

Die Nahrung

Food

*Chemie
Biochemie
Mikrobiologie
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Professor MUDr. Josef MAŠEK DrSc. 70 Jahre alt

Am 8. März 1978 begeht Professor MAŠEK, Vorstand des Forschungszentrums für Metabolismus und Ernährung im Institut für klinische und experimentelle Medizin, Prag, seinen 70. Geburtstag. Nachdem die Verdienste Prof. MAŠEKS um die Ernährungswissenschaft bereits vor 5 Jahren in dieser Zeitschrift ausführlich gewürdigt worden sind, sei an dieser Stelle nur auf einige neuere Arbeiten und Ergebnisse hingewiesen:

Die Arbeiten über die Fettsucht wurden abgeschlossen; auf diesem Forschungsgebiet war Prof. MAŠEK von 1967 bis 1976 der Initiator in den sozialistischen Ländern; von Prof. MAŠEK wurden die Empfehlungen für die tägliche Nährstoffaufnahme überarbeitet und erweitert, und es wurden Prognosen für die Entwicklung des Lebensmittelverbrauchs während der wissenschaftlich-technischen Revolution neu erarbeitet. Die gemeinsam mit HRUBÁ, HEJDA, NERADILOVÁ und anderen Wissenschaftlern durchgeführten Arbeiten über die Bedeutung des Vitamin C in der Ernährung wurden abgeschlossen. Besonders hingewiesen sei hierbei auf die Hypothese über den Einfluß dieses Vitamins auf die Leistung des Gehirns (1975 Preis der Medizinischen Akademie in Paris) und auf akute Infektionen des Respirationstraktes.

Neben seiner wissenschaftlichen Tätigkeit übt Professor MAŠEK noch immer eine Reihe sehr bedeutender Funktionen aus. So leitet er seit 1973 das tschechoslowakische Nationalkomitee der IUNS und ist seit 1974 Vorstand des Lehrstuhls für innere Medizin am Institut für ärztliche Fortbildung; er ist Mitglied des Kollegiums der medizinischen Wissenschaften der Tschechoslowakischen Akademie der Wissenschaften.

Prof. MAŠEK ist eine Vielzahl von Ehrungen zuteil geworden. So wurde er 1975 zum korr. Mitglied der Tschechoslowakischen Akademie der Wissenschaften ernannt; er ist Ehrenmitglied der Gesellschaften für Ernährung in Bulgarien, Ungarn, der DDR und der Slowakei und gehört zahlreichen ausländischen Gesellschaften an.

Die Beziehungen zwischen Prof. MAŠEK und dem Zentralinstitut für Ernährung in Potsdam-Rehbrücke und der Zeitschrift „Die Nahrung“, der er als Mitherausgeber angehört, sind seit Jahrzehnten sehr eng; ihm gebührt an diesem Tage unser besonderer Dank. Wir wünschen Professor MAŠEK auch weiterhin Gesundheit und Schaffenskraft für die Bewältigung seiner zahlreichen verantwortungsvollen Aufgaben.

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Depletion of glucose in Egyptian egg white before dehydration

S. K. SHEHAB, N. M. DARWISH and M. A. SADEK

Glucose was completely removed from egg white in 9 h by using *Streptococcus lactis* and 0.2% yeast extract at pH 6.0 and 30 °C. A distinct objectionable odour was developed accompanied by a change in the appearance of egg white. Using *Aerobacter aerogenes* at pH 7.0 and 37 °C, glucose depletion was completed after 3 to 4 h depending on the initial number of bacteria used. The undesirable changes in odour and appearance of egg white were not observed. *Saccharomyces cerevisiae* succeeded, in presence of 0.2% yeast extract, in depleting sugar in egg white in 9 h. The optimum pH for the reaction was in the range of 6.0 to 7.5 at 32 °C. Glucose oxidase powder of fungal origin was also used for glucose depletion. Glucose was completely removed after 8 h by adding 3.8 glucose oxidase units/100 ml egg white at pH 7.3 and 14.5 °C. 1.9 and 0.95 glucose oxidase units per 100 ml egg white were not enough for complete glucose removal. No objectionable odour or undesirable changes in egg white were observed.

Dehydration of liquid egg white without removal of glucose results in poor storage stability of dried product [1]. The aldehydic group of glucose reacts with the amino groups of protein resulting in browning and insolubility of the dried product (MAILLARD reaction). It was recommended that glucose must be removed prior to drying to retard this reaction [2, 3].

