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Chemical and microbial evaluation of Egyptian dried egg white prepared by different techniques

N. M. Darwish, S. K. Shehab and M. A. Sadek

Egg white was dehydrated (pan drying, spray drying) without and with glucose removal (using bacteria, yeast and enzyme). Although, moisture, protein and ether extract percentage in spray dried samples were, in general, in the same range as the imported Swiss made spray dried sample (as standard), yet the least moisture percentage was found in samples in which sugar was removed by yeast.

The standard sample had the best solubility (99.79%), followed by the spray dried sample desugarized by yeast (99.67%). Samples in which *Streptococcus lactis* was used gave the highest percentage of insoluble matter (4.24%). Pan dried and spray dried samples were similar in solubility.

The total microbial counts were found to decrease in egg white after drying (max. 4.9×10 org. in prepared samples, zero in the standard). *Salmonella* was absent in all dried samples.

There are variations in the type of protein fractions of liquid egg white when the enzyme treatment or controlled bacterial fermentation were used. When yeast fermentation was used a decrease in some protein fractions was noticed in comparison with the nondesugarized sample.

The dehydrated egg white proteins indicate that there was no difference between the different techniques applied for glucose removal, between drying methods and between prepared samples and imported one; 5 fractions were obtained in each sample.

All over the world, the demand for dehydrated egg white began to increase for many industries such as an adhesive for cork in bottles capsules in carbonated beverage plants, for coating of roasted coffee and for use in cake mixes.

Dehydration of egg white is carried out mainly by two ways, pan drying or drum drying and spray drying processes. The drying process should permit the retention of certain valuable properties of the fresh egg white such as solubility of the protein, aerating capacity, binding power and palatability.

Dehydration of liquid egg white without removal of glucose results in poor storage stability of dried product [1]. It was recommended that glucose must be removed prior to drying to retard this effect [2, 3]. Hamed et al. [4] found that natural fermentation of Egyptian egg white was unsuccessful in removing glucose. This contradicts the results of Stewart et al. [5] who stated that the fermentation process of egg white was completed in 80 h. The authors explained this difference to be due to the difference in both chemical and bacteriological composition of egg white in different

countries. They succeeded in depleting glucose in Egyptian egg white by using a crude enzyme complex which contained glucose oxidase and catalase and was prepared from *penicillium notatum*.

DARWISH et al. [6] studied the different methods that could be applied for depleting glucose in Egyptian egg white before dehydration. The present study was done to find out the best method that could be applied for preparing dried egg white in Egypt as a new industry. Egg white was dehydrated with and without glucose removal. Glucose removal was carried out using bacteria, yeast and enzyme. Both pan drying and spray drying methods were used.

Evaluation of the final dried products was done by determining both chemical and microbial composition of prepared dried products in comparison with an imported dried sample.

Electrophoretic analysis of protein was carried out on liquid egg white before and after glucose removal and after dehydration.

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 [7]. Egg white was separated, using a commercial breaking tray and separator, blended and strained.

The imported egg white powder that was used for comparison was spray dried (Swiss made).

Pyridine/glacial acetic acid buffer. 8 ml pyridine was added to 2.5 ml glacial acetic acid and diluted to 1 l with water. The buffer was adjusted to pH 5.5.

Veronal/oxalic acid buffer. 10.3 g sodium diethylbarbiturate was dissolved in 1 l water. pH was adjusted to 7.5 by 0.05 M oxalic acid solution.

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

- Bacterial fermentation using *Streptococcus lactis* in presence of 0.2% yeast extract at pH 6.0 and 30 °C [6]. Glucose depletion was completed in 9 h.
- Bacterial fermentation using *Aerobacter aerogenes* at pH 7.0 and 37 °C [6]. Glucose depletion was completed in 3 to 4 h.
- Yeast fermentation using *Saccharomyces cerevisiae* in presence of 0.2% yeast extract at pH 6—7.5 and 32 °C. Glucose depletion was completed in 9 h [6].
- Enzymatic method in which a crude enzyme complex containing glucose oxidase and catalase (prepared from *penicillium notatum*) was used. The enzyme concentration used was 2.25 glucose oxidase units/100 ml egg white at pH 6—7.3 and 14.5 °C and in presence of 0.2 ml of 30% H_2O_2 [4] Glucose depletion was completed in about 11 h.

