Utilizing the Functions of Hydrocolloids as Fat Mimetic to Enhance the Properties of Low-fat Domiati Cheese

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Abstract

Regarding the rising demand of eating healthy displayed by consumers over the past few years, dairy market has been increasing its supply in producing low-fat cheese. As well known, the reduction of fat in cheese milk causes a significant decrease in organoleptic properties and cheese yield, thus dairy scientists have a continuous challenge to counteract these defects and satisfy the consumer’s requests. This work was undertaken to investigate the possibilities to improve the characteristics of low-fat Domiati cheese by using hydrocolloids as fat mimetic. Mixture of kappa carrageenan, locust bean and xanthan gums have been added to cheeses milk. Upon using different concentrations of Hydrocolloids, low-fat cheese showed a significant increase in the physiochemical characteristics, yield, and moisture. Furthermore, organoleptic properties obtained were both highly acceptable and comparable to full-fat cheese. These positive effects were also sustained during the 75 days ripening period where sensory scores were closely similar to those obtained from full-fat cheese.

Keywords: Domiati cheese; Low-fat; Hydrocolloids; Fat mimetic; Cheese yield

Abbreviations: FFC: Full-fat Cheese; LFC: Low-fat Cheese; LFH: Low-fat Cheese Containing Kappa Carrageenan Locust Bean and Xanthan Gums

Introduction

Domiati cheese (Gbnah Beeda) is one of the most unique cheeses both in Egypt and the Middle East. It is usually made from bovine milk where the milk is salted at the first step of its manufacturing process, Sodium chloride are usually added in ranges of 5% to 14% of milk (W/W) depending on the consumer preferences and manufactures season. Based on the huge production and consumption rate of this cheese in the Egyptian markets (about 75% of total cheese production), Domiati cheese is rated the most popular soft white pickled cheese in Egypt [1-3]. Over the last decade, as a result of growing healthy interest of consumers and the increased awareness from the health and nutrition associations and society, the market demand of reduced-fat products in general has become more than just a trend. Therefore, the production of low-fat cheese has been a constant preoccupation, that resulted in the significant growth of low-fat cheese production and thereby the overall growth of the low-fat cheese market [4,5]. Fats are well known to play a critical role in the flavour, texture, and appearance of food products. It quickly becomes obvious that developing low-fat products with a quality matching that of their full-fat types is a quite difficult task when one is replacing fat with alternative ingredients [6]. When dealing with cheese, reducing, or lowering its fat content adversely affects both its flavour and texture, therefore the development of low-fat cheese with comparable properties of full-fat cheese is often challenging [7,8]. In efforts to increase the likeability of low-fat cheese and overcome these defects, various suggestions and trials have been proposed. Fat replacers have been strongly recommended, regarding their effects on cheeses composition, component recoveries, cheeses yield, and yield recovery [9].

Fat replacers play a similar role of fat in cheese with a reduction in the caloric value which thereby possibly decreases the consumer’s susceptibility to coronary heart diseases [10]. Generally, the ingredients of fat replacers are fats, proteins, or carbohydrates, giving similar physiochemical properties as milk fat [11], they are often categorized into two groups: Fat substitutes which are fat based indigestible macromolecules with a caloric value lower than that of milk fat, or fat mimetic often called protein and / or carbohydrate based fat replacers which are polar, water sol compounds having different chemical structures in contrast to fat [4]. Hydrocolloids are one of the biopolymers derived from natural sources such as plant, animals or microorganisms. When the biopolymer is carbohydrate based then it is often referred to as Hydrocolloids; due to their large number of hydroxyl groups, hydrocolloids demonstrate high hydrophilic properties and affinity for binding to water molecules. Owing to their structure and physiochemical properties to mimetic milk fat and their functional properties such as thickening, gelling, emulsifying and stabilization, the use of hydrocolloids as fat replacers has been extended in manufacturing cheese and other foods [12,13].

