bands of liquid egg samples. 1. LWE, 2. LWE, 3. LWE, 4. 1000 g/kg-Y, 5. 900 g/kg-Y, 6. 800 g/kg-Y, 7. 700 g/kg-Y, 8. 600 g/kg-Y, 9. 500 g/kg-Y, 10. 400 g/kg-Y, 11. 300 g/kg-Y, 12. 200 g/kg-Y, 13. 100 g/kg-Y, 14. 0 g/kg-Y, 15. marker. *Y* Yolk, *LWE* liquid whole egg

consistent with the results presented in the literature (Awadé and Efstathiou, 1999). Since ovalbumin (540 g/kg) and conalbumin (120 g/kg) are the most abundant proteins in the albumen, they can be easily monitored (Alleoni and Antunes, 2004; Belitz et al., 2009; Pipich et al., 2008). On the other hand, the amount of other albumen proteins (ovomucin 35 g/kg, lysozyme 34 g/kg, etc.) were considerably low compared to the ovalbumin and conalbumin; therefore, it was concluded that determining them in the gel image is not a practical procedure. However, much more proteins could be described in the egg yolk compared to the albumen. As can be seen from the gel image in Fig. 1, the egg yolk (lane 4) comprises a number of polypeptides with a variety of molecular weights ranging from 20 to 200 kDa. In order to identify these polypeptides in the gel image, the results obtained from different studies in the literature were utilized, which were listed in Table 1S. While the LDL apoproteins were determined at 120 kDa (apovitellenin Va) (Naderi et al., 2014), between 60 and 70 kDa (Anton et al., 2000; Freschi et al., 2011), between 50 and 60 kDa (apovitellenin III) (Naderi et al., 2014; Strixner and Kulozik, 2013), and 20 kDa (apovitellenin II) (Strixner and Kulozik, 2013), HDL apoproteins were identified at 79 kDa (Le Denmat et al., 2000) and 30 kDa (Le Denmat et al., 2000; Strixner and Kulozik, 2013). The other free proteins; α-livetin, α-, β- livetins, and phosvitin were observed between 50 and 60 kDa (Naderi et al., 2014; Strixner and Kulozik, 2013), between 38 and 40 kDa (Navidghasemizad et al., 2014), and 45 kDa (Le Denmat et al., 2000), respectively.

When the gel image (Fig. 1) has been monitored, it can be seen that protein band intensity changes depending on yolk:white ratio. More specifically, when the ratio of the yolk in liquid egg is high, the band intensity of the yolk proteins is also high. On the other hand, when the ratio of the egg white in liquid egg is high, the band intensity of the albumen proteins is high. It can be concluded that these band intensities are directly related to the concentration of proteins; and thus, the band intensities can be used to detect the egg yolk:white ratio in the liquid egg. In order to determine yolk:white ratio in the liquid egg, one protein band representing the concentration of egg white and another protein band representing the concentration of the yolk were selected to construct the calibration curve. The ratio of the band intensities was used to eliminate the errors that can be encountered in the case of using single protein band intensity in the gel image. In Fig. 1, ovalbumin (45 kDa) and apovitellenin Va (120 kDa) were selected for representation of the albumen and the yolk, respectively. They were preferred due to their high intensity bands in the albumen (lane 14) and the yolk (lane 4) gel profiles. Furthermore, there is no protein band at 120 kDa in the albumen lane (14), so this band represents only the yolk concentration. Although ovalbumin and phosvitin bands are present at 45 kDa, phosvitin (lane 4) can be neglected because of its low band intensity. As seen in Fig. 1, when the albumen concentration increases in the liquid egg, ovalbumin band intensity (45 kDa) also increases. Similarly, an increase in the yolk concentration results in an increase in the apovitellenin Va band intensity (120 kDa). Band volume intensity of the egg proteins was determined by using an image analysis program. The detected protein bands of the samples were indicated in Fig. 1S. A protein band graph (Fig. 2S) of each lane was formed with pixel position of the bands versus intensity. Then, the band volume intensity of each protein in the corresponding lane was determined.