Methods of dehydration

Spray drying method. A laboratory spray dryer system "Nubilosa" was used. Liquid egg white was first centrifuged, then pasteurized at 50 °C. This helps, too, in accelerating the dehydration rate. Egg white was then atomized under an atomizing pressure of 2 kg/cm². The inlet temperature of the spray dryer was 140 °C and the outlet temperature 70 °C. The produced powder was packed in air tight glass containers.

Pan drying method. Liquid egg white was spread in a thin film of about 0.2 cm thickness using 15 cm diameter petri dishes. The plates were kept on a water bath at 50 ± 1 °C. The dehydration time was about 2 h. The dried egg white was scrapped, ground and kept in air tight glass bottles.

Chemical evaluation

This involved the determinations of moisture, sugar, protein, ether extract and insoluble matter in both prepared and imported dried egg white. Electrophoretic patterns of egg whites protein were also determined before and after dehydration.

Sugar was determined using the modified SOMOGYI method [4].

Moisture, total protein and ether extract were determined by the methods described by the A.O.A.C. [8] uzing KJELDAHL method for protein determination and SOXHLET apparatus with ethyl ether for ether extract determination. Moisture content was determined by drying the samples in an electric oven for 3 h at 105 °C.

Percentage of insoluble matter in dried egg white was determined using 1.5 g samples by dissolving in water and centrifuging at 2000 r.p.m. for 20 min. The precipitate was dissolved again in water and centrifuged. The insoluble matter was washed into tared aluminium dish and dried at 105—107 °C to constant weight.

For electrophoretic analysis, both fresh and dried egg whites were diluted with pyridine : acetic acid buffer solution (1:3 v_w/v). This mixture was centrifuged at 3500 r.p.m. and the precipitate containing impurities was discarded. The supernatant solution was subjected to electrophoresis.

Paper electrophoresis. Whatman No. 1 filter paper stripes 2 × 40 cm were used. The apparatus used consisted of an electrophoretic chamber of the hanging type and a stabilizing power unit. The electrophoretic run was carried out for 18 h at 5 °C and pH 7.5 using veronal/oxalic acid buffer. The current potential used was 250 V and 10 mA/cm of the paper width. Paper stripes were dried at 100 °C for 1/2 h and stained with bromophenol blue (1 g in 1 l methanol) for 20 min. Excess dye was washed out with 5% acetic acid followed by methanol. Stripes were dried on filter paper at room temperature. The separated zones were evaluated by direct scanning of the filter paper using BECKMAN scanner.

Disc electrophoresis. This method was carried out on polyacrylamide following the standard method of DAVIS [9]. Gel tubes, 5 mm diameter and 11 cm length were used. The samples were added in 0.02 ml of 10% (w/v) sucrose. The run was carried out at 5 °C and pH 8.3. Bands were developed using 7% (v/v) acetic acid containing 1% amidoblack for 1 h and then kept for 48 h in 7% acetic acid solution.

Microbiological evaluation

This evaluation included the determination of viable count of total bacteria, yeast, coliform and detection of salmonella.

The total count of bacteria and yeast was determined following the technique recommended by SCHARF [10]. Total plate count agar medium was used for bacterial count and malt agar medium for yeast count. Coliform bacteria were determined using MCCONKEY's broth medium [11]. The most probable number (M.P.N.) of coliform organisms was calculated using specific tables [12]. The detection of salmonella was carried out using the method recommended by AFDOUS [13].

Results and discussion

Table 1 represents the chemical analysis of the prepared spray dried egg white samples in comparison with the imported one. Although the moisture, protein and ether extract percentages were in the same range in all samples, yet, the moisture content of samples, in which yeast was used, was slightly lower than the rest. The slightly higher percentages of protein and fat in prepared samples in comparison with the imported one might be due to differences in chemical composition of eggs.

The insoluble matter percentage in dried egg white is of considerable importance to consumers. It is clear from Table 1 that the imported egg white had a better property, concerning solubility, followed by the sample in which sugar was removed by yeast fermentation. Samples in which *Str. lactis* was used gave the highest percentage of insoluble matter (4.24%), which indicates that the method used, in this case, for glucose depletion had some effect on the solubility of the end product.

