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Athletes Nourishment: Introducing Novel L-Glutamine Fortified Dairy Products

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ABSTRACT

L-glutamine (Gln) supplementation may provide beneficial effects for individuals special athletes. Gln fortified functional dairy products namely: plain probiotic set yoghurt, mango yoghurt, strawberry yoghurt, and karish cheese were produced in the present study. Most trials of Gln fortified probiotic yoghurt and fruit yoghurt resulted in chemical, physical and sensory characteristics close or similar to the control, and some were characterized with better attributes than the control. Linear increase in acetaldehyde and diacetyl concentration was detected when Gln was added to yoghurt. A slightly decrease in pH was noticed in yogurt enriched with Gln. In case of karish cheese, five treatments were prepared containing 1-5% Gln. It was found that Gln increased the moisture content of cheese. Yield, gross composition, texture profile analysis and sensory evaluation of Karish cheese fortified with Gln were illustrated in this study.

Keywords: L-glutamine, yoghurt, Karish cheese, Functional dairy products, Athletes

INTRODUCTION

Functional foods designed for athletes have emerged as a novel sector of special purposes food products. The concept of functional food is about the role of food in the health-enhancing and health-maintenance. These foods are now designed infants, adolescents, athletes etc. (Hasler, 2005; Katan, 2017).

Among all dairy products in the Egyptian market, plain yoghurt, fruit-yoghurt, and karish cheese are the most consumed. Yoghurt gains its popularity from its variety of nutritional characteristics or textural characteristics. Karish cheese is a traditional Egyptian low fat cheese, manufactured from skimmed milk, which is consumed fresh (Robinson and Tamime, 1996). It is preferred to wide range of consumers as a result of its nutritional value, reasonable price, chemical composition and its limited fat content. Such attributes make yoghurt and karish cheese preferred as functional food.

L-glutamine (Gln) is classified as a non-essential amino acid. It is synthesized in the body from glutamic acid and ammonia in an energy requiring reaction (Watford *et al.*, 2000). There are few data on Gln as food supplement. However, some studies have proved its efficiency, protective and supportive role, especially among athletes (Ziegler *et al.*, 1990; Piattoly, 2005). Significant reduction in Gln blood-concentration was noticed in cases of stress, clinical trauma, starvation, or prolonged exercise (Castell, 2002). Conversely, the concentration of blood Gln increased in athletes after short-term exercise (Poortmans *et al.*, 1974; Parry-Billings *et al.*, 1992). Gln levels in muscle fall in a dose-dependent manner to the degree of stress. Furthermore, plasma Gln levels decline during and after prolonged training. The amount of Gln released by the skeletal muscle under stressful situations is greater than the amount found in the intracellular pool and incorporated into proteins. On the other hand, Gln may improve the hydration status of the skeletal muscle, resulting in an increase in cellular volume. The increase in cell volume could be an anabolic signal for the muscle cell, which may increase muscular strength (Haussinger *et al.*, 1994; Antonio and Street, 1999).

Because of decline of the intracellular Gln concentrations in a dose dependent manner (i.e. the greater the stress, the greater the decline), it could be concluded that chronic exercise training would increase the requirements for L-glutamine. Based on such available data, it seems reasonable to state that Gln supplementation may provide beneficial effects for individuals special athletes. Despite parenteral administration is recommended for Gln in clinical trials, the gastrointestinal tract absorbs Gln expeditiously (He *et al.*, 2016). So, oral supplementation would be an effective vehicle of delivering the Gln dosage required by athletes (Piattoly, 2005). Therefore, the objectives of this study were production of L-glutamine fortified functional dairy products with acceptable sensory attributes

MATERIALS AND METHODS

Strains source and culturing conditions

Probiotic lactic acid bacteria (LAB) cultures used in this study (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptocccus thermophilus* STY1) were obtained from the Faculty of Agriculture, Saba Bacha, Alexandria University culture collection (FASBAU). All strains were maintained for long preservation on MRS slant agar (De Man *et al.*, 1960) (Biolife, Italy) for further study, Bacterial growth from slant culture was reactivated in 10 ml broth medium at 37 °C for 24 h at anaerobic conditions.

Materials

1. Fresh full fat buffalo's milk was obtained from the local market in Alexandria Governorate.
2. L-glutamine (Gln) 99%, (Supreme Formulation LLC, USA) was used.
3. Mango and strawberry fruits were purchased from markets of Alexandria Governorate,

Solubility and recrystallization test of L-glutamine.

