低温化における水分蒸発から推定された茹でた卵白，ゲル状ゼラチンおよびコンニャク中の自由水および結合水の量

Bound and Free Water in Boiled Egg White, Gelatin-gel and Konjak Estimated by Moisture Evaporation under Cold Temperature

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Key Words : free water, bound water, egg white, gelatin-gel, konjak, moisture evaporation
キーワード：自由水、結合水、卵白、ゲル状ゼラチン、コンニャク、水分蒸発

Summary

When boiled egg white was left under cold temperature as in an ordinary refrigirator, there were observed two stages of moisture evaporation, i.e., the fast and the slow one. We had described in a short scientific correspondence (Nature, 1990) that it was due to the free and bound water in the proteins. This paper aims its full description with another results on gelatin-gel and konjak. There are known about 40 proteins in egg white. Neglecting the ninor ones, the average molecular mass of the egg proteins was estimated as 51,000, and that of the amino acids, 110. It showed that there were 7 and 0.7 grams of free and bound water per gram of the proteins, respectively. In 5% gelatin-gel, there was 0.3 gram of bound water per gram of gelatin, corresponding 1.5 moles per one amino acid as its average molecular mass was estimated as 90. The difference between the amounts of the bound water in the egg white proteins and gelatin was suggested to be due to rather the residual moieties of the amino acids in the proteins, but not to the hydrophobic nature in regard to the solubility of the amino acids composing the proteins. The model of free and bound water in protein and konjak was schematized. In konjak composed of konjak mannan, the bound water was 1.5 moles per mole of glucose or mannose, the conponents of the carbohydrate. The amounts of the bound water in these materials were discussed, compared to those measured by NMR.

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Introduction

For moisture in mixtures or single materials, it is known that there are generally contained the bound and free water[1-4]. For the measurement of them, NMR is well used [5-6]. However, when we put some varieties of materials, especially food materials under cold temperature as in an ordinary refrigerator, we observed two stages of moisture evaporation and indicated that this was due to the difference between the bound and free water in an example of boiled egg white. This method was quite different from that by NMR, and very simple by means of the weight measurement[7].

As it is well known, there are about 40 proteins in egg white, in which molecular weights for the main components are known[8]. Based on this we estimated an average molecular weight of the proteins, and induced the amounts of bound and free water in terms of grams or moles per gram or mole of the proteins in boiled egg white, respectively. The concept was expanded to the contents of bound and free water in amino acid moieties in the proteins. However, the above report was so simple that full explanations had been required. This paper is for this purpose, including the result of bound water in gelatin free of other components of proteins, and konjak, a food of undigestable carbohydrate of konjak mannan.

Methods

1. Moisture evaporation of boiled egg white under cold temperature

Eggs on the market were hard boiled. The shell was removed, the content was cut vertical, and the egg yolk was removed. The part of boiled egg white was put on aluminum foil folded zigzag in a petri dish as shown in Figure 1. It was put in an ordinary refrigerator. Its weight was intermittently measured until the weight change was not observed.

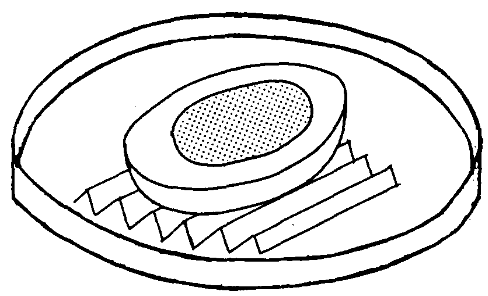


Figure 1. Boiled egg white on aluminum foil folded zigzag under cold temperature for moisture evaporation. By simply leaving the boiled egg white as sich on petri dish in an ordinary refrigerator, the moisture evaporates. A similar treatment was done for 5% gelatine-gel and konjak.

2. Estimation of an average molecular weight of egg white proteins

As stated before, there are about 40 proteins in egg white and some main components are known with their molecular weights[8]. To estimate the average molecular weight of egg white proteins on this data, the main components were tabulated as in Table 1. Considering the molecular masses(MW) of the main ones and neglecting the rests that compose minor components, the average molecular mass was calculated from the following formula:

$$\text{average molecular mass} = \frac{\Sigma(\text{content})\times(\text{MW})}{\Sigma(\text{content})}$$

3. Estimation of the average molecular mass of amino acids in egg white proteins

At present for any materials, if proteins are contained, the amino acids are analyzed, especially in the field of food sciences. Amino acid compositions are now tabulated in the food composition table with other nutrients[9]. According to this, the amino acid composition of the egg white is shown as in Table 2. As amino acids in proteins are linked by peptide bond, their moieties are smaller by one water molecule per peptide bond than the free amino acids of the decomposed proteins. However, when both the weight composition of each amino acid and the average molecular mass of the proteins are known, the number for each amino acid can be calculated. Consequently, the average molecular mass of the amino acids in the proteins can be induced. In such way, the average molecular mass of the amino acids in the egg white proteins was calculated (Table 2).

