



## BIOLOGICAL EVALUATION OF COW'S MILK YOGHURT FORTIFIED WITH DIFFERENT SOURCES OF MILK PROTEINS

[13]

Mervat S. Youssef<sup>1</sup>; M.S. Masoud<sup>1</sup> and A.E. Fayed<sup>2</sup>

1. Regional Center for Food & Feed, Agriculture Research Center, Giza, Egypt
2. Food Science Department, Faculty of Agriculture, Shoubra El-Kheima, Ain Shams University, Cairo, Egypt

**Keywords:** Skim milk powder, Sodium caseinate, Whey protein concentrate, Yoghurt starter culture micronutrients, ultrafiltration, Blood plasma, Mineral bioavailability

### ABSTRACT

The aim of this study was essentially to evaluate the biological quality of cow's milk yoghurt fortified with nil (control) or 1.0% milk protein supplied either from skim milk powder (SMP), sodium caseinate (NaCn), dried whey protein concentrate (DWPC) or by concentrating the milk itself through the ultrafiltration (UF) process to obtain the same protein elevation level.

The obtained results revealed that SMP-fortified yoghurt was characterized with the highest contents of dry matter (DM), lactose/DM, ash/DM, titratable acidity, acetaldehyde and bacterial counts of yoghurt starter culture. It contained also the highest levels of Ca, P, K, Mg, Cu and Mn. Highest Fat/DM% was found in UF-yoghurt and highest pH value was found in NaCn-Yoghurt., which contained also the highest Na level. The highest K and Fe levels were found in DWPC-yoghurt, which possessed also the highest levels of thiamin (B<sub>1</sub>), like as in the UF-one, and riboflavin (B<sub>2</sub>).

Biologically, the highest true digestibility and net protein utilization were achieved in rats fed on DWPC-yoghurt. However, rats fed on SMP- yo-

ghurt exhibited the highest biological value of protein. Best blood picture, as indicated from white and red blood cells, hemoglobin and hematocrit, was accomplished in rats fed on UF- yoghurt followed by those fed on DWPC-one. The profile of blood plasma of rats indicated that DWPC-yoghurt caused the highest level of total protein, albumin (A) and globulin (G). Nevertheless, the highest A/G ratio was occurred due to feed whether on SMP-, UF- or control- yoghurt.

Furthermore, rats fed on DWPC-yoghurt gained the best mineral bioavailability as indicated from the highest levels of Ca, P, K, Mg, Cu, Fe and Zn in their blood plasma. The same was valid for UF-yoghurt with respect to P, Mg and Fe and for SMP-yoghurt towards only the K absorption.

As a conclusion, UF-technique offers the best possibility for yoghurt quality improvement whether *via* facilitate the supplementation with WPC or UF-milk retentate to enhance both compositional content and biological quality of yoghurt.

### INTRODUCTION

Yoghurt is the most famous fermented dairy product in world and well-being has existed in many civilizations for a long time.

Cow's milk, which is the main obtainable raw milk supply for dairy industries in many countries, is relatively characterized with thin body because

of its solids deficiency and its obtained yoghurt is usually suffering from a pronounced weak consistency and wheying off defects. In some countries, the use of non dairy stabilizers in yoghurt making for consistency improvement is governed by legislative regulation (FAO/WHO, 1976). The use of such non dairy ingredients in yoghurt is not legally permitted in Egypt (EOSQC, 2005) as well as in many other countries (Fayed *et al* 1996 and Tamime & Robinson, 1999). Therefore, solids enrichment became necessary when yoghurt was designed to be made from cow's milk for overcoming such disadvantages. Thermal treatment was the old traditional procedure applied to prevent such defects by heat solids concentration. Nevertheless, the excessive thermal process to reach the prospective solids concentration affects the nutritive benefits of milk. Solids elevation has been also achieved by the direct addition either of skim milk powder (Lankes *et al* 1998; Folkenberg & Martens, 2003 and Husein, 2006), total milk proteinate (Fayed *et al* 1996 and Omar & Abou El-Nour 1998), whey protein concentrate (Abd El-Salam *et al* 1991; Remeuf *et al* 2003 and Husein, 2006). Moreover, the technique of membrane filtration, especially the ultrafiltration, has successfully contributed with an efficient role to avoid such faults in yoghurt quality (Chapman *et al* 1974; Jepsen, 1979; Abrahamson & Holmèn, 1980; Hagagg & Fayed, 1988 and Husein, 2006). However, little information is rather available about the biological comparison between such products in relation to their protein supplementation procedures.

For that in view, the objective of the present research was to find out how some fortification procedures with milk protein might influence the cow's milk yoghurt attributes with emphasis on the biological quality.

## MATERIALS AND METHODS

### Materials

Fresh cow's milk (3.50% fat and 3.27% protein) was obtained from Higher Institute of Agric. Co-operation, Shoubra El-Kheima at Faculty of Agriculture, Ain Shams University. Skim milk powder (SMP, 36% protein) made in Denmark and dried whey protein concentrate (DWPC, 82% protein) made by SEK DATABLAD, Hvidovre and Viborg, Denmark, were obtained from the local market. Sodium caseinate (NaCn, 84% protein, Lactovit Co. Germany) was obtained from

Arab Dairy Co., Cairo, Egypt. Lyophilized mixed yoghurt starter culture (YSC) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from Chr. Hansen's laboratories, Copenhagen, Denmark.

### Experimental procedures

Five treatments including the control were designed, where cow's milk was firstly fortified with nil (control) or 1.0% milk protein whether directly by adding the suitable quantity expressed on the base of the protein content of SMP, NaCn or DWPC, or by the concentration of milk itself to reach the same protein elevation level by the ultrafiltration (UF) technique at 50°C (as recommended by Maubois *et al* 1971) using CARBOSEP UF-unit (type 2S 37, France) with zirconium oxide membrane area 1.68 m<sup>2</sup> after its previously heat treatment at (72°C/ 2 min.) prior UF processing.

