Denaturation of Spray-dried Egg Yolk During Processing and Storage

Kyuya NAKAGAWA¹, †, Shuji ADACHI¹, Akihiro HANDA²

¹ Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku Kitashirakawa Oiwakecho, Kyoto 606-8502, Japan
² R&D Division, Kewpie Corporation, 2-5-7 Sengawa-cho, Chofu-shi, 182-0002 Tokyo, Japan

Egg yolk powder is industrially produced by spray-drying fresh raw egg yolk separated from shell eggs. Stress due to heat and dehydration causes denaturation of egg yolk proteins, so it largely affects the quality level of the resultant dried powders. In this study, denaturation kinetics of dried egg yolk powder were measured under isothermal and iso-humidity conditions. The denaturation level was evaluated by using color index, viscosity values of the slurry (made from the dried powder dispersed in water) and the agglomerated particle sizes. Fresh egg yolk was spray-dried and the resultant quality levels of the dried powders were evaluated in the same manner. Some of these quality factors would not simply relate with the temperature and RH. It was suggested from the kinetic parameters and the spray-drying experiments that the residence times of products in the specific parts of a drying system (e.g. atomizer, chamber, pipe for transport, collector etc.) would have critical impacts on the product qualities. The kinetic parameters predicted that even under a relatively low RH conditions, a rate of a quality change would significantly increase when products are subjected to higher temperature above threshold.

Keywords: spray-drying; denaturation; kinetics; egg yolk

1. Introduction

Quality control is always an issue in food manufacturing, and of interest for food engineers. Egg powder production is an industrialized process, where pre-treated egg white, egg yolk or whole egg separated from shell eggs are spray-dried to convert into powder forms. As expected, stress due to heat and dehydration causes denaturation of egg proteins, so it largely affects the quality level of the resultant dried powders. Dry egg powders are widely used in food industry for manufacturing bakery food, mayonnaise, confections, ice cream etc. Dried eggs gain significant advantages in transportation, handling, storage, uniformity and microbiological safety as in the case of common dried foods [1,2]. Qualities are strongly affected by the selected drying method and conditions, so numbers of researches have been conducted to clarify the relationships between processing parameters (e.g. temperature, humidity, air velocity etc.) and product qualities such as color, microstructure, retention of antioxidants, etc. [2-6] Important physicochemical properties of egg powders that change during storage are commented in the publication of Rao and Labuza (2012) such as turbidity, solubility, residual denaturation enthalpy, surface hydrophobicity, sulphhydril content and aggregation[7]. Some changes in properties affects expected functionality of the product. It is useful if a final product property can be predicted by counting possible stress given to the product during processing and storage. Our interests in this study is to apply denaturation kinetics of dried egg collected under isothermal and iso-humidity condition to predict denaturation degree as a result of processing and storage where temperature and humidity change dynamically. Considering rather slow denaturation rate (or deterioration rate) of egg white, major stress would be given during long time storage. Egg yolk mainly contains lipid (triacylglycerol and phospholipid), protein, carbohydrates and some minor components such as vitamins and minerals. It rather easily receives thermal and dehydration stress, so a processing gives certain damages to the product [8-10].

In this study, commercial dried egg yolk powder was employed for deterioration test to obtain denaturation kinetics data measured under isothermal and iso-humidity conditions. The denaturation level was evaluated by using color index, viscosity values of the slurry (made from the dried egg yolk powder) dispersed in water and the agglomerated particle sizes. Fresh egg yolk was spray-dried and the resultant quality levels of the dried powders were evaluated in the same manner. Some of these quality factors would not simply relate with the temperature and RH. It was suggested from the kinetic parameters and the spray-drying experiments that the residence times of products in the specific parts of a drying system (e.g. atomizer, chamber, pipe for transport, collector etc.) would have critical impacts on the product qualities. The kinetic parameters predicted that even under a relatively low RH conditions, a rate of a quality change would significantly increase when products are subjected to higher temperature above threshold.
powder dispersed in water) and the agglomerated particle sizes. Fresh egg yolk was spray-dried under various conditions and the resultant quality levels of the dried powders were evaluated in the same manner.