Solubility test was done according to the method of Shriner *et al.* (1956) with minor modification. Thirty-two different Gln concentrates ranged from 2% to 10%, with gradient of 0.25%, were prepared in distilled water. All samples were shaken vigorously for 2 min and heated gradually up to 60 ºC in water bath. Once all Gln was dissolved the current temperature was recorded. Subsequently, temperature was reduced gradually, and the solution was stored either at 4 or 25 ºC for 24h. Afterwards, Gln-crystals were detected visually and under light microscope.

Yoghurt Manufacture

Yoghurt was prepared by lactic acid fermentation of pasteurized (80°C /5s) buffalo's milk.. Starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles*) was added at 42 ºC and well mixed. The inoculated milk was poured in cups, 100 g each, and incubated at 42 ºC for about 3 h to reach pH 4.5-4.6, followed by cooling at 4 °C. Different six treatments were prepared using five different Gln concentrations, in addition to the control. Fruits-yoghurt was prepared by adding 20% (w/w) of mango or strawberry-sucrose mixture (9:1) at the bottom of the yoghurt cup.

Karish Cheese Manufacture

Karish cheese was made from skimmed buffalo's milk (0.5% fat) after pasteurized at 75 °C for 15 s. Milk was divided into six equal portions (five liters each), the first part was considered as control. While five Gln concentrations were added to the other five portions. The milk achieved acid coagulation in 4–5 h using L. delbrueckii ssp bulgaricus and S. thermophilus. After complete coagulation (pH 4.6–4.4), the curd was scooped into a mat to drain under gentle pressure (one third of the weight of the cheese curd) for 18 h. At optimum moisture content, the cheese was cut into cubes and then cooling to 4 °C.

pH determination

pH values of the milk, and yoghurt samples were measured using a pH meter (Jenway 3505, England)

Viscosity

Viscosity was measured at 22 °C using viscometer (D.P. SELECTA, S.A. ST-2020R, Korea) using a speed ranged from 60 to 200 rpm with spindle R5. Viscosity expressed in mPa·s.

Chemical Analysis

Analysis of milk, was carried out using milk analyzer (3510 Laktostar, Funke Gerber, Berlin, Germany). Cheese samples were analyzed for their moisture, fat, protein, and ash according to Horwitz and Latimer (2000).

Yield Calculations

The yield of cheese was a mathematical expression for the quantity cheese obtained from a given quantity of milk according to the following formula:

Yield = (Cheese kg/Milk kg)

Rheological Properties of Cheese

The textural properties of cheese samples were evaluated using texture analyzer (Stable Micro Systems ltd. Vienna court, Lammas TA.XT. Plus). Samples were taken from fresh cheeses and measured immediately. Cheese samples size was 30×30×30 mm. Speed was 1 mm/s and 10 mm was the distance of penetration. Samples were allowed to stand at ambient temperature for at least 20 min prior testing. The probe used was backward extrusion ring 40mm part code: A/BE. Data were collected on computer and the texture profile parameters were calculated from texture analyzer and computer interface. The texture profile parameters were obtained and calculated as describe by Mochizuki (2001).

Sensory evaluation

Ten experienced panelists participated in the evaluation of dairy products were chosen for the assessment of the sensory attributes of yoghurt and karish cheese samples. Samples of yoghurt (100 g cups) and karish cheese (5×5 cm pieces) were placed on white plates and presented to the panelists in a random order. The samples were evaluated organoleptically after 1 day of manufacturing. All of the sensory attributes assessed by the panelists were rated using a 5-point scale where the worst = 1 and the best = 5. The assessment procedure of the sensory attributes of yoghurts was divided into: smell, appearance, textural properties, and flavour and mouth feel. Concerning karish cheese, panelists were also instructed to report any defects that they notice in appearance, texture and flavour according to the (IDF Standard 99A, 1987) guidance for the sensory evaluation of soft cheese.

RESULTS AND DISCUSSION

Solubility and recrystallization of L-glutamine (Gln)

Results in Figure (1) show that a linear line was established to allow the prediction of temperature needed to dissolve certain concentration of Gln. at low concentrations (up to 2%) Gln was soluble at 25°C. The temperature needed to dissolve 3.5, 5.0, 7.5, and 10% Gln was 36, 40, 50, and 60 °C, respectively. Studying of solubility for Gln is of importance for dairy industry. Most of dairy product such as yoghurt and karish cheese are usually stored under cooling, which may allow Gln crystals to form in cases of high concentrations. However, during manufacturing of dairy products, they are subjected to different heat treatments, which may affect the solubility of Gln significantly. Depending on the nature of the solute, the solubility may increase or decrease under direct effect of temperature (Tremblay *et al.*, 2005).