Hydrocolloids have been used in different dairy products for different functions. For example, Abd Elhamid [14] used carboxymethyl cellulose to enhance the properties of low-fat Domiati cheese; while...
Romeih et al. [6] used different commercial hydrocolloids as fat replacers to produce low-fat white-brined cheese. To counteract the negative effects of fat reduction by using guar gum and xanthan gum as fat replacers in low-fat Mozzarella cheese [10] and using carrageenan in low-fat Oaxaca cheese [13]. Furthermore, the effect of using protein-based and carbohydrate-based fat replacer on rheological and sensory properties low-fat fresh Kashar cheese have been demonstrated by Koca and Metin [9], in addition, low-fat white pickled cheese were produced by using some protein or carbohydrate based fat mimetic [4], evaluating different fat replacers like modified corn starch and xanthan gum on low-fat Mozzarella cheese [15]. Recently, hydrocolloids were also used to stabilize structure, enhance viscosity and alter textural characteristics of different types of cheese such as in processed cheese with vegetable fats to enhance the rheological properties [16]. Jana et al. [17] studied the effect of using xanthan gum-locust bean gum, carrageenan on texture profile of casein based Mozzarella cheese analogue, while Černíková et al. [18] used them in processed cheese as a replacement of traditional emulsifying salts.

Based on our knowledge, there are no recent studies about using this mixture of hydrocolloids (K-carrageenan, Locust bean and Xanthan gums) with low-fat Domiati cheese; therefore, the main objective of this study is to evaluate the functionality of hydrocolloids throughout storage as fat mimetic in low-fat Domiati cheese.

Materials and Methods

Cheese making procedure

Five different treatments of cheese were made, each treatment was manufactured in triplicate, and six litres of milk were used for each replicate. Full-fat Domiati cheese (FFC) was manufactured using full-fat milk purchased from a local farm (Alexandria, Egypt) (T.S% = 11.6 ± 0.3, Fat% = 3.5 ± 0.13, Protein% = 3.1 ± 0.04, pH = 6.7 ± 0.01). After cooling the milk to 38°C, and then supplemented with 8% sodium chloride. In case of low-fat milk, 3% NaCl was added to the milk. The milk was heated to 40°C, and then inoculation of the mesophilic mixed culture Streptococcus thermophilus + Lactobacillus delbrueckii ssp. bulgaricus (Danisco, Copenhagen, Denmark) the milk was held at the same temperature for approximately 30 min for starter maturation. The milk was coagulated using the calf rennet (Chr. Hansen, Denmark), after dissolving in water. After 2-3 hours of coagulation, each curd was then transferred to a small mould under an initial pressure for whey expulsion for 24 hours. After pressing, the curd was cut into small blocks, placed in airtight plastic containers, and covered with a 12% pasteurized filtered brine solution. The blocks were stored at 23°C to 25°C for 24 h, and then all cheese containers were sealed and stored in a cold room at 5°C to 6°C for the ripening period of 75 days.

Cheese analysis

Moisture of different cheese treatments were determined by using oven drying method [20]. Fat content of milk and cheese samples were determined by the Gerber method [21]. Protein contents of the milk and cheese were determined by measuring total nitrogen using the Kjeldahl method [20] by converting the value to protein percentage using the factor of 6.38. The pH of milk and cheese samples was measured using a digital pH meter (Hanna pH 211, Hanna instruments, Woonsocket, RI, USA).

Superficial yields were elaborated as the weight of cheese divided by the weight of used milk [22]. All the analysis measurements were done in triplicate at days of 0, 15, 30, 45, 60 and 75 of ripening.

Sensory evaluation

In the present study Domiati cheese products were evaluated targeted appearance, flavour, body texture and odour by regular score panel according to the scoring sheet of Abou et al. [23] and the total scores were presented. Twenty panellists had evaluated the cheese in two consumers sensory test panel, for organoleptic assessment of the cheeses at days of 0, 15, 30, 45, 60 and 75 of ripening. Each panelist evaluated all samples during all storage period and has been instructed with the regular protocols of test panel.

