Artigo Original
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00354

Effect of macromolecules on the viscosity of whole condensed milk

Aryele Nunes da Cruz Encide SAMPAIO[1], Camila koutsodontis Cerqueira CÉZAR[1], Evelyn Fernanda Flores CARON[1], Gustavo Nunes de MORAES[1], Fernanda RAGHIANTE[2], Fabio Sossai POSSEBON[1], Juliano PEREIRA[1], Otávio Augusto MARTINS[1]*

Abstract

The viscosity of a fluid refers to the resistance to flow, such as in the case of whole condensed milk. Condensed milk is a product created by partially dehydrating milk, concentrated milk, or reconstituted milk, and then sugar is added. Its fat and protein content can be adjusted to meet specific characteristics. The aim of this study was to evaluate the effect of macromolecules on the viscosity of various brands of whole condensed milk. The brands of whole condensed milk analyzed were classified as LCA, LCB, LCC, LCD, and LCE, with a total of 50 samples from different batches. The viscosity was measured using the Ford cup method, which was validated through tests for linearity, detection limit, quantification limit, and repeatability. The physicochemical tests included viscosity, soluble solids (ºBrix), moisture, protein, lipids, RMF, carbohydrates, total caloric value, and SNG. The main findings showed that the LCC, LCD, and LCE brands had high levels of macromolecules (protein, lipids, and carbohydrates) and viscosity. Consequently, it was concluded that high concentrations of macromolecules in whole condensed milk result in increased viscosity of the product.

Keywords: ford cup; macromolecules; physicochemical analysis.

Practical Application: High concentrations of macromolecules have the effect of increasing the viscosity of whole condensed milk.

1 INTRODUCTION

The rheological properties of foods are essential for numerous reasons, among which the following stand out: (a) a knowledge of the flow behavior and deformation properties of foods is essential in the design and sizing of equipment such as belt conveyors, pipes, storage tanks, sprayers, or pumps for food handling. Viscosity is also used to estimate and calculate momentum, heat, and energy transport phenomena; (b) rheological data can be very interesting to modify the manufacturing process or formulation of a final product so that the texture parameters of the food are within the range considered desirable by consumers; (c) rheological studies can provide information that facilitates a better understanding of the structure or distribution of molecular components of foods, especially macromolecular components, as well as predicting structural changes during processes such as conditioning and elaboration to which they are subjected; and (d) continuous viscosity measurements are increasingly important in many food industries to control the proper functioning of the production process, as well as the quality of raw materials, intermediate, and finished products (Berk, 2009; Navas, 2006).

The concept of viscosity involves the problems of fluid flow, treated by rheology, as a measure of the frictional resistance that a moving fluid offers to an applied shear force. Therefore, viscosity can be defined as resistance to the gradual deformation of a medium and is related to the difference in the rate of shear deformation in a fluid medium. Viscosity is due to friction between neighboring particles moving at different speeds and depends on temperature and confining pressure for most media (Barnes, 2000; Hack, 2021; Hughes, 2006; Moore, 1976).

Viscosity can be independent (Newtonian or ideal viscous medium) or dependent (non-Newtonian medium) on shear strain rate and time. Temperature and confining pressure can also influence. Gases, fluids, foods (condensed milk and honey), and many terrestrial materials behave viscously (Atkins & Paula, 2014; Hack, 2021; Moore, 1976).

Viscosity depends on the physicochemical characteristics and temperature conditions of the material. Therefore, a viscometer is a device used to measure a material's resistance to flow through friction or flow time. There are several methods for determining viscosity. The most common ones use rotary, orifice, and capillary viscometers (Brasil, 2008).

Received: 17 June, 2024.
Accepted: 10 July, 2024.
[1]*Universidade Estadual Paulista "Júlio de Mesquita Filho", School of Veterinary Medicine and Animal Science, Food Inspection Laboratory, Botucatu, São Paulo, Brazil.*
[2]*Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Uberlândia, Minas Gerais, Brazil.*
*Corresponding author: otavio.a.martins@unesp.br
Conflict of interest: nothing to declare.
Fundig: Laboratory of Applied Physical Chemistry to Food, the Public Nutrition Guidance Service (SOAP), and the Support Foundation for Veterinary Hospitals of Unesp (FUNVET).

Determining the viscosity of fresh milk and condensed milk uses an orifice viscometer (Almeida et al., 2017). Condensed milk is a product resulting from the partial dehydration of milk, concentrated milk, or reconstituted milk, with added sugar, and its fat and protein contents can be adjusted solely to meet the characteristics of the product (São Paulo, 2018).

Viscosity can be interpreted as resistance to flow. The greater the intermolecular forces or the size of the molecules (macromolecules) that are flowing, the greater the internal friction and therefore the greater the viscosity. Viscosity is the resistance that a fluid food presents to flow, such as condensed milk. This resistance is defined as the internal friction that results from the movement of one layer of fluid about another (Atkins & Paula, 2014; Moore, 1976).

This work aimed to evaluate the effect of macromolecules on the viscosity of whole condensed milk from different brands sold in the Botucatu region, São Paulo, Brazil.

