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MILK JAM OR DULCE DE LECHE: PHYSICOCHEMICAL CHARACTERIZATION

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ABSTRACT
Physicochemical characterization of Dulce de Leche (DL) or milk jam, a type of sweetened concentrated milk especially popular in South America, was carried out on six commercial brands produced in Turkey. One of the samples was prepared using sheep milk while the others were purchased from local retailers. Solid content, protein, fat, ash, pH and lactic acid content were determined. Lightness, yellowness and redness as color parameters of the DL samples were evaluated. Carbohydrate profile and contents in DL samples were also determined by HPLC. The data were treated using Principal Component Analysis (PCA). Extensive variability among all the parameters evaluated was observed, as a result of using different DL production procedures in the dairies. In this regard, PCA was shown to be useful to separate the DL samples with distinct physicochemical characteristics and to assess the influence of different production techniques on the properties of DL samples.
Keywords: Dulce de Leche, physicochemical analysis, milk jam, principle component analysis.

SÜT REÇELİ VEYA DULCE DE LECHE: FİZİKOKİMYASAL KARAKTERİZASYONU

ÖZ
Özellikle Güney Amerika'da popüler olan şekerli konsantre sütün bir tipi Dulce de Leche (DL) veya süt reçelinin fizikokimyasal karakterizasyonu, Türkiye'de üretilen altı ticari ürün ile gerçekleştirilmiştir. Örneklerden biri koyun sütü kullanılarak hazırlanmış, diğerleri yerel perakendecilerden satın alınmıştır. Kuru madde, protein, yağ, kül, pH, laktik asit içeriği ve renk parametreleri belirlenmiştir. Renk parametreleri olarak "lightness", "yellowness" ve "redness" değerlendirilmiştir. DL örneklerindeki karbonhidrat profili ve içeriği ise HPLC ile belirlenmiştir. Veriler, Temel Bileşen Analizi (TBA) kullanılarak işlenmiştir. Değerlendirilen tüm parametreler arasında, işletmelerde farklı DL üretim prosedürlerinin kullanımının bir sonucu olarak, geniş aralıkta bir değişkenlik gözlenmiştir. Bu bağlamda, TBA'nın, DL örneklerinin özellikleri üzerine farklı üretim tekniklerinin etkisinin belirlenmesinde ve belirgin fizikokimyasal karakteristiklere sahip DL örneklerinin ayırt edilmesinde kullanılabileceği gösterilmiştir.
Anahtar kelimeler: Dulce de Leche, fizikokimyasal analiz, süt reçeli, temel bileşen analizi.

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INTRODUCTION

Dulce de Leche (DL) or milk jam or milk caramel is a sweetened concentrated milk product, which is popular in some South American countries. In recent years, DL products have started to be on the market, increasingly, with the name of "milk jam" in Turkey. Çanakkale is the one of the important cities in the country, in which is produced large amount of cow, sheep and goat milk, and dairy products. DL is consumed especially as a spread, as a dessert and at breakfast and can be used for confectionery and ice-cream manufacture in Turkey. In DL production, milk and sucrose mixture is concentrated by boiling at atmospheric pressure to a solids content of approximately 70 %. In some case, sucrose is partially replaced by glucose to avoid crystallization. Maillard browning is the main reaction between sugars and milk proteins during the production of DL (Malec et al., 1999). Sodium bicarbonate ($NaHCO_3$) is added during production in order to avoid casein coagulation and favor the Maillard reaction, responsible for its typical brown color and flavor.

DL is a complex biopolymeric matrix, and proteins and carbohydrates are the two main construction materials of the DL (Ranalli et al., 2016). Typical composition of DL is moisture (30 %, max), milk solids not fat (24 %, min), milk fat (6-9 %), protein (5 %, min) and ash (2 %, max) (Zalazar and Perotti, 2011). According to Resolution No 12/78 of the National Norms and Standards for Foods, moisture and sugar (excluding lactose) contents of Dulce de Leche must be 30 g / 100 g and 60 g / 100 g, respectively (Silva et al., 2015).

Although DL is composed of milk, sucrose, $NaHCO_3$ and other additives, it can exhibit distinct characteristics because of different processing parameters. Using different milk such as goat and / or sheep and / or their mixture in DL production have also direct influence on the physiochemical properties of the final product.