Statistical analysis

The experimental design was completely randomized design (CRD) in factorial arrangement using three replications; all data are presented as mean ± SD for three replicates for each sample. The Students’ tests were performed using XLSTAT 2007 (Addinsoft 1995e2007, New York, USA) when data were compared with controls. Univariate analysis of variance (ANOVA) was applied using Stat graphics 16.1.11 (Stat Point Technologies, Inc., Virginia, USA) when multiple comparisons were performed. The differences were considered significant at P<0.05.

Results and Discussion

Effect of fat mimic on the physicochemical attributes of cheese

Milk fat is one of the major components in milk and most important components in cheeses as well. It is trapped in the protein matrix during cheeses making [24]. Table 1 shows fat percentage and protein content for full- and low-fat Domiati cheese after manufacturing and during ripening period. When fat level in cheese milk was reduced from 3.5% to 2%, fat contents in cheese reduced significantly. Full-fat cheese milk showed the highest fat cheese content among all treatments, whereas low-fat cheeses with or without hydrocolloids showed lower significant fat content, in addition, the hydrocolloids significantly attributed to the fat content of cheese treatments, using different levels of fat mimetic (Kappa carrageenan, Locust bean and Xanthan gums) increased the fat content respect to the control low-fat cheese; however there were no significant differences among the different concentrations of fat replacers. Similar results were reported by Sattar et al. [10] and Salazar et al. [25]. During ripening period, the fat content of all cheeses increased significantly (Table 1). This increase was continuously achieved until the end of ripening days. This phenomenon may be due to the reduction of moisture content during storage of ripening period [8,26]. In case of hydrocolloids treatments; however, the fat content increased with ripening period, the values were significantly lower than in the control low-fat cheese starting after 30 days of storage and continued with same trend until the 75 days of storage. This could be a result of higher water binding capacity of the added hydrocolloids, and the lower rate of moisture losses during ripening. This variation
was clear by calculating the fat increasing in rate from 1 to 75 days, as it were 27.97%, 17.36%, 14.18% and 13.52% for LFC, LFH1, LFH2, and LFH3 respectively. Regarding protein content, reducing fat content in cheeses led to an increase in protein content. Full-fat cheese showed the lowest protein content (18.50 ± 0.10) among all samples and this may be due to the higher moisture content. Some other studies have previously reported similar results [4,9,14,27-29]. Adding mixture of hydrocolloids as fat mimetic didn’t significantly affect the cheese protein content.

Among all cheeses samples, a decrease in proteins content was observed with the increase in the ripening period as a result of degradation, where a significant decrease was observed after 45 days of storage. Comparing the cheese containing fat mimetic as fat replacers with low-fat cheese, the protein decreasing rate was somewhat higher regarding the high moisture content, which generally increases the enzyme activity [4, 6]. One of the important characteristics of cheese curd is the moisture content, which affects many other factors either physically such as yield and texture or chemically and nutritional values based on dry weight. Table 2 shows the moisture content and their behaviour during the ripening period. Through the presented differences in moisture content between the full-fat cheese and the low-fat cheese, inverse relationship between fat content and moisture content was observed. This relationship could be attributed to their nitrogen content, where higher protein content of reduced-fat cheeses may have contributed to an increased water-binding capacity of the cheese matrix, subsequently increasing moisture content [4,6,14]. Furthermore, moisture content had been improved by the effects of adding the mixture of hydrocolloids. This significant increase is due to higher water binding capacity of the Kappa carrageenan, Locust bean and Xanthan gums [7,12,15,16]. In addition, the highest concentration of fat replacers (0.75 g/kg of milk) showed the highest moisture content among all cheese treatments; whereas increase the level of hydrocolloids mixture, the moisture contents increased non-significantly [10].

