Functional Stirred Yoghurt Supplemented with Green Coffee Powder: Health-promoting Effects on Hypercholesterolemia and Obesity

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الزبادي الوظيفي المدعم بمسحوق القهوة الخضراء: التأثيرات المعززة للصحة على ارتفاع كوليسترول الدم والسمنة

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ملخص البحث

تم إجراء البحث الحالي من أجل: 1. دراسة تأثير المستويات المختلفة (0.50 ، 1.50 و 3.0٪) من مسحوق البن الأخضر (GCP) على الزبادي المخفوق بروبيوتيك. 2. تقييم تأثير الخصائص الفيزيوكيميائية والوظيفية والحسية والغذائية للزبادي المخفوق الناتج على بعض التأثيرات المعززة للصحة في الفئران التي تعاني من ارتفاع كوليسترول الدم والسمنة.أدت إضافة اللبن الزبادي مع مسحوق القهوة الخضراء إلى خفض نسبة الحموضة والدهون و معدل إنفصال الشرش. كما زاد زمن التحفيز المشترك، وإجمالي المواد الصلبة، ودرجة الحموضة، والرماد، ومحتوى البروتين الكلي مع زيادة التدعيم بمسحوق القهوة الخضراء مقارنةً بالعينة الضابطة، ولم يكن هناك فرق معنوي في إجمالي محتوى الأحماض الدهنية المتطايرة للزبادي المخفوق من المعالجات المختلفة خلال فترة التخزين. كانت جميع المعالجات مقبولة من قبل المحكمين. كما أدى تدعيم الزبادى بمسحوق القهوة الخضراء إلى انخفاض وزن الجسم والمأخوذ الغذائى للفئران تحت الدراسة المصابة بارتفاع كوليسترول ودهون الدم.أدى إعطاء الفئران المصابة بإرتفاع كوليسترول الدم 20 جم/ يوم من اللبن الزبادي المخلوط مع البروبيوتيك المضاف إليه مسحوق القهوة الخضراء لمدة اسبوعين إلى تقليل حدة التغيرات المرضية في أنسجة الكبد والكلى نتيجة الإصابة بإرتفاع كوليسترول الدم والحفاظ على وظائف الكبد والكلى بالإضافة إلى تحسين التغيرات في مستوى دهون الدم.

الكلمات المفتاحية: الزبادي الوظيفي ، مسحوق القهوة الخضراء ، خصائص جودة الزبادي ، ارتفاع كوليسترول الدم

Functional stirred yoghurt supplemented with green coffee powder: Health-promoting effects on hypercholesterolemia and obesity

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Abstract:

The current research was conducted to: 1) Investigate the impact of different levels (0.50, 1.50 and 3.0%) 0f green coffee powder (GCP) on probiotic stirred yoghurt. 2) Evaluate the effect of the physiochemical, functional, sensory and nutritional properties of the resultant stirred yoghurt on some health-promoting effects in hyperlipidemia and obesity rats. The yoghurt supplemented with GCP decreased the acidity, fat and synersis. The co-agulation time, total solids, pH, ash and total protein content increased with the increase of GCP supplementation as compared to the control sample. There was no significant difference in the Total Volatile Fatty Acids (TVFAs) content of stirred yoghurt from different treatments during the storage period. All the treatments had been acceptable by the panelists. The yoghurt supplemented with GCP reduced body wieght and food intake in under study hyperlipidemia rats. Feeding the hypercholesterolemia rats on 20 g/per day probiotic stirred yoghurt supplemented with GCP for six weeks decreased the severity of pathological changes in hepatic and renal tissues and preserved both liver and kidney functions, as well as ameliorated the changes in the lipid profile.

Keywords: Functional yogurt, green coffee powder, quality properties of yoghurt, hypercholesterolemia

Introduction:

Yoghurt is the most consumed healthy and nutritious food around the world. Research shows that most people in developing or underdeveloped countries suffer from nutritional diseases particularly obesity and hyperlipidemia, and enriched dairy products can dramatically reduce these diseases.

Green coffee powder (GCP) is considered a good source of bioactive compounds that have high antioxidative properties. Cardiovascular disease is one of the most important morbidity causes occurring primarily as a result of accelerated atherosclerosis **(Hasni & Kaplan, 2020).**

Milk is not a natural source of soluble or insoluble fiber in the human diet **(Urashima *et al.*, 2009)**.Yoghurt is among the most common dairy products consumed around the world **(Loveday *et al.*, 2013)**. There is no fiber in yoghurt and dairy products. Fiber is a component of the cell wall of fruits, grains, seeds, and vegetables. Supplementation of yoghurt or dairy products with fiber is of increasing interest to create functional foods with health benefits and improve their functionality, and complement their healthy properties **(Tamime *et al.*, 1999)**.

Dönmez *et al.*, (2017) investigated the effect of added GCP and green tea powder (GTP) on syneresis behavior and consistency of yogurts, and concluded that GCP and GTP behaved differently in acidified gel net-works of yogurt and modified its rheological behavior, as they have different profiles and concentrations of polyphenols.

Jeong, *et al.*, (2018) evaluated the effects of GTP on the fermentation and bioactive properties of yoghurt. Results indicated that addition of GTP can enhance the beneficial health effects of yoghurt by increasing its antioxidant activity, LAB growth and anti-inflammatory effects.

Consumption of products that contain a high percentage of fiber prevents or decreases hypercholesterolemia **(Dhingra *et al.*, 2012)** and obesity **(Van Dam & Seidell, 2007)**.

Coffee is a product consumed daily in the world by all social classes. It is one of the most commonly consumed beverages around the world due to its stimulative effect and good taste **(You *et al.*, 2011)**.

Obesity, in recent decades, has become a serious clinical disease caused by following a high-fat diet. WHO defines obesity as abnormal or excessive fat accumulation. Additional evidence suggests that obesity is related to epidemiological diseases including diabetes, heart disease, stroke, arthritis, inflammation, and cancers **(Osborn & Olefsky, 2012)**.

The current present study was conducted to investigate the effect of GCP addition to probiotic stirred yoghurt on the physiochemical properties and sensory evaluation of yogurt. It also aimed to evaluate the nutritional aspects of the obtained products on the hypercholesterolemia and obese rats.