2. Materials and methods

2.1 Materials

Commercial dried egg yolk powder (Y1-F, Henningsen Foods Inc., USA) was employed in this work for deterioration test. Fresh egg yolk (gifted from Kewpie Corp.) was used for spray-drying experiment within 48 h from its production. All the other chemicals used in this work were analytical grade.

2.2 Deterioration test

Dried egg yolk powder was desiccated with a saturated solution of NaCl (75% of relative humidity), Mg(NO₃)₂ (59%RH) and LiCl (11%RH) at 4°C for three days for equilibration. After equilibration, certain amount of powder was taken out from the desiccator, and immediately wrapped by aluminum foil. These wrapped powder specimens were set in a thermal block (made by aluminum, with holes for setting test tubes) of which temperature was controlled by external temperature devise at 50, 60, 70 and 75°C. Specimens were deteriorated at these temperatures for selected time durations, and immediately cooled down in an ice bath in order to stop further deteriorations. After specimens were taken out from the ice bath, they were equilibrated at room temperature and unwrapped for use in subsequent analyses. All analyses below were conducted at least triplicate.

2.3 Color analysis

Color of the deteriorated specimens were analyzed by a color meter (SA4000, NIHON DENSOKU INDUSTRIES, Co., Ltd., Japan). The apparatus was calibrated with a standard white before use. Then powders were placed in a container, the sample is put on the analysis area and coved to reduce the effect of external light. The machine gives four parameters: L for brightness, a for color (green to red), b for color (yellow to blue) and ΔE, color difference between the sample and the standard.

2.4 Viscosity measurement

The deteriorated egg yolk powder was mixed with water in the weight ratio of 1: 1.25 (egg yolk: water) to make a slurry. The viscosity of this slurry could reflect the degree of deterioration. A fixed amount of the slurry was placed in a rotary viscometer (TV-20, Toki Sangyo Co., Ltd., Japan), and the temperature was stabilized at 25°C with the water jacket surrounding the sample stage. The viscosity was started to measure at a speed of 0.3 rpm, and then the speed was augmented until the increment of the viscosity value was not observed (until around 2 rpm). Considering that the mixture is a non-Newtonian fluid, the stabilized value of viscosity was read. The viscometer is calibrated with a standard solution, 2668 mPa s at 25°C for a speed of 2 rpm.

2.5 Agglomerated particle size analysis

Agglomerated particle sizes were analyzed by image analysis. This analysis counts insoluble fractions of the agglomerated protein particles. The obtained values are expected to be corresponded to the degree of hydrophobicity [11]. Specimen powders were dispersed in water (egg yolk to water ratio at 1: 10) and observed with a microscope. Based on the obtained images, the mean particle sizes were estimated using image analysis software (ImageJ 1.40 g). At least 100 particle samples were counted from three different images of each specimen. A circular equivalent diameter was calculated to evaluate the sizes of particles.

2.6 Spray-drying fresh egg yolk

Fresh egg yolk was mixed with the same volume of water and spray-dried with a spray-drier (LB-8, Ohkawara Kakohki Co., Ltd., Japan) equipped with a rotary atomizer (Fig. 1). The inlet temperature was set to 120, 130 and 150°C, and the fresh egg yolk mixed with water (volume ratio at 1:1) was fed with a flow rate of 50 or 70 ml/min. Atomization rate was set around 15000 rpm. The resultant moisture content of the spray-dried powder was measured by using moisture analyser (MS-70, A&D Co., Ltd., Japan).

![Fig. 1 Spray-drying apparatus.](image-url)
3. Results and discussion

3.1 Color analysis results

One could observe a general trend that the brightness, $L$, of the deteriorated egg yolk powder slightly decreased with deterioration time. And this diminution slows down as decreasing temperature of the deterioration test. Furthermore, the gap between the various curves decreased with temperature. Samples desiccated at 75% RH were slightly darker than the other samples, indicating that the adsorbed moisture affected the change in color with time. The color change would mainly be caused by the Maillard reaction, where sugars and protein components react with each other, and water in the reaction system gives mobility for the reactants. As shown in Fig. 2, samples prepared at higher relative humidity had a more intense red color and the value of $a$ increased with time and temperature. The higher the temperature, the faster the red color intensity increased. Here, significant influence of the desiccation RH condition was not visible except the one prepared at 75% RH.