After solubility test was done, the solutions were stored either at 4 ºC or 25 ºC for 24h for recrystallization test. Recrystallization depends on the decrease of the solubility of a solid in a solvent at lower temperature. At 4 ºC concentrates from 2 to 3.5% Gln did not build up crystals, while few crystals were detected at 3.75% Gln under the light microscope. All concentrates containing 4% Gln or higher, have built up crystals easily seen by naked eye. On the other hand, at room temperature all concentrates up to 4% Gln did not form any crystals. However, concentrates over 4.25% formed crystals easily seen by naked eye as shown in Table (1).

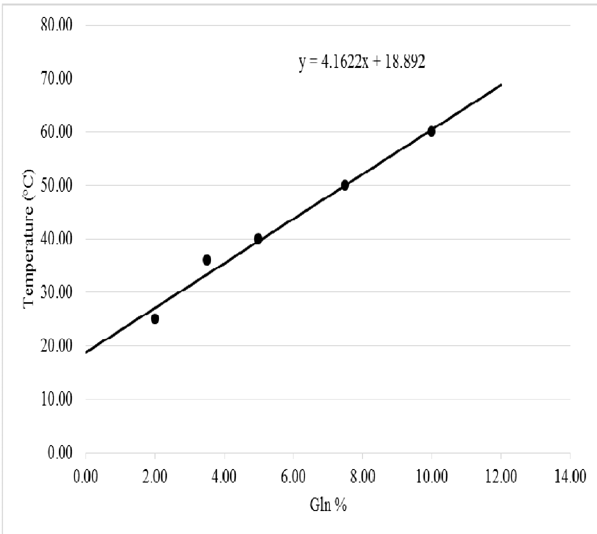

Figure 1. Solubility test of L-glutamine in distilled water from 2% to 10%

Milk Analysis

Analysis of buffalo milk used in yoghurt and karish cheese production is shown in Table (2).

Table 1. Recrystallization test of L-glutamine concentrations from 2% to 10% at 4ºC and 25ºC.

Gln concentrations%	Crystallization	
	4ºC	25ºC
2.00	-	-
2.25	-	-
2.50	-	-
2.75	-	-
3.00	-	-
3.25	-	-
3.50	-	-
3.75	+	-
4.00	++	-
4.25	++	++
4.50	++	++
4.75	++	++
5.00	++	++
5.25	++	++
5.50	++	++
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8.00	++	++
8.25	++	++
8.50	++	++
8.75	++	++
9.00	++	++
9.25	++	++
9.50	++	++
9.75	++	++
10.00	++	++

- : No crystallization was detected
+ : Few crystals were detected by light microscope.
++ : More crystals were detected by naked eye.

Table 2. Chemical composition (%) and density of buffalo's milk used in yoghurt and Karish cheese.

	Milk for production of	
Component	**Yoghurt**	**Karish cheese**
Fat	6.39	0.56
SNF	11.28	12.27
Protein	3.73	4.13
Lactose	5.41	5.77
Density	1.0355	1.0457

Yoghurt Fermentation Time

Yoghurt fermentation time was monitored for the control and Gln-treated samples. It ranged from 2h and 25min to 3h and 17min. The lowest value was for the control, while the longest period was for samples containing 5% Gln. Data in Table (3) showed that, the incubation periods of yoghurt trials production were altered gradually under the effect of Gln. Every increase in Gln concentration by 1% altered the fermentation time by 7-12 min (10 min in average). The incubation periods of fruit-yogurt were nearly similar to plain-yoghurt.

Yoghurt pH

pH of plain-yoghurt samples incubating the control, 1, 2, 3, 4, and 5% Gln were 4.43, 4.44, 4.44, 4.38, 4.38 and 4.38, respectively. All fruit-yoghurt samples showed similar or close pH to plain-yoghurt. The difference ranged between ± 0.08 as shown in Table (3).

Acidification of milk is the most important step in yoghurt production. It controls most chemical and rheological characteristics of the final product (Dalgleish and Law, 1989; Lucey, 2004). The applied treatments did not result in great change in the final pH, in contrast with the control. Therefore, it could be concluded that, Gln did not affect the final pH of yoghurt (Table 3).

Table 3. Incubation periods, pH value, viscosity, Acetaldehyde and Diacetyl concentration in plain, mango and strawberry yogurt supplemented with different concentrations of L-glutamine (Gln).