Band volume intensities of the ovalbumin and apovitellenin Va proteins were selected according to their pixel position in the lane. The measured band volume intensities of these proteins between the lanes from 14 to 4 are presented in Table 2S. These lanes were used in the construction of the calibration curve that contains liquid egg with a concentration of yolk from 0 (lane 14) to 1000 g/kg (lane 4). As can be seen in Table 2S, a decrease in the band volume intensity of ovalbumin was observed depending on the increase in the yolk concentration in the liquid egg. There was also an increase in the band volume intensity of apovitellenin Va; however, a band volume intensity of apovitellenin Va (120 kDa) in the albumen sample was not detected as seen in Fig. 1 (lane 14). Therefore, the intensity value was set as 0. On the other hand, the band volume intensity at 45 kDa in the yolk sample (lane 4) could be measured thanks to the presence of phosvitin protein. The ratio of band volume intensities (120/45 kDa) of apovitellenin Va and ovalbumin proteins was determined using the intensity values provided in Table 2S. Then, the calibration curve given in Fig. 3S was constructed using the ratios of the band volume (120/45 kDa) and the concentration of the yolk (from 0 to 1000 g/kg) in the liquid egg samples. A high coefficient of determination value (R^2= 0.992) was obtained from the model. In addition, limit of detection (LOD) and limit of quantification (LOQ) were calculated as 62 g/kg and 187 g/kg, respectively. Along with the increase in the yolk concentration, an increasing trend was also observed in the ratio values of the band volume.

In order to use the model for prediction of yolk:white ratio in different LWE samples, its accuracy was validated using different liquid egg samples with known actual yolk:white ratio (n = 11, including different shell eggs in the size of small, medium, and large). For that purpose, SDS-PAGE analysis of these new samples was performed, and SDS-PAGE image of these liquid egg samples was demonstrated in Fig. 2. Similar to the previous (Fig. 1) analysis, the band volume intensities of the proteins for

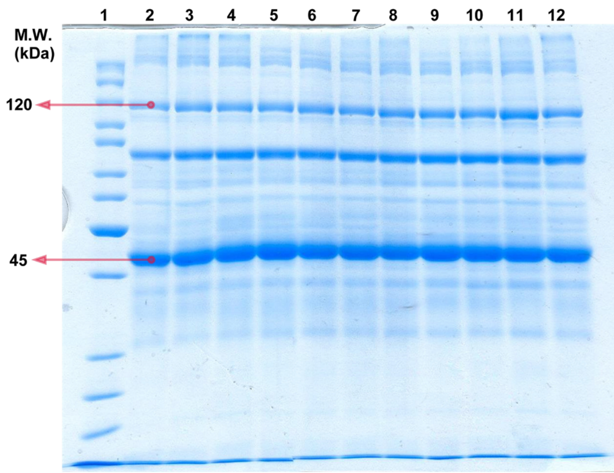

Fig. 2 SDS-PAGE gel image of the protein bands of eleven liquid egg samples (from lane 2 to 12)

each lane (from 2 to 12) in Fig. 2 were also obtained using the image analysis program. Then, the ratio of the band volume intensity of apovitellenin Va and ovalbumin proteins (120/45 kDa) of each sample was calculated. By substituting the ratio of band volume intensities of these proteins in the model, concentration of the yolk (g/kg) in the samples was predicted. The actual and the predicted concentration of the yolk (g/kg) in eleven liquid egg samples are listed in Table 3S. The yolk concentration of all samples was higher than the LOQ value (> 187 g/kg). In addition, these eleven egg samples do not recognize calibration samples (three different shell eggs) so that they were new sample for the calibration model. As can be seen in Table 3S, yolk concentration of 90.9% of these samples (ten of eleven samples) was predicted with a relative error less than 10%. In another study by Goni et al. (2008), prediction accuracy of the method was evaluated based on the relative error of results to be less than 10%.

Determination of yolk:white ratio in LWE containing egg white

An SDS-PAGE analysis was performed using the samples of LWE containing extra egg white. Extra egg white was added to LWE (normal yolk:white ratio, 30(%):70(%)) at the ratio of 65%, 55%, 40%, 30%, 20%, 10%, and 5% of egg yolk. After addition of extra egg white, yolk(%):white(%) ratios of the samples were obtained as 10.5:89.5, 13.5:86.5, 18:82, 21:79, 24:76, 27:73, 28.5:71.5, 30:70. °Brix value of the samples was measured to obtain prior information about liquid egg content. °Brix value of the samples is provided in Table 1. °Brix value of LWE was measured as 25.8. As can be seen in Table, as egg white was added in LWE, a decrease in °Brix value was occurred gradually. The reason for this decline is that the egg white contains less dry matter content (121 g/kg) than egg yolk (513 g/kg) (Belitz et al., 2009). After addition of over 10% egg white, Brix value of the samples decreased below 24. The SDS-PAGE image of these samples is presented in Fig. 3, where it can be seen that while the albumen concentration increases (from lane 9 to 2) in the liquid egg, the band intensity of apovitellenin Va (120 kDa) decreases due to the decrease in the yolk concentration. However, no considerable change in the band intensities of ovalbumin and phosvitin proteins (45 kDa) was observed. The band volume intensity of the proteins for each lane (from 2 to 9) was determined with the image analysis program. The measured band volume intensities of apovitellenin (120 kDa) and ovalbumin (45 kDa) are listed in Table 4S. As presented in the table, the band volume intensity of apovitellenin Va increases (from lane 2 to 9) due to the decrease in the albumen concentration. Considering the band at 45 kDa, similar values were obtained in the band intensity volume.