Material and methods:

Materials

Fresh cow's milk was collected from a local farm (Zagazig, Sharkia, Egypt). GCP was purchased from Misr Cafe Co. 10th of Ramadan City, Egypt. Freeze dried DVS ABT-5 Probio-Tec® cultures containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium* BB-12 were obtained from Christian Hansen Laboratory Copenhagen, Denmark, by Misr Food Additives (MIFAD), Egypt.

Chemical analysis of GCP

The powder was analyzed in the central laboratory for soil, food, and feedstuff (CLSFF), Faculty of Technology and Development, Zagazig University, Egypt, for Total solids, fat, total protein contents, Ash and dietary fiber of samples were determined according to **AOAC (2007**). Carbohydrate content was calculated by difference TS- (fat + protein+ ash) according to **Guzmán-González *et al.*, (1999)**.

Antioxidant activity as radical scavenging activity (RSA) was measured by bleaching of the purple coloured solution of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) according to the method of **Hatano *et al.*, (1988)**. The absorbance was determined compared to the control at 517 nm according to **Gülcin *et al.*, (2004)**.

The total phenolic content of the GCP was determined using Folin-Ciocalteu colorimetric method **Yilmaz & Gokmen, (2013**), and the results were expressed as mg gallic acid equivalent (GAE)/100 g dry weight.

Manufacture of probiotic stirred yogurt

Probiotic stirred yoghurt supplemented with GCP was manufactured according to **Tamime & Robinson, (1999)** method. The fat content of cow's milk was reduced to 1% by separating cream using person square. Milk was divided into four equal portions (each 2 L). The first portion was served as a control sample. GCP was added to the second (T1), third (T2), and the fourth (T3) portions at 0.5, 1.5, and 3% respectively. After heat treatment at 90°C for 10 min, the different treatments were cooled to 42°C and inoculated with an ABT5 starter culture (0.025%), and then the portions were incubated at 37°C until the pH value reached 4.6. After coagulation, yoghurt treatments were stirred with an electric mixer (Moulinex, France) for 3 min at a low speed (less than 20 rpm), and the stirred yoghurt was dispensed into plastic cups (200 mL) and closed with the covers. Finally, the different treatments were immediately transferred to cold storage (6±1 °C).

Syneresis measurement

Twenty-five grams of yoghurt samples were weighed on a 125 mm filter paper placed on the top of a funnel. The syneresis of whey was carried out by gravity and the quantity of whey collected in the flask of known weight. The drainage time and temperature were 120 minutes and 25°C respectively according to **Sahan *et al.*, (2008).**

Chemical analysis of yoghurt supplemented with GCP

Yoghurt samples PH change during storage were measured by using a laboratory pH meter (HANNA, Instrument, Portugal). The acidity, total solids, fat, and total protein contents were determined according to **AOAC, (2007)**. The total volatile fatty acids (TVFAs) were assessed according to **Kosikowski., (1984)**. All samples were analyzed when fresh and after 5, 10 and 15 days from the manufacturing process with triple replicates.

Sensory evaluation of yoghurt supplemented with GCP

Ten trained panelists from the staff members of the Food and Dairy Technology, Faculty of Technology and Development, Zagazig University, Egypt used a quality rating scorecard: The evaluation of taste (7 points), consistency (5 points), flavor (5 points), color (3 points), and total (20 points) according to **Lisak *et al.*, (2012)**. All samples were evaluated sensually when fresh and after 5, 10 and 15 days of production.

In vitro characteristics of yoghurt supplemented with GCP

Animals

Thirty adult western strain male healthy albino rats (weighting, 90-120 g), purchased from the National Research Center (Giza, Egypt) were used. The rats were housed as groups (6 rats for each) in wire cages under hygienic conditions in an air-conditioned at the animal house (Faculty of Pharmacy, Zagazig University).

Diet of the experiment

Standard basal diets were obtained from the central animal house of the National Research Center (Giza, Egypt). The standard diet consisted of 22% protein, 3.5% fat, 12% fiber, 60% carbohydrates, and 2.40% ash. Additionally, a high cholesterol

diet was prepared using the basal diets described above which was ground and supplemented with cholesterol (1%), bile salts (0.25%) and fats (15%) according to **Zulet *et al*., (1999).**

Experimental design and animal groups

The rats were divided into the following groups:

Group (A): A normal control group (negative control group, 6 rats). The rats of this group were fed on the basal diet and tap water for (6 weeks).

Group (B): Twenty-four rats fed on a hypercholesterolemia diet for 4 weeks to make hypercholesterolemia rats. After that, the rats were divided into 4 sub-groups (6 rats for each) fed on experimental diets and tap water for 6 weeks as follows;

Sub-group B1: A positive control group (non-treated group) fed on the basal diet.

Sub-group B2: Rats fed on the basal diet and yoghurt without GCP.

Sub-group B3: Rats fed on the basal diet and 20g/per day of yoghurt supplemented with 0.5% GCP daily.

Sub-group B4: Rats fed on the basal diet and 20g/per day of yoghurt supplemented with 3% GCP daily.

Samples collection

At the end of the experiment period (6 weeks), the rats were fasted for 12 h. They were anesthetized and blood samples were collected from the portal vein heparinized centrifuge tubes. The plasma was separated by centrifugation at 3000 rpm for 10 min at the room temperature and kept in plastic vials stored at -20°C until they were analyzed. The organs (liver, kidney, and heart) were collected directly after the slaughtering of rats at the end of the experimental period and preserved in 10% neutral buffered formalin, dehydrated in ascending concentrations of aqueous ethyl alcohol, then cleared in xylol, and embedded in paraffin

wax. The samples were divided at 5 µm in thickness and stained with hematoxylin and eosin for microscopic examination according to **Bancroft & Stevens, (1990)**

Biological evaluation

Determination of body weight gain and feed efficiency ratio

The body weight gain (BWG) and feed efficiency ratio (FER) were determined according to **Chapman *et al.*, (1959)** using the following equation:

$$(\text{BWG}\%) = \frac{Final\ Weight\_\ Initial\ Weight}{Initial\ Weight} * 100$$

$$(\text{FER}) = \frac{Body\ \ Weight\ gain}{Food\ intake\ (g)}$$

Kidney function

Serum urea nitrogen was determined at 550 nm according to **Fawcett & Soctt., (1960)**. The serum creatinine was determined at 510 nm as reported **Larsen, (1972)**.