During the ripening period as shown in Table 2, somewhat decreases of moisture content among all cheeses samples have been observed until the end of ripening period. As an indicator of cheese acidity, the influence of fat mimetic on the mean pH values is shown in Table 2. The pH values have been analysed after the cheese manufacturing and continuously monitored until the end of ripening to follow the changes in cheeses acidity.

Reducing fat content in cheese milk didn’t significantly affect the pH value, in addition, there was somewhat lower values in case of using different concentration of fat mimetic but was not significant, similar studies reported that using Kappa carrageenan, Locust bean and Xanthan gums didn’t have significant effect on cheese pH. Kavas et al. [4], Sattar et al. [10] and Hanaková et al. [16] found similar results in their studies about different fat replacers. During ripening, there was a slight decreasing pattern of pH values among all cheeses, this decrease was very clear after 45 days of ripening and continues till the end of ripening especially in case of adding hydrocolloids as fat mimetic. The decreasing of pH value during ripening is a result of the chemical composition change [30]. The gradual decrease of pH value during ripening may be attributed to combination of the content of mineral content such as calcium and phosphorus, furthermore, the ratio of salt to moisture is also a very important factor [31]. In addition, during the ripening of cheese, changes of biochemistry may have occurred due to the different proteolysis and metabolism of the milk components and the residual lactose in the cheeses curd, these changes are important factors that affect the pH value during ripening of cheese [32]. Since the moisture content is important to the different chemical reactions that occur in cheese curd during ripening, and as well-known that cheese with fat mimetic retained higher moisture than corresponding treatments without adding, thus could explain the noticed higher decrease of pH value in these cheeses treatments. These changes in pH values during the ripening could partly explain that the decrease of moisture content might be due to the concentration of curd as a result of developed acidity during ripening period which helps to eject the whey from the curd [33,34].

**Effect of fat mimetic on the attitude of cheese yield**

Cheese yield is a very important parameter in the cheese industry and it highly affects the manufactures decision in choosing different additives, fat replacers, and other ingredients.

It’s well known that cheese is a concentrated product mainly formed of proteins and fats. As a consequence, cheeses yield is highly affected by fats and casein content of the milk [35]. Cheeses yield as presented in Figure 1 showed a significant reduction with decreasing fats content in cheeses, 28.87 ± 0.42% and 26.89 ± 0.14% for full- and low-fat cheese respectively. Although it is true that fats in cheese are replaced by moisture, but an overall reduction in yield is inevitable in the production of cheese from low-fat milk because the total amount of fats removed is not equal to the amount of moisture added [6,36]. In addition, the increase of fats content of milk in case of full-fat

<table>
<thead>
<tr>
<th>Table 1: Fat and protein content for different types of Domiati cheese during 75 days of ripening.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cheese treatments</strong></td>
</tr>
<tr>
<td><strong>Fat %</strong></td>
</tr>
<tr>
<td>FFC</td>
</tr>
<tr>
<td>LFH1</td>
</tr>
<tr>
<td>LFH2</td>
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<tr>
<td>LFH3</td>
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<tr>
<td>LFH1</td>
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<tr>
<td>LFH2</td>
</tr>
<tr>
<td>LFH3</td>
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<tr>
<td><strong>Protein %</strong></td>
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<tr>
<td>FFC</td>
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<tr>
<td>LFH1</td>
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<tr>
<td>LFH2</td>
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<tr>
<td>LFH3</td>
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</tbody>
</table>

Data are means ± SD; n=3
* indicates significantly different (P < 0.05) with respect to the first control (FFC) in the same time of ripening
** indicates significantly different (P < 0.05) with respect to the second control (LFC) in the same time of ripening
*** Different letters following means in cheese with hydrocolloids treatments denote significant differences (p<0.05)
cheese leads to reduction of whey syneresis from cheese curd during manufacturing [37,38]. The cheeses yield increased significantly and proportionally by adding fat mimetic with different concentration to reach 28.74 ± 0.33, 30.14 ± 1 and 31.57 ± 0.52 for LFH1, LFH2 and LFH3, respectively. This influence may be related to the high water-binding property of the mixture of Kappa carrageenan, locust bean and xanthan gums [12,16]. The uses of hydrocolloids as fat replacers have been known for their effects in improving both texture and yield [10,14,28,39].