2 MATERIALS AND METHODS

2.1 Samples

Five brands of whole condensed milk sold in Botucatu City, São Paulo, Brazil, were evaluated. Brands of whole condensed milk were classified as LCA, LCB, LCC, LCD, and LCE. In total, 10 samples from different batches of each brand were analyzed for a total of 50 samples. The samples were sent to the Physical-Chemistry Laboratory of Foods of Animal Origin of the Public Food Guidance Service (SOAP) of the Department of Animal Production and Preventive Veterinary Medicine of the Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Brazil. Each assay was performed in triplicate (n = 150). The results were expressed as mean ± standard deviation.

2.2 Viscosity determination

The viscosity determination of the samples was carried out using a Gehaka® Ford cup viscometer. The Ford cup viscometer met the standards of and ABNT (1986) and ASTM (2023). The equipment measurement and testing procedure followed the manufacturer's recommendations contained in the manual.

To choose the appropriate orifice number for the Ford cup, three samples of the same brand of condensed milk with the same manufacturing batch were used. The sample flow time was determined for each orifice number (numbers 3, 4, 5, 6, 7, and 8), and the mean and standard deviation of time (in seconds) were calculated. Based on this, the best orifice for analysis was adopted with the following characteristics: (a) the test that obtained a standard deviation ≤ 3% of the average number of seconds and (b) a flow time between 20 and 100 s according to the manufacturer's manual (Gehaka, 2000).

Validation of viscosity determination in whole condensed milk was carried out to verify whether the methodology was suitable for the intended purpose. The parameters used were linearity through the standard curve, limit of detection, limit of quantification, and repeatability (Brasil, 2011; 2014; 2017; SBM, 2022).

The appropriate hole in the Ford cup was selected based on the above study. The LCA, LCB, LCC, LCD, and LCE samples were homogenized at 25°C ± 0.5°C. The Ford cup has been properly leveled. The hole was closed with the finger, and the cup was filled with the sample to the highest level without bubbles. The excess sample was removed using a flat glass plate. The finger was removed from the hole, the glass was flat, and the stopwatch was started. When the first interruption of the flow occurred, the timer was stopped. The time (in seconds) was applied to the formula (mm^2/s) provided by the manufacturer according to the selected orifice, where viscosity = mm^2/s; t = seconds; and 1 mm^2/s = 1 cST; therefore, 1 cSt = 0.000001 m^2/s = 1 mm^2/s (Gehaka, 2000).

2.3 Determination of Soluble Solids by Refractometry

The determination of soluble solids (°Brix) was carried out using the refractometer (Leica® 1375331L0). Before determining the °Brix of the samples, the refractometer was properly calibrated according to the manufacturer's manual. Add a drop of sample to the prism of the refractometer. Soon after, he lowered the prism lid and read the shadow line on the scale. The result was expressed in %.

2.4 Moisture determination

A volume of 5 g of the sample was weighed in a porcelain capsule previously heated in an oven at 105°C for 2 h, cooled in a desiccator with silica to room temperature, and weighed. The capsule with the sample was heated in an oven at 105°C for 6 h, then placed in a desiccator with silica at room temperature, and then weighed. The calculation was performed using the following formula (Equation 1):

$$\text{Moisture (g/100 g)} = (100 \times N) / M \quad (1)$$

where:

N = g of moisture (mass loss in g);

M = g of the sample (Brasil, 1981; 2005a; 2022; IAL, 2008).

2.5 Protein determination (Kjeldahl method)

A volume of 0.5 g of the homogenized sample was added onto tissue paper and then transferred to the Kjeldahl tube. Furthermore, 10 mL of sulfuric acid (1.820–1.825 or 1.84 g/mL) and about 1 g of the catalytic mixture were added. The tube was sent to the (TE-007 Tecnal®) digester block at 400°C for ± 4 h in the hood until the solution became blue-green and free of undigested material (black dots). Let it cool for some time and then the tube was transferred to the nitrogen distiller (TE-0364 Tecnal®). The tapered end of the soda was dipped in 10 mL of 0.05 M sulfuric acid, contained in a 250 mL Erlenmeyer flask with four drops of 0.2% methyl red indicator. The 40% sodium hydroxide solution was added to the Kjeldahl tube containing the digested sample, using a funnel with a tap, until a slight excess of base was achieved. The sample was heated to boiling point and distilled until approximately 150 mL (± 5 min) of distillate was

obtained. The sample was titrated in excess of 0.05 M sulfuric acid with a 0.1 M sodium hydroxide solution (Equation 2).

$$\text{Protein (g/100 g)} = (V \times 0.14 \times f)/M \quad (2)$$

Where:

V = difference between the volume (mL) of 0.05 M sulfuric acid solution used and the volume (mL) of 0.1 M sodium hydroxide solution used in the titration;

M = g of the sample;

f = 6.25 (AOAC, 1995; Brasil, 1981; 2005a; 2022; IAL, 2008).

2.6 Determination of lipids (Soxhlet method)

An amount of 2–5 g of the sample in a Soxhlet cellulose cartridge was oven-dried at 105°C for 1 h. Place a small portion of cotton wool in the cartridge. Transfer the cartridge to the Soxhlet (Tecnal® TE-044) extraction device. The reboiler flask at 105°C was attached to the extractor, a sufficient quantity of ethyl ether (± 80 mL) was added, and parts of the equipment were adapted appropriately. The sample was kept under continuous extraction heating for 6 h–8 h. Later, the reboiler flask was removed after recovering all the ethyl ether. The flask with the extracted residue was transferred to an oven at 105°C for 1 h. Then the sample was cooled in a desiccator to room temperature. Later, it was weighed and the heating operations were repeated for 30 min in the oven and cooled until a constant weight was reached (maximum 2 h) (Equation 3):

$$\text{Lipids (g/100 g)} = (N \times 100)/M \quad (3)$$

where:

N = g of lipids;

M = g of the sample (AOAC, 1995; Brasil, 1981; 2005a; 2022; IAL, 2008).