Traditional manufacturing in open kettles is the most widely used process in the industry (Figure 1). It's a classic batch process, and there is a general agreement that this process produces the best quality DL (Zalazar and Perotti, 2011).

One of the most important steps in production is heating or boiling step. During boiling, Maillard reaction occurs depending on the raw materials used, and the processing conditions such as pH, time and temperature; and the reaction provides a desirable color and a very pleasant flavor to the product.

Monitoring and evaluation of the physicochemical characteristics of the DL is important because these characteristics are strongly related to nutritional, technological and sensory quality of the product. However, there are few studies on the properties and composition of the DL in the literature (Ferreira et al., 2011; Gaze et al., 2015; Ranalli et al., 2012; Ranalli et al., 2016; Silva et al., 2015) and furthermore there are no studies on the DL produced in Turkey.

The objective of the present work was to characterize total six commercial DL brands, which one of them was produced in a certain dairy in Çanakkale and the others purchased from Çanakkale market. This study also proposes an approach to distinguish different DL samples using physicochemical analysis and principle component analysis (PCA).

MATERIALS and METHODS

Materials

Five of total six DL samples (1 – 5) were purchased in local retailers located in Çanakkale. 1 – 4 samples were cow milk DL and sample 5 was goat milk DL. Sample 6 was produced in a local dairy plant (Güntepe) by using sheep milk. Samples were transferred to the laboratory at room temperature. General composition and properties of the sheep milk used production of sample 6 were determined and presented in Table 1.

In preparation of sample 6, whole sheep milk, sucrose and sodium bicarbonate were used. According to traditional production method, the mixture was boiled during approximately 120 minutes, keeping a constant manual agitation. After boiling, sample was cooled to about 50^{o}C and packaged in glass containers (Figure 1).

Table 1. General composition of sheep milk used for DL (sample 6) production

Dry matter (g/100 g)	Fat (g/100g)	Protein (g/100g)	Lactic acid (g/100g)	pH
17.86 ± 0.23	7.35 ± 0.24	5.39 ± 0.17	0.165 ± 0.06	6.82 ± 0.09

* Values are means ± standard deviation.
Analyses were performed in triplicate.

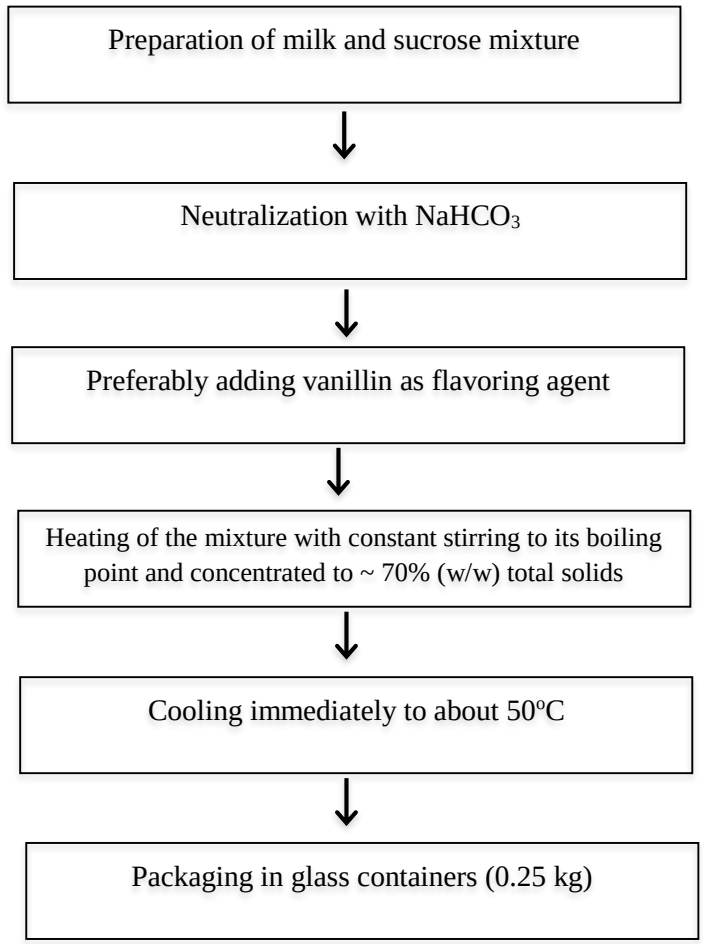

Figure 1. Traditional (batch) production of DL (in open kettles)

All DL samples is handmade, uses discontinuous processing.
Dry individual sugar standards (glucose, sucrose and lactose) were purchased from Sigma (Germany).