The yoghurt bases were prepared as described by Tamime & Robinson (1999) with adopting the manufacture conditions enacted by EOSQC (2005), where yoghurt milk was heat treated to 85°C for 5 min. followed by temperature adjustment to 42°C. Then, yoghurt milks were inoculated with 2% of freshly activated YSC, filled into 1 kg polystyrene containers, covered, and incubated until complete coagulation (through about 3 h.). Thereafter, the containers were transferred to the refrigerator (5±1°C), where they were kept to the next day for analyses. Three replicates were done for every treatment.

### Analytical methods

Dry matter (DM), fat, nitrogen, ash contents and titratable acidity (TA) were determined according to AOAC (2000). Lactose content was calculated by the difference. The acetaldehyde (AC) content was estimated as described by Lees and Jago (1969). The pH value was measured using a pH meter (HANNA Instruments, USA)

Levels of calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg) as major minerals; and zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) as minor minerals were determined using an Inductive Coupled Plasma (ICP)-plasma technique, Perkin Elmer-Optima 2000 DV as described in AOAC (2000).

The count of *Str. Thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were enumerated using in order M17 and MRS agar media as described

by **Gueimonde et al (2003)**. Both cultures were incubated anaerobically for 2 days at 37°C. The count was expressed as colony forming units (cfu)/g of product.

Thiamin (B<sub>1</sub>) and Riboflavin (B<sub>2</sub>) were measured as described by **Bognar (1992)**, using Beckman HPLC consisting of pump model 126, injector and data handling system. Perkin-Elmer fluorescence detector LC240 and C18 column 25 cm x 4.6 mm were used.

Biological assay was carried out using weighing age Albino male rats of 70-75 g caged individually in the metabolic cages, those were employed for rats comprised an upper living area with feeding system and below a device for the collection of urine and feces. The cages were similar in construction to that described by **Schiller (1960)**, although with a modified method. Acid-washed bottle jars with polyethylene stoppers were used for drinking water and collection of feces and urine (**Horszczaruk & Bock, 1963**) and maintained at 20-24°C and 45-55% relative humidity. Diets and water were provided fresh daily unless otherwise specified.

Five different diets were previously prepared according to the composition of the diet of **Eggum (1973)** for the 5-subjected experimental animal groups. The 5-diets were different not only in their protein sources but also in their nature as well as the all yoghurt samples were high in fat content in degree to over the recommended dietary allowance (RDA) of the rats. Thus, the calculation of the nutrients due to the composition of different 5-diets of the corresponding 5 animals groups was according to iso-caloric diet (40 Kcal/rat/day), where starch and oil are expensed in the nitrogen free diet. The rats fed 150 mg nitrogen and 10 g DM/rat/day. The amount of DM is adjusted with N-free diet supplied with vitamins and minerals.

To evaluate the effect of yoghurt samples on the absorption of nutritional minerals in blood plasma of rats, the procedure of **Eggum (1973)** was applied where rats were housed individually in metabolic cages distributed on 5-subjected experimental of 5-rats for each. The 5-groups were fed barley for one-day before starting the experimental to be adapted. The experiment was spread over for a period of 9 days. The animals were weighed at the beginning of the experiment and again at the end. During the experimental period urine of rats was collected in 50 ml of 5% H<sub>2</sub>SO<sub>4</sub> while their feces in 100 ml H<sub>2</sub>SO<sub>4</sub>. Nitrogen of urine and feces was estimated. At the end of experiment all rats were exposed to fasting period of

3 h. and accurately weighed according to **Waynforth & Flecknell (1992)**. Rats were anaesthetized with ether, blood samples were collected from plexieye *via* capillary tube, where, total protein and albumin of blood plasma were measured by the photometric system according to the biuret and bromocresol green methods in order (**Johnson et al, 1999**), while globulin was calculated by the difference between them. Complete blood picture was carried out according to **RDS (1995)** using Micro-Cobas automatic blood cell counter.

The parameters of the protein quality, namely true digestibility (TD) biological value (BV) and net protein utilization (NPU) for the 5-yoghurt samples which evaluated biologically *via* the corresponding 5-rats groups were calculated individually according to **Eggum (1973)**.

The obtained data were exposed to statistical analysis according to statistical analyses system user's guide (**SPSS, 1998**).

## RESULTS AND DISCUSSION

### Gross composition

The results displaying in **Table (1)** show that the fortification of yoghurt milk with protein led to significant differences ( $P < 0.01$ ) in all chemical composition depending on the procedure applied. The dry matter (DM), the lactose/DM and ash/DM contents were higher in SMP- yoghurt and lower, in order, in the control and UF- yoghurt, which contained the highest fat/DM%. That was because the UF process allows passing lactose as well as minerals and retains milk fat as done towards the true protein as stated by **Maubois et al (1971)**; **El-Hofi (1984)**; **Fayed (1986)**; **Haggag & Fayed (1988)** and **Husein (2006)**. The decreased fat content was found in SMP- and DWPC- yoghurts. The control contained the lower, while either DWPC- or NaCn-yoghurt possessed the highest protein/DM% due to the increased protein content of any of both fortifying ingredients.

### Bacterial population

Data of **Table (1)** indicate that the 1% protein fortification of yoghurt milk by adding SMP led to significant increment in the count either of *Str. thermophilus* or *Lb. delbrueckii* subsp. *bulgaricus* followed that supplemented by DWPC. That might be due to some special nutritional growth factors occurred indirectly through adding of such fortifying source, which perhaps meet the

Table 1. Chemical and bacteriological properties of cow's yoghurt elevated with 1.0% milk protein by different procedures