Table 1
Proteins of egg white and their molecular weight

proteins	content(%)	MW	product
avidine	0.1	68,300	3,415
conalbmine	12.5	81,300	1,016,250
flavoprotein	0.8	34,000	27,200
lysozome	3.5	15,650	53,999
ovalbumine	60.0	46,000	2,760,000
ovoglobuline	8.0	40,500	324,000
ovoglycoprotein	0.8	24,000	18,300
ovoinhibitor	0.8	46,500	37,200
ovomacroglobline	0.5	830,000	415,000
ovomucin	2.2	?	?
ovomucoid	11.1	28,000	308,000
papain-inhibitor	0.1	12,700	1,270
summation	100.3		5,068,500

About 40 protein are known in egg white(Bahr,J.W. and Bakst,M.R., in Reproduction in Farm Animals(ed. Hafez,E.S.E.) 5th edn. p379 (Lee and Febiger, philadelphia 1987). Considering the molecular masses of the main ones and neglecting the rest, the average molecular mass can be estimated as 51,000.

Table 2
Amino acids in egg white proteins and the average molecular weight of amino acids in the proteins

Amino acid	MW	content*1	Content*2	Number of aa*3
Ile	131.2	580	2,732	24.1
Leu	131.2	930	4,412	37.0
Lys	146.2	720	3,447	26.9
Met	149.2	410	1,969	15.0
Cys	121.2	320	1,616	7.3
Phe	165.9	620	3,016	20.5
Tyr	181.2	420	2,065	12.7
Thr	119.1	470	2,178	21.5
Trp	204.2	160	797	4.3
Val	117.2	760	3,512	35.4
His	155.2	260	1,255	9.1
Arg	174.2	610	2,987	19.1
Ala	89.1	650	2,832	39.8
Asp	133.1	1,100	5,194	45.1
Glu	147.1	1,400	6,709	52.0
Gly	75.1	380	1,577	27.6
Pro	115,1	380	1,750	18.0
Ser	105.1	670	3,032	34.8
summation			51,080	450.4

*1;amino acid composition (mg) in egg white (100g).
*2;amount(g) of amino acid in average molecular mass (51,000) of egg white proteins, where mass of water molecule in each peptide bond is reduced.
*3:number of each amino acid in average molecular mass (51,000) of egg white proteins.
From the result above, average molecular mass of amino acids was obtained as 110.

4. Experiment in gelatin-gel

Gelatin powder (product of Wako Pure Chemical Industries, LTD, Japan) was dissolved in water, heated and cooled(5% gelatin-gel). About 15 grams of the gelatin-gel was cut into thick plate and left in the refrigerator as the boiled egg white. The average molecular mass of the amino acids in gelatin was estimated from its amino acid composition[9].

5. Konjak

Konjak on the market as a food item was perchased, cut into a thick plate (about 15g), and left in the refrigerator as described above.

Results

1. The average molecular mass of egg white proteins

Disregarding the fraction of small number, the average moleclar mass of egg white proteins was estimated as 51,000. This value is a little bigger than that of ovalbumine, 45,000, the main component composing 60.0% of the proteins.

2. The average molecular mass of amino acids in egg white proteins

By reducing the molecular mass of water per peptide bond, the amounts of the composing amino acids were accumulated until the mass of 51,000. Subsequently, the amounts for each amino acid were induced (the values of the 4th column in Table 2). When it was divided by the mass of each corresponding amino acid in which mass of water molecule was reduced, number of each amino acid in the average molecular mass of the proteins could be obtained (the values of the 5th column in Table 2). The total number of amino acids was 450. From this, the average molecular mass of the amino acids in egg white proteins was induced as 110, neglecting the small fraction of the value shown in Table 2.