3.2 Viscosity measurement results

As in the case of color analyses, viscosity increased with the temperature of the deterioration test. The higher the temperature, the faster the viscosity increased (Fig. 3). We can also observe that the RH during desiccation clearly affected the kinetics of the viscosity change. Samples desiccated with higher RH had higher viscosity than the other when compared at the same temperature. Viscosity of the slurry would reflect the change in fluid friction with dispersed egg yolk particles. Protein components in the egg yolk particles could change its hydrophobicity as a cause of denaturation [8,9]. It thus affected the resultant viscosity values of slurries [10]. In other words, the present viscosity values could be fair indicators of a deterioration under thermal stress with controlled moisture contents.

Seeing from the trends in the viscosity value change in Fig. 3, they could be expressed by linear regressions. Fitting coefficients were determined by the least square

![Fig. 2 Color change during deterioration tests, L and a values.](image)

![Fig. 3 Viscosity change during deterioration tests (Solid lines are regression lines fitted with the slope coefficients ($k_{vis}$) in Table 1).](image)

© 2017 Japan Society for Food Engineering
method as summarized in Table 1. These values, namely kinetic constants, were plotted as functions of temperature and RH values to give a fitting surface (Fig. 5). This surface can be expressed by following polynomial equation.

\[
k_{\text{vis}}(T, RH) = 4.8 \times 10^{-1}T^2 + 1.0 \times 10^{-3}RH^2 + 4.6 \times 10^{-3}T \cdot RH - 5.3 \times 10^1 T - 3.2 \times 10^{-4} RH + 1.5 \times 10^1 \quad (1)
\]

Table 1  Kinetic constants of viscosity and particle size value change obtained by assuming zero order kinetics.

|        | 11%RH  | 59%RH  | 75%RH  |
|--------|--------|--------|--------|
| \(k\)  | \(\text{[Pa s/h]}\) | \(\text{[Pa s/h]}\) | \(\text{[Pa s/h]}\) |
| 50°C   | 14.2 \((R^2=0.94)\) | 18.4 \((R^2=0.88)\) | 19.4 \((R^2=0.88)\) |
| 60°C   | 26.8 \((R^2=0.97)\) | 30.9 \((R^2=0.94)\) | 72.6 \((R^2=0.97)\) |
| 70°C   | 108 \((R^2=0.99)\)  | 153 \((R^2=0.97)\)  | 179 \((R^2=0.83)\)  |
| 75°C   | 225 \((R^2=0.99)\)  | 255 \((R^2=0.87)\)  | 302 \((R^2=0.88)\)  |

|        | 11%RH  | 59%RH  | 75%RH  |
|--------|--------|--------|--------|
| \(k\)  | \(\text{[µm/h]}\) | \(\text{[µm/h]}\) | \(\text{[µm/h]}\) |
| 50°C   | 4.14 \((R^2=0.99)\) | 4.19 \((R^2=0.98)\) | 4.19 \((R^2=0.98)\) |
| 60°C   | 6.36 \((R^2=0.91)\) | 8.39 \((R^2=0.97)\) | 8.93 \((R^2=0.96)\) |
| 70°C   | 11.4 \((R^2=0.99)\) | 13.3 \((R^2=0.96)\) | 15.3 \((R^2=0.94)\) |
| 75°C   | 23.6 \((R^2=0.99)\) | 26.8 \((R^2=0.98)\) | 40.2 \((R^2=0.99)\) |

3.3 Particle size analysis results

As shown in Fig. 4, the trends in agglomerated particle size change were similar to the other quality factors. The mean sizes of the agglomerated particles increased with deterioration time and temperature. When the temperature was set at 50°C, the deviation among the samples desiccated at different RH were almost similar. If the particle agglomeration was caused by a certain denaturation of protein component, we can suggest that this types of denaturation did not occur in a significant way at a low temperature. The impact of RHs on the particle sizes increased with temperature, that is noticeable when comparison is made between 75% and 11%RH.