Property	Control	Source of milk protein elevation			
		SMP	UF	NaCn	DWPC
Dry matter (DM) %	12.75 <sup>d</sup>	15.13 <sup>a</sup>	14.53 <sup>b</sup>	13.78 <sup>c</sup>	13.80 <sup>c</sup>
Fat/DM <sup>a</sup>	30.21 <sup>b</sup>	24.43 <sup>d</sup>	35.25 <sup>a</sup>	25.37 <sup>c</sup>	24.99 <sup>d</sup>
Protein/DM %	28.97 <sup>d</sup>	30.44 <sup>c</sup>	31.74 <sup>b</sup>	33.00 <sup>a</sup>	33.03 <sup>a</sup>
Lactose/DM <sup>a</sup>	28.89 <sup>d</sup>	31.57 <sup>a</sup>	21.65 <sup>c</sup>	29.05 <sup>b</sup>	29.97 <sup>b</sup>
Ash/DM %	6.20 <sup>c</sup>	6.95 <sup>a</sup>	5.51 <sup>c</sup>	6.77 <sup>b</sup>	6.90 <sup>d</sup>
Acidity % <sup>**</sup>	0.73 <sup>c</sup>	1.00 <sup>a</sup>	0.85 <sup>b</sup>	0.80 <sup>c</sup>	0.85 <sup>b</sup>
pH value	4.50 <sup>c</sup>	4.45 <sup>c</sup>	4.75 <sup>b</sup>	4.77 <sup>b</sup>	4.85 <sup>b</sup>
AC ( $\mu$ mol/100g)	308 <sup>c</sup>	445 <sup>a</sup>	347 <sup>c</sup>	233 <sup>d</sup>	420 <sup>b</sup>
Str. ( $\times 10^7$ )	19.50 <sup>c</sup>	25.12 <sup>a</sup>	19.91 <sup>c</sup>	18.20 <sup>d</sup>	22.39 <sup>b</sup>
Lb ( $\times 10^6$ )	19.06 <sup>c</sup>	22.91 <sup>d</sup>	18.62 <sup>d</sup>	17.38 <sup>e</sup>	21.38 <sup>b</sup>

The means with the same letter did not significantly differ ( $P > 0.05$ )

SMP - Skim milk powder

UF - Ultrafiltration

NaCn - Sodium caseinate

DWPC - Dried whey protein concentrate

\* Calculated by the difference

\*\* Determined as lactic acid AC - Acetaldehyde

Str. *Streptococcus thermophilus*

Lb *Lactobacillus delbrueckii* subsp. *bulgaricus*

nutritional requirement of those strains. **Rasic and Kurmann (1978)** reviewed that the protein containing sulphur amino acids influenced favorable the growth of *Lactobacillus* sp. by lowering the oxidation/reduction potential through increasing groups made available by heating. Likewise, **Dave and Shah (1998)** found that, the fortification of yoghurt with 2% WPC supported the growth of *Str. thermophilus* and multiplication of this organism was faster, which could have been the reason for the shorter incubation time needed to reach pH of 4.5 for samples. Similar observations were reported by **Husein (2006)**.

### Biochemical properties

The protein fortification resulted in significant increase in titratable acidity (TA) and acetaldehyde (AC) content of yoghurt, but delayed the reduction in the pH value, except of that fortified with SMP, (Table, 1). This phenomenon could be ascribed to the strengthened buffering capacity gained as the protein content raised. Similar findings were reported by **Brule et al (1974)**; **Mocquot (1979)**; **Green et al (1981)**; **Haggag & Fayed (1988)** and **Husein (2006)**. Moreover, **Ra-**

**sic & Kurmann (1978)** and **Estevez et al (1988)** confirmed that the increased solids content by adding SMP enhanced favorably the production of AC of yoghurt. However, SMP caused the highest TA and AC contents; and the lowest pH value of yoghurt among other fortification supplies. That could be due to the relatively high lactose content of SMP *vis a vis* other protein sources studied. Many authors stated that, the fortification with milk powder can lead to excessive acidity (**Hamdy et al 1972**; **Tamime & Deeth, 1980**; **Lankes et al 1998**; **Tamime & Robinson, 1999** and **Husein, 2006**). Further, **Rasic & Kurmann (1978)** reported also that the rate of AC production is highly dependent on the acidity level. The control exhibited the lowest figures in these respects DWPC-yoghurt took place the second order in both two properties, whilst UF-yoghurt had AC content lower than that of DWPC one. The TA% of UF-yoghurt did not vary from those fortified with NaCn or DWPC. While, the pH value of NaCn- yoghurt was higher than other samples. The pH value whether of UF- or DWPC- yoghurt occupied the second order. The control and SMP-yoghurt had similar lowest pH values.



### Micronutrients situation

The yoghurt fortification with milk protein *via* SMP was associated also with minerals elevation, where its yoghurt exhibited the highest levels of Ca, P and Mg. While, due to the minerals permeability towards UF membrane whether during the preparation of whole-milk retentate or previously applied during DWPC preparation from whey, UF-yoghurt had the least levels of K, Na and Mg as well as the DWPC fortified yoghurt came in the second order in all minerals studied except of K (Table, 2). With exception of Na and K, NaCn-fortified yoghurt possessed the lowest minerals values.

UF-yoghurt ranked the first order in relation to Cu and Zn contents while DWPC-fortified yoghurt had the highest levels of Fe. Fukuwatari *et al* (1982), Glover (1985) and Fayed *et al* (1995) found that, minerals such as Ca, Mg, and P as well as trace elements such as Cu, Fe and Zn are partly or wholly bound to protein; only those bound are UF-retained and concentrated whether in the milk retentate or in the WPC.

All protein-fortification procedures studied led to impart the yoghurt higher vitamins levels. DWPC- or UF-yoghurt contained the highest value of thiamin (B<sub>1</sub>). Likewise, DWPC- yoghurt appeared the highest riboflavin (B<sub>2</sub>) content (Table, 2). Renner & Abd El-Salam (1991) reviewed that, when compared with RDA, the WPC appears to be a significant source of B<sub>1</sub>, B<sub>2</sub> and B<sub>12</sub>, and a good source of pantothenic, because 80-90% of B<sub>1</sub>, 70-80% of B<sub>2</sub>, 40-70% of B<sub>12</sub> and 55-75% of pantothenic acid are transferred during cheesemaking into the whey. Moreover, Glover (1971) confirmed that the UF-retention percent of thiamin was 62% for whole milk and 33% for whey. Likewise, the corresponding figures for riboflavin were 61 and 50%, respectively.

