Effects of freeze-drying and spray drying processes on functional properties of phosphorylation of egg white protein

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Abstract: Egg white protein (EWP) was phosphorylated with Sodium Tripolyphosphate (STP) at pH 4.5. Freeze drying and spray drying were used for drying purpose and the effects of these drying methods on the functional properties were investigated. The functional properties of native and modified proteins were also determined. The results demonstrated that phosphorylation of EWP markedly improved its functional properties, and that it was more effective for the food industry. The freeze-dried STP-EWP powders were superior in terms of solubility, emulsion stability, water holding capacity, oil and water absorption capacity and heat gel strength than spray-dried STP-EWP powders. The results in viscosity showed no significant differences between freeze-dried and spray dried. The spray-dried powders were better in terms of foaming ability and foam stability than freeze-dried powders. However, the spray drying required the longest time to produce. Freeze drying was found to be the best method in terms of production of modified EWP powders with superior functional properties.

Keywords: freeze drying, spray drying, egg white protein, phosphorylation, functional properties

DOI: 10.3965/j.ijabe.20150804.1942

Citation: Liu L L, Wang H, Ren G Y, Duan X, Li D, Yin G J. Effects of freeze-drying and spray drying processes on functional properties of phosphorylation of egg white protein. Int J Agric & Biol Eng, 2015; 8(4): 116－123.

1 Introduction

Egg white protein (EWP) is an important source of highly nutritional and functional ingredients in a wide variety of food products. The functional property of protein refers to its specific effects in the process and the storage of food[1,2]. To EWP, it is the reflection of the functional properties: solubility, emulsification, foamability and gelation. For further industrial uses, it is desirable to improve their functional properties.

Many attempts have been made to develop a rational molecular design using chemical and enzymatic modifications of proteins to improve their gelling, water-holding capacity, foaming and emulsifying properties. Chemical modification of protein is very important in biological systems, as well as in vitro. Modification occurs unintentionally during food processing, storage and cooking and intentionally in modification of proteins for improved functionalities (color, flavor, taste, texture and solubility). One of the most important intentional modifications of proteins is phosphorylation[3,4]. The phosphorylation method is applicable for practical use, because pyrophosphate is permitted as a food additive in food industry in many countries. Over the past few decades, several phosphorylation methods have been reported by some researchers[5-9]. However, these phosphorylation methods posed some problems, making them very

Received date: 2015-05-13 **Accepted date:** 2015-07-22

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difficult to be practically applied.

The volume of liquid egg and egg white used in formulations increases constantly. In 2011, approximately 40% of all eggs produced in the USA were further processed for food service, manufacturing, retail and export. Although the production of frozen eggs has leveled out, some growth has been noted in dried egg production. The physicochemical and functional properties of the phosphorylation of EWP (STP-EWP) are strongly influenced by processing conditions and the method of manufacture. Drying is an important process which helps in converting the food solutions into dry solid form[10,11]. The dry solid forms of materials commonly have longer shelf-life, smaller volume requirement, and can be transported and handled with relative ease. The drying process inhibits the enzymatic degradation and limits the microbial growth. Drying is one of the oldest methods of processing and preserving food for later use. It is a complex operation involving heat and mass transfer which may cause change of food quality.

Spray-drying has been widely used for drying heat sensitive drugs and foods because time that materials exposure to high temperature is short, normally ranging from 5 s to 30 s, but solvents can be evaporated rapidly from the sprayed droplets. However, rapid solvent evaporation may influence and modify the crystalline structure of some substances. EWP usually dried by spray-drying. However, the dramatic increase in surface combined with temperatures above 60°C over several days leads to thermal denaturation of proteins. That is why the usage of spray-dried EWP in commercial food processing is rather limited[12]. As a consequence of this, spray-dried STP-EWP shows enormous impairment in important functional properties. As numbers of drying methods are available to produce STP-EWP and these drying methods should be chosen depending on their drying efficiency and quality of the dried products. It is reported that freeze-drying proteins better retain their native structural form because they suffer less thermal and water evaporation related stresses. The freeze dried particles have higher porosity and shrink less[13]. However, the spray-drying process causes more shrinkage in particles and produces denser particles. These structural and morphological differences in dried products bring about significant differences on the functional properties of food proteins[14]. Phosphorylation of EWP markedly improved its functional properties such as solubility, emulsifying property, foaming ability and foam stability[1,2,6]. These functional properties greatly affect the texture and sensory perception in products[15]. As mentioned above, the drying process can bring about structural changes in STP-EWP and ultimately affect the quality of food in which the STP-EWP is incorporated.