Formerly sugar was depleted from egg white by permitting a spontaneous fermentation to take place. STUART et al. [4] found that the bacteria present in this case were usually of the genera *Aerobacter* and *Escherichia*. The experimental results of AYRES [5] indicated that glucose could be removed from egg albumen by using *Saccharomyces cerevisiae*, *Streptococcus lactis* and *Aerobacter aerogenes*.

The experiments of CARLIN et al. [6] on the effect of removal of glucose by glucose oxidase revealed that treated dried albumen rehydrated readily and had no off flavour or odour.

HAMED et al. [7], trying to prepare dried egg white as a new industry in Egypt, used a crude enzyme complex prepared from *Penicillium notatum* for depleting glucose from chicken egg white before drying. It was found during their work that natural fermentation of egg white was unsuccessful in removing glucose, which contradicts the results of STEWART et al. [8].

The authors explained the difference to be due to the difference in both chemical and bacteriological composition of eggs in different countries. So it was the purpose of the present study to try several methods for depleting glucose in Egyptian egg white before dehydration. This was done in an attempt to establish the best method for glucose depletion that would give dried egg white of high quality. However the evaluation of the final dried products will come next.

Glucose removal from egg white was done using both active dry yeast (*Saccharomyces cerevisiae*) and bacteria (*Streptococcus lactis* and *Aerobacter aerogenes*). Besides, another experiment, similar to that of HAMED et al. [7], was carried out in which the source of enzyme was changed, to find out the effect of changing source of enzyme on the rate of glucose removal.

Materials and methods

Chicken eggs were obtained from the poultry farm of the Animal Husbandry Dept., Ministry of Agriculture, Cairo, Egypt. The eggs were first washed, then examined by candling [9].

The egg whites were blended in a waring blender and forced through fine screen to break the fibrous structures and remove pieces of shells.

Bacterial Fermentation

Strains of *Streptococcus lactis* and *Aerobacter aerogenes* that were isolated from milk and identified in Food Technology & Dairy Laboratory, National Research Center, were used in this study. Determinations of the total count of bacteria and yeast were carried out using the technique of SCHARF [11].

Yeast fermentation

Active dry yeast (*Saccharomyces cerevisiae*) was obtained from the Egyptian Company for Yeast and Starch. The "resting cell" technique [10] was applied to avoid as much as possible the yeasty flavour of the product.

Enzymatic oxidation

Glucose-oxidase powder of fungal origin (crude, containing catalase) with an activity of about 3.8 glucose-oxidase units per mg was obtained from B.D.H. Chemicals, England. 0.1 g of the enzyme powder was dissolved in 100 ml acetic acid buffer (pH 7.3). 0.25 ml, 0.5 ml and 1.0 ml of this enzyme solution were used for glucose depletion. The method used for depleting glucose from egg white was that of HAMED et al. [7].

Glucose was determined using the modified Somogyi method of HAMED et al. (7).

Yeast extract that was prepared and standardized especially for use in bacteriological culture media was obtained from Difco Laboratories, U.S.A.

Results and discussion

Glucose depletion by *Streptococcus lactis*

Table 1 shows the effect of adding *Str. lactis* on glucose concentration in egg white at pH 6.0 and 30 °C. It shows that, regardless of the initially inoculated number of bacteria, the use of *Str. lactis* was not effective in the complete removal of glucose. The drop in sugar content after 9 h was from 57 to 61% of the initial concentration. During the fermentation, a distinct objectionable odour was developed accompanied by a change in the appearance of egg white.

Table 1
Removal of glucose from liquid egg white by using *Streptococcus lactis* at pH 6.0 and 30 °C

time [h]	Trial I		Trial II	
	Total count [org./ml]	Glucose content [mg/100 ml]	Total [org./ml]	Glucose content [mg/100 ml]
0	52×10^4	269	31×10^5	269
3	94×10^4	249	62×10^5	231
6	108×10^5	214	40×10^6	190
9	111×10^6	101	99×10^6	114

The insufficient sugar removal might be due to the deficiency of the medium in some essential nutrients, required for complete growth of the test organism. These results are in agreement with those obtained by AYRES [5].

Table 2 shows the effect of adding different levels of yeast extract to the medium. The sugar content was markedly decreased especially after 6 to 9 hours of incubation period. This decrease was much more obvious with higher levels of yeast extract. The presence of glucose in egg white could not be detected after 9 h when a level of 0.2% yeast extract was used. The undesirable changes in odour and appearance were noticed here too.

