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Nutritional, chemical, syneresis, sensory properties, and shelf life of Iranian traditional yoghurts during storage

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2 **and shelf life of Iranian traditional yoghurts during**  
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24

25        **Abstract**

26        Tuluq and Torba yoghurts are traditional concentrates from Iran. Physicochemical,  
27        nutritional, and sensory properties of these yoghurts were studied along 60 days of storage.  
28        Results showed that, both pH and percentage of free whey decreased significantly ( $P < 0.05$ ),  
29        while titratable acidity, total solid, salt, protein and fat content increased ( $P < 0.05$ ) during  
30        storage. The yoghurt lipolysis decreased during the first 30 days and then increased during  
31        the storage. The indexes pH 4.6-soluble nitrogen/total nitrogen and non-protein nitrogen/total  
32        nitrogen in yoghurt samples decreased during first 30 days, possibly due to removing of low  
33        molecular weight nitrogenous compounds of Tuluq and Torba bags at late storage and then  
34        increased. Considerable  $\alpha_{s1}$ - and  $\beta$ -casein degradation occurred in Tuluq yoghurt. This might  
35        be due to endogenous surface bacteria and yeasts activities on Tuluq bag. It was concluded  
36        that Tuluq yoghurt had long shelf-life and high quality, being a valuable dairy product.

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38        **Keywords:** sensory properties, lipolysis, proteolysis; electrophoresis;  $\beta$ -casein

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## 42 **1. Introduction**

43 Fermentation is one of the oldest methods used to extend milk shelf-life by converting  
44 it into yoghurt. However, the short shelf-life of commercial yoghurts is still a major issue in  
45 milk processing. Different techniques have been reported to increase the shelf-life and  
46 improve quality of yoghurts over storage (Tamime & Robinson, 2000).

47 In the Middle East and Balkan regions, several concentrated yoghurts are traditionally  
48 produced: “Tuluq yoghurt” in Iran, “Torba yoghurt” in Turkey and “Labneh” in Arabian  
49 countries (Al-Kadamany, Khattar, Haddad, & Toufeili, 2003; Özer, 2006). Tuluq yoghurt has  
50 a long shelf-life (2 months) with desired organoleptic properties probably due to its lowered  
51 moisture content and the nature of its storage bag. This natural storage bag gave the product  
52 name “Tuluq”, as this term means sheepskin and goatskin bags, that are used for traditional  
53 concentrated yoghurt packaging and cheese ripening, respectively.

54 Tuluq and Torba yoghurts can be made from sheep’s, goat’s and cow’s milk and their  
55 processing is unique. The yoghurt whey is adsorbed within sheepskin and goatskin bags  
56 (Tuluq bag) or cloth bag (Torba bag) during production and storage at 4 °C (Tamime &  
57 Robinson, 2007). Therefore, as the whey seeped through the Tuluq bag and its evaporation  
58 occurs, the total solid levels and yoghurt acidity increase. The bag-retained yoghurt becomes  
59 concentrated and water activity is lowered to 0.7, and as a result shelf-life is extended.

60 Tuluq yoghurt is characterized by an acidic flavour, creamy colour and smooth texture,  
61 with a desirable taste crossing between sour cream and Lighvan cheese (a traditional Iranian  
62 brined curd cheese from sheep’s milk). Different methods have been used to produce  
63 concentrated yoghurt, including ultrafiltration, reverse osmosis and centrifugation (Özer,  
64 2006). However, the industrial application of these techniques for the manufacture of  
65 concentrated yoghurt is rather limited due to the transfer of whey proteins and minerals in  
66 ultrafiltration to permeate and high processing cost (Özer, 2006), while Tuluq and Torba

67 yoghurts are traditionally produced and can be commercialized as a typical product in  
68 countries where they are not produced.

69 Several aspects are considered to define the quality of yoghurts, such as total solid  
70 content and drainage temperature. It is reported that lowering drainage temperature (between  
71 2 and 10 °C) results in a higher production yield (Hamad & Al-Sheik, 1989). In Turkey and  
72 Arabic countries, the concentrated yoghurt is packaged into plastic containers, but in Iran the  
73 Tuluq yoghurt is kept in Tuluq bags during storage.

74 Lipolysis and proteolysis are major biochemical events with high beneficial impact on  
75 physicochemical and sensory attributes of traditional yoghurt and cheese. Therefore, lipolysis  
76 and proteolysis lead to precursor formation of a whole range of flavour and odour compounds  
77 in traditional yoghurt and cheese (Hernandez et al., 2009). Physicochemical and sensory  
78 properties of cloth bag concentrated yoghurts from Lebanon have been studied (Al-  
79 Kadamany et al., 2002, 2003).

80 The application of appropriate methods to manufacture traditional concentrated yoghurt  
81 is essential for higher acceptability with good physicochemical, sensory and nutritional  
82 characteristics. The purpose of the present study was to evaluate the changes in  
83 physicochemical parameters, lipolysis, proteolysis and sensory attributes of Tuluq and Torba  
84 yoghurts during storage.

## 85 **2. Materials and methods**

### 86 *2.1. Preparation of bags*

87 Tuluq and Torba bags were prepared from sheepskin and cotton cloths, respectively.  
88 Firstly, to reduce post contamination and the animal flavour, the Tuluq bags were filed with  
89 yoghurt, salt (1 g/100 g), mint (0.1 g/100 g), tarragon (0.1 g/100 g) and thyme (0.1 g/100 g)  
90 for 24 h. After overnight storage, the bags were thoroughly washed with water. Torba bags

91 were also washed before use. Fig. 1 shows the Tuluq bag before and after production of  
92 Tuluq yoghurt.