However, there have been no published reports on the effects of spray-drying and vacuum-freeze drying on the functional properties of STP-EWP. Therefore, the objective of this paper was to examine the effects of spray-drying and freeze drying on the solubility, emulsifying ability and stability, viscosity, foaming ability and stability, water and oil absorption capacities, heat induced gel strength.

2 Materials and methods

2.1 Materials

All shell eggs were purchased from Denis Supermarket (Luoyang, China). Native protein (N-EWP) was prepared as follows: egg white, separated from infertile eggs (less than 10 days after laid), was homogenized, acidified to pH 4.5 with 1 M HCl, and then centrifuged. The supernatant obtained was diluted with an equal volume of distilled water, dialyzed and then lyophilized. All reagents used were of analytical grade.

2.2 Preparation of STP-EWP

STP-EWP was prepared according to the method proposed by Hirofumi et al. with some modifications[7].

2.3 Drying treatment

Spray-drying: Spray-drying was carried out using a Nitro Utility spray dryer with a 0.5 mm nozzle (GEA Nitro Process Engineering, Denmark). The spray-dried STP-EWP powder was collected and is referred to as STP-EWP-S.

Freeze drying: For preparing freeze-drying samples, a Beta2-8LD type vacuum freeze-dryer (Christ, Germany) was used to prepare freeze-dried STP-EWP. The dried

STP-EWP was obtained and referred to as STP-EWP-F.

2.4 Determination of solubility

Solubility properties of protein samples were investigated according to the method of Ortiz and Wagner[16] with slight modifications. Protein suspensions of 1% (w/v) were prepared in distilled water and stirred for 1 h by a magnetic stirrer. The pH values of different suspensions were adjusted to 2.0-8.0 (0.01 M citric acid and 0.01 M NaOH). The mixtures were stirred at room temperature for 30 min and centrifuged at 3 400 r/min for 15 min. The supernatant was diluted and determined by the amount of soluble protein with the method of Lowry et al.[17].

2.5 Determination of emulsifying properties

Emulsifying properties of protein samples were measured according to the method of Pearce and Kinsella[18]. The emulsifying activity index (EAI) and the emulsion stability index (ESI) were calculated by equations (1) and (2), respectively.

$$\text{EAI } (m^2/g) = (2\times 2.303\times A_{500})/[0.25\times \text{Cpp weight (g)}] \quad (1)$$

where, A_{500} represents the absorbance at 500 nm and 0.25 is the oil volume fraction.

$$\text{ESI (min)} = A_0\times \Delta t/\Delta A \quad (2)$$

where, $\Delta A=A_0-A_{10}$, Δt=10 min, A_{10} and A_0 represent the absorbance values at 10 min and at the start of the experiment (zero time) respectively, at 500 nm wavelength.

2.6 Determination of viscosity properties

The viscosities were determined by the method of Puppo and Anon[19] with slight modifications. The viscosities of protein suspensions of 2% (w/v) prepared in 0.05 M Tris with respective pH as needed were determined by NDJ-5S digital viscosimeter at room temperature.

2.7 Determination of foaming properties

20 mL of 0.5% protein solution was prepared in a buffer and homogenized at 16 000 r/min for 2 min using the homogenizer specified in the preceding section. The homogenized sample was immediately transferred into a 25 mL cylinder and the total volume was noted after 30 s. The foaming ability (FA) was calculated using Equation (3) given below:

$$\text{FA } (\%) = [(A-B)/B]\times 100 \quad (3)$$

where, A is the volume after homogenizing (mL); B is the volume before homogenizing (mL). The homogenized sample was left to stand at 20°C for 3 min and the volume of this sample was recorded. Foam stability (FS) was calculated by Equation (4).

$$\text{FS } (\%) = [(N-M)/M]\times 100 \quad (4)$$

where, N is the volume of the foam after standing (mL); M is the volume of the foam before homogenizing (mL).