2.7 Determination of fixed mineral residue

In total, 2 g of the sample was weighed in a porcelain capsule previously heated in a muffle furnace at 550°C for 2 h, cooled in a desiccator with silica to room temperature, and weighed. The ashes of the sample were placed on a heating plate at a low temperature. The carbonized sample was incinerated in a muffle furnace at 550°C for 6 h until it turned white or slightly grayish, cooled in a desiccator with silica to room temperature, and then weighed. The calculation was carried out with the following formula (Equation 4):

$$\text{RMF (g/100 g)} = (100 \times N)/M \quad (4)$$

Where:

N = g of ash;

M = g of the sample (Brasil, 1981; 2005a; 2022; IAL, 2008).

2.8 Determination of carbohydrates

The values obtained in the determinations of protein (g/100 g), lipids (g/100 g), moisture (g/100 g), and RMF (g/100 g) were used. The values obtained were applied to the proximate composition formula (Equation 5) (Brasil, 1981; 2005a; 2013; 2022; IAL, 2008):

$$\text{carbohydrates (g/100 g)} = [100 - (\text{protein} + \text{lipids} + \text{moisture} + \text{RMF})] \quad (5)$$

2.9 Calories

The caloric values of 1 g of protein (4 kcal), lipids (9 kcal), and carbohydrates (4 kcal) were used. The values obtained for protein (%), lipids (%), and carbohydrates (%) were applied to the following formula (Equation 6) (Brasil, 1981; 2005b; 2013; 2022; IAL, 2008):

$$\text{Calorie (kcal/100 g)} = [(\text{protein} \times 4) + (\text{lipids} \times 9) + (\text{carbohydrates} \times 4)] \quad (6)$$

2.10 Non-fat solids

The values obtained in the determination of moisture (g/100 g) and lipids (g/100 g) were applied to the following non-fat solids formula (SNG) (Equation 7) (Brasil, 1981; 2005a; 2013; 2022; IAL, 2008):

$$\text{(g/100 g)} = [100 - (\text{moisture} + \text{lipids})] \quad (7)$$

2.11 Statistical analysis

The values of the tests obtained from the samples carried out in triplicates were statistically analyzed by analysis of variance (ANOVA) through a completely randomized design or randomized test and complemented with the Tukey test to compare means, considering 5% significance (Montgomery, 2020).

3 RESULTS

The best holes to use in the Ford cup for determining the viscosities of the LCA, LCB, LCC, LCD, and LCE brands are numbers 6 and 7 (Table 1). The orifice number 6 was chosen. The viscosity formula was then used according to the manufacturer's manual: viscosity (mm^2/s) = 14.92t - 15.56. The data analyzed for the holes were presented as a coefficient of variation (CV) of 13.94% and a p-value of 0.0001.

Tables 2–4 depict the validation of the viscosity method through linearity parameters; LD/LQ; and repeatability, respectively. In the study of linearity, the correlation coefficients (r), linear (a), and angular (b) were 0.9906, -117.1581, and 15.4315, respectively (Table 2). The limits of detection (LD) and quantification (LQ) were 3.08 and 9.80 mm^2/s, respectively (Table 3). In the repeatability study, three CVs were obtained: 0.0536%, 0.0845%, and 0.0578% (Table 4).

Table 1. Mean ± standard deviation of flow time (in seconds) of the three samples of whole-type condensed milk sold in Botucatu City, São Paulo, Brazil, from the same date of manufacture from holes numbered 3, 4, 5, 6, 7, and 8 of the Ford cup according to the manufacturer's manual. Statistical analysis was complemented with the Tukey test at 5% significance.

Ford cup hole number	Sample 1	Sample 2	Sample 3	Mean ± standard deviation
3	800	910	1022	910.67 ± 111.00[1] d*
4	552	703	719	658.00 ± 92.15[1] c
5	457	462	463	460.67 ± 3.21[2] b
6	93	93	93	93.00 ± 0.00[2] a
7	99	100	95	98.00 ± 2.65[2] a
8	107	99	111	105.67 ± 6.11[3] a

[1]Standard deviation above 10% of the average recommends changing the orifice and measuring the Ford cup; [2]Standard deviation <3% of the mean demonstrates that the hole can be used without correction; [3]Standard deviation of 3–10% of the average must be corrected using a measurement curve; *CV = 13.94% and $p < 0.0001$.

Table 2. Linearity of the standard curve for determining the viscosity (mm²/s) of whole condensed milk (LCI) sold in Botucatu City, São Paulo, Brazil. The abscissa axis = concentration (g/100 g) of LCI aqueous solution and the ordinate axis = viscosity (mm²/s) of the solutions.