The trends of the yoghurt micronutrients are in coincidence with those reviewed by Renner (1983); Green *et al* (1984); Renner *et al* (1989); Scott (1989); Renner & Abd El-Salam (1991) and Youssef (2000).

### Nutritional protein quality

Concerning the quality of yoghurt protein from the nutritional view point in relation to the fortification procedure, DWPC-yoghurt gained the best true digestibility (TD) % (Table, 3). Renner & Abd El-Salam (1991) reviewed that, WPC has a high and balanced amino acid profile, and its

TD% has been found to be 97-100%. Moreover, the decrease of casein portion by adding WPC led to inhibit the formation of a very firm and only slowly decomposable coagulum and consequently enhance markedly the passage of the milk through the stomach. Boirie *et al* (1997); Fruhbeck (1998) and Jacobucci *et al* (2001) showed that, casein was absorbed slowly and promoted post-prandial protein deposition by inhibition of protein breakdown without excessive increase in blood amino acid concentration. On the other hand, whey protein was absorbed very fast and rapidly stimulated protein synthesis but also acid oxidation. The investigators classified casein and whey protein, as slow and fast metabolizing proteins respectively. UF-yoghurt ranked the second order followed by NaCn-yoghurt, which did not vary from the unfortified one (control). SMP-fortified yoghurt showed the lowest TD%. That could be due to the expansive level of lactose associated with SMP adding (Table, 1). Renner & Abd El-Salam (1991) reported that, the TD% decreases with increased dietary lactose content. The biological value (BV) % of SMP-yoghurt protein was the highest. They confirmed that, although the TD% decreases, the BV% increases with increased dietary lactose content. Whereas, the fraction of absorbed nitrogen exerted as urinary urea nitrogen decreased due to that, the lactose delays the absorption of amino acids, thereby making their utilization more efficient. DWPC-yoghurt came in the second order of BV%. Renner & Abd El-Salam (1991) declared that, there is a clear difference between casein and whey protein which has the highest BV% (104%). UF-yoghurt seemed BV% higher either than NaCn-yoghurt or the control.

The net protein utilization (NPU) % which is the multiplication yield of both of TD% and BV% was higher in DWPC-yoghurt followed by UF-one. Jacobucci *et al* (2001) elucidated that the essential amino acid scores, compared with FAO/WHO (1991) is higher (essentially 1.0) for WPC and lower (0.87) for casein. Moreover, they found that no differences should exist in ratios of the rats weight gain/protein consumption between WPC and casein. Samples, either of SMP or the control ranked the third order, while NaCn-yoghurt caused the lowest NPU. That was clearly reflected on the growth rate of experimental rats fed thereon. Where, animals fed on UF or WPC-yoghurt grown better than those fed on the control, SMP or NaCn-yoghurt, respectively. The overall trends of these results agree with those explained

Table 2. Minerals, trace elements and vitamins of cow's yoghurt elevated with 1.0% milk protein by different procedures

Component	Control	Source of milk protein elevation			
		SMP	UF	NaCn	DWPC
Minerals (g/kg)					
Ca	1.444 <sup>d</sup>	2.232 <sup>a</sup>	1.542 <sup>c</sup>	1.393 <sup>c</sup>	1.661 <sup>b</sup>
P	1.117 <sup>d</sup>	1.676 <sup>a</sup>	1.244 <sup>c</sup>	1.185 <sup>d</sup>	1.359 <sup>b</sup>
K	1.157 <sup>c</sup>	2.080 <sup>a</sup>	1.118 <sup>c</sup>	1.323 <sup>b</sup>	1.988 <sup>a</sup>
Na	0.383 <sup>c</sup>	0.551 <sup>b</sup>	0.383 <sup>c</sup>	0.624 <sup>a</sup>	0.505 <sup>b</sup>
Mg	0.118 <sup>c</sup>	0.183 <sup>a</sup>	0.117 <sup>c</sup>	0.113 <sup>c</sup>	0.163 <sup>b</sup>
Trace elements (mg/kg)					
Cu	0.363 <sup>c</sup>	0.505 <sup>a</sup>	0.498 <sup>a</sup>	0.397 <sup>b</sup>	0.410 <sup>b</sup>
Fe	0.240 <sup>d</sup>	0.339 <sup>c</sup>	0.586 <sup>b</sup>	0.218 <sup>d</sup>	0.781 <sup>a</sup>
Zn	5.082 <sup>d</sup>	7.559 <sup>b</sup>	8.607 <sup>a</sup>	5.810 <sup>c</sup>	6.192 <sup>c</sup>
Mn	0.066 <sup>c</sup>	0.107 <sup>a</sup>	0.083 <sup>c</sup>	0.072 <sup>d</sup>	0.094 <sup>b</sup>
Vitamins (mg/kg)					
B <sub>1</sub>	0.496 <sup>c</sup>	0.529 <sup>b</sup>	0.650 <sup>a</sup>	0.550 <sup>b</sup>	0.672 <sup>a</sup>
B <sub>2</sub>	1.539 <sup>d</sup>	1.733 <sup>b</sup>	1.681 <sup>c</sup>	1.527 <sup>d</sup>	1.980 <sup>a</sup>

The means with the same letter did not significantly differ ( $P>0.05$ )

SMP : Skim milk powder

UF : Ultrafiltration

NaCn: Sodium caseinate

DWPC: Dried whey protein concentrate

Table 3. Nutritional protein quality and growth rate of rats fed on cow's yoghurt elevated with 1.0% milk protein by different procedures

property	Control	Source of milk protein elevation			
		SMP	UF	NaCn	DWPC
True digestibility %	92.83 <sup>c</sup>	88.74 <sup>d</sup>	94.36 <sup>b</sup>	92.59 <sup>c</sup>	96.83 <sup>a</sup>
Biological value %	83.60 <sup>d</sup>	87.11 <sup>a</sup>	84.12 <sup>c</sup>	83.10 <sup>d</sup>	86.35 <sup>b</sup>
Net protein utilization %	77.61 <sup>c</sup>	77.30 <sup>c</sup>	79.38 <sup>b</sup>	76.94 <sup>d</sup>	83.61 <sup>a</sup>
Growth rate (g/day)	2.05 <sup>b</sup>	1.80 <sup>c</sup>	2.48 <sup>a</sup>	1.57 <sup>d</sup>	2.42 <sup>a</sup>

The means with the same letter did not significantly differ ( $P>0.05$ )

SMP : Skim milk powder

UF : Ultrafiltration

NaCn: Sodium caseinate

DWPC: Dried whey protein concentrate

by Renner (1983); Renner *et al* (1989); Scott (1989); Renner & Abd El-Salam (1991) and Youssef (2000).