2.8 Measurement of water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC was determined by the method of Chen et al[20] and OAC was measured according to the method proposed by Li et al.[15] with some modification. Briefly, 0.5 g sample (W_0) of N-EWP and STP-EWP was dispersed in 6 mL of soybean oil or deionized water in a pre-weighed 15 mL centrifuge tube using a vortex mixer. Sample was left to stand at 30°C for 30 min, and the volume (V_1) was recorded. The dispersion was centrifuged at 3000 g for 20 min. The supernatant was carefully poured into a 10 mL graduated cylinder, and the volume (V_2) was recorded. WAC or OAC (mL of oil or water per gram of protein) was calculated from the Equation (5).

$$\text{WAC/OAC} = (V_1 - V_2) / W_0 \quad (5)$$

2.9 Heat induced gel strength

Diluted protein samples (1.5 g freeze-dried protein samples (N-EWP and STP-EWP) + 4.5 g deionized water, 3 g liquid protein samples (N-EWP and STP-EWP)+3 g water) was heated at 80°C for 20 min in a water thermostatic bath 3041 (Kottermann, Germany), followed by cooling at 20°C for 10 min in a water bath. Heat induced gel strength was carried out using a penetrometer (OFD, Germany), equipped with upside down conical-frustum shaped stainless steel probe (diameter of upper base: 1.9 cm, diameter of lower base: 1.0 cm). The probe was manually placed on the protein surface (diameter: 2.7 cm) and allowed to penetrate the protein gel. The intensity of the penetration was registered on the penetrometer (mm). A deep penetration points out a very frail heat induced gel.

2.10 Scanning electron microscope (SEM)

The microstructure of N-EWP, STP-EWP-F and STP-EWP-S were studied using SEM (JSM-6390, JEOL

Ltd., Tokyo, Japan). The acceleration voltage used in SEM was 20 kV, beam current of 5×10^{-9} mA and the working distance was 15 mm. In order to improve the conductivity and image contrast, the samples were coated with a layer of gold in an argon atmosphere (20 mA, 13 Pa, 1.5-2.0 min). The microstructure of proteins were viewed and photographed at a standard instrument magnification of 5 000.

2.11 Statistics

The data were expressed as mean values with standard deviation, and the experiments were repeated three times. Significant differences between mean values are F determined by Student's t-test at the 5% significance level. The solubility, emulsifying properties, viscosity properties, foaming properties, WAC and OAC capacities were analyzed statistically by using SPSS software, version 14 (SPSS Inc., Chicago, USA).

3 Results and discussion

3.1 Solubility of the protein powders

The solubility of protein is an important property for its application in food processing. Effects of drying process on the solubility properties of native and modified egg white proteins within a pH range of 2.0-8.0 were presented in Figure 1. The results showed that the STP-EWP-F significantly change the solubility properties of N-EWP (p<0.05) at the given conditions. This might be because the attached phosphate groups in the modified protein molecules increased the electrostatic repulsions between protein molecules, which protein molecules could dissolve even in acidic conditions. It can be seen from Figure 1 that the solubility of STP-EWP-F is higher than the solubility of STP-EWP-S. The high water solubility of freeze-dried protein powders can be attributed to less thermal stress encountered in the freeze drying process, while the highest extent of thermal and dehydration stresses encountered by the spray dried protein powders[21,22]. The solubility of three samples (STP-EWP-F, STP-EWP-S and N-EWP) are relatively low at pH 5.0 while are higher at other pH values. The surface charge of amphiphilic molecules such as proteins depends on the pH and their solubility was the lowest or not at their isoelectric points. So the variation of solubility can be attributed to both net charge of egg white protein and surface hydrophobicity[23].