3. Moisture evaporation of boiled egg white under cold temperature —estimation of the bound and free water—

By simply leaving the boiled egg white in an ordinary refrigerator, the moisture evaporated as shown in Figure 2. All this section was a review from the reference 7. As the Figure shows, moisture evaporation took place in two stages;in the first stage, that showed a fast moisture evaporation, the boiled egg white lost its weight by 80%, and in the second, a slow one, by 8%. The rest, 12%, was the solid component. The difference of this evaporation rate was

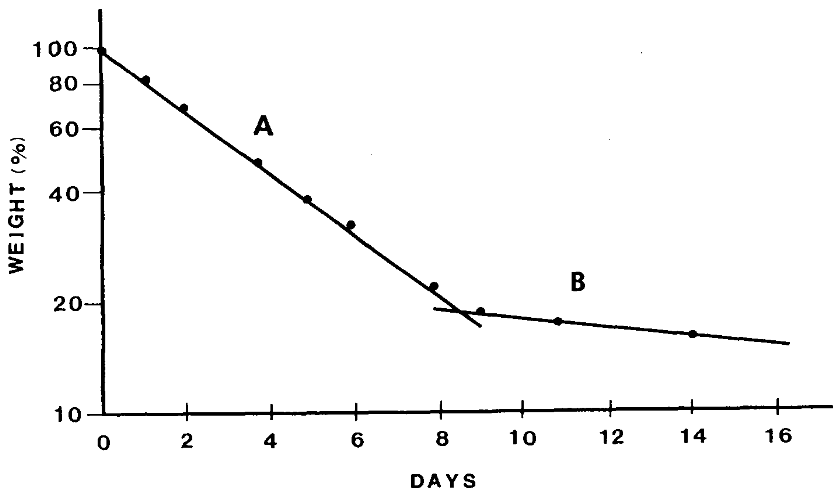


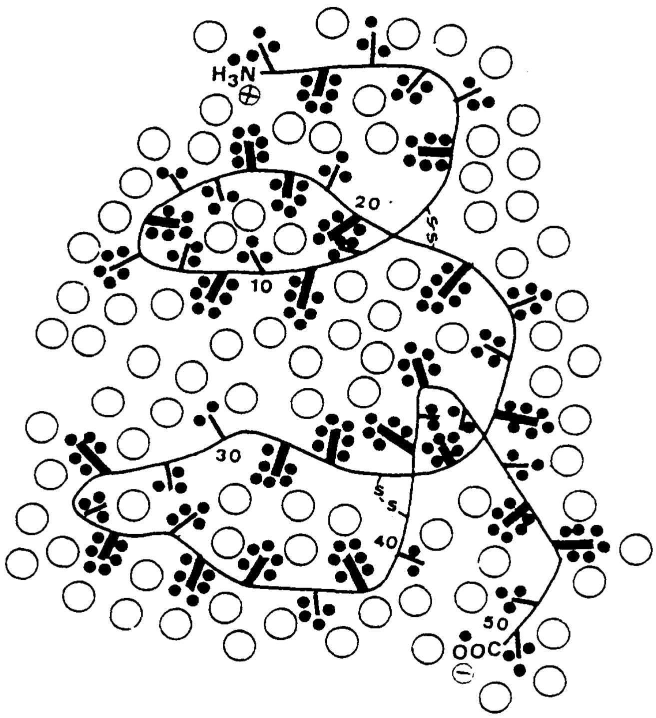
Figure 2. The pattern of moisture evaporation of the materials left under cold temperature as in a refrigerator. The two stages of moisture evaporation from the materials such as boiled egg white, 5% gelatin-gel and konjak took place. The fast evaporation is due to that of the free water(A), and the slow one, that of the bound water(B). For the weight of a half part of the boiled egg white, the fast evaporation took 10 days. (The firure was adopted with modification from the original in the reference 7).

due to the degree of the connection between water and the proteins. The fast moisture evaporation shows the loose connection between them, and the slow one, the tight one. The latter is due to the bound water, and the former to the free water. Assuming that the solid is composed of proteins, one gram of the protein was estimated to contain 7 and 0.7 grams of free and bound water, respectively. In other words, one mole of the proteins contains 19,000 and 1,900 moles of free and bound water.

As the average molecular mass of the amino acids in the proteins was estimated as 110, one amino acid contained 40 and 4 molar free and bound water, respectively.(Fig.3).

Figure 3. The model of bound and free water in the denatured proteins.