### Blood picture

The blood picture, that reflecting the physiological situation of animal as an intake function, indicated that rats fed UF-yoghurt possessed the excellent condition with regard to all parameters studied without any exception, namely, white and red blood cells (WBC and RBC), hemoglobin (HGB) and hematocrit (HCT). Rats fed on DWPC-yoghurt occupied the second order (Table, 4). These phenomena are, not haphazardly, corresponding to the significantly higher Fe and Cu contents found together in the two yoghurts (Table, 2). Renner *et al* (1989) explained that copper deficiency, like iron deficiency, causes anemia. Moreover, the availability of copper from milk can be improved by changing the ratio between casein and whey protein in favor of the latter. That is what has been actually carried out by UF- milk concentration or rather by DWPC adding to the yoghurt milk. Lesser figures of RBC and HCT were determined in the blood of rats when fed on SMP-yoghurt, NaCn-yoghurt or rather on the control. Both HCT values whether of SMP- or NaCn-yoghurt were under the recommended normal healthy range (36.0) reported by Bishop *et al* (1992).

### Blood plasma profile

Concerning the blood plasma profile, data given in Table (5) reveal that, rats fed on DWPC-

supplemented yoghurt gained the highest levels of albumin and globulin and hence total protein contents in their blood plasma followed by those fed on UF-yoghurt, the control, NaCn- and SMP-yoghurt. These phenomena could be explained with regard to the corresponding results of the protein qualities given in Table (3).

Regarding the minerals contents of blood plasma, it could be firstly noticed that, although the experimental diets were previously adjustably supplied with required minerals, the bioavailability of them were significantly differently valued in consequence of the source of milk protein with which rats-feeding yoghurts were fortified. Renner (1983) and Miller *et al* (1995) declared that, minerals bound to protein are in the forms most available and easily utilized by the body than when present in the ionic forms. The figures given in Table (5) indicated that blood plasma of rats fed on DWPC-yoghurt contained the highest levels of Ca, P, K, Mg, Cu, Fe and Zn. Renner *et al* (1989) and Renner & Abd El-Salam (1991) confirmed that, the bioavailability of Zn from casein-containing diets is lower than from diets containing whey protein or serum albumin. One reason for this difference is the binding of Zn to the negative charged phosphate groups of casein micelles. Another reason is the relatively low amino acid cysteine content of casein (0.6%) as compared to that of whey proteins (2.5%) and serum albumin (6.7%). The same was valid for the amino acid methionine. Likewise, the availability of Cu and Fe was associated with diets containing lactalbumin. Dael *et al* (2005) found that the apparent absorption of Ca, P, Fe and Zn from the whey proteins-adopted diets (casein: whey proteins, 40: 60)

Table 4. Blood picture of rats fed on cow's yoghurt elevated with 1.0% milk protein by different procedures

Property	Control	Source of milk protein elevation			
		SMP	UF	NaCn	DWPC
White blood cells ( $10^3/\text{mm}^3$ )	5.80 <sup>d</sup>	10.17 <sup>a</sup>	9.80 <sup>a</sup>	7.20 <sup>c</sup>	8.90 <sup>b</sup>
Red blood cells ( $10^6/\text{mm}^3$ )	6.42 <sup>c</sup>	6.30 <sup>c</sup>	7.72 <sup>a</sup>	6.09 <sup>d</sup>	6.89 <sup>b</sup>
Hemoglobin (g/dl)	12.6 <sup>b</sup>	11.9 <sup>b</sup>	13.7 <sup>a</sup>	12.1 <sup>b</sup>	12.6 <sup>b</sup>
Hematocrit %	36.6 <sup>b</sup>	35.3 <sup>c</sup>	41.2 <sup>a</sup>	34.5 <sup>c</sup>	36.7 <sup>b</sup>

The means with the same letter did not significantly differ ( $P>0.05$ )

SMP : Skim milk powder

UF : Ultrafiltration

NaCn: Sodium caseinate

DWPC: Dried whey protein concentrate

Table 5. Blood plasma profile of rats fed on cow's yoghurt elevated with 1.0% milk protein by different procedures

Property	Control	Source of milk protein elevation			
		SMP	UF	NaCn	DWPC
Total protein (g/dl)	5.25 <sup>b</sup>	4.49 <sup>c</sup>	4.45 <sup>a</sup>	4.68 <sup>c</sup>	5.48 <sup>a</sup>
Albumin (g/dl)	3.40 <sup>b</sup>	2.97 <sup>d</sup>	3.50 <sup>b</sup>	3.11 <sup>c</sup>	3.37 <sup>a</sup>
Globulin (g/dl)	1.85 <sup>b</sup>	1.52 <sup>c</sup>	1.95 <sup>b</sup>	1.57 <sup>c</sup>	2.11 <sup>a</sup>
A/G Ratio	1.84 <sup>ab</sup>	1.95 <sup>a</sup>	1.80 <sup>b</sup>	1.98 <sup>a</sup>	1.60 <sup>c</sup>
Mineral (ppm)					
Ca	51.920 <sup>d</sup>	70.98 <sup>c</sup>	74.70 <sup>b</sup>	43.88 <sup>c</sup>	76.07 <sup>a</sup>
P	177.65 <sup>b</sup>	166.40 <sup>c</sup>	180.60 <sup>a</sup>	165.15 <sup>c</sup>	181.05 <sup>a</sup>
K	121.30 <sup>c</sup>	169.80 <sup>a</sup>	146.85 <sup>b</sup>	123.95 <sup>c</sup>	168.55 <sup>a</sup>
Mg	10.719 <sup>c</sup>	15.015 <sup>b</sup>	16.700 <sup>a</sup>	11.380 <sup>c</sup>	16.545 <sup>a</sup>
Cu	0.720 <sup>b</sup>	0.340 <sup>a</sup>	0.602 <sup>c</sup>	0.634 <sup>c</sup>	0.888 <sup>a</sup>
Fe	2.993 <sup>c</sup>	3.468 <sup>b</sup>	3.650 <sup>a</sup>	3.559 <sup>b</sup>	3.611 <sup>a</sup>
Zn	1.062 <sup>d</sup>	1.259 <sup>c</sup>	1.390 <sup>b</sup>	0.868 <sup>c</sup>	1.735 <sup>a</sup>