Egg white in which glucose was removed by *Aer. aerogenes* was also dehydrated by pan-drying. Solubility of the dried product, in this case, was found to be 98.79% compared with 98.9% obtained by using spray-drying method.

Table 1
Chemical composition of prepared spray dried egg white in comparison with the imported sample

Content [%]	Imported spray dried egg white	Laboratory spray dried egg white				
		Without glucose removal	Glucose removed by enzyme	Glucose removed by yeast fermentation	Glucose removed by bacterial fermentation	
					Using *str. lactis*	Using *aer. aerogenes*
Moisture	7.45	8.04	7.42	6.43	7.95	7.85
Protein	88.50	89.01	90.50	90.04	89.56	89.70
Glucose	0.00	3.50	0.00	0.00	traces	0.00
Ether extract	0.16	0.22	0.21	0.20	0.22	0.20
Insoluble matter	0.21	0.74	2.99	0.33	4.24	1.10

Table 2 shows the microbial flora of the prepared spray dried samples and the imported dried egg white. It is clear that the lowest total microbial count was obtained in the imported spray dried egg white. The total bacterial count in dried egg white depends on the type and number of bacteria present just before dehydration, as well as on the way of handling egg white after dehydration. The type and number of bacteria in liquid egg white before dehydration depends, in turn, on the method employed for glucose removal and the time required for glucose depletion in egg white. These facts might explain why samples dehydrated without removing glucose were the lowest in total bacterial count.

DARWISH et al. [6] reported the bacterial counts in egg white, before and after glucose was removed by different methods. It was clear from the reported data that the highest count was found in egg white desugarized by *Aer. aerogenes*. This might be the reason for the high bacterial content of dried egg white in which glucose was removed by *Aer. aerogenes*.

The total bacterial counts decreased in general after dehydration. It is evident from Table 2 that the flora in the imported sample consisted mainly of sporeformers. Mean-

Table 2
Microbial count per gram of spray dried egg white

Micro-organism	Imported spray dried egg white	Prepared spray dried egg white				
		Without glucose removal	Glucose removed by enzyme	Glucose removed by yeast fermentation	Glucose removed by bacterial fermentation	
					Using *str. lactis*	Using *aer. aero genes*
Total count	2.0×10^2	4.0×10^2	14.0×10^2	14.0×10^2	13.0×10^2	45.0×10^3
Sporeformers	2.0×10^2	8.0×10	5.0×10^2	2.0×10^2	3.0×10^2	—
Coliform	0	4.9×10	4.9×10	1.1×10	1.1×10	3.3×10
Yeast	—	—	—	59.0×10	—	—
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative

while, sporeformers in the prepared samples were only from 14.3 to 35.7% of the total bacterial population. Coliforms ranged from 11 to 49 org./g in the prepared samples and zero in the imported one. Yeast count was 59.0 × 10 org./g in the dried sample desugarized by yeast fermentation. Salmonella was absent in all tested samples. GORMAN [14] reported that salmonella should be negative in dried egg white to follow the specifications and standards of F.D.C. regulations.

Disc electrophoresis was carried out on liquid egg white before and after glucose removal by enzyme, yeast and bacteria. 9 distinctive bands were obtained in all samples indicating the presence of similar protein fractions in all samples. These bands were identified according to FEENY et al. [15] as ovalbumin (3 fractions), conalbumin (2 fractions) and globulin (4 bands). Ovomucoid was not stained. It should be mentioned that removal of glucose by yeast caused a decrease in two fractions (decrease in color intensity of bands), one of which was ovalbumin and the other globulin. This might be due to the proteolytic enzymes found in yeast.