The thick and thin bars stand for the higher and lower degree of hydrophilic nature as the moieties of amino acids in proteins, respectively. One symbol of ● and ○ stand for one mole of bound water and more than 20 moles of free water, respectively. Although one mole of the egg white proteins in average is composed of about 450 amino acids, in this figure, however, the 50 amino acid moieties are drawn as a model with 200 moles of bound water in order to indicate 4 moles of bound water per one amino acid moiety in average. The molecules of free water were drawn ad libitum. Because of the denatured proteins, α-helices and β-structures were not drawn in this figure as they might be destructed. The symbol, -S-S-, shows the bond between cysteins, in which there would be no bound water or little of any because of its high hydrophobic nature. The numerals show the ordering number of the amino acids from the N-terminal. This model may fit the structure of the boiled egg white proteins, but for gelatin-gel because of its low contents of the bound water, the thin bars, the amino acid moieties that have lower degrees of hydrophilic nature, may increase in number.



4. Bound and free water in gelatin-gel

Five percent (5%) gelatin-gel left in the refrigerator lost its weight as moisture by 93.4% of all the weight in the first stage which lasted 10 days. The moisture evaporation of the second stage lasted another 5 days, in which, 1.6% of weight was lost. The average mass of the amino acids in gelatin was estimated as 90. From these results, 1 gram of gelatin contained 0.3 grams of bound water, and one amino acid contained 1.5 molar bound water. One gram of gelatin in 5% gelatine-gel contained 18.7 grams of free water, i.e., 93.5 molar free water per amino acid.

5. Bound and free water in konjak

Konjak (a product from the tuber of konjak;*Amorphophallus konjac*) left in the refrigerator lost its weight as moisture by 97.0% of all the weight in the first stage. In the second stage 0.5% of weight was lost. As konjak is composed of two molecules of mannose one molecule of glucose[12)], average mass of the monosaccharide in it could be estimated as 162, the value of the difference between the molecular mass of water, 18, and that of glucose or mannose, 180. From these results, 1 molar monosaccharide in konjak contained 1.5 moles of bound water.(Feg. 4)

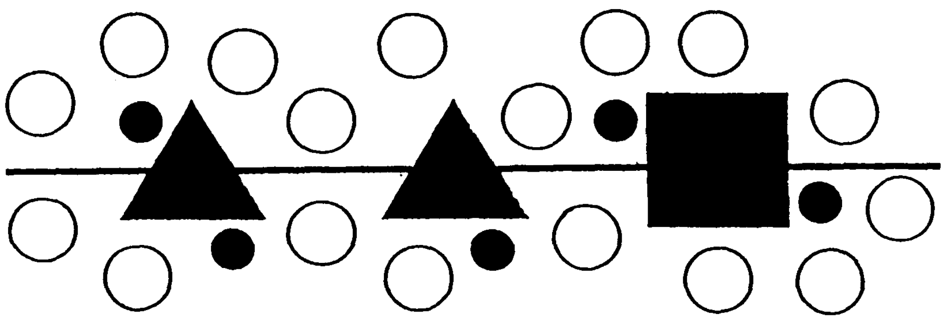


Figure 4. The model of bound and free water in Konjak. Konjak is composed of two moles of mannose and one mole of glucose in average. As there were estimated 1.5 moles of bound water per one monosaccharide, it would be schematized as in the figure, where the symbols of ▲ and ■ are mannose and glucose moiety, respectively. The symboles of ● and ○ are same to those in Fig. 3.

Discussion

When this moisture evaporation method was applied to some foods such as fish paste product, two types of tofu, Okinawa tofu and ordinary tofu, and some fruits such as apple and pear, etc., there took place two stages of moisture evaporation in each case.

Among the main components in foods, the one that contains most the amount of bound water is proteins. Effect of carbohydrates on it is very small, and fat would be out of question because of its hydrophobic nature. There would be bound water in inorganic substances. However, these contents are so small in food that their effect on the experiment can be neglected. When we applied konjak, that contains 6% ashes, to this experiment, the amount of bound water was small (0.17g of bound water/g of konjak, less than that of geletin), due to mainly the carbohydrates composing konjak, sugesting that the effect of inorganic substances were negligble.

In egg white contents of proteins, carbohydrates and ashes are 10.4, 0.9 and 0.7%, respectively. In this paper, the solid, that remained after all moisture evaporated, was regarded as proteins, because the effect of carbohydrates and inorganic substances on the amount of bound water was too small.