The means with the same letter did not significantly differ ( $P>0.05$ )

SMP: Skim milk powder

UF: Ultrafiltration

NaCn: Sodium caseinate

DWPC: Dried whey protein concentrate

or ( $\beta$ -Cn:  $\alpha$ -Lactalbumin, 40: 60) was higher than diets based only on casein. The highest levels of P, Mg and Fe were found also in blood plasma of rats fed on UF-yoghurt, while, SMP-yoghurt occasioned the highest level of K in the blood plasma of rats fed thereon. The next order for Ca absorption was recorded for rats fed on UF-, SMP-, control and NaCn- yoghurts, respectively. The increased Na level of NaCn-yoghurt as illustrated in Table (2) may led relatively to hinder the absorption of Ca by rats fed thereon. Miller *et al* (1995) explained that, high intakes of Na can increase urinary Ca excretion. The corresponding order for P was the control, SMP- and NaCn- yoghurts respectively. UF-yoghurt exhibited the second order in the K absorption followed by NaCn- and control yoghurts. The second order of the absorption of Mg, Cu, Fe and Zn were achieved in rats fed, in order, on SMP-, control, SMP- or NaCn- and UF-yoghurts.

As a conclusion, UF-technique offers the best possibility for yoghurt quality improvement whether *via* facilitate the supplementation with WPC or UF-milk retentate to enhance both com-

positional content and biological quality of yoghurt.

## REFERENCES

- Abd El-Salam, M.H.; S. El-Shibiny; M.B. Mahfouz; H.F. El-Dein; H.M. El-Atriby and V. Antila (1991). Preparation of whey protein concentrate from salted whey and its use in yoghurt. *J. Dairy Res.*, **58**: 503-510.
- Abrahamson, R.K. and T.B. Holmen (1980). Yoghurt from hyperfiltration, ultrafiltration and evaporated milk and from milk with added milk powder. *Milchwissenschaft*, **35**: 399-402.
- AOAC (2000). Association of Official Analytical Chemists. *Official Method of Analysis*. 25<sup>th</sup> Ed., Benjamin Franklin Station, Washington, USA.
- Bishop, M.L.; J.L. Duben-Engelkirk and E.P. Fody (1992). In: *Clinical Chemistry. Principles, Procedures, Correlations*. 2<sup>nd</sup> Ed., pp. 182-214. pub. by Lippincott Co. Philadelphia, USA.
- Bognar, A. (1992). Determination of vitamin B<sub>12</sub> in food by high performance liquid chromatogra-