Physicochemical analyses
General composition (moisture, protein and ash) was determined according to the recommendations of the Association of Official Analytical Chemists (AOAC, 2005). Moisture was determined gravimetrically by oven drying until constant weight. The ash content was determined gravimetrically after incineration of 3 g sample at 550ºC in a muffle furnace. Protein content was calculated using the total nitrogen determined by Kjeldahl method with subsequent multiplication by the factor 6.38. The pH and the percentage of lactic acid were determined. The pH was measured in a pH meter (Sartorius, TE214S,

Germany) by direct insertion of the electrode in the sample. The lactic acid was measured by titration with sodium hydroxide using phenolphthalein as indicator.

Sugar analyses by HPLC

The analyses of sugars (lactose, sucrose and glucose) were performed by high performance liquid chromatography (HPLC) according to the Association of Official Analytical Chemists (AOAC, 2006). All standards (lactose, sucrose and glucose) were dissolved in deionized water and serially diluted to concentration of 50 μg/ml. They were prepared daily. 10 g DL was weighted into centrifuge bottle and added 50 ml petroleum ether. Then the sample was centrifuged for 15 min at 1800 rpm. Supernatant was decanted and discarded, and extraction was repeated. Precipitate was pulverized with a glass rod and added 100 g deionized water and weighted and placed in 85°C water bath for 25 minute. And then it was cooled to room temperature and added deionized water to original weight. It was centrifuged for 10 min at 2000 rpm until withdrawing portion of clear supernatant. The clear supernatant was filtered through 0,45 μm filter (Sartorius, GmbH, Germany).

The HPLC analysis of sugars was performed on an Agilent 1200 series HPLC system consisting of a detector RID 1200 (Agilent Technologies). Filtered samples were injected (50 μm) into the HPLC using a Luna Amino column (100A° pore size, Phenomenex, Torrance, CA, USA), 250 mm x 4.6 mm i.d. with 5μm particle size.

The mobile phase was acetonitrile:water 80:20 (v/v) at flow rate of 1.5 ml/min. HPLC chromatograms were obtained using Agilent ChemStation software.

Color measurement

The color parameters were determined by using a spectrophotometer (M-3600d, Minolta Co., Japan) at room temperature. The results were expressed in L* (lightness; 0 = black, 100 = white), a* (+a = redness, - a* = greenness) and b* (+b = yellowness, - b* = blueness) values.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using the ANOVA procedures of the IBM-SPSS statistics software (version 23.0). Principle Component Analysis (PCA) was applied using the mean values of the physicochemical, sugar and color results. The matrix data set was composed of 6 lines and 14 columns. The statistical package was R project (FactoMineR and factoextra R package).

RESULTS and DISCUSSION

Table 2 shows average protein, ash, moisture and total sugar as well as the average pH and lactic acid of the samples. It was reported that typical composition of DL is moisture (30 %, max), milk solids not fat (24 %, min), ash (2 %, max) and protein (5 %, min) (Zalazar and Perotti, 2011). Furthermore, faced with the need to establish standards for DL, some countries have created an identity and quality regulation (BSR, 1997), establishing the above mentioned limits for DL. Therefore, high moisture (especially for sample 3) and low protein (for samples 2 - 5) contents were found in the present study. Samples 3 - 5 did not comply with the typical composition values for DL when its typical composition was taken into account.

Sample 6 produced from sheep milk presented the highest protein content and the lowest moisture content. And the lowest protein content was estimated in the DL sample 5 produced from goat milk. Protein values in the cow milk DL samples (1 - 4) ranged from 3.27 to 6.43, respectively, with a significant variability ($P < 0.05$).