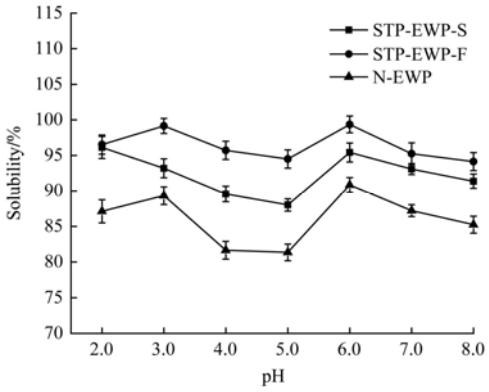


Figure 1 Effect of different drying methods on the solubility of native and modified proteins at a pH range of 2.0 to 8.0

3.2 Emulsifying properties

As big molecule, egg white protein has a lot of polar and unpolar groups, tends to be absorbed between the disperse oil phase and the continuous water phase. Thus, it can emulsify the mixture of oil and water and prevent the oil congregating. Figure 2 showed the effects of drying process on the EAI and ESI of native and modified protein. The high emulsifying capacities in three protein samples suggest that they were suitable for application in food and pharmaceutical products as emulsifiers[24]. Generally, the emulsifying activities of modified egg white proteins (STP-EWP) were better than that of N-EWP, thus, phosphorylation had significant influence on the emulsifying activities of EWP (p<0.05). The low solubility and the high degree of proteins denaturation in spray dried samples led to the lowest EAI in STP-EWP-S, due to solubility and net charge of proteins could tremendous influence their emulsifying properties[15]. The stability of oil-in-water emulsions depends on the adsorption of proteins at the oil-water interface during homogenization. And the formation of a protective protein-rich membrane inhibits coalescence of the oil droplets[23,25]. As shown in Figure 2, the ESI (indicates the emulsion stabilizing capacity) of freeze-dried sample (STP-EWP-F) was significantly (p<0.05) higher compared to ESI of spray-dried sample (STP-EWP-S). The highest ESI of freeze-dried STP-EWP suggests that it is more stable at the interface and adequately flexible to form strong interfacial membranes which enhance the stability of emulsion[26]. On the other hand, the poor

molecular flexibility in the spray-dried STP-EWP (due to higher level of denaturation) means that they were lower in stabilizing the emulsion.

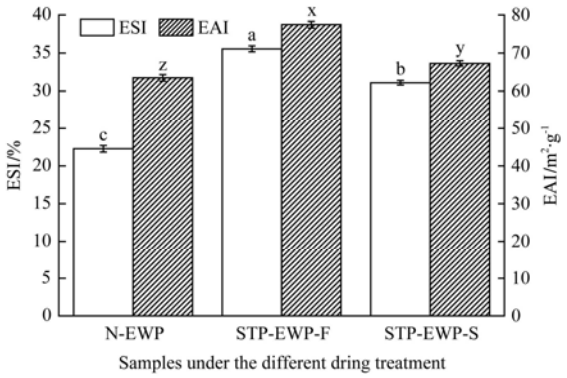


Figure 2 Effects of different drying methods on the emulsifying capacity and emulsifying stability of native and modified proteins (a, b, c indicate significant differences (p<0.05) and x, y, z indicate significant differences (p<0.05))

3.3 Viscosity properties

As shown in Figure 3, at the same concentration, the viscosity of N-EWP was higher than that of phosphorylated egg white protein (STP-EWP-F and STP-EWP-S), especially at pH 3.0-5.0 and 10.0-11.0 (p<0.05). The change of viscosity resulted from the combination of protein molecules and PO_4^{3-}. The PO_4^{3-} changed the shape of protein molecules and surface charges, which had a big impact effect on the hydration layers and the interactions of proteins. After spray-drying, the viscosity decreased dramatically. However, the loss in viscosity after freeze-drying was considerably smaller and had no significantly difference with that using spray drying (p>0.05). Since there was a positive correlation between the gel-like structure from the viscosity and the foam stability of egg white protein, it can be assumed that spray-drying definitely contributes to preserve the foam stability of egg white protein.