Sample tested	LCI concentration (g/100 g)	Mean ± standard deviation[1]
1	25	282.84 ± 1.03 mm²/s
2	40	566.32 ± 0.53 mm²/s
3	55	670.76 ± 0.72 mm²/s
4	70	879.64 ± 1.07 mm²/s
5	85	1.207.88 ± 2.00 mm²/s
6	100	1.476.44 ± 3.21 mm²/s
Linear coefficient (a)		-117.1581
Angular coefficient (b)		15.4315
Correlation coefficient (r)		0.9906
Line equation		Y = 15.4315X - 117.1581

[1]Orifice number 6 of the Ford cup and viscosity (mm²/s) = 14.92t - 15.56.

Table 3. Limits of detection (LD) and quantification (LQ) for determining the viscosity (mm²/s) of condensed milk sold in Botucatu City, São Paulo, Brazil. The orifice used in the Ford cup was number 6 with the following formula: viscosity (mm²/s) = 14.92t - 15.56.

Repetition	mm²/s
1	2.500
2	2.499
3	2.499
4	2.500
5	2.500
6	2.501
7	2.498
Mean ± standard deviation	2,499.57 ± 0.98
t (one-sided with 99% confidence)	3.143
LD (mm²/s)[1]	3.08
LQ (mm²/s)[2]	9.80

[1]$LD = t_{(n-1;\,1-\alpha)} \cdot s$; [2]$LQ = 10 \cdot s$.

Table 4. Repeatability for determining viscosity (mm²/s) of whole condensed milk sold in Botucatu City, São Paulo, Brazil. The orifice used in the Ford cup was number 6 with the following formula: viscosity (mm²/s) = 14.92t - 15.56.

Repetition	Test 1	Test 2	Test 3
1	2.077	2.250	2.388
2	2.076	2.251	2.388
3	2.077	2.252	2.389
4	2.074	2.255	2.389
5	2.075	2.255	2.387
6	2.076	2.252	2.385
7	2.075	2.253	2.388
Average	2,075.71 mm²/s	2,252.57 mm²/s	2,387.71 mm²/s
Standard deviation	1.1127 mm²/s	1.9024 mm²/s	1.3801 mm²/s
CV[1]	0.0536%[2]	0.0845%[2]	0.0578%[2]

[1]CV (%) = [standard deviation/mean] x 100; [2]Ideal = $CV < 10\%$.

The average viscosity test values (mm²/s) of the LCD whole condensed milk brand (2,498.20 mm²/s ± 1.37 mm2/s) were significantly higher ($p < 0.01$) than the LCA (2,075.38 mm²/s ± 1.00 mm²/s), LCB (2,253.67 mm²/s ± 1.38 mm²/s), LCC (2,386.60 mm²/s ± 1.06 mm²/s), and LCE (2,460.87 mm²/s ± 3.33 mm²/s) brands. The LCA whole condensed milk brand presented the lowest viscosity value statistically compared to the other brands evaluated (Table 5).

The whole condensed milk brands LCC (73.07 °Brix ± 0.58 °Brix), LCD (73.00 °Brix ± 0.74 °Brix), and LCE (73.07 °Brix ± 0.64 °Brix) presented significantly higher mean values ($p < 0.01$) compared to the brands LCA (65.87 °Brix ± 0.82 °Brix) and LCB (68.93 °Brix ± 0.87 °Brix) about soluble solids content. The LCA whole condensed milk brand had the lowest °Brix value statistically compared to the other brands (Table 6).

The LCB whole condensed milk brand (32.73 g/100 g ± 0.94 g/100 g) presented the highest significant mean value ($p < 0.01$) of moisture compared to the other mean values of the LCA (31.67 g/100 g ± 0.71 g/100 g), LCC (27.13 g/100 g ± 0.90 g/100 g), LCD (26.83 g/100 g ± 0.91 g/100 g), and LCE (26.93 g/100 g ± 0.83 g/100 g) brands. The LCC, LCD, and LCE brands statistically presented the lowest average moisture content values (Table 7).

The average protein content values of the whole condensed milk brands LCC (8.65 g/100 g ± 0.09 g/100 g), LCD (8.61 g/100 g ± 0.08 g/100 g), and LCE (8.63 g/100 g ± 0.07 g/100 g) were significantly higher ($p < 0.01$) than LCA (7.46 g/100 g ± 0.09 g/100 g) and LCB (7.46 g/100 g ± 0.10 g/100 g) brands. As reported, the lowest protein values were the LCA and LCB brands (Table 8).

The whole condensed milk brands LCC (7.92 g/100 g ± 0.11 g/100 g), LCD (7.93 g/100 g ± 0.08 g/100 g), and LCE (7.94 g /100 g ± 0.10 g/100 g) presented significantly higher mean values ($p < 0.01$) of lipid content compared to LCA (6.81 g/100 g ± 0.07 g/100 g) and LCB (6.81 g/100 g ± 0.07 g/100 g) brands. Therefore, the LCA and LCB brands had the lowest lipid values (Table 9).

The LCB whole condensed milk brand (2.03 g/100 g ± 0.12 g/100 g) demonstrated a significantly higher average value

Table 5. Mean ± standard deviation of viscosity analysis (mm^2/s) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance. The orifice used in the Ford cup was number 6 with the following formula: viscosity (mm^2/s) = 14.92t - 15.56.

Brands	Mean ± standard deviation
LCA	2,075.38 ± 1.00 a[1]
LCB	2,253.67 ± 1.38 b
LCC	2,386.60 ± 1.06 c
LCD	2,498.20 ± 1.37 e
LCE	2,460.87 ± 3.33 d

Values followed by different letters in the same column differ significantly (p<0.05).
[1]$p < 0.01$ and $CV = 2.60\%$.