Gln (%)	Yoghurt type	Incubation time (h:min)	pH	Viscosities (mPa.S.)	Acetaldehyde (ppm)	Diacetyl (ppm)
	Plain	2:25	4.43	1268	8.1	9.1
Control	Mango	2:23	4.32	1560	11.2	14.6
	Strawberry	2:26	4.40	1537	10.2	14.1
	Plain	2:35	4.44	1817	8.4	9.5
1%	Mango	2:31	4.45	2246	11.0	14.5
	Strawberry	2:33	4.42	2195	10.5	14.0
	Plain	2:48	4.44	1998	8.6	9.3
2%	Mango	2:45	4.40	2473	11.0	14.3
	Strawberry	2:45	4.41	2413	10.8	13.8
	Plain	3:00	4.38	1199	8.7	9.6
3%	Mango	3:00	4.41	1474	11.3	14.8
	Strawberry	3:00	4.42	1454	11.0	13.9
	Plain	3:07	4.38	1666	8.8	10.0
4%	Mango	3:05	4.43	2058	11.2	15.0
	Strawberry	3:05	4.45	2014	11.0	14.2
	Plain	3:17	4.38	1661	9.1	10.3
5%	Mango	3:15	4.44	2051	11.2	14.9
	Strawberry	3:15	4.46	2008	11.2	14.2

Viscosity

Viscosity was measured in yoghurt trials after manufacturing and cooling overnight. The values ranged between 1199 and 1998 mPa.S for plain-yoghurt and from 1474 to 2473 mPa.S for fruit-yoghurt (Table (3). All samples showed viscosity higher than the control, except for the trial containing 3% Gln. The highest value was recorded in the presence of 2% Gln. It was also noticed that fruit-yoghurt had higher viscosity values than plain yoghurt, and the samples containing mango was slightly higher than the samples containing strawberry.

Labropoulos *et al.* (1984) mentioned that viscosity value of yoghurt is one of the most important characteristics of yoghurt, and it is affected by many factors on one of them is fruit addition.

Flavour Compounds in Yoghurt

Gradual increase in acetaldehyde and diacetyl was detected in the treatments with added Gln, compared with the control. In case of plain-yoghurt, the acetaldehyde concentration ranged between 8.1 and 9.1ppm. The lowest value was detected in the control, while the highest was detected in the presence of 5% Gln. The same trend was noticed for diacetyl, where the control recorded 9.1 ppm and the samples containing 5% Gln achieved 10.3 ppm.

Concerning fruit-yoghurt, all trials achieved acetaldehyde and diacetyl concentrations higher than the plain-yoghurt, since the increase ranged between 25-45%. Samples containing mango was of higher contents than those containing strawberry. As illustrated in Table (3) all fruit-yoghurt samples (mango and strawberry) had slight increase of acetaldehyde and diacetyl in the presence of high concentration of Gln. These results are in agreement with the findings of Bills *et al*. (1972) and Cheng (2010).

Despite Gln slightly prolonged yoghurt manufacturing process, more flavour compounds were formed. Pollack and Lindner (1942) and He *et al.* (2016) stated that glutamine is of strong growth-promoting properties for certain lactic acid-producing bacteria. This information is in agreement with the acetaldehyde and diacetyl data in Table (3).

Karish Cheese Yield and Analysis

Five different Gln concentrations were add to the cheese milk. The highest yield of 258 g/kg milk was recorded in the presence of 1%Gln, and the lowest of 185 g/kg milk was achieved in the presence of 5%Gln. The rest values were 203, 217, 221, and 239 for trials 4%, control, 3%, and 2%Gln, respectively (Table 4). The yield was of about 2-20% higher in the cheese made with 1, 2, and 3% Gln, compared to the control. This is due to the ability of Gln to produce cheese with high water holding capacity. Such effect is similar to that noticed when exopolysaccharides (EPS) or EPS-producing culture was used as starter in soft cheese making like Mozzarella (Perry *et al*., 1997; Ricciardi and Clementi, 2000). The increase in cheese moisture was parallel to the increase in yield, indicating that the increase in yield was due to an increase in the moisture content and vice versa.

Moisture content of all cheese samples ranged from 77.52% to 84.79%. The lowest value was recorded in the samples containing 5% Gln, while the samples containing 1% Gln had the highest moisture. The moisture content in cheese samples increased when low concentrations of Gln (1 and 2%) were added, while decrease was noticed in samples made with higher Gln concentrations (4 and 5%). Slight changes were observed in fat, total protein, and ash of the control and experimental samples (Table 4).