Liver function

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined at 505 nm according to **Reitman & Frankel., (1957).**

Assessment of lipids profile

The total serum cholesterol was determined according to **NIHP, (1987)**. Furthermore, triglycerides in serum were estimated using the method described by **Triuder, (1969)**. The high-density lipoprotein (HDL) was determined according to the procedure reported by **Friedewald, (1972)** and **Grodon &**

Amer., (1977). The very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were determined according to the method of **Lee & Nieman., (1996)** as follows:

VLDL (mg/dl) = Triglycerides/5
LDL (mg/dl) = Total cholesterol - (HDL+VLDL)

All the parameters estimated in serum samples were carried out by using a spectrophotometer (model DU 4700) and analyzed using biodiagnostic kits.

Statistical analysis

The obtained results were evaluated statistically using analysis of variance as reported by **McClave & Benson., (1991)**. The other reported values were expressed as mean ±SD and ±LSD, two-tailed Student's t-test was used to compare between different groups. P value of less than 0.05 was considered statistically significant. SPSS was used.

Results and Discussion:

Chemical composition, total phenolic compounds (TPC) and radical scavenging activity (RSA) of GCP

Chemical composition of GCP was shown in **Table 1**. The results showed that total solids, fat/dry matter, protein/dry matter, carbohydrate, crude fibers and ash were 88.71, 5.85, 16.68, 64.96, 42.88 and 3.76 respectively.

Total phenolic compounds (TPC) in GCP reached 1900 mg GAE /100 g dry weight. These results are in agreement with that reported by **Beder-Belkhiri *et al*, (2018),** who found that TPC and caffeine of coffee were 690mg GAE/100g, and 0.96 mg/g respectively. Therefore, GCP is considered a good source of bioactive compounds that have high antioxidative properties. The radical scavenging activity of GCP reached 840.2%.

Table (1) Chemical composition of green coffee powder (GCP)

Parameter	Content
Moisture (%)	11.29±0.20
Fat/dry matter (%)	5.85±0.15
Protein/dry matter (%)	16.68±0.57
Carbohydrate (%)	64.96±0.36
Crude fibers (%)	42.88±3.20
Ash (%)	3.76±0.04
Total phenolic content (mg GAE/100 g)	1900±20.02
Radical scavenging acidity (%)	840.22±5.40

Physiochemical properties yoghurt supplemented with GCP

Coagulation time and syneresis rate

Table 2 shows that the control samples scored the lowest time of coagulation comparably with the other treatments. This may be attributed to the addition of GCP which delayed the coagulation process and this refers to the effect of TPC on the activity of starter culture strains **Teshome *et al.*, (2017)** found that the phenolic components presented in mango and papaya juices decreased the viability of lactic acid bacteria during storage.

Table 2 shows that the control samples had higher values of syneresis than those supplemented with GCP. There was a decrease in the syneresis of samples with an increase of GCP concentration. It was shown that the syneresis decreased during storage period (up to 15 days) because of the formation of new covalent bonds owing to the ability of polyphenols to interact covalently with proteins **(Richard *et al.*, 2006)**. Increasing the added mount of GCP improved the properties of the formed texture which led to a decrease in syneresis rate. The interaction of phenolics with proteins in stirred yoghurt supplemented with GCP was found to be well enough to strengthen the gel structure of yogurt, which led to a decreased syneresis rate. Similar results

were obtained by **Dönmez, *et al.*, (2017**) who reported that the addition of GCP decreased the syneresis rate in yogurt.

Chemical composition

Table 2 shows that the acidity of stirred yoghurt was not significantly affected by GCP addition until 0.50%, but the supplementation with 1.50 and 3.00% of GCP decreased the acidity of stirred yoghurt and there was no significant difference between T2 and T4. In addition, there were no significant differences in the pH values between stirred yoghurt supplemented with 0.50% of GCP and the control samples during the storage period. There were no significant differences in the pH values between stirred yoghurt samples supplemented with 1.50 and 3.00% of GCP and the control samples during the storage period.

Regarding the total solids content, it was shown that the total solids contents increased with the increase in GCP addition **(Table 2)**. A statistically significant difference among control and T2, T3 was observed. **Table 2** shows that there was no significant difference in the fat content of stirred yoghurt from different treatments during the storage period. In addition, the total protein content increased (no significant differences) with the increase in the GCP supplementation rate. Significant differences ($P \leq 0.5$) were found in the ash content with the increase in GCP supplementation as compared to the control sample. The obtained results showed that there was no significant difference in the TVFAs content of stirred yoghurt from different treatments during the storage period.

Table (2) Physiochemical characteristics of probiotic stirred yogurt supplemented with GCP