Ash values of the samples presented more variability as protein values did. It was also found that goat milk DL sample (5) had the highest ash content.

Moisture values of the samples (1 - 4) produced from cow milk (in the range of 26.36 – 37.58) varied significantly between the samples according to the different ingredients that could be included and different parameters in the production operated.

Table 2. Chemical composition and acidity values for the DL samples*

Samples	Protein (g/100 g)	Moisture (g/100 g)	Total sugar (g/100 g)	Ash (g/100g)	T. sugar in DM(g/100g)	Protein in DM(g/100g)	Lactic acid (g/100 g)	pH
1	$6.43^{e} \pm 0.01$	$30.94^{d} \pm 0.03$	$47.27^{c} \pm 0.23$	$1.88^{e} \pm 0.02$	$68.45^{cd} \pm 0.31$	$9.31^{e} \pm 0.01$	$0.349^{c} \pm 0.01$	$6.09^{a} \pm 0.01$
2	$3.27^{b} \pm 0.09$	$31.09^{d} \pm 0.03$	$45.06^{bc} \pm 0.60$	$1.70^{d} \pm 0.01$	$65.39^{c} \pm 0.84$	$4.74^{b} \pm 0.13$	$0.226^{b} \pm 0.00$	$6.30^{a} \pm 0.02$
3	$4.71^{d} \pm 0.06$	$37.58^{e} \pm 0.18$	$44.78^{bc} \pm 0.30$	$1.38^{a} \pm 0.01$	$71.74^{d} \pm 0.70$	$7.55^{d} \pm 0.11$	$0.098^{a} \pm 0.00$	$6.75^{b} \pm 0.07$
4	$4.40^{c} \pm 0.04$	$26.36^{b} \pm 0.25$	$43.36^{b} \pm 0.59$	$1.63^{c} \pm 0.01$	$58.88^{b} \pm 1.00$	$5.98^{c} \pm 0.03$	$0.371^{c} \pm 0.02$	$6.07^{a} \pm 0.09$
5	$1.76^{a} \pm 0.04$	$28.04^{a} \pm 0.16$	$38.94^{a} \pm 0.04$	$2.56^{f} \pm 0.01$	$54.11^{a} \pm 0.26$	$2.44^{a} \pm 0.01$	$0.386^{c} \pm 0.01$	$6.19^{a} \pm 0.04$
6	$9.00^{f} \pm 0.04$	$25.24^{c} \pm 0.42$	$45.19^{bc} \pm 1.55$	$1.55^{b} \pm 0.02$	$60.45^{b} \pm 2.20$	$12.03^{f} \pm 0.1$	$0.235^{b} \pm 0.02$	$6.31^{ab} \pm 0.13$

[a-f] Means with different letters in the same column are significantly different ($P < 0.05$) using Tukey's test.
* Values are means ± standard deviation.
Analyses were performed in triplicate.
(DM represents dry matter)

Regarding acidity, samples 1, 4 and 5 were statistically ($P < 0.05$) close, with lactic acid values of 0.35, 0.37 and 0.39, respectively, while sample 3 was significantly different from the other DL samples with its low acidity ($P < 0.05$). It was found that pH has a strong correlation with L* (lightness) (r=0.723, $P < 0.01$). Decreasing lightness with decreasing pH indicated that non-enzymatic browning reactions occurred at higher rates. It's known that at pH= 5 - 6, there is active participation of the sugars in the Maillard reaction, increasing the tendency to browning (Oliveira et al., 2009).

As shown in Table 3, differences were also observed for all sugars between samples ($P < 0.05$). Sample 1 showed the highest lactose content while sample 4 and 6 presented the highest sucrose values. Only samples 1 and 2 include glucose while this sugar was not found in the other DL samples, suggesting heterogeneity of the technological processing of DLs.