However the effects of concentration was not significant between the mixture of Kappa carrageenan, locust bean and xanthan gums treatments, but the LFH3 which exhibited the highest concentration of hydrocolloids (0.75 g/kg of milk) showed a significantly higher yield value in respect to the full-fat cheese directly after manufacturing [37,38]. The cheeses yield increased significantly and proportionally by adding fat mimetic with different concentration to reach 28.74 ± 0.33, 30.14 ± 1 and 31.57 ± 0.52 for LFH1, LFH2 and LFH3, respectively. This influence may be related to the high water-binding property of the mixture of Kappa carrageenan, locust bean and xanthan gums [12,16]. The uses of hydrocolloids as fat replacers have been known for their effects in improving both texture and yield [10,14,28,39].

End of ripening. The low-fat cheese with the added mixture of Kappa carrageenan, locust bean and xanthan kept their attitude and presented significantly higher yield values and lower decreasing rates than the control low-fat cheese and even than high fat cheese in some cases. In addition, yield values were also in proportion with the hydrocolloids concentration, where the end of ripening period (75 days) the cheese yield was 22.65 ± 0.46%, 23.41 ± 0.19% and 24.00 ± 0.18% for LFH1, LFH2 and LFH3, respectively. While it was 21.81 ± 0.32 and 20.98 ± 0.32 for high and low-fat cheese respectively.

Effect of fat mimic on the sensory attitude of cheeses

Even though Domiati cheese is often consumed after ripening, sensory evaluation directly after production is very important and reflects its sensory attitude during the ripening period. The results of sensory evaluation out of 100 scores for cheese are presented in Table 3, cheeses with lower fats usually have less pronounced flavour, possibly because of its dilution in reduced and low-fat cheeses because of excessive moisture retention [24,42]. The fats in cheese are responsible for most of the flavours [8] therefore when fats are decreased, the cheeses' flavour decreases [4,14].

![Figure 1: Mean of yield of FFC (black bars), LFC (grey bars), LFH1 (striped bars), LFH2 (white bars) and LFH3 (checkered bars) Domiati cheese during 75 days of ripening.](image)

![Table 2: Moisture content and pH values for different types of Domiati cheese during 75 days of ripening.](table)

**Table 2: Moisture content and pH values for different types of Domiati cheese during 75 days of ripening.**

<table>
<thead>
<tr>
<th>Moisture %</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFC</td>
<td>60.28 ± 0.13</td>
<td>58.47 ± 0.17</td>
<td>57.69 ± 0.33</td>
<td>56.15 ± 0.19</td>
<td>54.19 ± 0.24</td>
<td>53.30 ± 0.55</td>
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<tr>
<td>LFH1</td>
<td>58.82 ± 0.14</td>
<td>57.52 ± 0.44</td>
<td>55.59 ± 0.51</td>
<td>53.65 ± 0.50</td>
<td>51.99 ± 0.69</td>
<td>50.66 ± 0.33</td>
</tr>
<tr>
<td>LFH2</td>
<td>60.67 ± 0.41$^{**}$</td>
<td>58.67 ± 0.56$^{**}$</td>
<td>56.70 ± 0.56$^{**}$</td>
<td>55.40 ± 0.45$^{**}$</td>
<td>54.40 ± 0.35$^{**}$</td>
<td>52.55 ± 0.44$^{**}$</td>
</tr>
<tr>
<td>LFH3</td>
<td>61.90 ± 0.26$^{**}$</td>
<td>59.50 ± 0.23$^{**}$</td>
<td>58.71 ± 0.35$^{**}$</td>
<td>57.22 ± 0.13$^{**}$</td>
<td>55.62 ± 0.33$^{**}$</td>
<td>53.96 ± 0.17$^{**}$</td>
</tr>
</tbody>
</table>

 Data are means ± SD; n=3

* indicates significantly different (P < 0.05) with respect to the first control (FFC) in the same time of ripening.