The trends in the agglomerated particle size value change, they could be expressed by linear regressions. The kinetic constants were determined by the linear least square fitting and listed in Table 1. These values were plotted as functions of temperature and RH values to give a fitting surface (Fig. 5) as written by following

Fig. 4  Change in agglomeration particle size during deterioration tests (Solid lines are regression lines fitted with the slope coefficients \((k)\) in Table 1).

Fig. 5  Fitting surfaces of kinetic constants.
Denaturation of egg yolk

3.4 Spray–drying fresh egg yolk

Fresh egg yolk was spray-dried under various drying conditions, and the denaturation levels of the resultant powders were analyzed in terms of the color index, viscosity values of the slurry and the agglomerated particle sizes. The results of the analyses were summarized in Table 2.

It was likely that the the residential time of powder in the drying chamber was influential on the resultant denaturation levels. One can see a rough trend that the denaturation level was higher when the operation time was longer even the operation was carried out with the same inlet temperature and flow rate, suggesting that the denaturation after drying was not negligible. Run 5 and 6 was carried out with the same operation condition, same outlet temperature, but with different operation time. Here, as expected, shorter residence time of the dried powder in the collector resulted in better quality parameters. This can be further confirmed in the comparison between Run 3 and 4, where ice bath was applied for Run 4 to cool down the collector temperature. This cooling resulted in decrease in slurry viscosity and slight decrease in agglomeration size (In Run 4, outlet temperature was slightly lower than Run 3.). When the inlet temperature and the feed flow rate was set at 130°C and 70 mL/min, the outlet temperature was at around 68°C (RUN 6). The outlet temperature decreased to 52°C by changing the inlet temperature to 120°C. Assuming that the dried powder stayed in the drying chamber and outlet pipes at these temperatures for 10 seconds at RH of 25% (for simplicity), the increase in slurry viscosity and agglomerate size at 68°C and 52°C could be estimated (by using the kinetic constant values in Table 1) to 0.24 Pas/0.025 µm and 0.042 Pas/0.012 µm, respectively. One cannot clearly confirm whether this estimation is reflected in the experimental values, however, the temperature range over 60°C may have a risk in the rapid change of the resultant qualities. Even under a relatively low RH conditions, a rate of a quality change at 70°C for several seconds could be equal to the rate at 50°C for more than 10 min. Based on the present kinetic constants, storing a powder at 50°C and RH of 25% for 10 min would give 2.5 Pas of slurry viscosity and 0.7 µm of agglomerate size increase. However, the difference appeared in the experimental values were clearly small in slurry viscosity and large in agglomerate size.

Let us estimate the denaturation levels of the egg yolk as a result of spray-drying by postulating temperature and RH history in the time course of processing. A virtual temperature and RH scenario can be deduced by reference to former publications [12,13]. First, the egg yolk was stocked in a reservoir tank at room temperature approximately around 15 min (in the present spray-drying test, total operation time was around 30 min, so this corresponds to a mean value). The RH=100% was applied to the RH value in solution. Solution in the tank (temperature was at 20°C) was continuously transferred to the atomizer, where the solution was immediately heated up in the channel of the atomizer of which temperature is

Table 2   Spray-drying conditions and quality parameters of the obtained powders.

| Run Nº | Rotation rate [rpm] | Inlet [ºC] | Flow rate [mL/min] | Outlet [ºC] | Collector [ºC] | Operation time [min] | Color, value a | Viscosity [Pa.s] | Agglomerate size [µm] | Moisture content [kg-water/kg-powder] |
|--------|---------------------|------------|-------------------|-------------|----------------|---------------------|----------------|----------------|---------------------|-------------------------------------|
| 1      | 15313               | 120        | 50                | 62          | 54             | 40                  | 7.41           | 0.28            | 11.9                | 0.059                               |
| 2      | 15371               | 120        | 70                | 52          | 57             | 28                  | 7.87           | 0.30            | 9.8                 | 0.056                               |
| 3      | 14996               | 130        | 50                | 78          | 60             | 30                  | 7.40           | 0.53            | 17.8                | 0.044                               |
| 4*     | 15255               | 130        | 50                | 71          | 50             | 27                  | 8.33           | 0.39            | 17.1                | 0.056                               |
| 5      | 14996               | 130        | 70                | 68          | 50             | 7                   | 8.49           | 0.41            | 18.3                | 0.038                               |
| 6      | 14751               | 130        | 70                | 68          | 50             | 19                  | 8.66           | 0.44            | 22.2                | 0.037                               |
| 7      | 15237               | 150        | 50                | 80          | 60             | 27                  | 7.18           | 6.4             | 16.0                | 0.032                               |
| 8      | 16200               | 150        | 70                | 75          | 46             | 16                  | 8.97           | 7.3             | 19.9                | 0.049                               |