Table 3. Sugars (lactose, sucrose and glucose) in DL samples obtained by HPLC

Samples	Lactose (g/100 g)	Sucrose (g/100 g)	Glucose (g/100 g)
1	$10.87^{e} \pm 0.05$	$35.47^{ab} \pm 0.26$	$0.93^{b} \pm 0.07$
2	$9.71^{d} \pm 0.13$	$33.18^{a} \pm 0.39$	$2.17^{c} \pm 0.09$
3	$7.00^{c} \pm 0.28$	$37.78^{cd} \pm 0.59$	-
4	$2.50^{a} \pm 0.18$	$40.86^{de} \pm 0.76$	-
5	$5.41^{b} \pm 0.09$	$33.53^{a} \pm 0.13$	-
6	$2.70^{a} \pm 0.13$	$42.49^{e} \pm 1.68$	-

[a-e] Means with different letters in the same column are significantly different ($P < 0.05$) using Tukey's test.
* Values are means ± standard deviation.
Analyses were performed in triplicate.

Color measurements were carried out in order to detect differences between both production techniques and determine deviations according to the use of additives. A change in color can be an indication for non-enzymatic browning reactions, which occur during heating. As shown in Table 4, significant differences ($P < 0.05$) were observed for all samples with respect to the color measurements. The differences among the samples are probably due to the differences in protein and carbohydrate composition and the changes in time and temperature according to the

each production protocol as well as the differences of milk type used in the manufacturing. Sample 4 showed the lowest lightness and yellowness parameters tending to be darker (Table 4). This sample was found to be significantly different from the other samples. This result indicated that Maillard and caramelization reactions occurred most intensively in the sample 4. When DL is produced by a prolonged processing time and / or by an excess of neutralizing agent, dark color can appear. On the other hand, this characteristic is very much desirable in DL used in ice cream manufacture (Zalazar and Perotti, 2011).

Table 4. Color parameters of the DL samples (L*; lightness, a*; redness and b*; yellowness)

Samples	L*	a*	b*
1	$48.68^{c} \pm 0.21$	$6.49^{c} \pm 0.49$	$17.24^{cd} \pm 1.65$
2	$46.23^{c} \pm 0.91$	$6.66^{c} \pm 0.07$	$13.82^{bc} \pm 0.05$
3	$57.36^{d} \pm 0.45$	$1.73^{a} \pm 0.10$	$11.58^{b} \pm 0.35$
4	$30.66^{a} \pm 0.28$	$3.55^{b} \pm 0.04$	$3.30^{a} \pm 0.02$
5	$43.48^{b} \pm 0.98$	$8.36^{d} \pm 0.29$	$17.63^{d} \pm 1.10$
6	$48.25^{c} \pm 0.50$	$6.92^{c} \pm 0.35$	$14.55^{bcd} \pm 0.73$

[a-d] Means with different letters in the same column are significantly different ($P < 0.05$) using Tukey's test.
[*] Values are means ± standard deviation.
Analyses were performed in triplicate.

Color parameters varied between the DL samples according to the formulation, different type of milk used in production, different additives and manufacturing technique. In the previous study, lightness values, detected on nine commercial DL sample representative of Argentinean market, ranged between 31.2 and 42.5 (Pauletti et al., 1992). In the present work, the L* value is somewhat lower for only sample 4 (30.66) while the L* values are higher in the other samples (43.48 – 57.36).

Furthermore, the highest lightness and the lowest redness values of sample 3 (Table 4) could indicate that the non-enzymatic browning reactions occurred at the lowest level in this sample.

Figure 2 and 3 show the PCA results performed with the data of physicochemical, sugar and color analysis of the DL samples. In order to separate the DL samples, PCA was used and it was demonstrated that the use of the analysis made it possible to reveal the differences among the samples. In present study, three main components (Dim 1 – Dim 3) were used, which explained 84.6 % of the variation. The first and second components (Dim 1 and Dim 2) contributed to 39.6 % and 28 % of the data, respectively. The third component (Dim 3) contributed to 17 % of the data. The need of using three dimensions suggests that there is a wide variability in the parameters of the DL production.

Dim 1 is positively associated with moisture, total sugar content, pH and L* value, which are important in the characterization of sample 3. The negative quadrant of Dim 1 was influenced by lactic acid, ash content and a* value and these variables characterized sample 5 (Figure 2).
Dim 2 is negatively associated with protein and sucrose contents. These variables were important for the characterization of sample 4 and sample 6. The positive quadrant of Dim 2 was influenced by glucose, lactose and b* value and the parameters characterized sample 2 (Figure 2).