Table 2

Effect of *Streptococcus lactis* in presence of different levels of yeast extract on rate of glucose removal from egg white at pH 6.0 and 30 °C

Time [h]	0.05 g yeast extract per 100 ml egg white		0.1 g yeast extract per 100 ml egg white		0.2 g yeast extract per 100 ml egg white	
	Total count [org./ml]	Glucose content [mg/100 ml]	Total count [org./ml]	Glucose content [mg/100 ml]	Total count [org./ml]	Glucose content [mg/100 ml]
0	53×10^5	432	53×10^5	432	53×10^5	432
3	70×10^6	416	80×10^6	405	109×10^6	318
6	103×10^6	324	112×10^6	351	35×10^7	86
9	48×10^7	108	113×10^7	86	30×10^7	0

Glucose depletion by *Aerobacter aerogenes*

Table 3 shows the rate of glucose removal from egg white by using *Aer. aerogenes* at pH 7.0 and 37 °C. It is clear that complete removal of glucose was successful in all cases and that the increase in the initial number of bacteria decreased the time required for glucose to reach the zero level. No objectionable odour or detectable changes in liquid egg white was observed. This is due to the fact that the fermentation duration was much shorter than that required by *Str. lactis*.

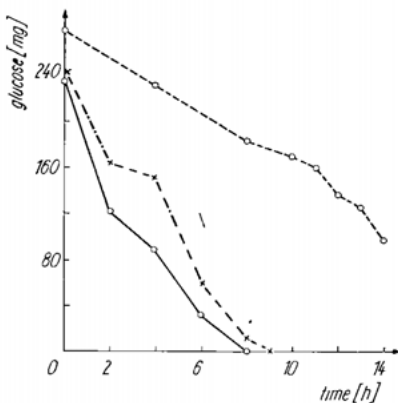


Fig. 1.

Fig. 1. Effect of adding different levels of *Saccharomyces cerevisiae* with 0.1% yeast extract at pH 7.5 and 32 °C

Saccharomyces cerevisiae: 0.1% ○---○, 0.2% ×---×, 0.3% ○—○

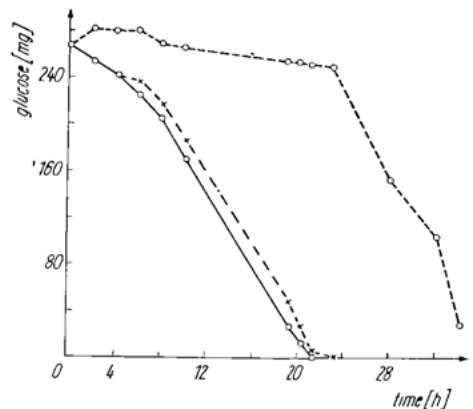


Fig. 2.

Fig. 2. Effect of the holding pH on the rate of glucose removal in egg white at 32 °C using 0.1% *Saccharomyces cerevisiae* and 0.1% yeast extract

pH values: 9.35 ○---○, 7.5 ×---×, 6.0 ○—○

Table 3
Removal of glucose from egg white by *Aerobacter aerogenes* at pH 7.0 and 37 °C

Time [h]	First Trial			Second Trial			Third Trial			Fourth Trial			Fifth Trial		
	Total bact. count [org/ml]	Glucose content [mg/100 ml]		Total bact. count [org/ml]	Glucose content [mg/100 ml]		Total bact. count [org/ml]	Glucose content [mg/100 ml]		Total bact. count [org/ml]	Glucose content [mg/100 ml]		Total bact. count [org/ml]	Glucose content [mg/100 ml]	
0	202×10^5	240		140×10^5	240		27×10^5	240		21×10^5	240		150×10^4	240	
1	—	71		—	175		—	136		—	175		—	192	
2	190×10^6	45		155×10^6	81		140×10^6	71		150×10^5	103		196×10^5	146	
3	85×10^7	0		70×10^7	0		90×10^7	13		60×10^6	13		40×10^6	22	
4	—	—		—	—		115×10^7	0		35×10^7	0		150×10^6	0	

Glucose depletion by yeast fermentation

Fig. 1 shows the effect of adding different levels of *Saccharomyces cerevisiae* on the glucose content of egg white. It is clear that increased yeast level was effective in accelerating the fermentation process. The sugar content of egg white reached the zero level in 8 h using 0.3% active dry yeast, and 9 h using 0.2% of the organism. Using 0.1% level of the yeast, the sugar content was still 97 mg/100 ml after 14 h of fermentation.