*+ indicates significantly different (P < 0.05) with respect to the second control (LFC) in the same time of ripening.

**+ Different letters following means in cheese with hydrocolloids treatments denote significant differences (p>0.05).

<table>
<thead>
<tr>
<th>pH</th>
<th>0</th>
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<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
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</thead>
<tbody>
<tr>
<td>FFC</td>
<td>5.33</td>
<td>5.29</td>
<td>5.25</td>
<td>5.16</td>
<td>5.14</td>
<td>5.09</td>
</tr>
<tr>
<td>LFH1</td>
<td>5.29</td>
<td>5.25</td>
<td>5.25</td>
<td>5.16</td>
<td>5.14</td>
<td>5.06</td>
</tr>
<tr>
<td>LFH2</td>
<td>5.17$^{**}$</td>
<td>5.13$^{**}$</td>
<td>5.06$^{**}$</td>
<td>4.98$^{**}$</td>
<td>4.93$^{**}$</td>
<td>4.88$^{**}$</td>
</tr>
<tr>
<td>LFH3</td>
<td>5.22$^{**}$</td>
<td>5.12$^{**}$</td>
<td>5.08$^{**}$</td>
<td>4.98$^{**}$</td>
<td>4.90$^{**}$</td>
<td>4.80$^{**}$</td>
</tr>
</tbody>
</table>

* indicates significantly different (P < 0.05) with respect to the FFC in the same time of ripening

** indicates significantly different (P < 0.05) with respect to the LFC in the same time of ripening

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**Table 3:**

<table>
<thead>
<tr>
<th>Ripening period (Days)</th>
<th>0</th>
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<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
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<td>60.28 ± 0.13</td>
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<td>57.69 ± 0.33</td>
<td>56.15 ± 0.19</td>
<td>54.19 ± 0.24</td>
<td>53.30 ± 0.55</td>
</tr>
<tr>
<td>LFH1</td>
<td>58.82 ± 0.14</td>
<td>57.52 ± 0.44</td>
<td>55.59 ± 0.51</td>
<td>53.65 ± 0.50</td>
<td>51.99 ± 0.69</td>
<td>50.66 ± 0.33</td>
</tr>
<tr>
<td>LFH2</td>
<td>60.67 ± 0.41$^{**}$</td>
<td>58.67 ± 0.56$^{**}$</td>
<td>56.70 ± 0.56$^{**}$</td>
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<td>53.96 ± 0.17$^{**}$</td>
</tr>
</tbody>
</table>

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** indicates significantly different (P < 0.05) with respect to the second control (LFC) in the same time of ripening.

---

**Figure 1:** Mean of yield of FFC (black bars), LFC (grey bars), LFH1 (striped bars), LFH2 (white bars) and LFH3 (checkered bars) Domiati cheese during 75 days of ripening.
In addition, supplementing the low-fat milk used in cheeses making with fat mimetic including kappa Carrageenan, locust bean and Xanthan gums led to higher scores on the evaluated attributes due to the moisture content enhancement, thereby resulting in a decrease in the differences noted between the sensory evaluation of full and low-fat cheese. Nevertheless, this positive effect of fat replacers was more noticeable and significant during the ripening period than it was during the first day. During the ripening, total quality of scores increased significantly in all cheeses whether controlled or treated but with different rates, this enhancement could be attributed to chemical changing and texture enhancing during the ripening of Domiati cheese [14,34,40]. Considering fats content, full-fat cheese kept its evaluation higher than the control low-fat cheese during all the ripening period and showed significant differences value at 75 days of ripening as similar results were obtained by Madadlou et al. [8] who reported that low-fat White cheeses received lower flavour and texture scores than full-fat cheeses. Nevertheless, cheeses with fat replacers showed surprising increase in sensory evaluation and scored the highest increasing rates during the ripening period. This increase resulted in higher significant sensory evaluation as compared with same cheeses without fat replacers. Moreover, the sensory evaluations of these treatments were very close to those in full-fat cheese; Nevertheless, this positive effect of fat replacers was in the differences noted between the sensory evaluation of full and low-fat Domiati cheese during 75 days of ripening.