Dim 3 is positively associated with protein, total sugar, a* and b* values. These variables were important in the characterization of sample 1, positioned on the positive quadrant of Dim 3 (Figure 3).

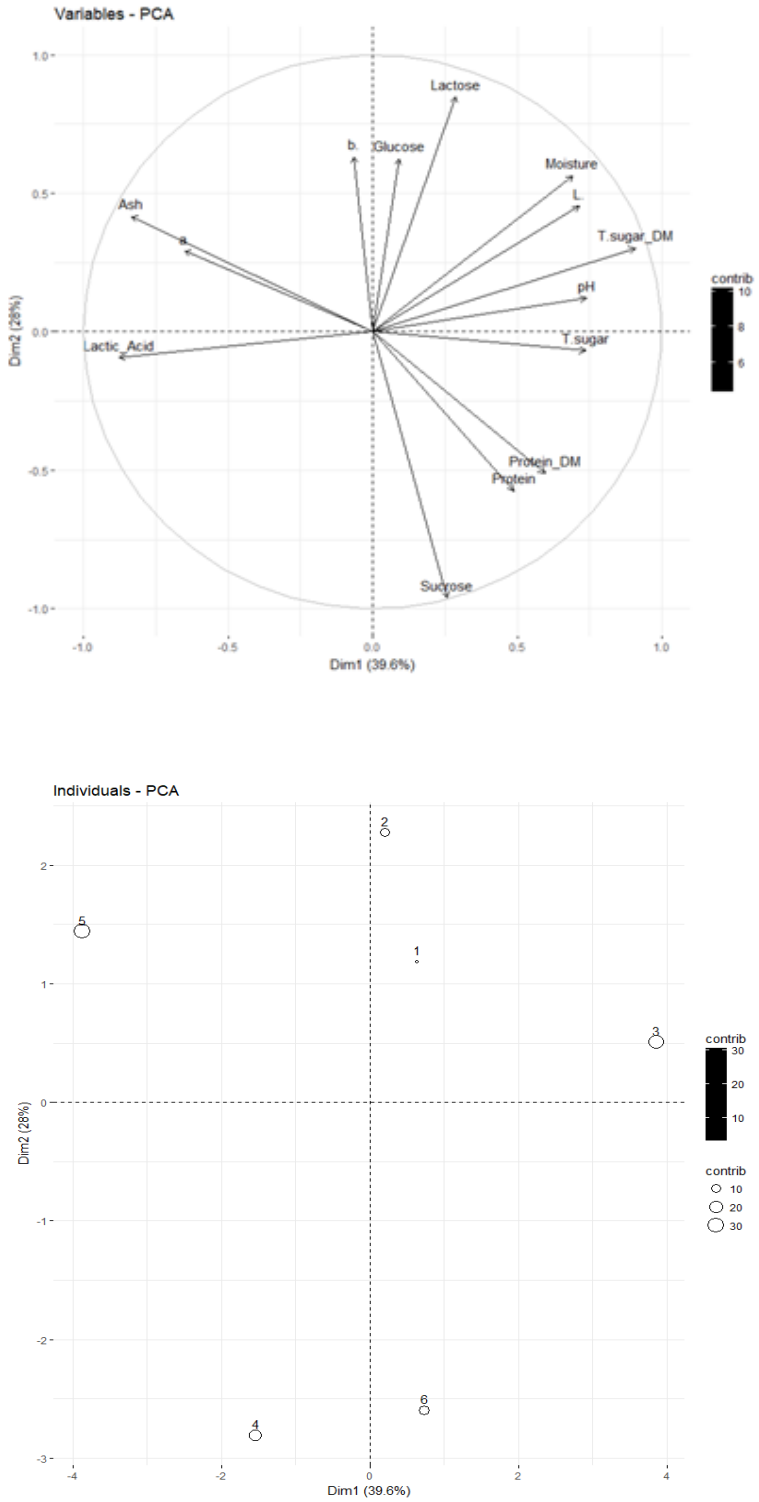

Figure 2. Principle component analysis (PCA) of DL samples, Dim 1 x Dim 2, of parameters (Variables-PCA) and samples (Individuals-PCA)