- phy and post-column derivatization. *Fresenius J. Anal. Chem.* **343**: 155-156.
- Boirie, Y.; M. Dangin; P. Gachon; M-P. Vasson; J.L. Maubois and B. Beaufrere (1997).** Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc. Natl. Acad. Sci., USA*, **121**: 14930-14935.
- Brule, G.; J.L. Maubois and J. Fauquant (1974).** Contents of mineral elements in products from ultrafiltered milk. *Lait*, **54**: 600-615.
- Chapman, H.R.; V.E. Bines; F.A. Glover and P.R. Skudder (1974).** Use of milk concentrated by ultrafiltration for making hard cheese, soft cheese and yoghurt. *J. Soc. Dairy Technol.*, **27**: 151-155.
- Dael, P. Van; P. Kastenmayer; J. Clough; A-R. Jarret; D.V. Barclay and J.C. Maire (2005).** Substitution of casein by  $\beta$ -casein or whey protein isolate by  $\alpha$ -lactalbumin does not affect mineral balance in growing rats. *J. Nutr.*, **135**: 1438-1443.
- Dave, R.I. and N.P. Shah (1998).** Ingredient supplementation effect on viability of probiotic bacteria in yoghurt. *J. Dairy Sci.*, **81**: 2804-2816.
- Eggum, B.O. (1973).** A Study of Certain Factors Influencing Protein Utilization in Rats and Pigs. pp. 17-21. Ph.D. Thesis, Institute of Animal Science. Royal Veterinary and Agriculture Univ., Copenhagen, Denmark.
- El-Hofi, M.A. (1984).** Use of Ultrafiltration in the Manufacture of Domiati Cheese for Salt Containing Wheys. pp. 47-59. Ph.D. Thesis, Justus Liebig univ., Giessen, Germany.
- EOSQC (2005).** Egyptian Organization for Standardization and quality control. *Egyptian Standard No. 1000 Yoghurt*. Cairo, Egypt.
- Estevez, C.R.; A. Goicoechea and S. Jimenez-Perez (1988).** Aroma development in yoghurt made by different methods. *Deutsche Molkerei-Zeitung*, **109**: 733-735.
- FAO/WHO (1976).** In: Joint Food Agricultural Organization / World Health Organization Committee of Government Experts on **The Code of Principles Concerning Milk and Milk Product. Report of the 18<sup>th</sup> Session, No. C x 5 / 70**, FAO, Rome, Italy.
- FAO/WHO (1991).** In: Joint Food Agricultural Organization / World Health Organization Committee of Government Expert **Consultation on Protein Quality Evaluation**. Report, Bethesda, Maryland, USA., 4-8 Dec. (1989). FAO, Food and Nutrition Paper, No. 51. Rome, Italy.
- Fayed, A.E. (1986).** Protein Fortification of Some Dairy Products. pp. 40-44. Ph.D. Thesis, Fac. of Agric. Ain Shams Univ., Cairo.
- Fayed, A.E.; Z.H. Zidan, A.A.K. Abou Arab and M.N.I. Magdoub (1995).** Ultrafiltration membrane permeability of some milk contaminants. *Int. Dairy J.* **5**: 569-576.
- Fayed, A.E.; I.M. Roushdy and A.I. Metwally (1996).** Physical and organoleptic properties of yoghurt made from standardized buffalo's milk fortified with total milk proteinate. **6<sup>th</sup> Conf. Agric. Dev. Res., Ain Shams Univ., Cairo, Dec. 17-19. Annals Agric. Sci., Sp. Issue**, pp. 109-124.
- Folkenberg, D.M. and M. Martens (2003).** Sensory properties of low fat yoghurts. Part A: Effect of fat content, fermentation culture and addition of non fat dry milk on the sensory properties of plain yoghurts. *Milchwissenschaft*, **58**: 48-51.
- Fruhbeck, G. (1998).** Slow and fast dietary proteins. *Nature*, **39**: 843-845
- Fukuwatari, Y.; M. Tomita; Y. Tamura; T. Mizota; I. Kiyosawa and M. Kuboyama (1982).** Membrane permeability of trace metal elements in whole milk by ultrafiltration. **XXI Int. Dairy Congr.**, **1**: 447.
- Glover, F.A. (1971).** Concentration of milk by ultrafiltration and reverse osmosis. *J. Dairy Res.*, **38**: 373-379.
- Glover, F.A. (1985).** Ultrafiltration and Reverse Osmosis for the Dairy Industry. **Reading National Institute for Research in Dairying (NIRD) Technical Bulletin No.15**.
- Green, M.L.; F.A. Glover; E.M.W. Scurlock; R.J. Marshall and D.S. Hatfield (1981).** Effect of use of milk concentrated by ultrafiltration on the manufacture and ripening of Cheddar cheese. *J. Dairy Res.*, **48**: 333-341.
- Green, M.L.; K.J. Scott; M. Anderson; M.C.A. Griffin and F.A. Glover (1984).** Chemical characterization of milk concentrated by ultrafiltration. *J. Dairy Res.*, **51**: 267-278.
- Gueimonde, M.; L. Alonso; T. Delgado; J.C. Bada-Gancedo; C. Reyes-Gavilan (2003).** Quality of plain yoghurt made from refrigerated and CO<sub>2</sub>-treated milk. *Food Res. Int.*, **36**: 43-48.
- Haggag, H.F. and A.E. Fayed (1988).** Production of Zabady from ultrafiltered buffalo's milk. *Food Chem.*, **39**: 29-36.
- Hamdy, A.; L.A. El-Koussy and R. Abd El-Latif (1972).** A study on the fermented milk (Zabadi). *Agric. Res. Review*, **50**: 159-168.
- Horszczaruk, F. and H-D. Bock (1963).** Eine modification des von K. Schiller vorgeschlagene

Stoffwechselkafig fuer Ratten. *Ztschr. Versuchstierk.*, 2: 126-131.

Husein, Y. A. (2006). *Studies on Yoghurt Quality Improvement*. pp. 48-60. M.Sc. Thesis, Fac. of Agric. Ain Shams Univ., Cairo.

Jacobucci, H.B.; V.C. Sgarbieri; N.F.G.P. Dias; P. Borges and C. Tanikawa (2001). Impact of different dietary protein on rat growth, blood serum lipids and protein and liver cholesterol. *Nutr. Res.*, 21: 905-915.

Jepsen, S. (1979). Membrane filtration in the manufacture of cultured milk products, yoghurt, cottage cheese. *Cultured Dairy Products J.*, 14: 5-8.

Johnson, A.M.; E.M. Rohlfis and L.M. Silverman (1999). Proteins., In: *Tietz Textbook of Clinical Chemistry*. pp. 477-540. Ed. by Burtis, C.A. and E.R. Ashwood., Pub. by W.B. Saunders Co., Philadelphia, USA.

Lankes, H.; H.B. Ozer and R.K. Robinson (1998). The effect of elevated milk solids and incubation temperature on the physical properties of natural yoghurt. *Milchwissenschaft*, 53: 510-513.

Lees, G.J. and G.B. Jago (1969). Methods for the estimation of acetaldehyde in cultured dairy products. *Aust. J. Dairy Technol.*, 24: 181-185.

Maubois, J. L.; G. Moquot; J.L. Thapon; M.C. Humilier; A. Chopin; H. Gouedranche; C. Dupas; E. Blance-Patin; M. Piot and J. Fauquant (1971). Preparation of cheese from a concentrate obtained by ultrafiltration of milk. *Lait*, 51: 495-533.

Miller, G.D.; J.K. Jarvis and L.D. McBean (1995). Dairy Foods and Osteoporosis. In: *Handbook of Dairy Foods and Nutrition*. pp. 93-133. CRC Press Inc., Boca Raton, Florida, USA.

Moquot, G. (1979). Present potentialities of ultrafiltration in cheese making. *Proc. 1<sup>st</sup> Biennial Marshall Int. Cheese Conf., Madison, Wisconsin*, 10-14 Sept., 603-610.

Omar, M.M. and A.M. Abou El-Nour (1998). Rheological properties of yoghurt enriched with whole milk protein. *Egypt. J. Dairy Sci.*, 26: 77-88.

Rasic, J.L. and J.A. Kurmann (1978). In: *Yoghurt, Scientific Grounds, Technology, Manu-*

*facture and Preparations*. pp. 90-185. Tech. Dairy Pub. House, Copenhagen, Denmark.

RDS (1995). *Roche Diagnostic Systems, User's Manual*. pp. ABX Hematologie, Montpellier, Brussels, Belgium.

Remeuf, F.; S. Mohammed; I. Sodini and J.P. Tissier (2003). Preliminary observation on the effect of milk fortification and heating on microstructure and physical properties of stirred yoghurt. *Int. Dairy J.*, 13: 773-782.