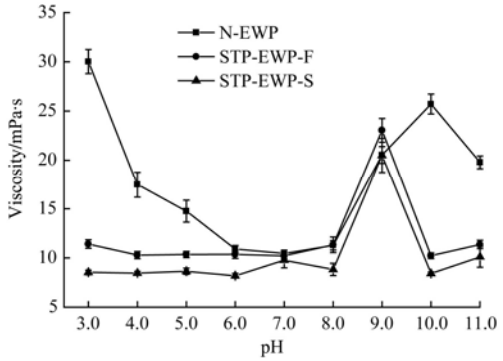


Figure 3 Effects of different drying methods on the viscosity of native and modified proteins at the pH value of 3.0 to 11.0

3.4 Foaming properties

Figure 4 showed the effects of different drying methods on the foaming ability and foam stability of EWP. As seen from Figure 4, the foaming ability and foam stability of freeze dried and spray dried samples were quite similar, but spray-dried samples were higher compared to freeze-dried samples (p>0.05), because spray drying might lead to more unfolding of the proteins, thus making them have more surface activities[12]. In addition to adsorption at air/water interface during bubbling, the solubility, conformational change and rearrangement at the interface, as well as the ability of form cohesive viscoelastic film through intermolecular interactions are basic requirements for a protein to be a good foaming agent[26].

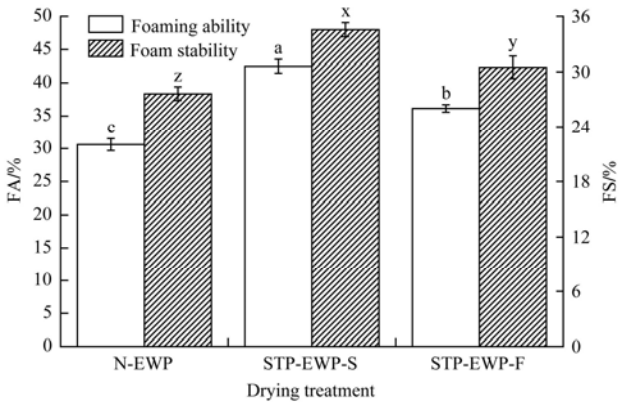


Figure 4 Effects of different drying methods on the foaming capacity and foam stability of EWP proteins (a, b, c indicate significant differences (p<0.05) and x, y, z indicate significant differences (p<0.05))

3.5 Water and oil absorption capacities and gel strength

As observed in Table 1, modified EWP (STP-EWP-F and STP-EWP-S) showed significant changes in water and oil absorption ability and heat induced gel strength in comparison to N-EWP (p<0.05). The proteins obtained from both drying methods exhibited good water absorption capability which can be attributed to the exposure of polar groups which have substantial effect on the amount of water absorbed[27]. The proteins obtained from freeze-dried exhibited a much stronger capacity of absorbing water than that from spray-dried powders (p<0.05). It is most probably due to highly compact/condensed and less porous particle morphology of the spray dried protein powders. Oil absorption

capacity is an important functional property of proteins used in the manufacture of meat and confectionery products. It can be affected by various factors such as the type of protein and manufacturing process used to produce it. Freeze-dried samples (STP-EWP-F) showed a significantly higher oil absorption capacity compared to the spray-dried samples (STP-EWP-S) (p<0.05) and the oil absorption capacity of freeze dried protein powder was 10 times higher than its water absorption capacity (Table 1). This can be attributed to the fact that the freeze dried powders have highly porous structure which facilitates the higher and faster absorption of oil. The process (such as freeze drying) favoring the formation of porous structure usually facilitates the preferential migration/diffusion of proteins[25]. This explains why spray-dried STP-EWP, as the spray dried powders, have lower oil absorption capacity compared with freeze-dried powder due to more compact and less porous. As shown in Table 1, there were significant differences between STP-EWP-F and STP-EWP-S (p<0.05). Therefore, spray drying treatment led to feeblish heat induced protein networks and higher penetration values.

Table 1 Influence of drying methods on water and oil absorption capacities and heat induced gel strength

Sample	Water absorption capacity /(g·g^{-1})	Oil absorption capacity /(mL·g^{-1})	Penetration values /mm
N-EWP	0.65±0.06^{c}	3.12±0.07c	3.0±0.02^{a}
STP-EWP-F	1.37±0.03^{a}	10.21±0.13^{a}	1.8±0.05^{c}
STP-EWP-S	0.89±0.01^{b}	6.89±0.15^{b}	2.5±0.08^{b}

Note: $^{a, b, c}$ indicate significant differences (p<0.05).