Fig. 2 shows the effect of hydrogen ion concentration on the rate of glucose removal from egg white using *Saccharomyces cerevisiae*.

The sugar content of egg white was zero after 21 h at pH 6.0 and 23 h at pH 7.5. At pH 9.35 the desugaring process was not completed after 34 h.

Table 4
Change of total yeast count during glucose removal from egg white with and without adding yeast extract

time [h]	Total count	
	without yeast extract	in presence of 0.1% yeast extract
0	16×10^2	50×10^3
4	60×10^3	23.5×10^4
6	160×10^3	55×10^4
8	22×10^4	60×10^4
10	50×10^4	80×10^4

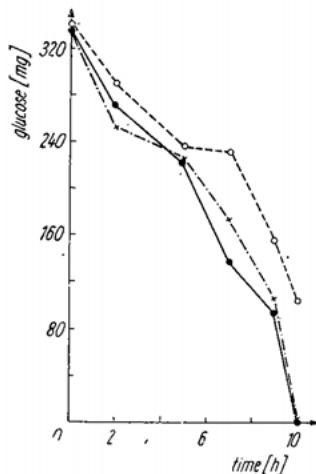


Fig. 3.

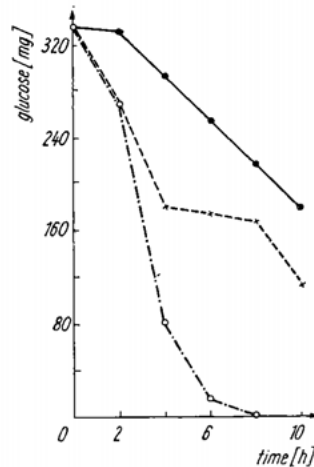


Fig. 4.

Fig. 3. Effect of adding yeast extract on the rate of glucose removal using 0.2% *Saccharomyces cerevisiae* at pH 7.5 and 32 °C

yeast extract: 0.1% ●—●, 0.2% ×—×, 0.3% ○—○, 0.0% ○—○

Fig. 4. Effect of adding different levels of enzyme on the rate of glucose removal at pH 7.3, 14.5 °C and 0.45 ml H₂O₂

Enzyme level: 0.25 ml ●—●, 0.50 ml ×—×, 1.0 ml ○—○

Fig. 3 shows the effect of adding yeast extract on the rate of glucose removal using 0.2% *Saccharomyces cerevisiae* at pH 7.5 and 32 °C. It is clear that the presence of yeast extract decreased the time required for complete removal of sugar. Changing the level of yeast extract from 0.1 to 0.2% did not affect the fermentation rate.

Table 4 shows the change in total yeast count during the desugaring process of egg white and the effect of adding 0.1% yeast extract on that count. It is clear that addition of yeast extract to the fermentation medium activated the yeast cells. This was demonstrated by the increase in the total count and consequent decrease in the desugaring time.

Enzymatic oxidation

Fig. 4 shows the effect of adding different levels of glucose oxidase enzyme obtained from B.D.H. Chemicals to 100 ml liquid egg white. It is clear that the glucose level was zero after 8 h using 1 ml of the enzyme dilution (3.8 glucose oxidase units), while there was still glucose detected in egg white up to 10 h using either 0.5 or 0.25 ml of the enzyme dilution.

The glucose is converted to gluconic acid by molecular oxygen in presence of the enzyme. The hydrogen peroxide formed is broken down in presence of catalase in the dioxygenase enzyme system to yield molecular oxygen, making it available for oxidation of additional quantities of glucose. The addition of hydrogen peroxide to the reaction mixture is a convenient method for supplying oxygen to the enzymatic oxidation of glucose to gluconic acid.

The optimum pH for glucose oxidase activity varies from 4.2 to 7.8 depending upon the source of the enzyme preparation [12]. The pH, temperature and hydrogen peroxide concentration that were used in the present study correspond to the optimum conditions according to HAMED et al. [7]. So the only difference between this experiment and that of HAMED's et al. was the source of enzyme. In their experiment the crude enzyme complex was prepared from the micellia of *Penicillium notatum* and was obtained from the Biochemistry Dept., National Research Center, Cairo, Egypt.