### 93 2.2. Production of concentrated yoghurts

94 Concentrated yoghurts were made from cows' milk by a traditional procedure similar to  
95 that reported by Robinson and Tamime (1994), with modifications as described below.  
96 Briefly, the fresh milk was obtained from the Animal Science Research Center, University of  
97 Tabriz, Iran. Milk was pasteurized by heating up to 90 °C for 10 min, then cooled to 45 °C,  
98 and inoculated with 3% starter culture, 1-day old yoghurt (*Streptococcus thermophilus* and  
99 *Lactobacillus delbrueckii subsp. Bulgaricus*) in equal proportions. The milk was maintained  
100 3.5 h at 43 °C ± 0.1 until the pH reached 4.7. Resulting yoghurt was cooled to 4 °C and  
101 mixed with 1.2 g/100 g salt. Concentrated yoghurts were made by whey removal from  
102 yoghurt inside Tuluq or Torba bags for 37 h at 4 °C. After this concentration period, yoghurt  
103 samples were kept at 4 °C for 60 days in Tuluq and Torba bags and called Tuluq or Torba  
104 yoghurts, respectively. Physicochemical properties and lipolysis of yoghurt samples were  
105 analysed every 10 days, proteolysis and sensory properties were assayed every 30 days.

### 106 2.3. Physicochemical properties

107 Total solids, protein, fat, ash and salt content of concentrated yoghurts were determined  
108 according to Marshall (2005). Total nitrogen was determined by micro-Kjeldahl procedure  
109 using a Kjeldahl apparatus (model: Tecator, Foss, Germany), and the crude protein content  
110 determined by multiplying the total nitrogen content by the conversion factor of 6.38. The pH  
111 values were determined using a pH-meter model Kent Hanna (USA). Titratable acidity (g  
112 lactic acid/ 100 g) was determined by titrimetric methods (Marshall, 2005).

113 Syneresis degree, expressed as proportion of free whey, was measured according to the  
114 method used by Al-Kadamany et al. (2003). A 20 g sample of control and concentrated  
115 yoghurts were layered on a 10 cm diameter Whatman (#2) filter paper that was fitted into a

116 Buchner funnel, and vacuum filtered for 10 min. Syneresis, expressed as free whey  
117 percentage, and calculated as follows:

$$118 \quad \% \text{ Free whey} = \frac{m_{\text{yoghurt initial}} - m_{\text{yoghurt after filtration}}}{m_{\text{yoghurt initial}}} \times 100$$

### 119 *2.3. Determination of lipolysis degree*

120 Lipolysis degree of yoghurt samples was determined using ethanolic titration according  
121 to method reported by Nuñez, Garcia-Aser, Rorríguez-Martin, Medina, & Gaya (1986).  
122 Briefly, 10 g of samples were macerated with 6 g anhydrous Na<sub>2</sub>SO<sub>4</sub> in a mortar and  
123 transferred with 60 mL diethyl ether to a 100 mL screw-capped bottle. The homogenate was  
124 stirred for 1 h, with ultrasonification for 30 s at 15 min intervals, decanted and the supernatant  
125 filtered through Whatman No. 1 paper. The precipitate in the bottle was resuspended in three  
126 successive 20 mL portions of diethyl ether, decanted and filtered. The total solvent was  
127 titrated with 0.1N ethanolic KOH solution. After titration the solvent was evaporated to  
128 dryness and fat was weighed. Free fatty acids (FFA) in yogurt were expressed as meq/100 g  
129 fat.

### 130 *2.4. Determination of pH 4.6-soluble nitrogen (SN) and non-protein nitrogen (NPN)* 131 *fractions*

132 The pH 4.6-soluble nitrogen (SN) and non-protein nitrogen (NPN) fractions of the  
133 yoghurt samples were quantified by the procedure of Kuchroo and Fox (1982). In addition,  
134 the SN/TN (total nitrogen) and NPN/TN were also calculated.

### 135 *2.5. Electrophoresis analysis*

136 Casein fractions degradation was studied using PAGE following the method of  
137 Andrews (1983). Casein samples were prepared as described by Kaminarides and Koukiassa  
138 (2002) and staining was carried out by the method of Shalabi and Fox (1987).

### 139 *2.6. Sensory properties*

140 The effect of storage time on the sensory properties of concentrated yoghurts was  
141 determined by twelve experienced panellists (eight females, four males; age 20-30 year) who  
142 were familiar with the Tuluq and Torba yoghurts. On the descriptive scale, intensity of  
143 flavour, texture and appearance attributes were determined on a 5-point scale where '5'  
144 corresponded to 'very strong' and '0' corresponded to 'none'. On a 9-point hedonic scale for  
145 overall flavour acceptability, '9' corresponded to 'excellent' and '1' corresponded to  
146 'unacceptable'. Score coefficient for all attributes was '2', but animal-like/ foreign attribute  
147 had '4' score coefficient. The overall acceptability was obtained as the sum of the scores of  
148 the acceptable attributes (surface brightness, surface smoothness, firmness, mouth-feel, and  
149 overall flavour) judged. Sensory assessments were clearly defined to the panellists according  
150 to Bodyfelt, Tobias, and Trout (1988). All assessments were determined in duplicate, in  
151 individual cabinets equipped with daylight.

## 152 2.7. Statistical analyses

153 Data were subjected to an analysis of variance according to a repeated measures  
154 experimental design with the MIXED procedure of the statistical analysis software. Least  
155 square means was used to determine the groups significantly different from each other. A  
156  $P < 0.05$  was considered to indicate statistical significance. All data were determined in  
157 triplicate and reported as means  $\pm$  standard errors.