Properties	Storage period (day)	Treatment			
		C	T1	T2	T3
Coagulation time per (min)		147±0.65^{d}	158±0.74^{c}	164±0.81^{b}	178±0.36^{a}
Syneresis	Fresh	34.20±0.47^{a}	31.04±0.37^{b}	29.88±0.65^{b}	28.00±0.93^{b}
	5	32.20±0.64^{a}	29.88±0.28^{a}	28.21±0.289^{b}	24.67±0.68^{c}
	10	29.55±0.36^{a}	23.21±0.25^{b}	18.21±0.47^{c}	19.00±0.29^{c}
	15	25.00±0.37^{a}	21.30±0.22^{b}	17.88±0.32^{c}	16.00±0.5^{c}
Acidity (lactic acid %)	Fresh	1.25±0.30^{a}	1.22±0.10^{a}	1.14±0.09^{b}	1.01±0.22^{b}
	5	1.28±0.12^{a}	1.24±0.05^{a}	1.17±0.20^{b}	1.06±0.10^{b}
	10	1.29±0.02^{a}	1.28±0.30^{a}	1.22±0.09^{ab}	1.18±0.13^{b}
	15	1.30±0.11^{a}	1.30±0.24^{a}	1.24±0.05^{ab}	1.22±0.22^{b}
pH value	Fresh	4.37±0.32^{b}	4.38±0.21^{b}	4.42±0.32^{a}	4.45±0.01^{a}
	5	4.32±0.22^{b}	4.35±0.40^{ab}	4.39±0.21^{a}	4.43±0.30^{a}
	10	4.30±0.23^{b}	4.33±0.25^{b}	4.37±0.34^{a}	4.41±0.11^{a}
	15	4.28±0.04^{b}	4.30±0.33^{a}b	4.34±0.11^{a}	4.39±0.47^{a}
Total solids (%)	Fresh	12.24±0.17^{b}	13.56±0.29^{b}	14.42±0.22^{a}	14.48±0.29^{a}
	5	12.71±0.14^{b}	14.19±0.23^{a}	14.68±0.39^{a}	15.04±0.40^{a}
	10	12.94±0.11^{b}	14.53±0.32^{a}	15.03±0.25^{a}	15.19±0.21^{a}
	15	13.98±0.18^{b}	14.71±0.21^{ab}	15.17±0.32^{a}	16.70±0.32^{a}
Ash (%)	Fresh	0.75±0.54^{b}	0.80±0.22^{a}	0.83±0.17^{a}	0.85±0.23^{a}
	5	0.80±0.09^{ab}	0.85±0.11^{a}	0.89±0.07^{a}	0.91±0.02^{a}
	10	0.82±0.04^{ab}	0.86±0.06^{a}	0.90±0.04^{a}	0.93±0.01^{a}
	15	0.86±0.01^{b}	0.92±0.04^{a}	0.95±0.04^{a}	0.96±0.03^{a}
Fat (%)	Fresh	1.50±0.14^{a}	1.43±0.12^{a}	1.47±0.18^{a}	1.45±0.11^{a}
	5	1.50±0.31^{a}	1.60±0.42^{a}	1.57±0.12^{a}	1.50±0.16^{a}
	10	1.60±0.36^{a}	1.65±0.11^{a}	1.75±0.33^{a}	1.67±0.59^{a}
	15	1.65±0.12^{a}	1.70±0.21^{a}	1.70 ±0.29^{a}	1.70±0.22^{a}
Total protein (%)	Fresh	3.62±0.21^{a}	3.64±0.23^{a}	3.66±0.21^{a}	3.70±0.29^{a}
	5	3.66±0.23^{b}	3.72±0.54^{a}	3.75±0.48^{a}	3.78±0.21^{a}
	10	3.72±0.26^{a}	3.74±0.23^{a}	3.80±0.25^{a}	3.84±0.24^{a}
	15	3.72±0.25^{b}	3.77±0.65^{ab}	3.84±0.27^{a}	3.86±0.21^{a}
Total volatile fatty acids: (0.1 N-NaOH/100) g)	Fresh	7.67±0.21^{a}	7.88±0.37^{a}	8.15±0.31^{a}	8.20±0.47^{a}
	5	8.76±0.45^{a}	9.29±0.32^{a}	9.30±0.54^{a}	9.35±0.41^{a}
	10	9.96±0.52^{a}	10.00±0.17^{a}	10.10±0.32^{a}	10.50±0.32^{a}
	15	11.03±0.34 ^{a}	11.10±0.21^{a}	11.25±0.15^{a}	11.44±0.41^{a}

C: control stirred yogurt, T1: stirred yogurt supplemented with 0.5% GCP, T2: stirred yogurt supplemented with 1.5% GCP and T3, stirred yogurt supplemented with 3.00 % GCP.

Sensory evaluation

The average score points given for taste, consistency, flavor and color characteristics of stirred yoghurt treatments are shown in **Table 3**. The results showed that there were no significant differences among the control sample and all the experimental samples in taste and flavor evaluation. Stirred yoghurt supplemented with GCP recorded the lowest scores in color evaluation especially the yoghurt supplemented at the ratio of 1.50 and 3.00%. Many researchers evaluated the effect of dietary fibers on dairy products and yoghurt quality. The addition of fiber decreased the overall flavor quality. These results agree with that reported by **Fernández-García *et al.*, (1998)**. From the data in **Table 3**, it can be concluded that all the treatments had been acceptable by the panelists.

Table (3) Sensory evaluation of stirred yogurt supplemented with GCP

Parameter	Storage period (day)	Treatment			
		C	T1	T2	T3
Taste (7 points)	Fresh	6.19±0.32	6.24±0.21	5.64±0.34	5.48±0.28
	5	5.94±0.43	6.14±0.25	5.74±0.22	5.96±0.23
	10	6.44±0.24	6.08±0.22	5.95±0.14	6.21±0.43
	15	5.81±0.54	6.17±0.34	5.70±0.21	6.17±0.54
Consistency (5 points)	Fresh	4.40±0.21	4.47±0.32	4.49±0.54	4.49±0.23
	5	4.22±0.32	4.45±0.23	4.73±0.36	4.91±0.27
	10	4.12±0.38	4.92±0.52	4.95±0.37	4.98±0.21
	15	4.05±0.43	4.61±0.21	4.68±0.46	4.69±0.28
Flavor (5 points)	Fresh	4.09±0.50	4.14±0.43	3.54±0.57	3.38±0.23
	5	3.84±0.20	4.04±0.23	3.64±0.34	3.86±0.52
	10	4.34±0.32	3.98±0.38	3.85±0.31	4.11±0.20
	15	3.71±0.43	4.07±0.32	3.60±0.26	4.07±0.28
Color (3 points)	Fresh	2.60±0.21	2.31±0.28	2.15±0.32	2.00±0.43
	5	2.69±0.65	2.29±0.34	2.30±0.27	2.28±0.32
	10	2.68±0.34	2.42±0.35	2.58±0.18	2.06±0.36
	15	2.64±0.53	2.40±0.24	2.43±0.15	2.41±0.17
Total (20 points)	Fresh	$17.28±0.20^{a}$	$17.16±0.27^{a}$	$15.82±0.32^{b}$	$15.35±0.64^{b}$
	5	$16.69±0.34^{a}$	$16.92±0.28^{a}$	$16.41±0.35^{a}$	$17.01±0.29^{a}$
	10	$17.58±0.73^{a}$	$17.04±0.23^{a}$	$17.33±0.21^{a}$	$17.36±0.61^{a}$
	15	$16.21±0.34^{b}$	$17.25±0.28^{a}$	$16.41±0.24^{a}$	$17.34±0.27^{a}$

C: control stirred yogurt, T1: stirred yogurt supplemented with 0.5% GCP, T2: stirred yogurt supplemented with 1.5% GCP and T3, stirred yogurt supplemented with 3.00 % GCP.

Biological evaluation

Body weight and food intake

Green coffee phytochemicals show a tendency to reduce body weight **(Igho *et al.*, 2011)**. It was shown that both the body weight and food intake in hypercholesterolemia rats had increased during the feeding period **(Table 4)**. At the end of the feeding period, the final body weights of hypercholesterolemia rats were significantly higher than the rats fed on basal diet and basal diet plus stirred yoghurt and/or stirred yoghurt supplemented with GCP. These results are similar to those reported by **Ngongang *et al.*, (2016)** who found that the highest increase in body weight was recorded in the groups of rats fed on a hypercholesterolemia diet only.