The ratio of the band volume intensity (120/45 kDa) was determined using the intensity values given in Table 4S. Yolk concentrations of the samples were predicted utilizing the model, where the ratio intensity values were used. The actual and the predicted concentration of the yolk (g/kg) in the samples are provided in Table 1. The relative error values have demonstrated that the model can predict the yolk concentration with quite high accuracy (error values less than 10%) for the samples containing additional egg white up to 30%. However, the model loses its validity when the yolk concentration of the samples with additional egg white is 40, 55, and 60% (the obtained error values were higher than 10% at these rates). This may be due to the fact that the band intensity value of apovitellenin Va (120 kDa) could not be measured correctly because of decreasing band volume (lane 2, 3, and 4). In addition, actual yolk concentration of these samples is below the LOQ value (< 187 g/kg). However, it can be safely concluded that the model is applicable in predicting yolk concentration of LWE samples containing more than 200 g/kg yolk concentration.

As a result of two analyses (°Brix control and SDS-PAGE), °Brix value and yolk:white ratio of the samples changed as extra egg white was added in LWE. Therefore, change in liquid egg yolk:white ratio was demonstrated with two different analyses supporting each other.

Determination of yolk:white ratio in LWE containing water

An SDS-PAGE analysis of LWE samples containing water was conducted. Water was added to LWE at the ratio of 65%, 55%, 40%, 30%, 20%, 10%, and 5% (w/w) of egg yolk content (30%). °Brix value of the samples containing

Table 1 Predicted concentration of yolk in the samples containing egg white

Lane	Additional egg white (%)	°Brix value	Actual concentration of the yolk (g kg − 1)	Predicted concentration of the yolk (g kg^{-1})	RE[a] (%)
2	65	17.7	105	159	50.9
3	55	19.6	135	191	41.1
4	40	21.2	180	210	16.4
5	30	23.1	210	216	2.7
6	20	23.8	240	258	7.4
7	10	24.7	270	272	0.8
8	5	25.2	285	294	3.0
9	0	25.8	300	301	0.2

[a]Relative error

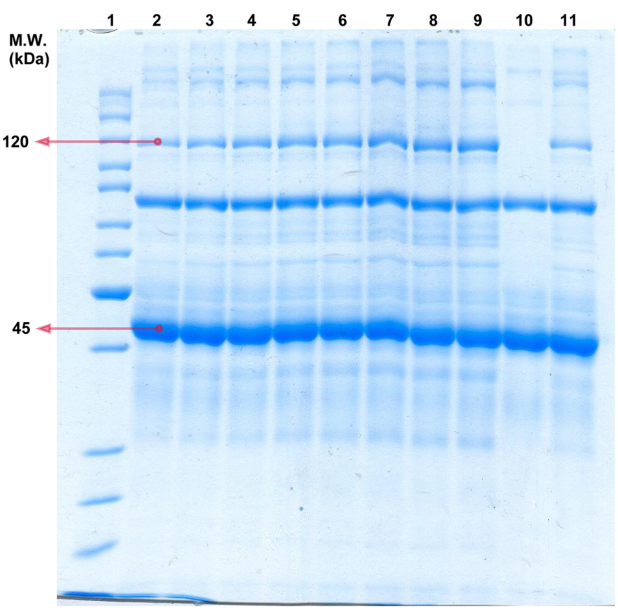

Fig. 3 SDS-PAGE gel image of protein bands of liquid egg samples containing extra egg white (%) instead of yolk content. 1. Marker, 2. 65%—EW, 3. 55%—EW, 4. 40%—EW, 5. 30%—EW, 6. 20%—EW, 7. 10%—EW, 8. 5%—EW, 9. LWE. *EW* Egg white, *LWE* liquid whole egg