Table 6. Mean ± standard deviation of soluble solids content (°Brix) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	65.87 ± 0.82 a[1]
LCB	68.93 ± 0.87 b
LCC	73.07 ± 0.58 c
LCD	73.00 ± 0.74 c
LCE	73.07 ± 0.64 c

Values followed by different letters in the same column differ significantly (p<0.05).
[1]$p < 0.01$ and $CV = 1.04\%$.

Table 7. Mean ± standard deviation of moisture content (g/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	31.67 ± 0.71 b[1]
LCB	32.73 ± 0.94 c
LCC	27.13 ± 0.90 a
LCD	26.83 ± 0.91 a
LCE	26.93 ± 0.83 a

[1]$p < 0.01$ and $CV = 2.97\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

Table 8. Mean ± standard deviation of protein content (g/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	7.46 ± 0.09 a[1]
LCB	7.46 ± 0.10 a
LCC	8.65 ± 0.09 b
LCD	8.61 ± 0.08 b
LCE	8.63 ± 0.07 b

[1]$p < 0.01$ and $CV = 1.03\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

Table 9. Mean ± standard deviation of lipid content (g/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	6.81 ± 0.07 a[1]
LCB	6.81 ± 0.07 a
LCC	7.92 ± 0.11 b
LCD	7.93 ± 0.08 b
LCE	7.94 ± 0.10 b

[1]$p < 0.01$ and $CV = 1.16\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

($p < 0.01$) of fixed mineral residue (RMF) compared to the other LCA (1.84 g/100 g ± 0.16 g/100 g), LCC (1.66 g/100 g ± 0.14 g/100 g), LCD (1.72 g/100 g ± 0.17 g/100 g), and LCE (1.75 g/100 g ± 0.19 g/100 g) brands. However, the LCC and LCD brands presented the lowest average RMF values (Table 10).

The whole condensed milk brands LCC (54.64 g/100 g ± 0.96 g/100 g), LCD (54.90 g/100 g ± 0.86 g/100 g), and LCE (54.75 g /100 g ± 0.95 g/100 g) resulted in significantly higher average values ($p < 0.01$) of carbohydrate content compared to LCA (52.22 g/100 g ± 0.74 g/100 g) and LCB (50.96 g/100 g ± 1.02 g/100 g) brands. The LCB brand had the lowest average carbohydrate value (Table 11).

The whole condensed milk brands LCC (324.42 kcal/100 g ± 3.21 kcal/100 g), LCD (325.44 kcal/100 g ± 3.60 kcal/100 g), and LCE (324.96 kcal /100 g ± 03.77 kcal/100 g) presented significantly higher mean values ($p < 0.01$) of calories compared to the LCA (300.04 kcal/100 g ± 3.33 kcal/100 g) and LCB brands (295.00 kcal/100 g ± 3.74 kcal/100 g). Therefore, the LCB brand had the lowest total calorie value (Table 12).

The whole condensed milk brands LCC (64.95 g/100 g ± 0.98 g/100 g), LCD (65.24 g/100 g ± 0.90 g/100 g), and LCE (65.13 g /100 g ± 0.84 g/100 g) demonstrated significantly higher mean values ($p < 0.01$) in SNG content compared to LCA (61.52 g/100 g ± 0.67 g/100 g) and LCB (60.45 g/100 g ± 0.98 g/100 g) brands. The LCB brand had the lowest average SNG value (Table 13).

The CVs of the viscosity, soluble solids, moisture, protein, lipids, RMF, carbohydrates, calories, and SNG tests were 2.60, 1.04, 2.97, 1.03, 1.16, 8.70, 1.70, 1.13, and 1.39%, respectively (Tables 5–13). The CVs demonstrated that the data obtained were homogeneous and stable in the experiment.

4 DISCUSSION

The Ford cup viscometer is a classic instrument used to control the kinematic viscosity of fluids, such as paints, varnishes, resins, honey, condensed milk, yogurt, and others (Barbosa & Rodrigues, 2004). This equipment is normally made of polished aluminum and has a hole through which the fluid drains, made of stainless steel and polished on the inside, with or without a level, and leveling feet that ensure lightness and accuracy during measurements. ABNT (1986; 2015) established that the Ford cup viscometer must be calibrated using at least three oils of known

Table 10. Mean ± standard deviation of the RMF content (g/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	1.84 ± 0.16 b[1]
LCB	2.03 ± 0.12 c
LCC	1.66 ± 0.14 a
LCD	1.72 ± 0.17 a
LCE	1.75 ± 0.19 ab

[1]$p < 0.01$ and $CV = 8.70\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

Table 11. Mean ± standard deviation of carbohydrate content (g/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	52.22 ± 0.74 b[1]
LCB	50.96 ± 1.02 a
LCC	54.64 ± 0.96 c
LCD	54.90 ± 0.86 c
LCE	54.75 ± 0.95 c

[1]$p < 0.01$ and $CV = 1.70\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

Table 12. Mean ± standard deviation of the total caloric value (kcal/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	300.04 ± 3.33 b[1]
LCB	295.00 ± 3.74 a
LCC	324.42 ± 3.21 c
LCD	325.44 ± 3.60 c
LCE	324.65 ± 3.77 c

[1]$p < 0.01$ and $CV = 1.13\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

Table 13. Mean ± standard deviation of SNG (g/100 g) of different brands of whole condensed milk (LCA, LCB, LCC, LCD, and LCE) sold in Botucatu City, São Paulo, Brazil. Statistical analysis was complemented with the Tukey test at 5% significance.