## 158 3. Results and discussion

### 159 3.1. Physicochemical properties

160 Changes in composition are shown in Fig. 2. The results revealed that both treatments,  
161 storage and their interactions had significant ( $P < 0.01$ ) effects on pH and titratable acidity  
162 values in yoghurt samples during 60 days of storage. The pH of Tuluq yoghurt significantly  
163 decreased over the 60 days of the storage period (from 4.26 to 4.13,  $P < 0.05$ ), while the  
164 Torba yoghurt revealed a decrease during the first 30 days of storage (from 4.16 to 4.09),



165 followed by an increase (from 4.09 to 4.56) (Fig. 2a). A similar trend was reported by Yildiz-  
166 Akgül (2018) who observed that pH values slightly decreased during storage time of Torba  
167 yoghurt from 3.66 to 3.39 after 14 days of storage. In addition, Moschopoulou et al. (2018)  
168 noticed that the greatest changes in pH and acidity took place within the first week of storage  
169 and resulted from residual lactose fermentation. This behaviour might be due to whey  
170 drainage during Torba yoghurt production and storage, withdrawing chemical compounds  
171 from the yoghurt, including acidic ones. However, an increase in the count of starter culture  
172 and psychrotrophic bacteria has led to an increase in proteolysis and production of released  
173 amines, which can increase the pH of the yoghurt samples. There was a corresponding  
174 increase in titratable acidity values of yoghurts, which are indicative of acid-producing  
175 microorganisms (Fig. 2b).

176 These pH and titratable acidity values are in accordance with previously published data  
177 (Al-Kadamany et al., 2002; Al-Kadamany et al., 2003; Güler, 2007; Şenel, Atamer, Gürsoy,  
178 & Öztekin, 2011) (Fig. 2a and b). Özer (2006) verified that the count of viable lactic acid  
179 bacteria cells numbers in concentrated yoghurt was on average higher than that of plain  
180 yoghurt. Therefore, the high population of lactic acid bacteria present in concentrated  
181 yoghurts can lead to a high acid production, which may explain the increase in titratable  
182 acidity during storage (Fig. 2b).

183 On the other hand, the treatment, the storage time and their interactions had significant  
184 effects on total solid and salt content of Tuluq and Torba yoghurts ( $P < 0.01$ ). In Tuluq and  
185 Torba yoghurts, an increase in the total solid (from 17.23 to 35.67 g/100 g and from 16.98 to  
186 37.47 g/100 g, respectively) and in salt content (from 0.29 to 0.51 g/100 g and from 0.25 to  
187 0.57 g/100 g, respectively) occurred due to drainage of free whey during its production and  
188 storage period (Fig. 2c and d), respectively. In addition, Tamime and Robinson (2007)

189 reported that the high salt content of concentrated yoghurt improves the shelf-life of the  
190 product.

191 Fig. 3 shows protein and fat content, syneresis and lipolysis variations measured in  
192 yoghurt samples during its storage. Results showed that the treatment, the storage time and  
193 their interactions had significant effects on protein and fat content and lipolysis of Tuluq and  
194 Torba yoghurts ( $P < 0.01$ ). The protein and fat content increased steeply in Tuluq and Torba  
195 yoghurts, from about 5-6 g/100 g to 11-12 g/100 g and from about 6-7 g/100 g to 17-18 g/100  
196 g, for protein and fat, respectively, possibly due to free whey separation occurred during the  
197 storage period (Fig. 3a and b). From nutritional point of view, Tuluq and Torba yoghurts are  
198 products rich in protein and fat content and have a better digestibility compared to original  
199 milk.

200 The syneresis of Tuluq (from 33.2 g/100 g to 18.6 g/100 g) and Torba (from 33.2 g/100  
201 g to 18.1 g/100 g) yoghurts gradually decreased during the storage period (Fig. 3c). This  
202 finding is in agreement with data reported by Yildiz-Akgül (2018) who observed that  
203 syneresis slightly decreased during storage time of Torba yoghurt from 2.13 mL to 1.74 mL,  
204  $P > 0.05$ , after 14 days of storage. The syneresis in set yoghurts has been linked with particles  
205 rearrangements of making up the casein gel network during incubation and storage period  
206 (Lucey, 2002). However, at the present study syneresis has been also directly related to the  
207 percentage of total solid in concentrated yoghurts and the increase in the total solid reduced  
208 the syneresis during storage. Tamime and Robinson (2000) reported that buffalo's milk  
209 yoghurt containing 20% total solid had a better texture, mouthfeel and a reduced syneresis  
210 than milk yoghurt with less total amount of solids.

211 The lipolysis degree of Tuluq and Torba yoghurts decreased from 0.39 to 0.30 meq/100  
212 g and from 0.40 to 0.26 meq/100 g fat during the first 30 days of storage, respectively. This  
213 behaviour might be due to separation of short-chain free fatty acids during drainage. These

214 outcomes are in agreement with data reported by Şenel et al. (2011) who found a sharp  
215 decrease was observed in the levels of individual free fatty acid (FFAs) in the strained  
216 yoghurt on the 15th day of storage. The decrease in the level of FFA may be associated with  
217 catabolism of FFA by microorganisms (Şenel et al., 2011). Then, Tuluq and Torba yoghurts  
218 lipolysis increased from 0.30 meq/100 g to 0.40 meq/100 g and from 0.26 meq/100 g to 0.45  
219 meq/100 g fat due to the action of starter and non-starter bacterial lipases on yoghurt fat  
220 during the last 30 days storage, respectively (Fig. 3d). A similar trend was reported by Şenel  
221 et al. (2011) who observed that after 15th day of storage, the levels of FFAs remained almost  
222 unchanged or increased slightly. On the contrary, Yildiz-Akgül (2018) noticed that the  
223 content of most of the FFAs on the last day of storage was higher than that of FFAs on the  
224 first day of storage. According to Kesenkas (2010), these differences may be attributed to the  
225 catabolism of FFA by yeast and mould contaminants. Lipolysis is agreed to be one of the  
226 primary biochemical events significantly affecting the shelf-life of many dairy products  
227 (Şenel et al., 2011). In addition, it is also an important phenomenon in determining the  
228 characteristic aroma and flavour of dairy products