Besides, the heat induced gel strength proved to be highly dependent on the moisture content (dry based) in the dried STP-EWP-F powder. As presented in Figure 5, there was a linear correlation between the penetration value and the moisture content. Aside from increasing the microbiological stability, the moisture contents should be less than 6%, at which below 5.8% the water was completely bounded and the STP-EWP proteins were stabilized in the so-called glass condition during thawing[28]. At higher changes of moisture contents, white egg proteins lead to a loss in the ability of producing a stable three-dimensional network. As a consequence of this, the penetration value increases.

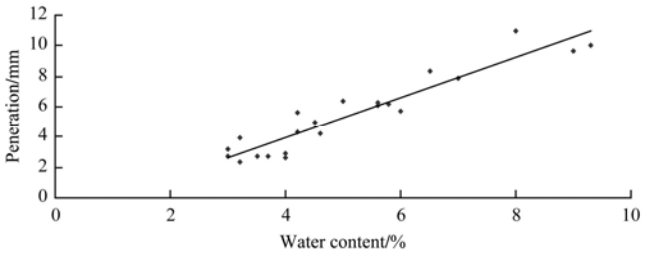
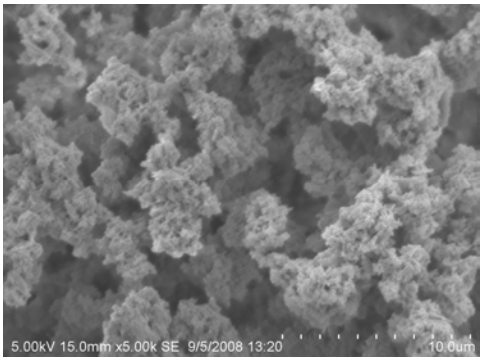
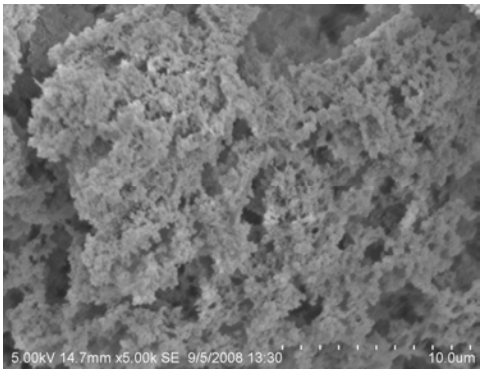


Figure 5 Correlation between the dry basis moisture content in the dried STP-EWP-F and the heated induced gel strength

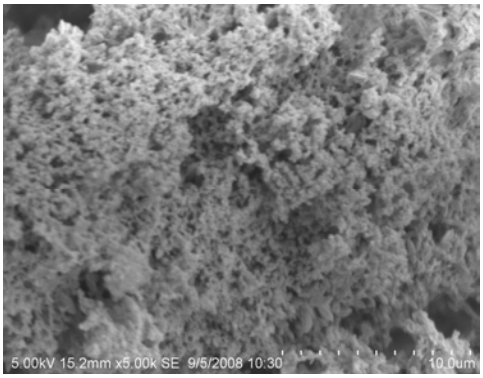
SEM analysis (Figure 6) showed that the N-EWP has a porous network structure, loose and irregular. The microscopic structures of STP-EWP-F and STP-EWP-S were very compact, the surfaces of them appeared very smooth, only at higher magnification we could observe its microstructure characteristics.



a. N-EWP



b. STP-EWP-F



c. STP-EWP-S

Figure 6 SEM micrographs of N-EWP, STP-EWP-F and STP-EWP-S

4 Conclusions

Different drying methods such as freeze drying and spray-drying had different effects on the functional properties of STP-EWP powders. The STP-EWP-F powders were superior in terms of solubility, emulsion stability, viscosity, water holding capacity, oil and water absorption capacity and heat gel strength than STP-EWP-S powders. The STP-EWP-S powders were better in terms of foaming ability and foam stability than STP-EWP-F. However, it required longer time to produce. SEM analysis showed that the microscopic structures of STP-EWP-F and STP-EWP-S are very compact, their surfaces appear very smooth. It is proved that freeze drying is the best method in terms of production of STP-EWP powders having superior functional properties.

Acknowledgements

The authors acknowledge that this work was supported by Special Fund for Agro-scientific Research in the Public Interest of China. No. 201303084).

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