*In the operation of Run 4, ice bath was applied to the collector exterior in order to cool it down.
close to the inlet air temperature (ca 130°C). Residence time of the solution was several seconds, but it allows rapid temperature increase up to around 70°C considering heat transfer rate. Solutions were subsequently atomized in the drying chamber where the product temperature and RH dramatically reduced due to the removal of water in a few seconds. The temperature of the dried product increased to the outlet temperature (ca 70°C), then transferred from the chamber to the collector via the transportation pipes of which temperature reached over 50°C. Final products were stored in this collector approximately more than 20 min where the RH was held at around 25%. Slurry viscosity and agglomeration degree were roughly estimated by adopting the kinetic constants given by the equation (1) and (2) with assumption of zero order kinetics to the above scenario. The resultant values for the slurry viscosity and agglomeration degree were 7.3 Pas and 20 µm, respectively. These values were not too far from the experimentally obtained values. It was interesting to note that this calculation predicts that the major quality loss would be due to the residence in drying chamber and storage collector. It is worth noting that the residence times of products in the specific parts of a drying system (e.g. atomizer, chamber, pipe for transport, collector etc.) have quite important meaning in order to estimate the resultant quality. Here we must recognize again an importance of modeling approach for spray-drying that covers whole processing steps [14–16]. Furthermore, detailed understanding of a food deterioration kinetics is of critical importance in predicting resultant qualities of dried food products.

4. Conclusion

In this study, the deterioration kinetics of dried egg yolk powder desiccated under constant relative humidity (RH) was measured under isothermal and iso-humidity conditions. Fresh egg yolk was spray-dried and the resultant quality levels of the dried powders were evaluated. Product qualities were evaluated in terms of viscosity values of the slurry (made from the dried powder dispersed in water) and the agglomerated particle sizes. Some of these quality factors would not simply relate with the temperature and RH. It was suggested from spray-drying experiments that the residence times of products in the specific parts of a drying system (e.g. atomizer, chamber, pipe for transport, collector etc.) would have critical impacts on the product qualities. Even under a relatively low RH conditions, a rate of a quality change might dramatically increase when products are subjected to higher temperature above threshold.