### 229 3.2. Proteolysis

230 The levels of classical nitrogen fractions in yoghurts during 60 days storage are shown  
231 in Table 1. The results showed that treatments, storage and their interactions had significant  
232 effects on TN and SN/TN ratio of Tuluq and Torba yoghurts ( $P < 0.01$ ), but there is no  
233 significant differences ( $P > 0.05$ ) between treatments on NPN/TN ratio. The TN of Tuluq and  
234 Torba yoghurts significantly increased ( $P < 0.05$ ) due to whey remotion from Tuluq and  
235 Torba bags (Table 1). This result is in disagreement with data found by Moschopoulou et al.  
236 (2018) who did not observe significant differences on TN between 1 and 28 days of storage  
237 in sheep, cow and goat milk yoghurt.

238 In dairy products, the determination of the level of SN/TN indicate the index of primary  
239 proteolysis. In Tuluq and Torba yoghurts, the SN/TN decreased from 14.1% to 7.9% and  
240 from 10.4% to 6.2% during first 30 days, respectively. This behaviour might be due to  
241 separation of low molecular weight nitrogenous compounds of Tuluq and Torba bags. This  
242 finding is in disagreement with data reported by El-Zahar, Chobert, Dalgalarrrondo, Sitohy,  
243 and Haertlé (2004) who observed that during the storage of yogurt up to 14 days, the amount  
244 of free amino groups increased with the increase of storage time up to maximal value after 7  
245 days. In addition, Hrnjez et al. (2014) showed an increase in proteolysis ranged from 12% to  
246 18% during 14 days of storage of cow milk yoghurt. Moreover, Sah, Vasiljevic, McKechnie,  
247 and Donkor (2015) also found an increase in proteolysis of various cow milk yoghurts within  
248 28 days. Finally, Politis and Theodorou (2016) reported that the water soluble nitrogen of  
249 commercial sheep and cow milk yoghurts increased by 50% within 18 days. On the contrary,  
250 Donkor, Henriksson, Singh, Vasiljevic, and Shah (2007) noticed that although free amino  
251 groups increased substantially during the first 24 h of yoghurt life, the increase from day one  
252 to day 30 was very limited. In this regards, Moschopoulou et al. (2018) also did not observe  
253 statistically significant proteolysis during 28 days of storage of sheep, cow and goat milk  
254 yoghurt. Then, the SN/TN ratio increased from 7.9% to 15.4% and from 6.2% to 14.2%  
255 during storage, for Tuluq and Torba yoghurts, respectively (Table 1). The results also showed  
256 that Tuluq yoghurt had higher SN/TN ratio than Torba yoghurt at 1 and 60 days. El-Zahar et  
257 al. (2004) reported similar SN values in fresh yoghurt and attributed them to lactic acid  
258 bacteria activity that led to an increase of soluble nitrogenous compounds during storage. The  
259 SN/TN ratio only gives an idea about proteolysis extension, but not on the composition of the  
260 soluble nitrogen. It would therefore, be possible that proteolysis in the yoghurts resulted in  
261 various breakdown products, although the total content of these were about the same (Wit,  
262 Osthoff, Viljon, & Hugo, 2005).

263 The secondary proteolysis can be evaluated through the index NPN/TN during cheese  
264 and yoghurt storage (Hesari, Ehsani, Khosroshahi, & McSweeney, 2006). The NPN/TN ratio  
265 of Tuluq and Torba yoghurts significantly ( $P < 0.05$ ) decreased during first 30 days of storage  
266 (from 10.8% to 7.9% and from 8.1% to 4.5%, for Tuluq and Torba yoghurts, respectively)  
267 and followed by a significant ( $P < 0.05$ ) increase till 15.38% and 14.19%, for Tuluq and  
268 Torba yoghurts, respectively) (Table 1). According to reports of El-Zahar et al. (2004) in  
269 probiotic yoghurt and Hesari et al. (2006) in Lighvan cheese, there are complex proteolytic  
270 and peptidolytic systems of microorganisms, both starter and nonstarter that are responsible  
271 for secondary proteolysis during storage time. Our results showed that Tuluq yoghurt had the  
272 highest degree of proteolysis during storage time, probably due to Tuluq bag endogenous  
273 surface bacteria, yeasts and enzymes, responsible for this effect.

274 Urea-PAGE electrophoretograms of the pH 4.6-insoluble fraction of yoghurts of Trial 1  
275 after 30 and 60 days of storage are shown in Fig. 4. In yoghurts, starter cultures hydrolysis  
276  $\alpha_{s1}$ - and  $\beta$ -casein as primary proteolysis, while  $\gamma$ -casein is accumulates. Electrophoretic  
277 pattern showed that proteins hydrolysis increased and were found to match the SN/TN ratio.  
278 The degradation of  $\alpha_{s1}$ - and  $\beta$ -casein in concentrated yoghurts (Fig. 4) was clearly indicated  
279 by the decrease in the bands intensity with the subsequent formation of the degradation  
280 products. This outcome is in agreement with data reported by El-Zahar et al. (2004) who  
281 observed that all proteins were gradually degraded during the cold storage of the yogurts,  
282 being the  $\alpha$ -lactalbumin was more hydrolyzed than  $\beta$ -lactoglobulin during yogurt storage. At  
283 the end of storage period (14 days), the relative quantity of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin  
284 was reduced by about 23-31% and 20-29%, respectively (El-Zahar et al., 2004).