Fig. 1, 2, 3 and 4 show the electrophoretic pattern of liquid egg white using paper electrophoresis. This technique did not succeed in separating the different fractions,

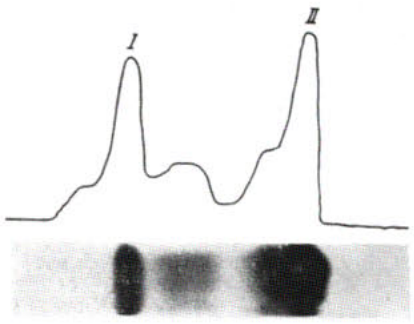

Fig. 1. Paper electrophoretic pattern for liquid egg-white without glucose removal
I — Conalbumin, II — Ovalbumin

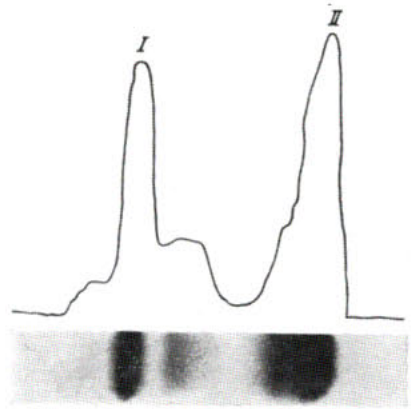

Fig. 2. Paper electrophoretic pattern for liquid egg-white desugared using bacterial fermentation

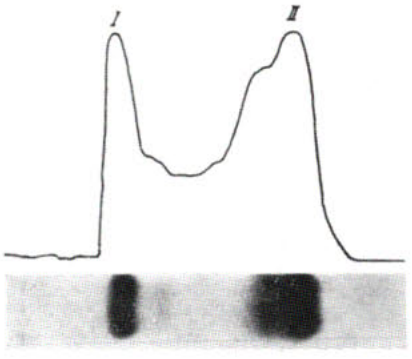

Fig. 3. Paper electrophoretic pattern for liquid egg-white desugared by yeast fermentation

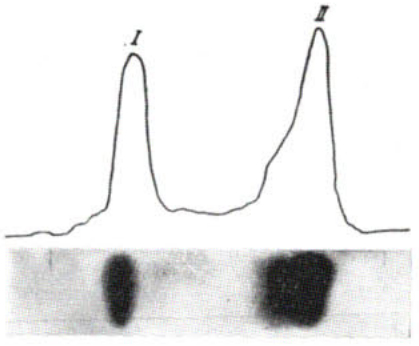

Fig. 4. Paper electrophoretic pattern for liquid egg-white desugared by enzyme treatment

that were obtained by disc electrophoresis. The main proteins found were ovalbumin and conalbumin, although it was hard to separate the different fractions in each one. Small peaks located between these two highest peaks represent the different fractions of globulin.

When disc electrophoresis was carried out on dehydrated egg white, the same kinds of protein fractions were obtained in all spray dried samples, with and without glucose removal, pan dried ones and the imported one. They were 5 fractions, and there was no clear difference between different samples.

These facts lead us to the conclusion that both pan drying and spray drying methods had nearly the same effect on protein pattern of egg white and that there was no difference, in this respect, between the prepared samples and the imported one.

Zusammenfassung

N. M. DARWISH, S. K. SHEHAB und M. A. SADEK: Chemische und mikrobiologische Bewertung von in Ägypten nach verschiedenen Verfahren hergestelltem Trockeneiweiß

Eiklar wurde ohne und unter Entfernung von Glucose mittels Bakterien, Hefen oder Enzymen getrocknet (Pfannentrocknung, Sprühtrocknung).

Wassergehalt, Proteingehalt und Ätherextrakt der sprühgetrockneten Proben entsprachen etwa den Werten einer als Standard verwendeten Probe (Schweiz); am niedrigsten lag der Wassergehalt in mit Hefe entzuckerten Proben.

Die Standardprobe hatte die beste Löslichkeit (99,79%), es folgte die mittels Hefe entzuckerte Probe (99,67%). Mit *Streptococcus lactis* behandelte Proben hatten den höchsten Gehalt an Unlöslichem (4,24%). Pfannen- und sprühgetrocknete Proben unterschieden sich nicht in der Löslichkeit.

Die Gesamtkeimzahl nahm beim Trocknen ab; sie betrug maximal $4{,}9 \cdot 10/g$ der getrockneten Proben (Null im Standard). Keine Probe enthielt Salmonellen.

Enzymbehandlung und kontrollierte Vergärung mittels Bakterien führte zu keinen Veränderungen der Proteinfraktionen des flüssigen Eiweißes. Bei Hefeanwendung war bei einigen Proteinfraktionen im Vergleich zur nicht entzuckerten Probe eine Abnahme festzustellen. Beim Trockeneiweiß zeigten die Proteine keine von der Technik der Glucoseentfernung oder der Trocknung abhängige Veränderungen; es gab auch keine Unterschiede zur Vergleichsprobe; jede Probe enthielt 5 Fraktionen.