**Conclusions**

Reducing fat in cheese milk and the addition of different hydrocolloids as fat replacers (including Kappa Carrageenan, locust bean and Xanthan gums), did affect the low-fat Domiati cheese in different ways during 75 days of ripening. The incorporation of hydrocolloids into cheeses had a positive effect on low-fat cheeses where it resulted in significant increases in moisture content, protein content and cheeses yield. Moreover, the sensory evaluation for fresh and during the ripening period showed that using selected hydrocolloids as fat replacers could increase the similarity between full and low-fat Domiati cheese. Finally, through the implementation of the conventional protocol to produce Domiati cheese by using low-fat milk and using hydrocolloids as fat mimetic, production of high quality low-fat Domiati cheese was successfully achieved where not only the absence of significant yield reduction was noted, but also the characteristics of full-fat cheese was well preserved. Thereby achieving the objective of meeting both the consumers need and the manufactures demands.

**Acknowledgement**

The authors are gratefully acknowledged Dr. Ola Nada (Alexandria University, Egypt) and Dr. Ahmed EI Ghannam (Damanhour University, Egypt) for providing language help during writing this manuscript.

### Table 3: Sensory evaluation out of 100 scores of different types of Domiati cheese during 75 days of ripening.

<table>
<thead>
<tr>
<th>Cheese Treatments</th>
<th>Ripening period (days)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFC</td>
<td>83.70 ± 4.74 *abcdef</td>
<td>84.20 ± 2.92 *abcdef</td>
<td>86.70 ± 5.37 *abcdfgh</td>
<td>89.65 ± 4.59 *abefgh</td>
<td>91.15 ± 2.60 *abcd</td>
<td>89.85 ± 5.15 *abcdfgh</td>
<td></td>
</tr>
<tr>
<td>LFC</td>
<td>76.60 ± 4.41 *a</td>
<td>77.00 ± 2.85 *a</td>
<td>82.30 ± 5.75 *abcdfgh</td>
<td>83.95 ± 3.80 *abcdfgh</td>
<td>86.70 ± 3.88 *abcdfgh</td>
<td>80.65 ± 5.44 *abcdfgh</td>
<td></td>
</tr>
<tr>
<td>LFH1</td>
<td>80.65 ± 4.64 *abcde</td>
<td>84.05 ± 3.85 *abcdef</td>
<td>82.65 ± 3.91 *abcdfgh</td>
<td>87.70 ± 2.83 *abcdfgh</td>
<td>89.75 ± 3.20 *abcdfgh</td>
<td>90.15 ± 3.01 *abcdfgh</td>
<td></td>
</tr>
<tr>
<td>LFH2</td>
<td>83.55 ± 4.22 *abcdef</td>
<td>86.15 ± 4.69 *abcdfgh</td>
<td>88.80 ± 2.61 *abcdfgh</td>
<td>89.70 ± 4.39 *abcdfgh</td>
<td>91.00 ± 2.32 *abcdfgh</td>
<td>92.90 ± 3.52 *abcdfgh</td>
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</tr>
<tr>
<td>LFH3</td>
<td>79.75 ± 5.21 *abcde</td>
<td>79.65 ± 5.91 *abcde</td>
<td>83.50 ± 3.07 *abcde</td>
<td>83.60 ± 4.52 *abcde</td>
<td>85.05 ± 3.52 *abcde</td>
<td>88.05 ± 5.61 *abcde</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD; n=20

* Different letters following means denote significant differences (p<0.05).

**References**