285 There were notable differences in electrophoretic patterns among the two concentrated  
286 yoghurt types. In Torba yoghurt, degradation of  $\beta$ -casein was negligible, while  $\alpha_{s1}$ -casein was  
287 considerably hydrolysed. Our results agree with those noticed by El-Zahar et al. (2004) who

288 observed that  $\alpha_{s1}$ -caseins were much more degraded during the storage period than  $\beta$ -casein.  
289 On the contrary, in Tuluq yoghurt, considerable  $\beta$ -casein degradation occurred, which may be  
290 due to endogenous surface bacteria and yeasts activities of Tuluq bag (Fig. 4). In this regard,  
291 El-Zahar et al. (2004) observed that  $\beta$ - and  $\alpha_{s1}$ -casein were reduced by about 18-23% and 18-  
292 25%, respectively, after 14 days of storage. According to Alichanidis, Anifantakis,  
293 Polychroniadou, and Nanou (1984), the high NaCl concentration and low pH of Feta cheese  
294 reduced the degradation of  $\beta$ -casein during storage. In addition, higher pre-treatment can  
295 make both  $\beta$ - and  $\alpha_{s1}$ -caseins more susceptible to proteolytic degradation due to expected heat  
296 induced denaturation (El-Zahar et al., 2004). This proteolysis increase can also reveal an  
297 increase the starter culture and psychrotrophic bacteria counts, which concur with the  
298 previously published data (Slocum, Jasinski, Anantheswaran, & Kilara, 1988).

#### 299 *4.3. Sensory properties*

300 Results of sensory evaluations of concentrated yoghurts are shown in Table 2.  
301 Modification of the sensory properties in Labneh from different milk (Rao et al., 1987),  
302 Labneh by some protein based fat replacers (Yazici & Akgun, 2004), concentrated yoghurt  
303 by a batch evaporator (Yeganehzad, Mazaheri Tehrani, & Shahidi, 2007), salted yoghurt  
304 (Güler, 2007) and Labneh by adding herbs (Tarakci, Temiz, & Ugur, 2010) has been reported  
305 by many researchers.

306 Often if the food appearance is unattractive, a potential consumer may never experience  
307 other sensory properties such as flavour and texture (Tarakci et al., 2010). In our study,  
308 overall flavour significantly ( $P < 0.05$ ) increased during the storage time from 7.34 to 8.14 in  
309 Tuluq yoghurt, whereas no significant differences were observed in overall flavour during the  
310 whole period in Torba yoghurt. This outcome is in disagreement with data reported by Şenel  
311 et al. (2011) who observed that aroma and flavour scores decreased during the storage time,  
312 especially after 15 day of storage. In addition, Hanif, Zahoor, Iqbal, and Ihsan-ul-Haq (2012)

313 also showed that mean flavour score of cow and buffalo milk yogurt decreased during storage  
314 time. According to Abrahamsen (1978), the decrease in flavor is correlated with the  
315 proteolytic activity of bacteria and the production of higher acidity. In addition, the loss of  
316 flavuor is attributed to fat and protein degradation (Mottar, Waes, Moersmans, & Naudts,  
317 1979) and development of slight sharp flavor produced by coliform bacteria, *clostridium*  
318 spp. and other microorganisms. Surface brightness, smoothness and mouth-feel attributes  
319 decreased in both yoghurt samples during the storage period. This outcome is in agreement  
320 with data reported by Hanif et al. (2012) who found that the mean scores for appearance  
321 decreased gradually during storage. The mean score for appearance decreased from 11.33 to  
322 5.66 in commercial yogurt, from 10 to 3.66 in cow milk yogurt and from 11.66 to 4.00 in  
323 buffalo milk yogurt after 15 days storage. A satisfactory yoghurt mouth-feel can be attained  
324 through the incorporation of high levels of total solid, fat, protein and flavour attributes  
325 (Özer, 2006).

326 The level of firmness in Tuluq and Torba yoghurts increased during the storage (Table  
327 2), which can be linked to the whey drainage from Tuluq and Torba yoghurts, leading to an  
328 increase of total solid of samples during production and storage period. Texture acceptability  
329 increased with increasing total solids significantly (Mahdian & Tehrani, 2007) because higher  
330 total solids increases gel firmness and reduce the degree of syneresis (Mohammeed, Abu-  
331 Jdayil, & Al-Shawabkeh, 2004). Our results are in disagreement with the findings of  
332 (Tarakci, & Kucukoner, 2003; Salwa, Galal, & Neimat, 2004; Hanif et al., 2012) who  
333 reported a decrease in score of body and texture of yogurt during storage.

334 The level of animal like/foreign, acid/sour, rancidity and yeasty/musty flavours  
335 gradually increased during the storage period (Table 2). In this regard, Salji, Sawaya, and  
336 Ayaz (1987) and Muir and Banks (2000) reported that the presence of lactic acid bacteria and  
337 post contamination microorganisms such as yeasts, moulds and psychrotrophic bacteria

338 coupled with undesirable packaging/storage conditions results in the development of off-  
339 flavours and other unacceptable physicochemical and organoleptic changes that eventually  
340 yoghurt becomes inconsumable.