Brands	Mean ± standard deviation
LCA	61.52 ± 0.67 b[1]
LCB	60.45 ± 0.98 a
LCC	64.95 ± 0.98 c
LCD	65.24 ± 0.90 c
LCE	65.13 ± 0.84 c

[1]$p < 0.01$ and $CV = 1.39\%$.
Values followed by different letters in the same column differ significantly (p<0.05).

kinematic viscosity, such that the viscometer's measurement range is covered between 20 s and 100 s.

Barbosa and Rodrigues (2004) also reported that standard deviations of measurement values must be determined and compared: (a) for standard deviations of up to 3%, the viscometer will be used without correction; (b) for standard deviations of 3–10%, they must be corrected using the calibration curve; and (c) for standard deviations above 10%, it is recommended to change the orifice and recalibrate the viscometer. In the present experiment, we calibrated the Ford cup with a sample of whole condensed milk to choose the best hole (3, 4, 5, 6, 7, and 8) and found that holes 6 and 7 satisfied the following criteria established by ABNT (1986; 2015), which are: (a) the measurement range was between 20 and 100 s and (b) the standard deviation of up to 3% of the calculated average.

Orifice number 6 of the Ford cup was chosen to determine the viscosity of the LCA, LCB, LCC, LCD, and LCE samples with the following formula established by the equipment manufacturer: $mm^2/s = 14.92t - 15.56$ (Gehaka, 2000). Almeida et al. (2011) used hole number 2 of the Ford cup to determine the viscosity of fresh milk.

The Pearson correlation coefficient (r) is a measure of the degree of linear relationship between two quantitative variables and is one of the criteria for approval of linearity (SBM, 2022). This coefficient varies between the values -1 and 1. The value 0 means that there is no linear relationship, the value 1 indicates a perfect linear relationship, and the value -1 indicates a perfect linear relationship, but the inverse, that is, when one of the variables increases and the other decreases. The closer it is to 1 or -1, the stronger the linear association between the two variables. In this work, the correlation coefficient was 0.9906, which indicates that the method developed presents a perfect linear relationship.

The LD of an individual analytical procedure is the smallest amount of analyte in the sample that can be detected but is not necessarily quantified under the conditions established for the assay. The LD for an analytical procedure may vary depending on the sample type. Therefore, it is necessary to ensure that all processing steps of the analytical method are included in determining this detection limit. The lowest acceptable concentration is considered to be the lowest concentration for which a degree of uncertainty can be considered satisfactory. Independent evaluations must be carried out on samples with a concentration equal to the determined detection limit (Perez, 2010; SBM, 2022). The LD of the experiment was 3.08 mm^2/s for determining viscosity.

The LQ of an individual analytical procedure is the smallest amount of the analyte in the sample that can be quantitatively determined with acceptable precision and accuracy under established experimental conditions. LQ is important for quantitative methods. The International Union of Pure and Applied Chemistry (IUPAC) proposes the value 10 as the standard value of the equation LQ = 10s. However, values 5 or 6 can also be adopted depending on the analytical rigor required (Perez, 2010; SBM, 2022). In the experiment, the value 10 was used to calculate the LQ and the value obtained was 9.80 mm^2/s.

Repeatability is the degree of agreement between the results of successive measurements of the same measurement carried out under the same measurement conditions. The repeatability limit is the maximum acceptable difference between two repetitions, that is, between two independent results, from the same test and in the same laboratory, under the same conditions (SBM, 2022). In the research, we assessed that the repeatability was satisfactory in the three evaluations, as the CVs were 0.0536, 0.0845, and 0.0578% because values were lower than 10% (the maximum suggested CV). In this work, viscosity values were higher in samples LCC (2,386.60 ± 1.06 mm^2/s), LCD (2,498.20 ± 1.37 mm^2/s), and LCE (2,460. 87 ± 3.33 mm^2/s), which presented the highest concentrations (g/100 g) of the macromolecules evaluated (proteins, lipids, and carbohydrates).

Macromolecules, which can be classified into proteins, carbohydrates, lipids, and nucleic acids, are of great interest to be explored in various industries, including pharmaceutical, biomedical, cosmetic, and food, due to their unique structural and functional characteristics. Therefore, the effective extraction of macromolecules (proteins, lipids, and carbohydrates) from their natural sources has always been a research topic of interest (Bilal & Iqbal, 2020; Jeevanandam et al., 2022; Ling & Hadinoto, 2022).

In this study, it was observed that the LCC, LCD, and LCE samples presented the highest protein concentrations (LCC: 8.65 g/100 g ± 0.09 g/100 g; LCD: 8.61 g/100 g ± 0 .08 g/100 g; and LCE: 8.63 g/100 g ± 0.07 g/100 g), lipids (LCC: 7.92 g/100 g ± 0.11 g/100 g; LCD: 7 .93 g/100 g ± 0.08 g/100 g; and LCE: 7.94 g/100 g ± 0.10 g/100 g), and carbohydrates (LCC: 54.64 g/100 g ± 0.96 g/100 g; LCD: 54.90 g/100 g ± 0.86 g/100 g; and LCE: 54.75 g/100 g ± 0.95 g/100 g). These data prove that the high concentrations of macromolecules evaluated (proteins, lipids, and carbohydrates) correspond to the high concentrations of viscosities determined in samples of whole condensed milk.