Liver function of rats fed on different experimental diets

Serum lipids

The obesity and hypercholesterolemia induce abnormally high levels of the blood and liver index including triglycerides, total cholesterol, LDL cholesterol, and low levels of HDL cholesterol. Blood serum lipid profiles of rats fed on different experimental diets are shown in **Table 4** which showed that the intake of 1% of cholesterol in the diet caused a significant increase in the total cholesterol in the rats of the positive control group in comparison with the other groups, indicating that hypercholesterolemia was successfully induced in the other groups. However, a reduction in the total cholesterol was observed in hypercholesterolemia rats that fed on yoghurt supplemented with GCP. The reduction most appeared with rats fed on yoghurt supplemented with GCP compared to the rats fed on yoghurt without any additives. Regular consumption of yoghurt with live cultures and probiotic strains was effective in

reducing serum cholesterol levels **(Vasiljevic & Shah., 2007)**. A recent study indicated that dietary fibers act as a key part of lipid-lowering **(Lim *et al.*, 2017)**. These results agreed with **(Ding *et al.*, 2020)**

Liver Functions

Table 4 shows that feeding rats on stirred yoghurt supplemented with GCP prevented the rise of mean serum alanine amino transaminase (ALT), and aspartate amino transaminase (AST) activities. The rate of decrease in the liver enzymatic activities was recorded 65 and 56.33 U/L for ALT and 111.67 and 106.67 U/L for AST with the rats fed on stirred yoghurt supplemented with 0.5% and 3.0% of GCP, respectively. Generally, it can be concluded that yoghurt supplemented with GCP significantly decreased the ALT and AST levels. These results agreed with **(Shahmohammadi *et al.*, 2017**).

Kidney functions

Table 4 shows that feeding rats on the stirred yoghurt supplemented with 0.5% and 3.0% of GCP prevented the rising of the mean serum creatinine and urea concentrations. The rate of prevention increased with the increase of the concentrations. The value of decreasing in the kidney function parameters was recorded as 0.82 and 0.72 mg/dl for creatinine as well as 77.20 and 72.67 mg/dl for urea after 6 weeks in the rats fed on yoghurt supplemented with.0.5 and 3.00% of GCP respectively. These results agreed with **(Ghalehkandi *et al.*, 2012)**.

Table (4) Biological evaluation of rats fed on different experimental diets

Parameter	Negative Control (A)	Positive Control (B1)	Yogurt (B2)	Green coffee treatment		LSD 0.05
				0.5% (B3)	3% (B4)	
BWG %	120.50±16.29 [a]	172.45±33.61 [a]	145.08±26.19 [a]	123.87±51.99 [a]	101.99±42.22 [a]	65.93
Food intake (g/day)	16.18±0.74 [b]	22±2.00[a]	16.08±1.36 [b]	15.53±1.05 [b]	15.07±0.60 [b]	2.28
FER (g)	0.139±0.139 [a]	0.152±0.004 [a]	0.193±0.025 [a]	0.140± 0.053 [a]	0.129±0.054 [a]	0.066

Kidney function of rats fed on different experimental diets						
Creatinine (mg/dl)	0.68±0.01 ^{e}	0.98±0.01 ^{a}	0.86±0.01 ^{b}	0.82±0.01 ^{c}	0.72± 0.03 ^{d}	0.03
Urea	68±2.65 ^{d}	93.67±4.73 ^{a}	82.33±3.06 ^{b}	77±2.0 ^{c}	72.67± 2.52 ^{cd}	5.70
Liver function of rats fed on different experimental diets						
ALT (u/l)	46.67±5.51 ^{e}	84±2 ^{a}	72.33±1.53 ^{b}	65± 1.4 ^{c}	56.33± 2.52 ^{d}	6.25
AST (u/l)	100±2 ^{d}	129±8 ^{a}	117.33±1.53 ^{b}	111.67±3.06 ^{bc}	106.67± 2.08 ^{cd}	7.46
Serum lipid of rats fed on different experimental diets						
Total cholesterol	141±3.61 ^{d}	246.33±5.51 ^{a}	185±5 ^{b}	164.67±5.51 ^{c}	150.67± 9.29 ^{d}	11.07
Triglycerids	118.67±7.37 ^{d}	158±6 ^{a}	137.33±1.53 ^{b}	129±1 ^{c}	122.67± 2.52 ^{cd}	8.14
HDL-C	57±1 ^{a}	33±3.46 ^{d}	42.67±1.15 ^{c}	51±1.73 ^{b}	53.67± 1.15 ^{ab}	3.52
LDL-C	60.27±3.87 ^{e}	182.4±6.26 ^{a}	114.87±5.28 ^{b}	87.86±7.40 ^{c}	72.47± 9.07 ^{d}	12.04
VLDL-C	23.73±1.47 ^{d}	31.6±1.2 ^{a}	27.47±0.31 ^{b}	25.8± 0.2 ^{c}	24.53± 0.50 ^{cd}	1.63
AI	1.47±0.11 ^{c}	6.54±0.82 ^{a}	3.00±0.64 ^{b}	2.24± 0.22 ^{bc}	1.81± 0.15 ^{c}	0.88

Results are means ± standard deviation

Group (A): Normal control group (control negative group), Group (B_1): Control positive group (non-treated group) rats fed on basal diet, Group (B_2): Rats fed on basal diet + stirred yogurt, Group (B_3): Rats fed on basal diet + stirred yogurt containing 0.5% of green coffee powder and Group (B_4): Rats fed on basal diet + stirred yogurt containing 3.0 % of green coffee powder.

Organs histology

Liver histology

Liver is responsible for the metabolism and detoxification of most components that enter the body **(Nunez, 2006)**. The results showed pathological changes in hepatics and renal tissues characterized by inflammatory cellular infiltration focal areas of necrosis in the hepatic parenchyma in liver tissue with the fatty change of hepatocytes. Additionally, various pathological alterations were observed in the renal tubules and glomeruli. Fatty liver refers to a large spectrum of diseases characterized by the accumulation of excess fat on the liver. A high-fat diet causes a variety of disorders. The current research proved that yoghurt supplemented with GCP can be used for the treatment of liver

diseases. The negative control liver sections showed normal central veins, hepatic sinusoids and hepatic cords. Photomicrograph of the liver (1) shows normal central veins (arrow) and hepatic cords (arrow head).