References

1) S.V. Jangam; An overview of recent developments and some R&D challenges related to drying of foods. Dry. Technol., 29, 1343-1357 (2011).
2) F. N. Fernandes, S. Rodrigues, C. Law, A. Mujumdar; Drying of exotic tropical fruits: A comprehensive review. Food Bioproc. Technol., 4, 163-185 (2011).
3) A. Wojdylo, A. Figiel, K. Lech, P. Nowicka, J. Oszmiański; Effect of convective and vacuum–microwave drying on the bioactive compounds, color, and antioxidant capacity of sour cherries. Food Bioproc. Technol., 7, 829-841 (2014).
4) A. Vega-Gálvez, K. Ah-Hen, M. Chacana, J. Vergara, J. Martinez-Monzó, P. García-Segovia, R. Lemus-Mondaca, K. Di Scala; Effect of temperature and air velocity on drying kinetics, antioxidant capacity, total phenolic content, colour, texture and microstructure of apple (var. Granny smith) slices. Food Chem., 132, 51-59 (2012).
5) C. H. Chong, C. L. Law, M. Cloke, L. C. Abdullah, W. R. Wan Daud; Drying models and quality analysis of sun-dried ciku. Dry. Technol., 27, 985-992 (2009).
6) K. J. Chua, A. S. Mujumdar, M. N. A. Hawlader, S. K. Chou, J. C. Ho; Batch drying of banana pieces — effect of step-wise change in drying air temperature on drying kinetics and product colour. Food Res. Int., 34, 721-731 (2001).
7) Q. Rao, T. P. Labuza; Effect of moisture content on selected physicochemical properties of two commercial hen egg white powders. Food Chem., 132, 373-384 (2012).
8) A. Handa, N. Kuroda; Functional improvements in dried egg white through the maillard reaction. J. Agr. Food Chem., 47, 1845-1850 (1999).
9) A. Handa, K. Hayashi, H. Shidara, N. Kuroda; Correlation of the protein structure and gelling properties in dried egg white products. J. Agr. Food Chem., 49, 3957-3964 (2001).
10) T. Jaekel, W. Ternes; Changes in rheological behaviour and functional properties of hen’s egg yolk induced by processing and fermentation with phospholipases. Int. J. Food Sci. Tech., 44, 567-573 (2009).
11) T. Jarungumlert, K. Nakagawa, S. Adachi; Influence of aggregate structure of casein on the encapsulation efficiency of β-carotene entrapped via hydrophobic interaction. Food Struct., 5, 42-50 (2015).
12) V. S. Birchal, L. Huang, A. S. Mujumdar, M. L. Passos; Spray dryers: Modeling and simulation. Dry. Technol., 24, 359-371 (2006).
13) D. Dobry, D. Settell, J. Baumann, R. Ray, L. Graham, R. Beyerinck; A model-based methodology for spray-drying process development. J. Pharm. Innov., 4, 133-142 (2009).
14) T. A. G. Langrish, D. F. Fletcher; Spray drying of food ingredients and applications of CFD in spray drying. Chem. Eng. Process.: Process Intensification, 40, 345-354 (2001).
15) D. Fletcher, B. Guo, D. Harvie, T. Langrish, J. Nijdam, J. Williams; What is important in the simulation of spray dryer performance and how do current cfd models perform? Appl. Math. Model., 30, 1281-1292 (2006).
16) M. W. Woo, W. R. W. Daud, A. S. Mujumdar, Z. Wu, M. Z. Meor Talib, S. M. Tasirin; CFD evaluation of droplet drying models in a spray dryer fitted with a rotary atomizer. Dry. Technol., 26, 1180-1198 (2008).
卵黄を噴霧乾燥する工程におけるタンパク質変性度の予測は、乾燥卵黄製造における品質維持に重要な課題の1つである。変性とかかわる現象は種々多様であり、変性度を1つの因子にて評価することは難しい。そこで本研究では乾燥卵黄の変性度を、加水した懸濁液の粘度、粉末の色彩度、加水した懸濁液の凝集粒子径の3つの観点から評価し、恒温、恒溼度の静的な条件下において測定した変性度変化速度を、噴霧乾燥過程にて進行する卵黄の変性の予測に適用することを目指した。

まず、比較的変性度の低い噴霧乾燥卵黄を、飽和塩溶液を用いてそれぞれ相対湿度11%，59%，75%に保持させたデシケータ中で、3日間4℃にて平衡化させた。平衡化させた粉末試料を直ちにアルミフォイルでラッピングし、50，60，70，75℃にて一定時間恒温処理した。恒温処理後の試料は速やかに氷浴中で冷却し変性の進行を止めた。得られた粉末試料の色彩度、試料に一定量加水し作製した懸濁液の粘度、加水した懸濁液の凝集粒子径を測定した。測定の結果、温湿度に依存した変性の進行を確認できた。懸濁液の粘度、凝集粒子径については温度、平衡化湿度が高いほど変化が速くなる0次的な変化を確認できたが、色彩度についてはその動力学が異なることが伺えた。液卵黄を等量の水と混合し、入り口温度120，130，150℃、フィード流量50，70 mL/min、アトマイザ回転速度15000 rpmの噴霧乾燥条件により粉末を作製し、得られた粉末試料についても先と同様の分析を行った。操作条件の変更に応じて最終的に得られる粉末卵黄の変性度も異なっていた。変性度値の比較より、乾燥に伴う脱水による変性の進行に加え、装置内部の特異的な部位（アトマイザ、出口配管、製品コレクタなど）における品質変化が無視できないほど大きいことが予見された。静的な条件で求めた変性度変化を用いた試算からも、低湿度の環境において一定の温度以上にて急激な変性度の増加が予測でき、製品品質に大きなインパクトを与えていくことが考えられた。