341 Our results revealed that storage ( $P < 0.01$ ) and treatments ( $P < 0.05$ ) had significant  
342 effects on overall acceptability of Tuluq and Torba yoghurts. However, the overall  
343 acceptability was not affected by the interaction (storage time  $\times$  treatments). Overall  
344 acceptability of yoghurts was negatively correlated with surface brightness, smoothness,  
345 firmness, mouth-feel and overall flavour attributes and positively correlated with animal  
346 like/foreign, acid/sour, rancidity and yeasty/musty flavours. Tuluq yoghurt showed the higher  
347 overall acceptability than Torba yoghurt after 30 and 60 days of storage (Table 2). It can be  
348 addressed to high level of total solid, fat, protein and overall acceptable flavour due to  
349 desirable physicochemical characterization, considerable proteolysis pattern and organoleptic  
350 properties during the storage.

#### 351 **4. Conclusion**

352 Results showed that application of different methods to manufacture of traditional  
353 concentrated yoghurt can be effective on physicochemical, lipolysis, proteolysis, organoleptic  
354 attributes and quality during storage. Physicochemical properties, proteolysis pattern and  
355 sensory scores of the Tuluq yoghurt were better than those from Torba yoghurt during the  
356 storage. It may be due to endogenous surface bacteria, yeasts and enzymes of Tuluq bag. The  
357 obtained results can be a considerable step to identify of new lactic acid bacteria strains in  
358 Tuluq bag. This study introduces Tuluq yoghurt as a valuable dairy product for its beneficial  
359 effects and unique flavour.

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485

486 **Caption to figures**

487 **Figure 1.** Tuluq bag 30 × 50 (A) before and (B) after Tuluq yoghurt production

488 **Figure 2.** Variation of (a) pH-values, (b) titratable acidity, (c) total solid and (d) salt  
489 content of Tuluq (■) and Torba (●) yoghurts during the storage period of 60 days under  
490 refrigeration. Data are means of triplicate determinations. Maximum standard errors of means  
491 were 0.058, 0.081, 1.965, and 0.064 for pH, titratable acidity, total solid and salt content,  
492 respectively. Error bars indicates standard error of triplicate measurements

493 **Figure 3.** Changes in (a) protein content, (b) fat content, (c) syneresis and (d) lipolysis  
494 content of Tuluq (■) and Torba (●) yoghurts during the storage period of 60 days under  
495 refrigeration. Protein, fat, syneresis, and lipolysis values are means of triplicate  
496 determinations. Maximum standard errors of means were 0.482, 1.76, 1.68, and 0.029 for  
497 protein, fat, syneresis and lipolysis content, respectively. Error bars indicates standard error  
498 of triplicate measurements.

499 **Figure 4.** Urea polyacrylamide gel electrophoretograms of (A) Tuluq and (B) Torba  
500 yoghurts after 1 (Lane 1), 30 (Lane 2) and 60 (Lane 3) days of storage period

501

**Table 1.** Classical nitrogen fractions of yoghurts during 60 days storage period

Properties <sup>A</sup>	Type of Yoghurt	Storage Time		
		1 Day	30 Day	60 Day
TN (g/100 g)	Tuluq	0.74±0.012 <sup>Bc</sup>	1.32±0.039 <sup>Bb</sup>	1.80±0.075 <sup>Ba</sup>
	Torba	0.91±0.013 <sup>Ac</sup>	1.58±0.033 <sup>Ab</sup>	1.96±0.039 <sup>Aa</sup>
SN/TN (%)	Tuluq	14.11±0.107 <sup>Ab</sup>	7.94±0.102 <sup>Ac</sup>	15.38±0.244 <sup>Aa</sup>
	Torba	10.41±0.109 <sup>Bb</sup>	6.23±0.061 <sup>Bc</sup>	14.19±0.103 <sup>Ba</sup>
NPN/TN (%)	Tuluq	10.83±0.101 <sup>Ab</sup>	7.93±0.084 <sup>Ac</sup>	12.26±0.174 <sup>Aa</sup>
	Torba	8.09±0.075 <sup>Bb</sup>	4.55±0.038 <sup>Bc</sup>	12.66±0.074 <sup>Aa</sup>

<sup>A</sup> Mean of three determinations ± standard error

<sup>a-c</sup> Nitrogen fractions level within each row during storage with different letters differ significantly ( $P<0.05$ )

<sup>A-B</sup> Nitrogen fractions level within each column with different letters differ significantly ( $P<0.05$ )