Table 4. Karish cheese yield and the chemical composition (%) of cheese with different L-glutamine (Gln) concentrations.

Gln (%)	Yield (g /kg milk).	Moisture	Fat	Protein	Ash
control	217	80.87	1.2	22.1	1.3
1%	258	84.79	1.1	19.9	1.3
2%	239	82.98	1.3	21.1	1.3
3%	221	81.16	1.4	22.0	1.2
4%	203	79.33	1.6	22.7	1.3
5%	185	77.52	2.1	23.3	1.1

Texture Profile Analysis (TPA) of Karish Cheese

Texture primary and secondary parameters (hardness, fracturability, gumminess, chewiness, adhesiveness, springiness, cohesiveness, and resilience,) of experimental karish cheeses samples (Figure 2). The textural attributes of foods play a major role in consumer appeal, buying decisions, and eventual consumption. However, TPA is the key instrumental method used to correlate with sensorial textural parameters.

It is also clear (Fig.2) that the hardness was lower in treatments containing 1, 2, and 3% Gln, compared with the control (409.7 g). The decrease in hardness may be due to the higher moisture and lower protein content. The addition of 4and 5% Gln increased hardness comparing either with the control or with the addition of 1, 2, and 3% Gln.

Concerning fracturability, gumminess, chewiness, and adhesiveness, similar trend of hardness results were recorded. Samples containing 1, 2, and 3%gln were less than the control, while samples containing 4 and5% Gln showed higher values than the control.

Springiness is the rate at which a deformed material goes back to its unreformed condition after the deforming force is removed. It is clear that springiness was either similar or slightly less than the control in all samples (Figure 2). In case of cohesiveness, all samples containing Gln showed values higher than the control. While resilience which is how well a product "fights to regain its original height", it had similar trend to springiness

Sensory evaluation of yogurt and Karish cheese

Panelists described plain-yoghurt containing Gln as creamy, soft, acidic, and light. All samples were similar or very close to the control (Figure 3). When 5% Gln was added little sandy mouthfeel was detected in the samples. In case of fruit yoghurt, samples were described as fruity, sugary, creamy and light, in contrast with plain yoghurt containing Gln. No sandy mouthfeel was detected in any fruit yoghurt samples containing Gln. Samples containing mango and 1-5% Gln showed better appearance and mouthfeel than the control (0% Gln), while flavour was slightly less. Strawberry-yoghurt containing 1-5% Gln gave sensory parameters close to the control (Figure 3). Whereas, fruit-yoghourt showed good sensory attributes. The plain Gln-yoghurt was more acceptable and its results were very close to the control.

The results of the sensory evaluation of Karish cheese are illustrated in Figure (3). Cheese made using Gln was described by panelists as cheese, acid, soft, and moist, which was similar or close to the control. Slight floury mouthfeel was detected in samples containing 5% Gln. These descriptions are consistent with the obtained TPA data.

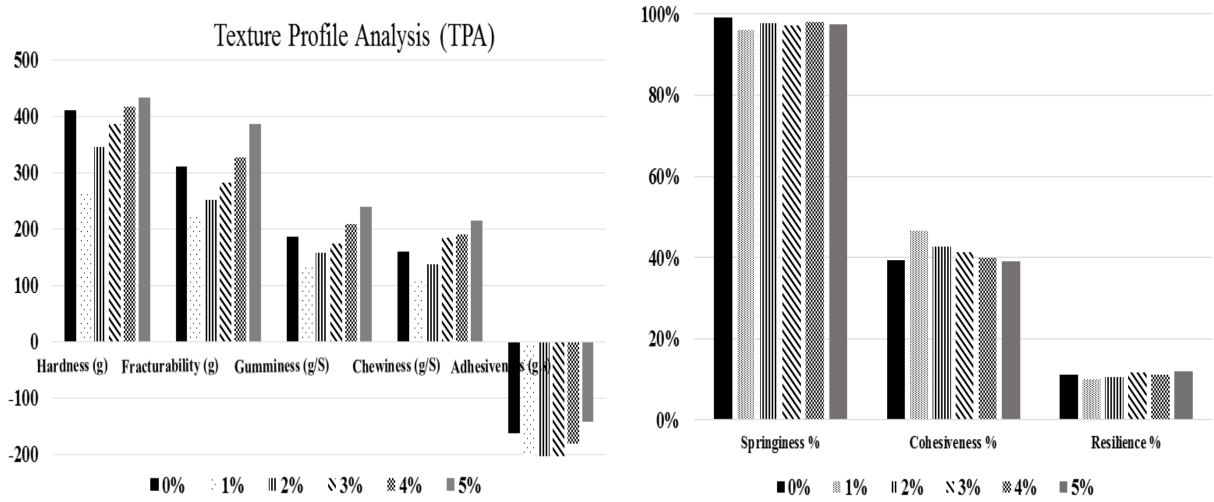

Figure 2. Texture Profile Analysis (TPA) of karish cheese made from skimmed buffalo's milk fortified with 1, 2, 3, 4, and 5% L-glutamine in comparison with the control.