The positive control (high-fat diet) liver sections showed a widely distributed fatty change represented by replacement of hepatocytes by large, clear and sharp vacuole with peripherally located nuclei. Round cell infiltration around the central vein and degenerative changes in few hepatocytes were also observed. Photomicrograph of the liver (2) shows fatty change within hepatic parenchyma (arrow heads) and round cell infiltration around the central vein (arrow).

Liver sections of rats that fed on yoghurt showed fat change which represented by replacement of hepatocytes by large, clear and sharp vacuole with peripherally located nuclei within a large number of hepatic parenchyma and round cells aggregations within hepatic sinusoids. The photomicrograph of the liver (3) reveals fat change within a large number of hepatic parenchyma (arrow heads) and round cell aggregations within hepatic sinusoids (arrow).

Liver sections of rats that fed on yoghurt supplemented with 1% GCP revealed degenerative changes of some hepatocytes mainly hydropic degeneration and fatty change beside the focal aggregated area of round cells within the hepatic parenchyma. The photomicrograph of the liver section (4) shows hydropic degeneration (curved arrow), fatty change (arrow head) and focal area of round cell infiltration (arrow).

Liver sections of rats that fed on yoghurt supplemented with 3% GCP showed normal hepatic parenchyma with a preserved lobular pattern, portal trade structures, vascular tree, kupffer cells and stromal component. The photomicrograph of liver sections (5) shows a normal vascular tree (star) and hepatic cord (curved arrow).

Kidney histology

The negative control group's kidney sections showed normal renal tubular epithelium and glomerular structures. The photomicrograph of the kidney (1) shows renal tubular epithelium (arrow head) and glomerular structures (arrow).

The positive control (high-fat diet) group's kidney sections revealed fatty change within renal tubular epithelium and tunica media of renal blood vessels. Degenerative changes in a moderate number of renal tubules, perivascular edema, and dilated tubular lumen were also detected. Photomicrograph of kidney (2) shows fatty change within the renal tubular epithelium **(**arrow heads) and tunica media of renal blood vessels (arrow), degenerative changes in a moderate number of renal tubules (curved arrow), perivascular edema (red star) and dilated tubular lumen (black star).

Kidney sections of rats that fed on yoghurt showed hemorrhage between renal tubules and vacuolated tunica media of renal blood vessels. The photomicrograph of kidney (3) shows hemorrhage between renal tubules (arrow head) and vacuolated tunica media of renal blood vessels (arrow). The kidney sections of rats which fed on yoghurt supplemented with 0.5% of GCP showed apparently normal most renal parenchyma. However, perivascular edema with eosinophilic infiltration was also observed. The photomicrograph of the kidney (4) shows perivascular edema with eosinophilic infiltration (arrow).

Kidney sections of rats that fed on yoghurt supplemented with 3% GCP showed apparently normal renal parenchyma. However, shrinkage of some glomeruli and round cell infiltration between tubules was also observed. The photomicrograph of kidney sections (5) shows shrinkage of some glomeruli (arrow) and round cell infiltration between tubules (curved arrow).

Heart histology

An array of health benefits have been attributed to the consumption of green coffee such as reduction of the

corresponding risk of cardiovascular disease **(Jesús *et al.*, 2017)**. Green coffee phytochemicals show a tendency to reduce visceral fat **(Igho *et al.*, 2011)**. Heart sections showed nearly normal histomorphology of cardiomyocytes in all examined sections. In the negative control group's heart sections, the photomicrograph of heart (1) shows a normal histomorphology of cardiomyocytes (arrow head). In the positive control group (high-fat diet), heart sections showed a fatty change within some cardiomyocytes and tunica intima of some cardiac blood vessels besides congested cardiac blood vessels were also observed. The photomicrograph of heart (2) shows fatty change within some cardiomyocytes (arrow heads) and tunica intima of some cardiac blood vessels (arrow) in addition to the congestion of some cardiac blood vessels (star).

Heart sections of rats that fed on yoghurt revealed hyaline degenerations in few myocardial bundles besides: vacuolated tunica intima of some congested cardiac blood vessels was also observed. The photomicrograph of heart (3) exhibits hyaline degenerations (arrow head) in few myocardial bundles, vacuolated tunica intima of some cardiac blood vessels (arrow).

Heart sections of rats that fed on yoghurt supplemented with 0.5% GCP revealed normal cardiomyocytes. However, congestion intramuscular and coronary blood vessels were observed. The photomicrograph of heart (4) shows the congestion of intramuscular and coronary blood vessels (arrows). Heart sections of rats which fed on yoghurt supplemented with 3% GCP show a normal histomorphology of cardiomyocytes. The photomicrograph of heart (5) demonstrates a normal histomorphology of cardiomyocytes (arrow head).

Figure (1) Liver, kidney, and heart histology of rats which fed on different experimental diets

Group (A): Normal control group (control negative group).

Group (B_1): Control positive group (non-treated group) rats fed on basal diet.

Group (B_2): Rats fed on basal diet + stirred yogurt.

Group (B_3): Rats fed on basal diet + stirred yoghurt containing 0.5 % of green coffee powder.
Group (B_4): Rats fed on basal diet + stirred yoghurt containing 3.00 % of green coffee powder.