Corroborating the results obtained, Almeida et al. (2017) reported that increasing the viscosity of milk causes a change in its consistency, resulting in a change in flow and making it difficult to escape. Cheng et al. (2019) reported that changes in protein concentration had a greater effect on viscosity data within each temperature evaluated than lipid concentration in milk samples. However, Yousefi et al. (2023) reported that the addition of chitosan-coated nanoliposomes led to an increase in the viscosity of milk samples. They also observed the formation of some particles deposited in the samples added with chitosan, which is attributed to the interactions of chitosan with milk proteins, particularly whey proteins (Yousefi et al., 2023). Toca et al. (2022) reported that lactose is an important component of milk and is the main carbohydrate. Perrone et al. (2008) assessed that the contents of proteins, lactose, and nonfat solids are decisive for the draining time of condensed milk; therefore, they are important variables for the composition of the final viscosity of the product (Perrone, 2011). The data demonstrate some similarities with the data of Perrone et al. (2008) and Perrone (2011) about the contents of soluble solids (°Brix), moisture (g/100 g), RMF (g/100 g), and SNG (g/100 g).

It was found that the LCC, LCD, and LCE samples presented the highest concentrations of soluble solids (LCC: 73.07 °Brix ± 0.58 °Brix; LCD: 73.00 °Brix ± 0.74 °Brix; and LCE: 73.07 °Brix ± 0.64 °Brix) and SNG (LCC: 64.95 g/100 g ± 0.98 g/100 g; LCD: 65.24 g/100 g ± 0.90 g/100 g; and LCE: 65. 13 g/100 g ± 0.84 g/100 g). The highest moisture and RMF concentrations were in the LCA (moisture: 31.67 g/100 g ± 0.82 g/100 g and RMF: 1.84 g/100 g ± 0.16 g/100 g) and LCB (moisture: 32.73 g/100 g ± 0.94 g/100 g and RMF: 2.03 g/100 g ± 0.12 g/100 g), which presented the lowest viscosity concentrations determined in the whole condensed milk samples evaluated.

The National Health Surveillance Agency (Brasil, 2005b) reported that the calculation of the total caloric value or energy value of food can be carried out with the following conversion factors: 4 kcal/g or 17 kJ/g, 4 kcal/g or 17 kJ/g, and 9 kcal/g or 37 kJ/g for carbohydrates, proteins, and lipids, respectively. The LCC, LCD, and LCE samples that presented the highest concentrations of protein, lipid, and carbohydrate macromolecules also demonstrated higher values for total calorie content. This demonstrates that samples with high levels of macromolecules correspond to high-calorie values. Atkins and Paula (2014) reported that highly viscous liquids flow slowly and slow the movement of objects through them. They also mentioned that high concentrations of macromolecules increase the viscosity of a solution. In our research, the whole condensed milk samples evaluated (LCC, LCD, and LCE) with high levels of macromolecules showed high viscosity values.

5 CONCLUSION

The brands of whole condensed milk, LCC, LCD, and LCE, presented the highest values of viscosities and macromolecules (proteins, lipids, and carbohydrates), and the high concentrations of macromolecules in whole condensed milk have the effect of increasing its viscosity.

REFERENCES

Almeida, M. B. M., Almeida, J. A. G., Moreira, M. E. L., & Novak, F. R. (2011). Adequacy of human milk viscosity to respond to infants with dysphagia: experimental study. *Journal of Applied Oral Science*, *19*(6), 554-559. https://doi.org/10.1590/S1678-77572011000600003

Almeida, M. B. M., Gomes Júnior, S. C., Silva, J. B., Silva, D. A., & Moreira, M. E. L. (2017). Study on viscosity modification of human and formula milk for infants with dysphagia. *Revista CEFAC*, *19*(5), 683-689. https://doi.org/10.1590/1982-021620171956017

Associação Brasileira de Normas Técnicas (ABNT) (1986). *NBR 5849 – Determinação de viscosidade pelo copo Ford*. ABNT.

Associação Brasileira de Normas Técnicas (ABNT) (2015). *NBR 5849 – Determinação de viscosidade pelo copo Ford*. ABNT.

Association of Official Analytical Chemists (AOAC) (1995). *Official methods of analysis of the Association of Official Analytical Chemists*. AOAC.

ASTM (2023). *ASTM D1200 - Standard Test Method for Viscosity by Ford Viscosity Cup*. ASTM International. https://doi.org/10.1520/D1200-23

Atkins, P., & Paula, J. (2014). *Atkins' Physical Chemistry* (10th ed.). OUP.

Barbosa, A. P., & Rodrigues, C. R. C. (2004). Nova metodologia para calibração de viscosímetros do tipo copo Ford. In *Rede Paranaense de Metrologia - METROSUL IV - IV Congresso Latino-Americano de Metrologia*.

Barnes, H. A. (2000). *A handbook of elementary rheology*. Institute of Non-Newtonian Fluid Mechanics, University of Wales.

Berk, Z. (2009). *Food process engineering and technology*. Elsevier.