**Table 2.** Grading scores for sensory attributes of concentrated yoghurts during the storage period†

Sample	Days	Sensory attributes									
		Surface brightness	Surface smoothness	Firmness	Mouth-feel	Animal like/Foreign flavour	Acid/Sour flavour	Rancidity flavour	Yeasty/Musty flavour	Overall flavour	Overall acceptability
Tuluq Yoghurt	1	8.71±0.49 <sup>a</sup>	8.28±0.41 <sup>a</sup>	8.01±0.33 <sup>b</sup>	9.14±0.40 <sup>a</sup>	15.42±1.23 <sup>a</sup>	8.28±0.28 <sup>a</sup>	8.28±0.28 <sup>a</sup>	8.14±0.25 <sup>ab</sup>	7.34±0.14 <sup>b</sup>	41.48±1.21 <sup>a</sup>
	30	8.00±0.49 <sup>a</sup>	8.86±0.27 <sup>a</sup>	8.61±0.27 <sup>a</sup>	9.14±0.27 <sup>a</sup>	18.85±0.82 <sup>a</sup>	8.57±0.33 <sup>a</sup>	9.28±0.26 <sup>a</sup>	9.71±0.19 <sup>a</sup>	9.42±0.12 <sup>a</sup>	44.03±0.73 <sup>a</sup>
	60	7.86±0.41 <sup>a</sup>	8.14±0.33 <sup>a</sup>	9.95±0.25 <sup>a</sup>	9.00±0.28 <sup>a</sup>	18.85±0.63 <sup>a</sup>	9.28±0.26 <sup>a</sup>	9.57±0.23 <sup>a</sup>	9.28±0.34 <sup>b</sup>	8.14±0.24 <sup>ab</sup>	43.09±0.46 <sup>a</sup>
Torba Yoghurt	1	8.00±0.36 <sup>a</sup>	8.00±0.42 <sup>a</sup>	8.01±0.28 <sup>b</sup>	8.86±0.50 <sup>a</sup>	16.85±1.30 <sup>a</sup>	7.43±0.25 <sup>b</sup>	7.00±0.28 <sup>b</sup>	7.00±0.35 <sup>b</sup>	7.14±0.17 <sup>a</sup>	40.01±0.76 <sup>a</sup>
	30	7.71±0.28 <sup>a</sup>	8.28±0.28 <sup>a</sup>	8.76±0.23 <sup>a</sup>	8.86±0.34 <sup>a</sup>	17.71±1.01 <sup>a</sup>	8.00±0.36 <sup>a</sup>	8.71±0.34 <sup>b</sup>	9.14±0.27 <sup>b</sup>	7.48±0.23 <sup>a</sup>	41.09±0.38 <sup>a</sup>
	60	7.14±0.27 <sup>a</sup>	7.14±0.27 <sup>a</sup>	10.00±0.23 <sup>a</sup>	8.71±0.27 <sup>a</sup>	18.57±0.66 <sup>a</sup>	7.67±0.82 <sup>b</sup>	9.28±0.39 <sup>a</sup>	9.43±0.25 <sup>a</sup>	7.14±0.23 <sup>a</sup>	40.13±0.51 <sup>a</sup>
Significance		NS	NS	*	NS	NS	*	*	*	*	NS

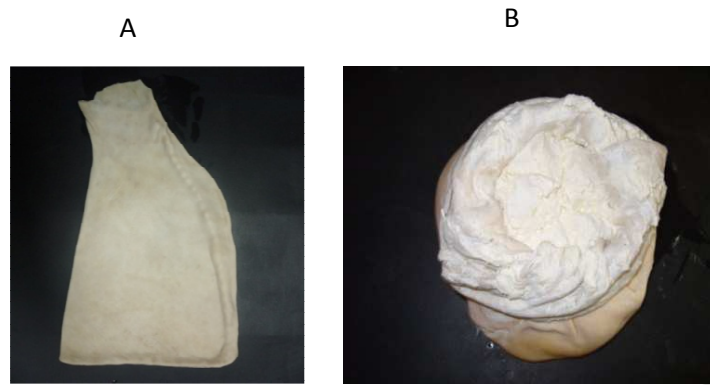
*P*, significant level; NS, non-significant

† Values are the mean of twenty-four determinations made by twelve individual assessors on yoghurts; Mean of determinations ± standard error

<sup>a,b</sup> Means with different superscript within columns for each yoghurt were significantly different from each other during storage period

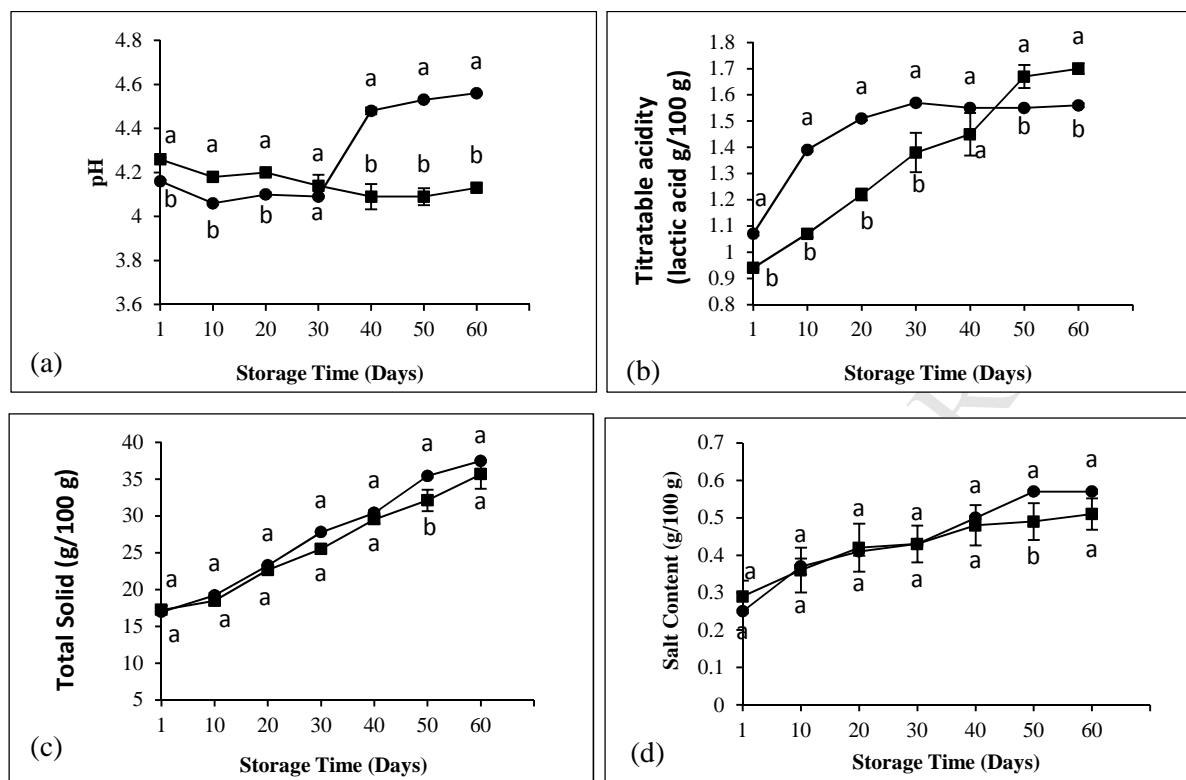
Significance: NS = not significant; \**P*<0.05