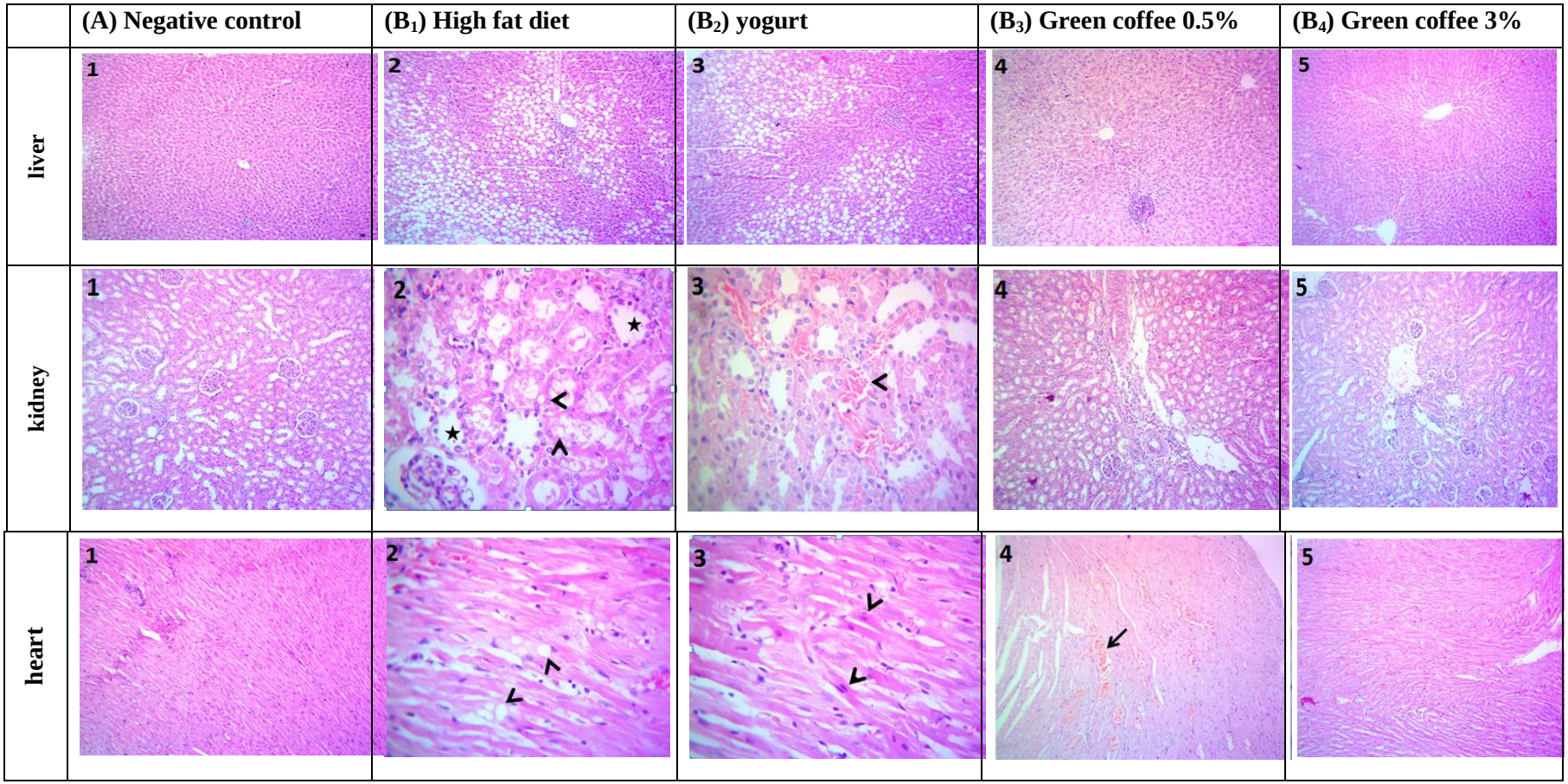

Figure (1) Liver, kidney, and heart histology of rats fed on different experimental diets

Conclusion:

It can be concluded that consumption of functional stirred yoghurt supplemented with GCP reduces high cholesterol and treats of obesity. Finding indicated that yoghurt supplemented with GCP consumption enhanced the nutritional value and phytochemical levels in food consisting of significant levels of the following: total phenolic and bioactive compounds

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References:

AOAC (2007). Association of Official Analytical Chemists-Official Method of Analysis. (18thEd.), Benjamin Franklin Station Washington, DC, USA.

Bancroft, J.D. and Stevens, A. (1990). Theory and practice of histopathological techniques. Churchill Livingstone, London.

Beder-Belkhiri, W.; Zeghichi-Hamria, S. Kadria, N.; Boulekbache-Makhloufa, L. Cardosod, S.; Oukhmanou-Bensidhouma,S. and Madania, K. (2018). Hydroxycinnamic acids profiling, in vitro evaluation of total phenolic compounds, caffeine and antioxidant properties of coffee imported, roasted and consumed in Algeria. Mediterranean Journal of Nutrition and Metabolism 11, 51–-63

Chapman, D.G., R. Gastilla and J.A. Campbell, (1959). Evaluation of protein in food I.A. Method for the

determination of protein efficiency ratio. Can. J. Biochem, Physiol., 37: 679-686.

Dhingra, D., Michael, M., Rajput, H., and Patil, R.T. (2012). Dietary fiber in foods: a review. Journal Food Science and Technology, 49(3), 255–-266.

Dönmez, Ö., Mogol, B. A., and Gökmen, V. (2017). Syneresis and rheological behaviors of set yoghurt containing green tea and green coffee powders. Journal of dairy science, 100 (2), 901-907.

Ding, F., Ma, B., Nazary-Vannani, A., Kord-Varkaneh, H., Fatahi, S., Papageorgiou, M., ... & Han, D. (2020). The effects of green coffee bean extract supplementation on lipid profile in humans: A systematic review and meta-analysis of randomized controlled trials. Nutrition, Metabolism and Cardiovascular Diseases, 30(1), 1-10.

Fawcett, J.K. and Soctt, J.E. (1960). A rapid and precise method for the determination of urea. J. Clinc. Path.13, 156:159 .

Fernández-Garía, E.; McGregor, J.U. and Traylor, S. (1998) The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yoghurt, J. Dairy Sci. 81, 655–-663.

Friedewald, W.T; Levy, R. I. and Fredrickson, D. S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 18: 499-502.

Ghalehkandi.J,;Yahya, E.; Ramin, N. Salama,N. and Nobar, T. (2012). Effect of Garlic (Allium sativum) Aqueous Extract on serum values of Urea, Uric-Acid and Creatinine compared with Chromium Chloride in Male Rats. Annals of Biological Research, 3 (9):4485-4490

Grodon, T. and Amer, M. (1977): Determination of HDL, J. American of Medicine. 62(5):707-714.

Gülçin, I., Küfrevioğlu, Ö. İ., Oktay, M. and Büyükokuroğlu, M. E. 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). Journal of ethnopharmacology 90(2-3):205-215.

Guzmán-González, M., Morais, F., Ramos, M. and Amigo, L. (1999). Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model system. I: Use of whey protein concentrates, milk protein concentrates and skimmed milk powder. J. the Sci. F. and Agri., 79 (8): 1117-1122.