Bilal, M., & Iqbal, H. M. (2020). Biologically active macromolecules: Extraction strategies, therapeutic potential, and biomedical perspective. *International Journal of Biological Macromolecules*, *151*, 1-18. https://doi.org/10.1016/j.ijbiomac.2020.02.037

Brasil (1981). Ministério da Agricultura. Laboratório Nacional de Referência Animal. *Métodos analíticos oficiais para controle de produtos de origem animal e seus ingredientes* (Vol. II – Métodos físicos e químicos). LANARA.

Brasil (2005a). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Métodos físico-químicos para análise de alimentos* (4th ed.). Ministério da Saúde.

Brasil (2005b). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Rotulagem nutricional obrigatória - Manual de orientação às indústrias de alimentos - 2a versão atualizada*. Agência Nacional de Vigilância Sanitária, Ministério da Saúde.

Brasil (2008). Agência Nacional de Vigilância Sanitária. *Guia de controle de qualidade de produtos cosméticos* (2nd ed.). Anvisa.

Brasil (2011). Ministério da Agricultura, Pecuária e Abastecimento. *Guia de validação e controle de qualidade analítica: fármacos em produtos para alimentação e medicamentos veterinários*. MAPA/ACS.

Brasil (2013). Ministério da Educação. Universidade Federal Rural de Pernambuco. Colégio Agrícola Dom Agostinho Ikas (CODAI). *Produção alimentícia – análises físico-químicas dos alimentos*. Ministério da Educação, CODAI.

Brasil (2014). Ministério da Agricultura, Pecuária e Abastecimento. *Manual de validação, verificação/confirmação de desempenho, estimativa da incerteza de medição e controle de qualidade intralaboratorial*. MAPA/DEQ/CGAL.

Brasil (2017). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. RDC nº 166, de 24 de julho de 2017. Validação de métodos analíticos. *Diário Oficial da União*, *141*.

Brasil (2022). Ministério da Agricultura, Pecuária e Abastecimento. *Métodos Oficiais para Análise de Produtos de Origem Animal*. Ministério da Agricultura, Pecuária e Abastecimento.

Cheng, N., Barbano, D. M., & Drake, M. A. (2019). Effect of pasteurization and fat, protein, casein to serum protein ratio, and milk temperature on milk beverage color and viscosity. *Journal of Dairy Science*, *102*(3), 2022-2043. https://doi.org/10.3168/jds.2018-15739

Gehaka (2000). *Manual de instrução: Viscosímetro copo Ford*. Gehaka.

Hack, H. R. (2021). *Viscosity*. Engineering Geology. ESA, Faculty of Geo-Information Science and Earth Observation (ITC), University of Twente.

Hughes, B. (2006). *Drilling fluids; reference manual*. Baker Hughes Drilling Fluids.

Instituto Adolfo Lutz (IAL) (2008). *Métodos físico-químicos para análise de alimentos*. Instituto Adolfo Lutz.

Jeevanandam, J., Acquah, C., & Danquah, M. K. (2022). Biological macromolecules as antidiabetic agents. In: Nayak, A. K., Dhara, A. K., & Pal, D. (Eds.), *Biological Macromolecules* (pp. 229-241). Academic Press.

Ling, J. K. U., & Hadinoto, K. (2022). Deep eutectic solvent as green solvent in extraction of biological macromolecules: a review. *International Journal of Molecular Sciences*, *23*(6), 3381. https://doi.org/10.3390/ijms23063381

Montgomery, D. C. (2020). *Design and analysis of experiments* (10th ed.). Wiley.

Moore, W. J. (1976). *Físico-química* (2 vol.). Edgard Blucher.

Navas, J. S. R. (2006). Introducción a la reología de los alimentos. *ReCiTeIA*, *6*(1), 1-46.

Perez, M. A. F. (2010). Validação de métodos analíticos: como fazer? Por que ela é importante? *Instituto de Tecnologia de Alimentos*, *22*(3), 1-9.

Perrone, I. T., Silva, P. H. F., Reis, P. I., & Faria, L. M. G. C. (2008). Avaliação do método prático de determinação de viscosidade por escoamento e suas correlações com a composição do leite condensado. *Revista do Instituto de Laticínios "Cândido Tostes"*, *63*(362), 17-21.

Perrone, I. T. (2011). Tecnologias para fabricação de doce de leite, leite condensado e soro em pó. *Informe Agropecuário*, *32*(262), 24-33.

São Paulo (2018). Secretaria de Defesa Agropecuária do Estado de São Paulo. *Instrução Normativa SDA - 47, de 26/10/2018 – Regulamento Técnico de Leite condensado*. Secretaria de Defesa Agropecuária do Estado de São Paulo.

Sociedade Brasileira de Metrologia (SBM) (2022). Escola Nacional de Tecnologia Industrial Básica. *Validação de métodos de ensaio*. SBM.

Toca, M. C., Fernández, A., Orsi, M., Tabacco, O., & Vinderola, G. (2022). Lactose intolerance: myths and facts. An update. *Archivos Argentinos de Pediatría*, *120*(1), 59-66. https://doi.org/10.5546/aap.2022.eng.59

Yousefi, M., Jafari, S. M., Ahangari, H., & Ehsani, A. (2023). Application of nanoliposomes containing nisin and crocin in milk. *Advanced Pharmaceutical Bulletin*, *13*(1), 134-142. https://doi.org/10.34172/apb.2023.014