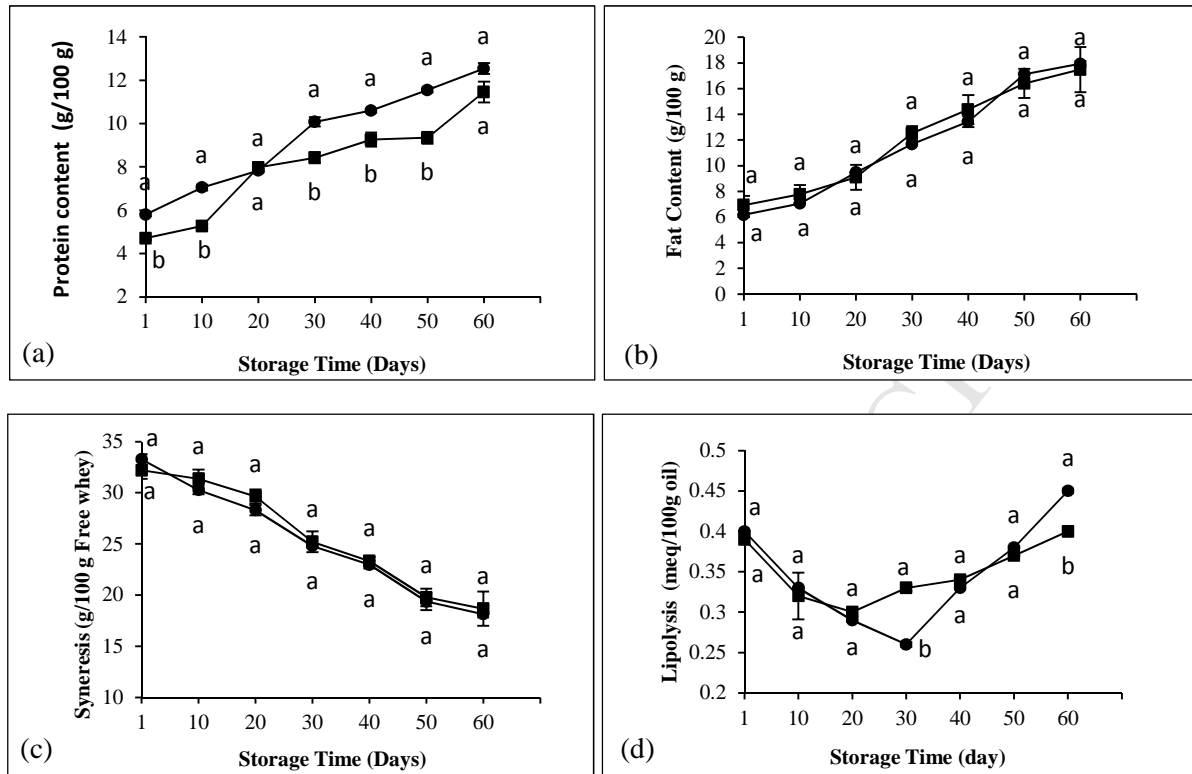


**Figure 1**

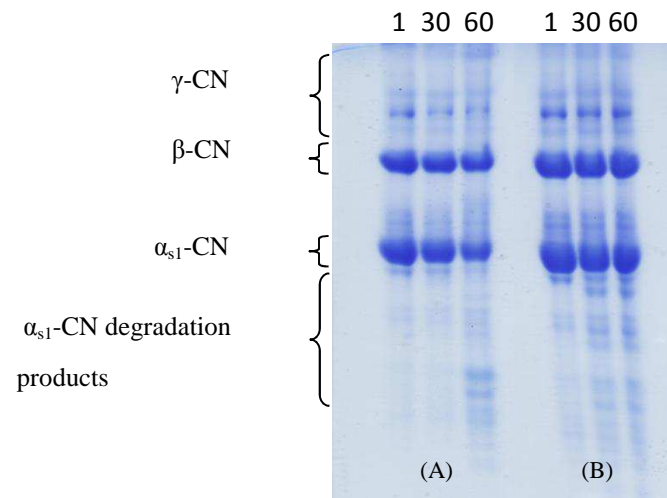
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**Figure 2**

<sup>a-b</sup> Values level between treatments with different letters differ significantly ( $P < 0.05$ )

**Figure 3**

<sup>a-b</sup> Values level between treatments with different letters differ significantly ( $P < 0.05$ )

**Figure 4**

**Highlights**

- ▶ Tuluq yoghurt had the highest degree of proteolysis during storage
- ▶ The yoghurt lipolysis decreased during the first 30 days and then increased

during the storage

- ▶ Considerable  $\alpha$ 1- and  $\beta$ -casein degradation occurred in Tuluq yoghurt

**Conflict of Interest**

Alirezalu, K. declares that she has no conflict of interest. Inácio, Rita S declares that she has no conflict of interest. Hesari, J. declares that she has no conflict of interest. Remize, F. declares that he has no conflict of interest. Saraiva, Jorge A. declares that he has no conflict of interest. Barba, Francisco J. declares that he has no conflict of interest. Sant'Ana, Anderson S. declares that he has no conflict of interest. Lorenzo, Jose M. declares that he has no conflict of interest.