extra water was given in Table 2. °Brix value of the samples decreased sharply as water was added in LWE due to the absence of dry matter in water. After addition of over 5% water, Brix value of the samples decreased below 24. The gel image of these samples is provided in Fig. 4, where it can be seen that the protein band intensities of the albumen and the yolk (from 2 to 9) decrease as water content of the samples increase. A decrease in the band volume of apovitellenin Va and ovalbumin was also observed in the lanes (from 2 to 9). The band volume intensity of the proteins in each lane (from 2 to 9) was determined with the image analysis program. The measured band volume intensities of apovitellenin (120 kDa) and ovalbumin (45 kDa) were listed in Table 5S. The band volume intensity of apovitellenin Va and ovalbumin (from lane 2 to 9) decreases due to the increase in the additional amount of water. The ratio of the band volume intensity (120/45 kDa) was determined using the intensity values given in Table 5S. The ratio of the band intensity does not change over the samples (from lane 2 to 9) since a simultaneous decrease in the band intensity was observed for both ovalbumin and apovitellenin Va proteins. Yolk concentration of the samples was predicted with the model using the ratio intensity values (120/45 kDa). The actual and the predicted concentrations of the yolk (g/kg) in the samples are presented in Table 2. Actually, addition of water to LWE samples caused a decrease in the both albumen and yolk concentrations. As a result, the actual yolk concentration decreased by addition of water. However, no difference among the predicted yolk concentration values was observed since the same ratios of the band intensity were used in the model.

As a result of Brix and SDS-PAGE analyses, addition of water caused decrease in Brix value of the samples but did not change the yolk:white ratio of liquid egg. Therefore, water adulteration in liquid egg could be detected applying these analyses. If yolk:white ratio of unknown liquid egg sample is desired to be checked from food producer, these analyses are performed. If both Brix and yolk:white ratio of liquid egg sample are different from expected values of LWE, egg white is added in liquid egg. However, if water is added in liquid egg, Brix value will change but yolk:white ratio will not.

In food applications, producers may demand liquid egg at different ratios of yolk:white (such as higher egg white content) to achieve food in different structures. However, food fraudsters might do adulteration during Brix adjustment of the liquid egg by using its own non-compositional substance (such as water). Therefore, it is highly critical to check whether the liquid egg is prepared as desired or not.

Table 2 Predicted concentration of the yolk in the samples containing water

Lane	Additional water (%)	°Brix value	Actual concentration of the yolk (g kg^{-1})	Predicted concentration of the yolk (g kg^{-1})
2	0	25.8	300	319
3	5	24.0	285	321
4	10	22.7	270	330
5	20	20.3	240	302
6	30	18.7	210	309
7	40	16.9	180	306
8	55	11.2	135	288
9	65	8.7	105	268

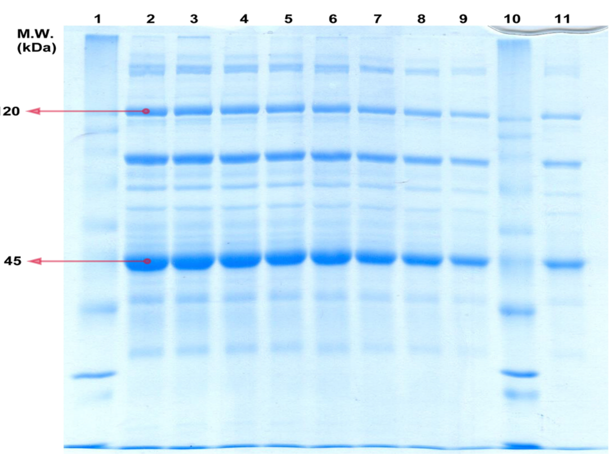

Fig. 4 SDS-PAGE gel image of protein bands of liquid egg samples containing extra water (%) instead of yolk content. 1. Marker, 2. LWE, 3. 5%—W, 4. 10%—W, 5. 20%—W, 6. 30%—W, 7. 40%—W, 8. 55—W%, 9. 65%—W. *W* Water, *LWE* liquid whole egg

To this end, the aim of the present work was to detect the LWE adulteration with water. It was demonstrated how the SDS-PAGE gel image and Brix value changes depending on the use of egg white or water in the liquid egg samples. Calibration curve obtaining a high determination of coefficient value (R^2 = 0.992) was formed. Yolk concentration of LWE unknown samples (n = 10) were predicted with a high success rate (relative error less than 10%) using the calibration model. Yolk concentration of LWE samples containing additional egg white or water were analyzed using SDS-PAGE and Brix measurements. If the liquid egg sample contains water instead of egg white, the yolk concentration will be in the nominal range as the yolk:white ratio does not change. Water addition in LWE resulted in a change in the Brix measurements (from 25.8 to 8.7) of the samples but not in the yolk concentration (between in the range of 268–330 g/kg) of the samples. However, when the liquid egg sample contains additional egg white, the yolk concentration will be less than the nominal range, which leads to a change in the yolk:white ratio. Therefore, addition of egg white in LWE led to decrease in both yolk concentration (from 301 to 159 g/kg) and Brix measurements (from 25.8 to 17.7) of the samples. This method is an ad hoc way of analyzing any unknown liquid egg sample in which the adulteration (with water) and yolk:white ratio can easily be detected. In conclusion, a combination of SDS-PAGE and Brix analysis is a promising technique to determine water adulteration and yolk:white ratio in liquid egg.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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