The enzyme concentrations used were 2.25, 3.00 and 3.75 glucose oxidase units per 100 ml egg white. It was found that glucose removal was completed in 11 h in all cases, and concluded that the enzyme level had no effect on the rate of glucose removal.

By comparing these results with present ones, it would be clear that in the present work, the enzyme concentration was not enough for complete glucose removal when 0.25 ml (0.95 glucose oxidase units) and 0.5 ml (1.9 units) of the enzyme dilution were used. When 1 ml of the enzyme dilution (3.8 units), a concentration comparable to that used by HAMED et al. [7], was used, glucose was completed in 8 h.

So, it is clear that whatever the source of the enzyme glucose oxidase might be, complete glucose removal from egg white could be done. Besides, it could be concluded from the present work and that of HAMED et al. [7], that under optimum conditions of pH, temperature and hydrogen peroxide concentration, there is a minimum for the enzyme concentration beyond which complete glucose depletion is impossible and above which the increase in the enzyme concentration does not have any effect on the rate of glucose removal.

Zusammenfassung

S. K. SHEHAB, N. M. DARWISH und M. A. SADEK: Entfernung von Glucose aus Eiklar ägyptischer Herkunft vor dem Trocknen

Glucose wird aus Eiklar bei Verwendung von *Streptococcus lactis* und 0,2% Hefeextrakt (pH 6,0; 30 °C) innerhalb von 9 h vollständig entfernt. Dabei entsteht ein unangenehmer Geruch, und das Aussehen verändert sich. Bei Verwendung von *Aerobacter aerogenes* (pH 7,0; 37 °C) dauert die Glucoseentfernung — abhängig von der zugesetzten Bakterienmenge — 3 bis 4 h. Unerwünschte Veränderungen im Geruch und Aussehen treten nicht ein. Mit *Saccharomyces cerevisiae* in Gegenwart von 0,2% Hefeextrakt dauert die Zuckerentfernung 9 h (pH-Optimum 6,0 bis 7,5 bei 32 °C).

Mit Glucoseoxydase (3,8 Einheiten/100 ml Eiklar) ist die Glucoseentfernung bei pH 7,3 und 14,5 °C in 8 h beendet; geringere Enzymmengen reichen für die Entzuckerung nicht aus. Unerwünschte Veränderungen in Geruch und Aussehen treten dabei nicht ein.

Резюме

С. К. Шехаб, Н. М. Дарвиш и М. А. Садек: Удаление глюкозы из яичного белка египетского происхождения перед сушкой

С помощью *Streptococcus lactis* и 2%-ного экстракта дрожжей (pH 6,0; 30 °C) глюкоза удаляется в течение 9 часов полностью из яичного белка. При этом возникает неприятный запах и изменяется внешний вид. При использовании *Aerobacter aerogenes* (pH 7,0; 37 °C), в зависимости от количества добавленных бактерий, удаление глюкозы происходит за 3 до 4 часа. Нежелательных изменений запаха и внешнего вида не наблюдаются. При использовании *Saccharomyces cerevisiae* в присутствии 0,2%-ного экстракта дрожжей удаление сахара длится 9 часов (pH оптимум 6,0 до 7,4 при 32 °C).

С глюкозидазой (3,8 единиц/100 мл яичного белка) удаление глюкозы при pH 7,3 и 14,5 °C закончена через 8 часов, более маленькие количества энзима для обессахаривания недостаточны. Нежелательных изменений запаха и внешнего вида при этом не наблюдаются.

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Technological evaluation of dried egg white prepared by different techniques

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Egg white was spray dried, using Egyptian fresh eggs, with and without glucose removal. Glucose depletion in egg white was carried out using bacteria, yeast and enzyme.

Foam capacity and stability of liquid egg white was found to decrease after glucose was removed by the different methods used. Foam capacity was also decreased by dehydration, except for egg white in which enzyme was used for glucose removal. Foam was more stable in samples desugared by yeast and least stable in enzyme treated egg white. Both pan drying and spray drying methods had the same effect on foam capacity and stability of egg white.

The pH value of liquid egg white was found to decrease by removing glucose and to increase by dehydration.

The specific volume data and organoleptic evaluation of angel food cakes in which dehydrated egg white, imported dried egg white and fresh egg white were used, showed that the best method to prepare dehydrated egg white is by using enzymatic treatment for glucose removal before dehydration.