At present pure proteins such as egg albumine and others are available. In this experiment, egg white containing a mixture of proteins was used. On the other hand, gelatin was also used a source of pure protein. Comparing the results obtained in egg white and gelatin, one gram of the latter contained 0.3 gram of bound water, i.e., 1.5 moles per an amino acid. This value is about one third to that in egg white. In 5% gelatin-gel, free water was 18.7g per gram of gelatin, i.e., 93.5 moles per an amino acid. The value of free water in this case, however, is not so meaningful as that of egg white, since it is variable according to the amount of water in the gel preparation, and it is not a natural product as egg white.

As it is well known, there are hydrophilic and hydrophobic amino acids in regard to their solubility in water[10-11] (Table 3). It is also true for proteins. Albumine is rather hydrophilic. But hydrophilic nature does not mean that it causes high amount of bound water. The amino acids highly contained in gelatin are glycine, proline and hydroxyproline, consisting 47% of all the weight. These amino acids are all hydrophilic (Table 3). Considering that the amount of bound water in gelatin was about one third to that in egg white, the amino acids that determine the amount of bound water would be the residual moieties of amino acids. It would be schematized as in Figure 2.

According to the measurement by NMR, the collagen contained 0.25 to 0.50g of bound water per one gram of collagen[5]. This value does not so differ from that in gelatin obtained by the moisture evaporation method.

Table 3
Solubility of Amino Acids in Water

Amino acid	Solubility(g/l)
Ala	166
Arg	150*
Asn	35.3*
Asp	4.5*
Cys	+++
Cys-Cys	0.11
Gln	48.1*
Glu	8.6
Gly	250
His	41.9
Hyp	288.7
Ile	41.2
Leu	24.3
Lys	536*
Met	33.8
Phe	29.6
Pro	207
Ser	250
Thr	141*
Trp	11.4
Tyr	0.45
Val	88.5

*Temperature giving the solubilites is 25° C, except for Arg(21° C), Asn (28° C), Asp(20° C), Gln(30° C), Lys(0° C) and Thr(52° C).
+++;freely soluble.
The data above were cited from Merk Index(7th ed.), but those of Lys and Thr were from Biochemical Data Book edited by Chemical Society of Japan (1989)

The amounts of bound water in gelatin and konjak was similar in terms of molar contents, 1.5 moles per the composing moiety for each material. Since both form a hard gel even in a high amount of water, the free water in these materials might be held tight in these molecules[13]. This is also true in the boiled egg white that forms a hard gel. In other words, in gel formation in egg white the denaturaliization of materials is essential. But as seen in seaweeds that form a natural gel, denaturalization is unnecessary.

By the moisture evaporation, transit state between bound and free water can not be detected, though it can be by MNR[5]. However, the amount of free and bound water can be visually traced it with an apparatus of refrigerator that is available everywhere. This is an advantage of this method, and further data accumulation sould be required.

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低温化における水分蒸発から推定された茹でた卵白、ゲル状ゼラチンおよびコンニャク中の自由水および結合水の量

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要　約

茹でた卵白を一般の冷蔵庫中で低温化におくと、急速および緩慢という２段階の水分蒸発が起こった。われわれはこの現象が卵白タンパク質中の自由水と結合水によるものとして報告した（Nature, 1990)。本文は、それにゲル状ゼラチンおよびコンニャクにおける結果を加え、ここにその詳細を総括的に述べることを意図したものである。卵白中には約40種のタンパク質があるといわれる。微量成分を無視すると、タンパク質およびその構成アミノ酸残基の平均分子量はそれぞれ51,000および110と推定された。このことは、自由水および結合水がタンパク質１グラム当たり、それぞれ７および0.7ｇあり、さらに１アミノ酸残基あたりそれぞれ40および４molesあることを示していた。一方、５％のゲル状ゼラチンでは、ゼラチン１ｇに対して結合水は0.3ｇであった。このことは、ゼラチン中の平均アミノ酸残基の分子量が90と算出されるため、１アミノ酸残基あたり1.5molesの結合水に相当する。茹でた卵白中のタンパク質とゼラチン中の結合水量の差は、それぞれの構成アミノ酸の水に対する親和性の違いというより、むしろ構成アミノ酸そのものの違いによると示唆された。他方、コンニャクマンナンと呼ばれる糖で構成されているコンニャクでは、結合水はその構成糖成分であるグルコースまたはマンノース１moles当たり1.5molesと算出された。本文には、茹でた卵白におけるタンパク質中のアミノ酸残基およびコンニャク中の構成糖成分に対するそれぞれの自由水および結合水の存在様式を模式図で示した。このような水の存在様式をＮＭＲによって推定されているが、それによる結果とも比較検討した。

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