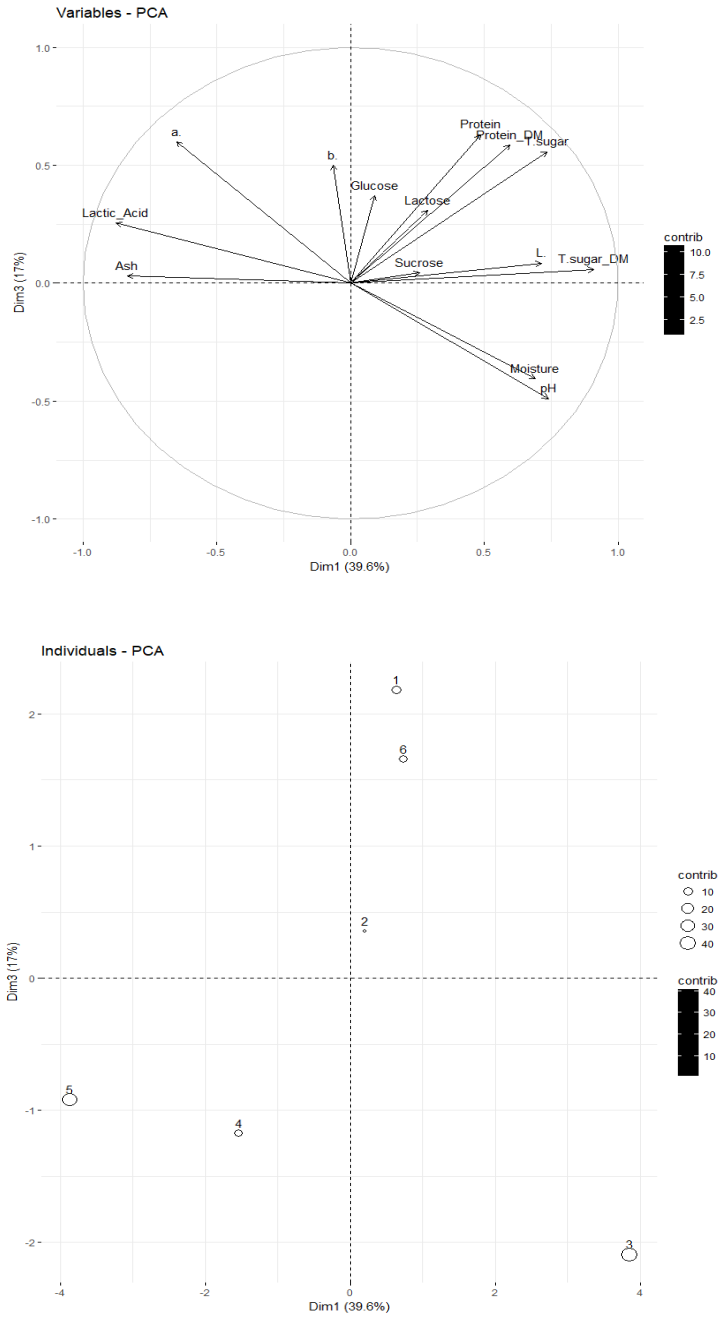

Figure 3. Principle component analysis (PCA) of DL samples, Dim 1 x Dim 3, of parameters (Variables-PCA) and samples (Individuals-PCA)

CONCLUSIONS

From the results obtained, it was observed that physicochemical properties of the DL samples have varied greatly. Color also presented differences between the samples. It is clear that the different properties in the DL samples related to the characteristics of milk, milk type used in production, method for obtaining milk, sweeteners and other additives and manufacturing process. With respect to the sugars, sucrose

exhibited to be the most predominant in the DL matrix as compared to lactose and glucose. In the present study, it was also demonstrated that the use of PCA made it possible to separate the DL samples with distinct physicochemical characteristics.

The use of DL, which already has a wide range of application in the food industry, has been also increasing as breakfast in Turkey and as an expected result of this; its production has been also increasing. Therefore, it becomes even more important to identify the properties of DL and evaluate the aspects involved in the identity of the product. In the further studies, the present work will be repeated in new commercial DL products that will be manufactured and furthermore, textural profiles and volatile compounds of the DL products will be determined.

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