Резюме

Н. М. Дарвиш, С. К. Шехаб и М. А. Садек: Химическая и микробиологическая оценка полученного в Египте различными способами сухого белка

Яичный белок с удалением или без удаления глюкозы сушился с помощью бактерий, дрожжей или энзимов (сущение в чашах или распылительной сушкой).

Содержание воды, содержание белка и эфирный и эфирный экстракт сушенных распылительной сушкой проб примерно соответствовали взятой в качестве стандарта пробе (Швейцария); самое низкое содержание воды наблюдалось в пробах, обессахаренных дрожжами.

Найлучшая растворимость (99,79%) наблюдалась в стандартной пробе, затем следовали обессахаренные дрожжами пробы (99,67%). Найбольшее содержание нерастворимых веществ (4,24%) обнаруживалось в обработанных *Streptococcus lactis* в пробах. Сушенные в чашах и распылительной сушкой пробы не отличались по растворимости. Общее число обсеменения снижается с сушкой; оно соответствовало максимально $4{,}9 \cdot 10/г$ сухой пробы (ноль в стандарте). Ни в одной пробе не обнаружены сальмонеллы.

Обработка энзимами и контролированное сбраживание с помощью бактерий не вызывает изменений белковых фракций растворимого белка. При обработке дрож-

жами в некоторых белковых фракциях обнаруживалось белке снижение по-сравнению с не обессахаренными пробами. В сухом белке не наблюдали никаких изменений, зависящих от технологии удаления глюкозы или сушения; нет и различий по отношению стандартной пробы; в каждой пробе имелись 5 фракций.

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The Relation between Ketone Bodies, Glucose and Minerals Contents in Bovine Blood under Various Physiological Conditions

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The interrelationship between the concentration of ketone bodies, glucose, minerals (Na, K, Ca, Mg, P, Cl) in serum and in TCA soluble whole blood under different physiological conditions in 110 cows was investigated.
There was a certain relationship (partly high significant) between the mineral content, ketone bodies and glucose levels in blood. However, there is no clear explanation for the qualification of blood minerals as an indicator of the content of ketone bodies and glucose in serum and whole blood. The age of animals and the seasons had no influence on the concentration of ketone bodies in serum and whole blood. Significant positive relationship was found between the time of lactation, pregnancy and serum glucose and negative relation with ketone bodies. There was no correlation between milk production and ketone bodies. The hours after feeding were positively correlated with ketone bodies.

Introduction

Ketosis, a wellknown disorder of metabolic disturbances, characterized by increased production of ketone bodies and decreased blood glucose content, is especially noticed in cows with high milk production. In USA yearly about 4% of the cows are ketotics mainly during a period from 10 days to 8 weeks post-partum (SHAW, 1956) with concomitant deficiency in glucose. The daily requirement of high productive cows is about 2 kg glucose. The mobilization of fat depots as a consequence of relatively low fodder consumption after pregnancy in relation to milk production affects the increase in ketone bodies (PATTERSON, 1966).

Beyond the critical period of post-partum, many various factors causing ketosis must be concidered. The role of minerals in ketosis is not well established. Deficiency of some trace elements such as Cobalt causes diminution of vitamin B_{12}-formation which is important for the conversion of propionate to succinate to glucose and could be a factor for the formation of ketosis. CORSE et al. (1970) have shown that vitamin B_{12} has a preventive effect on ketosis. Hypomagnesaemia has been reported to be associated with ketosis (BREIREM, 1949). Little is known in the literature about the relation between minerals and ketone bodies content in bovine blood. This work deals with investigations on the relationships between the levels of ketone bodies, glucose and major mineral content in blood of dairy cows and the variations associated with different physiological states.

Material and Methods

Blood samples were obtained during one year by jugular vein puncture from 110 dairy cows in different physiological conditions reared not uniformly in eight farms around Helsinki. The animals were mainly Ayrshires, although a few cows of Finnish native breed were included. To avoid haemolysis and hydrolysis of organic phosphate samples were centrifuged as soon as possible and sera were

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