Dehydration of egg white is carried out mainly by two ways, pan drying and spray drying processes. The drying process should permit the retention of certain valuable properties of the fresh egg white such as solubility, foaming capacity and palatability. It was recommended that glucose must be removed prior to drying to avoid MAILLARD reaction which results in browning and poor storage stability of products [1, 2]. Natural fermentation of Egyptian egg white was unsuccessful in removing glucose [3]. DARWISH et al. [4] studied the different methods that could be applied for preparing dried egg white as a new industry in Egypt. Egg white was dehydrated, with and without glucose removal. Glucose was removed by different methods including the use of bacteria, yeast and enzyme.

The present study is concerned with the technological evaluation of dried egg white, prepared by the different techniques mentioned before. It includes:

- Effect of drying on the foaming capacity and foam stability of egg white.
- As the hydrogen ion concentration is known to be an important factor in determining the foaming capacity of egg white, it was decided to study the effect of removing glucose and dehydration on the pH value of egg white.
- Effect of the drying process on the palatability of egg white.

Materials and methods

Chicken eggs were obtained from the poultry farm of the Animal Husbandry Dept., Ministry of Agriculture, Cairo, Egypt. They were washed, then examined by candling [5]. Egg white was separated, blended and strained. The imported egg white powder that was used for comparison was spray dried, Swiss made.

The methods used for glucose depletion in liquid egg white were:

- Bacterial fermentation using *Str. lactis* in presence of 0.2% yeast extract at pH 6.0 and 30 °C [6].
- Bacterial fermentation using *Aer. aerogenes* at pH 7.0 and 37 °C [6].

- Yeast fermentation using *Sacch. cerevisiae* in presence of 0.2% yeast extract at pH 7.3 and 32 °C [6].
- Enzymatic method in which a crude enzyme complex (prepared from *Penicillium notatum*) containing glucose oxidase and catalase was used [3]. The enzyme concentration used was 2.25 glucose oxidase units/100 ml egg white at pH 6.0 and 14.5 °C in presence of 0.2 ml 30% H₂O₂.

Dehydration of egg white

Egg white was dehydrated using both spray drying and pan drying methods [4].

Whipping test and pH determination

The foaming power of egg white was measured by a rough comparative device, by plunging a ruler into the foam that was still in the bowl in which it was beaten [7].

The pH value of the rehydrated egg white was measured using a "Pye" pH meter with Calomel and glass electrodes.

Technological evaluation of dehydrated egg white:

Angel food cake was used for measuring palatability of dried egg whites, prepared by spray drying method.

The basic recipe for angel food cake [8] was: 46 g flour, 126 g sugar, 122 g fresh egg white, 1.8 g cream of tartar.

The weight of rehydrated egg white, used in making the cake, was the same as that given in the recipe. Rehydration was made by dissolving 4 g dehydrated egg white in 26 g water [8, 9].

After following the method for mixing the ingredients [8], the dough was pured into ungreased 9 × 5 × 35 cm pan and baked at 180 °C for 30 min.

Cake volume was determined by the rape seed test. It is equal to the difference between the volume of seeds required to fill the pan containing the cake and that required to fill the empty pan. Specific volume in cm³/g was calculated.

Samples of the different cake treatments were evaluated by a taste panel.

Table 1 shows the kind of egg white that was used in the different kinds of treatments of angel food cakes. It should be mentioned that samples in which *Str. lactis* was used for the desugaring process had bad odour and unpleasant flavour that affected the cake from which it was prepared and that is why it was excluded from this experiment.

The experiment for the organoleptic evaluation of cake was run as a balanced incomplete block design, designated type V by COCHRAN et al. [10], with $k = 3$, $t = 5$ and $b' = 20$, as shown in Table 2. Thus, 3 samples of angel food cakes made from each of 3 different egg white treatments were baked on each of twenty days. In addition, a duplicate sample was made from just one of the given treatments. The judges did not know that there was a duplicate sample among the 4 samples presented to them. The duplicate was always presented as the last sample. The samples were presented to judges in the same order shown in Table 2. Score cards were offered to taste panel for judging.

Table 1
Kind of egg white used in the different treatments of angle food cakes

Treatment number	Kind of egg white used
0	Imported egg white
I	Dehydrated egg white in which glucose was removed by enzyme before dehydration.
II	Dehydrated egg white in which glucose was removed by yeast before dehydration.
III	Dehydrated egg white in which glucose was removed by <i>Aer. aerogenes</i> before dehydration.
IV	Fresh egg white.