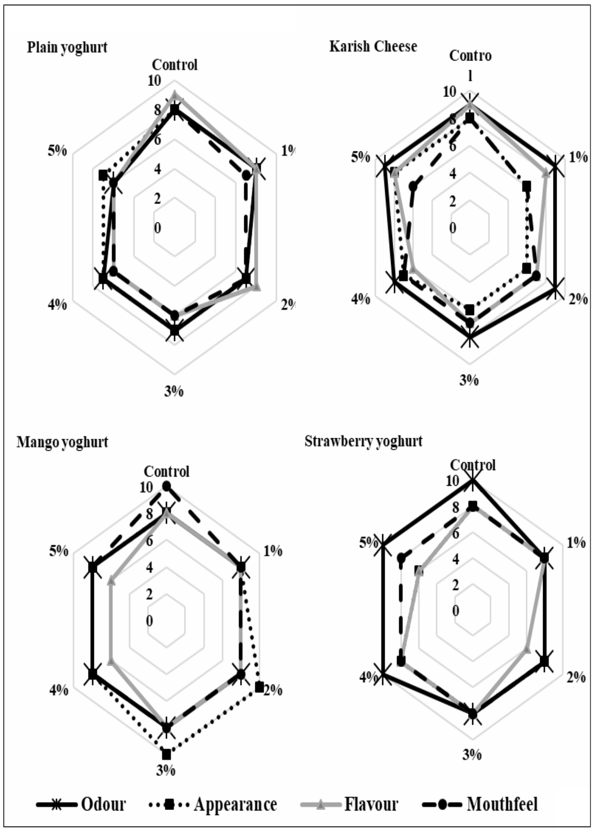

Figure 3. Sensory evaluation of plain, mango, strawberry yoghurt, and karish cheese made from buffalo's milk fortified with 1, 2, 3, 4, and 5% L-glutamine in comparison with the control.

CONCLUSION

It can be concluded that, functional L-glutamine fortified yoghurt, fruit yoghurt and karish cheese can be produced. All products showed very good chemical composition, technological characteristics, and acceptability up to 4% L-glutamine.

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تغذية الرياضيين: تقديم منتجات لبنية جديدة مدعومة بالجلوتامين

محمد أحمد السيد جمعة ، فايز خلف مراد خلف و ايمان حسين السيد عياد
قسم علوم الأغذية - كلية الزراعة – جامعة الإسكندرية

الحمض الأميني غير الأساسي الجلوتامين هو أكثر الأحماض الأمينية وفرة في البلازما وكذلك العضلات الهيكلية. يعاني الرياضيون من إنخفاض كبير في تركيز جلوتامين الدم بعد التمرين لفترة طويلة. قد يساهم الجلوتامين في مساعدة الأفراد خاصة الرياضيين. في هذه الدراسة تم إنتاج منتجات ألبان وظيفية معتمدة على الجلوتامين وهي: اليوجورت، يوجورت المانجو، يوجورت الفراولة، والجبن القريش. أظهرت معظم التجارب التي أجريت على اليوجورت المحتوي على الجلوتامين زيادة طفيفة في وقت التجبن في الزبادي المحتوي على الجلوتامين وأن الخواص الكيميائية والفيزيائية والحسية كانت قريبة أو مشابهة للعينات القياسية، وأظهرت بعض المعاملات خصائص أفضل من القياسية. لوحظ زيادة طردية في نسب الاسيتالدهيد والداي أسيتيل في عينات الزبادي المحتوية على الجلوتامين. أما الجبن القريش، تم تحضير خمس معاملات تحتوي على ١-٥٪ جلوتامين. وجد أن إضافة الجلوتامين أدى الي زيادة في محتوى الرطوبة في الجبن. في هذه الدراسة أوضح كل من الريع، والتركيب الإجمالي وتحليل القوام، والتقييم الحسي للجبن القريش قرب المعاملات من العينات القياسية.