Hasni, S.A. and .Kaplan **M. J. (2020)** Chapter 36 Mechanisms of vascular damage in systemic lupus erythematosus Systemic Lupus Erythematosus (Second Edition) Basic, Applied and Clinical Aspects, Academic press, Pages 325-331

Hatano, T., Kagawa, H., Yasuhara, T., And Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and pharmaceutical bulletin 36(6): 2090-2097.

Igho, O., Rohini, T., and Edzard, E. (2011). The use of green coffee extract as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. Gastroenterology Research and Practice, 31, 1-6.

Jeong, C. H., Ryu, H., Zhang, T., Lee, C. H., Seo, H. G., and Han, S. G. (2018). Green tea powder supplementation enhances fermentation and antioxidant activity of set-type yogurt. Food Science and Biotechnology. 27 (5) :1419-1427.

Jesús, S.G.; Luis, C.Z. and Daniel, A.J.V. (2017). Chlorogenic acid: recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome, Molecules 22, 358.

Kosikowski, F.V. (1984). Cheese and Fermented Milk Foods 2nd Ed, printing Brook tonalds. New York, 14817.USA.

Larsen, K., (1972). Creatinine assay by a reaction-kinetic principle. Clin. Chim. Acta, 41: 209.

Lee, R. and D. Nieman, (1996). Nutritional Assessment.2nd Ed., Mosby, Times. Mirror Co. St. Louis, Missouri.

Lim, M.Y., You, H.J., Yoon, H.S., Kwon, B., Lee, J.Y., Lee, S., Song, Y.M., Lee, K., Sung, J., and Ko, G., (2017). The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. Gut 66, 1031-1038.

Lisak, K.; Lenc, M. ; Jelicec, I. and Bozanic, R. (2012) Evaluation of the Strawberry Flavored yoghurt with Stevia and sucrose addition. Croatian journal of food Technology, Biotechnology and nutrition (special issue) 7, 39-43

Loveday, S. M.; Sarkar, A. and Singh, H.(2013). Innovative yoghurts: Novel processing technologies for improving acid milk gel texture. Trends Food Sci. Technol. 33:5–-20.

McClave, J.T., and Benson, P. G.(1991). Statistical for business and economics. Max Well Macmillan International editions. Dellen Publishing Co. USA.272-295.

Ngongang, E. F. T.; Tiencheu, B. ;Achidi,A.U., Fossi, B. T.; Shiynyuy,D. M.,; Womeni, H. M., and François, Z. N.(2016). Effects of Probiotic Bacteria from Yoghurt on Enzyme and Serum Cholesterol Levels of Experimentally Induced Hyperlipidemic Wistar Albino Rats. American Journal of Biology and Life Sciences. 4(6): 48-55

NIHP (1987): Detection, Evaluation and treatment of high cholesterol in adults, National Institute of Health Publication. 88:292.

Nunez, M. (2006). Hepatotoxicity of antiretrovirals: Incidence mechanisms and management, J Hepatol. 44:133.

Osborn, O, and Olefsky J.M. (2012). The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med; 18(3): 363-374

Reitman, A. and S. Franklel, 1957. A colorimetric method ffor the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. AmerJ. Clinc. Path, 28: 56-63

Richard, T., D. Lefeuvre, Descendit, A. Quideau, S. and Monti. J. P. (2006). Recognition characters in peptide-polyphenol complex formation. Biochim. Biophys. Acta 1760:951–-958.

Sahan, N., Yasar, K. and Hayaloglu, A. A.(2008). Physical, Chemical and Flavour Quality of Non-fat Yoghurt as Affected by a β-Glucan Hydrocolloid Composite during Storage. Food Hydrocoll., 22: 1291–-1297.

Shetty, K., Curtis, O. F., Levin, R. E., Witkowsky, R. and Ang, W. (1995). Prevention of Vitrification Associated with in vitro Shoot Culture of Oregano. (Origanum vulgare) by Pseudomonas spp. Journal of Plant Physiology 147(3-4): 447-451.

Shahmohammadi, H. A., Hosseini, S. A., Hajiani, E., Malehi, A. S., & Alipour, M. (2017). Effects of green coffee bean extract supplementation on patients with non-alcoholic fatty liver disease: a randomized clinical trial. Hepat Mon, 17(4), e12299.

Tamime, A.Y. and Robinson, R.K. (1999) Yoghurt Science and Technology. 2nd Edition, Woodhead Publishing Ltd., Cambridge.

Teshome, G., Keba, A., Assefa, Z., Agza, B., and Kassa, F.(2017). Development of Fruit Flavored Yoghurt with Mango (*Mangifera indica* L.) and Papaya (*Carica papaya* L.) Fruits Juices. Food Science and Quality Management, 67:40-45.

Triuder, p. (1969): En jymatic colorimetrie determination of triglycoride by GPO-PAP Method, Ann.Clin. Biochem.. 6: 24-27

Urashima, T. ; Kitaoka, M. ; Asakuma, S. and Messer, M.(2009),'Milk oligosaccharides', in Mcsweeney, P. L. H. and Fox, P. F. , Advanced Dairy Chemistry Vol. 3 Lactose, water, salts and minor constituents , 3rd ed., New York, Springer, 295–349.

Van Dam, R. M. and Seidell, J. (2007) Carbohydrate intake and obesity, Eur. J. Clin. Nutr.61, S75–-S99.

Vasiljevic, T., and Shah, N. P. (2007). Fermented milks - health benefits beyond probiotic effects. In R. C. Chandan, & Y. H. Hui (Eds.), Handbook of food product manufacturing (pp. 99-116).London, UK: Wiley.

You, D. C., Kim, Y. S., Ha, A.W., Lee, Y. N., Kim, S.M., Kim, C.H., Lee, S.-H., Choi, D., and Lee, J.M. (2011). Possible health effects of caffeinated coffee consumption on Alzheimer's disease and cardiovascular disease. Toxicological research. 27(1), 7-10.

Yilmaz, C., and Gokmen, V. (2013). Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions. Industrial Crops and Products.49:130-135.

Zulet, M.A., Barber, A., Garcin, H., Higueret, P. and Martinez, J.A. (1999). Alterations in carbo-hydrate and lipid metabolism induced by a diet rich in coconut oil and cholesterol in a rat mod-el. J. of the American College of Nutri-tion, 18(1), 36-42.