Renner, E. (1983). In: *Milk and Dairy Products in Human Nutrition*. pp. 90-256. W-GmbH, Volksw. Verlag, Munich, Germany.

Renner, E. and M.H. Abd El-Salam (1991). In: *Application of Ultrafiltration in the Dairy Industry*. pp. 217-314. Elsevier Applied Sci. Pub. London, UK.

Renner, E.; G. Schaafsma and K.J. Scott (1989). Micronutrients in Milk. In: *Micronutrients in Milk and Milk-Based Food Products*. pp. 1-69. Ed. by Renner, E., Elsevier Sci. Pub. LTD, Cambridge, UK.

Schiller, K. (1960). Ein Stoffwechselkafig fuer Ratten. *Ztschr. Tierphysiol. Tierernahrung Futtermittelk.*, 15: 305.

Scott, K.J. (1989). Micronutrients in Milk products. In: *Micronutrients in Milk and Milk-based Food Products*. pp. 71-123. Ed. by Renner, E., Elsevier Sci. Pub. LTD, Cambridge, UK.

SPSS (1998). *Statistical Package for Social Science*. SPSS Inc., North Michigan Avenue, Chicago, Illinois, USA.

Tamime, A.Y. and H.C. Deeth (1980). Yoghurt: Technology and biochemistry. *J. Food Prot.*, 43: 939-977.

Tamime, A.Y. and R.K. Robinson (1999). In: *Yoghurt, Science and Technology*. 2<sup>nd</sup> Ed., pp. 234-327. CRC Press, Wood Lead Pub. Ltd, Cambridge, UK.

Waynforth, H.B. and P.A. Flecknell (1992). In: *Experimental and Surgical Technique in the Rat*. 2<sup>nd</sup> Ed., pp. 100-202. Academic press Ltd, London, UK.

Youssef, M.S.H. (2000). *Studies on the Nutritional Value of Labneh Produced by Different Procedures*. pp. 29-131. Ph.D. Thesis, Fac. of Agric., Ain Shams Univ., Cairo.



## التقييم الحيوى ليوجهورت اللبن البقرى المدعم بمصادر مختلفة لبروتينات اللبن

[١٣]

مرفت سيد يوسف<sup>١</sup> - محمد سيد مسعود<sup>١</sup> - عاطف السيد فايد<sup>٢</sup>

١. المركز الإقليمي للأغذية والأعلاف- مركز البحوث الزراعية- الجيزة- مصر

٢. قسم علوم الأغذية- كلية الزراعة- جامعة عين شمس- شبرا الخيمة- القاهرة- مصر

الفرز المجفف قد أبدت أعلى قيمة حيوية للبروتين. وكانت أحسن صورة دم معبرا عنها بقيم كريات الدم الحمراء والبيضاء والهيموجلوبين والهيمكريت (النسبة الحجمية للكرات الدموية الحمراء بالدم) موجودة بالفئران المغذاه على يوجهورت الترشيح الفائق يليها المغذاه على اليوجهورت المدعم بمركز بروتينات الشرش. كما دل فحص بلازما الدم على أن اليوجهورت الأخير سبب إرتفاع نسبة البروتين الكلى والأليومين والجلوبيولين مع أن أعلى نسبة ألبومين/جلوبيولين كانت ببلازما دم الفئران المغذاه على اليوجهورت المدعم باللبن الفرز المجفف، الترشيح الفائق أو حتى غير المدعمة (الكنترول). علاه على ذلك كان معدل إمتصاص العناصر المعدنية الكالسيوم، الفسفور، البوتاسيوم، الماغنسيوم، النحاس، الحديد والزنك أعلى ببلازما دم الفئران المغذاه على اليوجهورت المدعم بمركز بروتينات الشرش. وهو نفسه ما حدث للفئران المغذاه على يوجهورت الترشيح الفائق بخصوص الفوسفور والماغنسيوم والحديد أو على اليوجهورت المدعم باللبن الفرز المجفف بخصوص إمتصاص البوتاسيوم فقط.

ومما سبق يمكن الاستنتاج بأن تقنية الترشيح الفائق تتيح الفرصة الأفضل لتحسين جودة اليوجهورت سواء عن طريق توفير التدعيم بمركز بروتينات الشرش أو مركز اللبن لتحسين كل من الجودة التركيبية والحيوية لليوجهورت.

أستهدف البحث أساساً تقييم الجودة الحيوية ليوجهورت اللبن البقرى المدعم بنسبة صفر (الكنترول) و ١% من بروتين اللبن من مصادر مختلفة شملت اللبن الفرز المجفف، كازينات الصوديوم، مركز بروتينات الشرش المجففة أو بتركيز اللبن نفسه بالترشيح الفائق لرفع نسبة البروتين إلى نفس النسبة المطلوبة.

ولقد أكدت النتائج المتحصل عليها أن اليوجهورت المدعم باللبن الفرز تميز بإحتوائه على النسبة الأعلى من الجوامد الكلية، اللاكتوز/المادة الجافة، الرماد/المادة الجافة، الحموضة، الاسيتالدهيد وأعداد بكتيريا بادئ اليوجهورت. كما أحتوى أيضا على النسبة الأعلى من الكالسيوم، الفسفور، البوتاسيوم، الماغنسيوم، النحاس والمنجنيز. وكانت أعلى نسبة دهن/المادة الجافة ليوجهورت الترشيح الفائق وكان أعلى رقم pH ذلك اليوجهورت المدعم بكازينات الصوديوم والذي أحتوى على أعلى نسبة صوديوم، في حين كانت أعلى نسبة من البوتاسيوم والحديد أيضا موجودة باليوجهورت المدعم بمركز بروتينات الشرش والذي أحتوى أيضا على أعلى نسبة من الثيامين (فيتامين ب١) مثله في ذلك مثل المدعم بالترشيح الفائق وكذلك الريبوفلافين (فيتامين ب٢).

من الناحية الحيوية، تحقق أعلى معدل هضم وصافى الاستفادة من النتروجين في الفئران المغذاه على اليوجهورت المدعم بمركز بروتينات الشرش. مع أن الفئران المغذاه على اليوجهورت المدعم باللبن